# KINETIC AND EQUILIBRIUM STUDIES OF THE REACTION OF 5,5'-DITHIOBIS(2-NITROBENZOATE) WITH HAEMOGLOBINS OF DOG AND DONKEY

**BY**

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## ABSTRACT

The CysF9[93]β sulphydryl group is an indicator for tertiary and quaternary structure change in haemoglobin. Allosteric effectors such as proton and inositol hexakisphosphate (inositol-$P_6$) influence its reactivity. This work was undertaken to study the effects of inositol-$P_6$ and pH on the kinetics and equilibrium of the reaction of CysF9[93]β of dog (*Canis familiaris*) and donkey (*Equus asinus*) haemoglobins with 5,5'-dithiobis(2-nitrobenzoate) (DTNB).

The number of sulphydryl groups in haemoglobin was determined by titrations with p-hydroxymercury(II)benzoate (pMB) and DTNB. The pseudo-first order kinetics of the reaction of haemoglobin with DTNB were studied at 25$^{o}$C, with and without inositol-$P_6$. Values of observed rate constant ($k_{obs}$) were plotted against [DTNB] to obtain the apparent second order forward rate constant, $k_F$. Equilibrium studies of the DTNB reaction were carried out at 25°C, with and without inositol-$P_6$. An equation was derived for the determination of the equilibrium constant of the reaction, $K_{equ}$, within a series of equilibria. Kinetics and equilibrium experiments were carried out on the oxy, carbonmonoxy and aquomet derivatives of the haemoglobins in the pH range 5.6 to 9.0.

The number of sulphydryl groups in dog haemoglobin reacting with DTNB and pMB were 2 and 4 per tetramer respectively, while those of donkey haemoglobin gave two sulphydryl groups per haemoglobin tetramer for both reagents. The plot of $k_{obs}$ against [DTNB] was linear at each pH, with a non-negligible positive intercept, indicating that the reaction of CysF9[93]β of dog and donkey haemoglobins with DTNB is a reversible process. The slope of each plot ($k_F$) varied with pH, giving different profiles for the three haemoglobin derivatives. Inositol-$P_6$ had a significant effect on $k_F$ for the two haemoglobins. The $K_{equ}$ showed strong pH dependences for all derivatives of the two haemoglobins. Inositol-$P_6$ had only a minor effect on the affinity of dog haemoglobin for DTNB. By contrast, it increased the affinity of donkey haemoglobin by two orders of

magnitude. These results could not be accounted for by changes in the tertiary conformation transition constant, $K_{rt}$ caused by inositol-$P_6$. Inositol-$P_6$ had little effect on $K_{rt}$ in the case of dog haemoglobin: $K_{rt}$ for stripped dog haemoglobin was calculated as 0.75 ± 0.13; in the presence of inositol-$P_6$ $K_{rt}$ became 0.70 ± 0.20. $K_{rt}$ for stripped donkey haemoglobin increased from 0.46 ± 0.02 to 0.83 ± 0.20 in the presence of inositol-$P_6$. This indicated that changes in tertiary structure govern the affinity of haemoglobin for DTNB.

**Keywords:** Dog and Donkey haemoglobins, Sulphydryl groups, 5,5'-dithiobis (2-nitrobenzoate), Tertiary structure transition.

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## DEDICATION

This thesis is dedicated to the Almighty God for His help.

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Benevolent Orighomisan Atolaiye

# CERTIFICATION

We certify that the work reported in this thesis was carried out under our supervision by Benevolent Orighomisan Atolaiye in the Department of Chemistry, University of Ibadan, Ibadan.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| α | Alpha subunit of haemoglobin |
| β | Beta subunit of haemoglobin |
| γ | Gamma subunit of haemoglobin |
| ATP | Adenosine triphosphate |
| 2,3-BPG | 2,3 – Bisphosphoglycerate |
| 2-DTP | 2,2-dithiobispyridine |
| Inositol-$P_5$ | Inositol-pentakisphosphate |
| Inositol-$P_6$ | Inositol-hexakisphosphate |
| Mb | Myoglobin |
| Hb | Haemoglobin |
| DTNB | 5,5'-dithiobis(2-nitrobenzoate) |
| TNB- | Chromophoric product of DTNB reaction; 5-thio(2-nitrobenzoate) |
| TNBH | Protonated form of $TNB^-$ |
| pMB | p-hydroxymercuri(II)benzoate |
| $P_{50}$ | Pressure at which haemoglobin is half saturated |
| $pO_2$ | Partial pressure of oxygen |
| AMP | Adenosine monophosphate |
| ADP | Adenosine diphosphate |
| $HbO_2$ | Oxyhaemoglobin |
| HbCO | Carbonmonoxyhaemoglobin |

| | |
|---|---|
| Hbmet | Aquomethaemoglobin |
| TNBH | Protonated form of TNB-, the chromophoric product of the DTNB reaction |

# CHAPTER ONE

# INTRODUCTION

## 1.1 Haemoglobin

Haemoglobin, Hb, the intracellular protein that gives red blood cells their colour, is one of the best-characterised proteins. It is the vital metalloprotein that conveys oxygen from the lungs to the tissues and facilitates the return of carbon dioxide from the tissues back to the lungs. In arterial blood, passing from the lungs through the heart to the peripheral tissues, haemoglobin is about 96% rich in oxygen. In venous blood returning to the heart, however, haemoglobin is about 64% saturated with oxygen. In addition to oxygen, haemoglobin transports hydrogen ion and carbon dioxide. The transport of oxygen by haemoglobin is based on the ability of its ferrous iron to combine reversibly with molecular oxygen instead of being irreversibly oxidized. Studies of the haemoglobin molecule have provided an understanding of the molecular events underlying biological function. They have provided fundamental insights into the structure-function relationships of proteins in general and the molecular basis of oxygen transport in particular.

Haemoglobins and the closely related protein, myoglobin, are without question two of the most important and abundant proteins of vertebrates. Every cubic centimetre of blood has five billion erythrocytes and each erythrocyte is packed with 280 million molecules of haemoglobin. Haemoglobin that can be readily isolated from the blood of any vertebrate is a heterotetramer of two α-globin and two β-globin polypeptides, with a haem group tightly bound to a pocket in each globin monomer. The movements and interactions of the α and β-globin subunits lead to the cooperative binding of oxygen to this heterotetramer, allowing it to pick up oxygen readily in the lungs and to unload it efficiently in the peripheral respiring tissues. The amino acid sequences of α and β globins are about 50% identical, regardless of which vertebrate species is the source. Myoglobin lacks the exquisite cooperativity of haemoglobins because it is a monomer, but its relationship to them is clear from the primary sequence and the virtually identical tertiary structure of its single chain to each of the four in haemoglobin.

Haemoglobins, being the most direct link between environmental conditions and body requirements, have experienced a major evolutionary pressure which has led to the development of a number of complex regulatory mechanisms intended to fulfil the physiological requirements of a given species. Investigation on the functional properties of haemoglobin from a diving mammal, the whale (*Balaenoptera acutorostrata),* indicated the existence of a sophisticated modulation mechanism based on the interplay of organic phosphates, $CO_2$ and temperature as required at varying oxygen availability at different depths (Giardina *et al*., 1992).

The functional characteristics of haemoglobin are basically derived from its subunit contacts, as well as from its interactions with effector molecules like chloride, carbon dioxide and organic phosphates such as adenosine triphosphate (ATP), 2,3-bisphosphoglycerate (2,3-BPG) and inositol pentakisphosphate, inositol-$P_5$ (Perutz, 1989). Four residues in each of the β-chains are known to constitute the organic phosphate binding site in human haemoglobins: ValNA1[1]β, HisNA2[2]β, LysEF6[82]β and HisH21[143]β (Arnone, 1972; Arnone and Perutz, 1974).

Myoglobin (Mb) is a relatively simple oxygen-binding protein found in almost all mammals, primarily in muscle tissue. It has a molecular weight of about 16,700 (Schenkman *et al*., 1997; Nelson and Cox, 2004). As a transport protein, it facilitates oxygen diffusion in muscle. Myoglobin is a monomeric haem protein that lacks cooperative oxygen binding; it serves as an intracellular storage site for oxygen in the muscle (Nelson and Cox, 2004). During periods of oxygen deprivation oxymyoglobin releases its bound oxygen which is then used for metabolic purposes such as glycolysis, pentose phosphate pathway and Krebb's cycle. Myoglobin accepts and stores the oxygen released by haemoglobin and transports it to the mitochondria.

Apart from haemoglobin and myoglobin, there are other oxygen binding proteins. Haemocyanin is the second most common oxygen transporting protein found in nature and is found in the blood of many arthropods and molluscs such as crabs, centipedes, slugs, octopuses, oysters, and squids. Haemocyanin uses copper prosthetic groups instead of iron haem groups and is blue in colour when oxygenated and colourless when deoxygenated (Sullivan *et al*., 1974; Jaenicke *et al*., 1999).

Some marine invertebrates and a few species of annelid use haemerythrin, an iron-containing non-haem protein, to carry oxygen in their blood. Haemerythrin appears pink or violet when oxygenated and colourless when deoxygenated (Klippenstein, 1980). It differs from other oxygen-binding proteins, such as

haemoglobin and haemocyanin, both in the polypeptide chain and in the metal complex used to bind oxygen reversibly. The two iron atoms in haemerythrin are bound to the imidazole rings of five histidine residues and the carboxylates of an aspartic acid and a glutamic acid (Stenkamp, 1994).

Erythrocruorin, found in many annelids, including earthworms is a giant free-floating blood protein containing numerous iron- and haem-bearing protein subunits bound together into a single protein complex with a molecular mass greater than 3.5 million Daltons (Royer Jr. *et al*., 2000).

Chlorocruorin is a giant extracellular oxygen-binding haem protein found in marine polychaete families. It contains an altered haem with an aldehyde group substituting the canonical three-ethenyl group (–CH=$CH_2$) and consequently appears as greenish red (Fox, 1949; Pallavicini *et al.*, 2001). It is very similar to erythrocruorin, but the haem group is significantly different in structure. The chlorocruorin prosthetic group differs only slightly from the haem of haemoglobin. Chlorocruorin appears green when deoxygenated and red when oxygenated (Gibson *et al.*, 1992).

Vanabin, also known as vanadium chromogen is found in the blood of sea squirts. A hypothesis states that it uses the rare metal vanadium in its oxygen binding prosthetic group (Ishit *et al*., 1995).

Leghaemoglobin, found in some plants and animals, regulates oxygen affinity via a mechanism involving a novel combination of haem pocket amino acids that lower oxygen affinity. Leghaemoglobin facilitates oxygen diffusion from outside the root to the obligate aerobes involved in nitrogen fixation inside the nodule. It however facilitates diffusion while maintaining a low (10μM) free oxygen concentration to prevent inhibition of bacterial nitrogenase complex. This plant haemoglobin therefore simultaneously fulfils the dual need for oxygen scavenging and transport (Kundu *et al.*, 2003).

## 1.2 Aims of this study

Of the 20 naturally occurring amino acids that proteins are composed of, the sulphydryl-containing amino acid cysteine (Cys) is the most reactive. The thiolate anion, $RS^-$, is over 500 times more nucleophilic than the corresponding alkoxy analogue, $RO^-$ (Streitweisser, 1956). This high reactivity of the thiolate anion has placed sulphydryl groups among the most important functional groups to be considered when investigating the reactivities, functions, mechanisms and conformations of biological macromolecules.

It has been demonstrated for a number of haemoglobins that the reaction of CysF9[93]β of haemoglobin with 5,5'-dithiobis(2-nitrobenzoate) (DTNB) is reversible (Okonjo *et al*., 2006; Okonjo *et al*., 2008; Okonjo *et al*., 2009; Okonjo *et al*., 2010). For any haemoglobin for which this is true this opens up the possibilities of measuring several parameters. The proton ($H^+$) and inositol hexakisphosphate (inositol-$P_6$) are allosteric effectors which affect the oxygen affinity of haemoglobin and the reactivity of the CysF9[93]β sulphydryl group. Organic phosphates regulate the oxygen affinity of haemoglobin in the red blood cell. Previous work on dog haemoglobin was done only on kinetics and on the assumption that the reaction of haemoglobin was irreversible. Most of the research carried out on donkey has been on evolution. The present work was planned with the following aims:

1. To test for the reversibility of the reaction of DTNB with dog and donkey haemoglobins.
2. If the reaction is reversible, to determine the kinetic and equilibrium constants of the DTNB reactions.
3. To test the effect of organic phosphates on the rate and equilibrium parameters of the DTNB reaction.
4. To determine the *pQs* of groups linked to the reaction of DTNB with CysF9[93]β of dog and donkey haemoglobins from pH dependence studies of their reactions with DTNB.
5. Since the DTNB reaction is coupled to the tertiary structure transition in haemoglobin, to determine the tertiary structure transition constant, $K_{rt}$, and the effect of organic phosphates on this parameter.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Protein primary structure

The unique characteristics of a protein are dictated by the linear sequence of its amino acids in the polypeptide chain. This is what constitutes its primary structure. The primary structure of a protein determines how it can fold and interact with other molecules in the cell to perform its function. Most human proteins are synthesised mainly from the various combinations of the 20 L-α-amino acids (Smith *et al*., 2005). The names and structures of the amino acids are shown in Table 2.1 and Fig. 2.1 respectively. In Fig. 2.1, the dotted boxes represent the side chains denoted by $R_i$.

Conventionally, amino acids are represented by a three-letter or one-letter abbreviation. The three-letter abbreviation uses the first two letters in the name plus the third letter of the name or the letter of a characteristic sound, such as Trp for tryptophan. The one-letter abbreviation uses the first letter of the name of the most frequent amino acid in proteins (for example, “A” for alanine). However, if the first letter has already been assigned to another amino acid, the letter of a characteristic sound is used (for example, “R” for arginine). A single-letter abbreviation is usually employed to represent each amino acid in a polypeptide sequence (Smith *et al*., 2005).

***Table 2.1**: Names and abbreviations of the twenty amino acids (Smith et al., 2005)*

| Name | Three-letter | One-letter | $pK_{a1}$(Carboxyl) | $pK_{a2}$(Amino) | $pK_{aR}$ (R group) |
|---|---|---|---|---|---|
| Alanine | Ala | A | 2.3 | 9.7 | |
| Arginine | Arg | R | 2.0 | 8.8 | |
| Asparagine | Asn | N | 2.2 | 9.0 | 12.5 |
| Aspartate | Asp | D | 1.9 | 9.6 | 3.9 |
| Cysteine | Cys | C | 2.0 | 10.3 | 8.4 |
| Glutamate | Glu | E | 2.2 | 9.7 | 4.1 |
| Glutamine | Gln | Q | 2.2 | 9.1 | |
| Glycine | Gly | G | 2.4 | 9.8 | |
| Histidine | His | H | 1.8 | 9.3 | 6.0 |
| Isoleucine | Ile | I | 2.4 | 9.7 | |
| Leucine | Leu | L | 2.4 | 9.6 | |
| Lysine | Lys | K | 2.2 | 9.0 | 10.5 |
| Methionine | Met | M | 2.3 | 9.2 | |
| Phenylalanine | Phe | F | 1.8 | 9.1 | |
| Proline | Pro | P | 2.0 | 11.0 | |
| Serine | Ser | S | 2.2 | 9.2 | 13.6 |
| Threonine | Thr | T | 2.1 | 9.6 | 13.6 |
| Tryptophan | Trp | W | 2.4 | 9.4 | |
| Tyrosine | Tyr | Y | 2.2 | 9.1 | 10.5 |
| Valine | Val | V | 2.3 | 9.6 | |

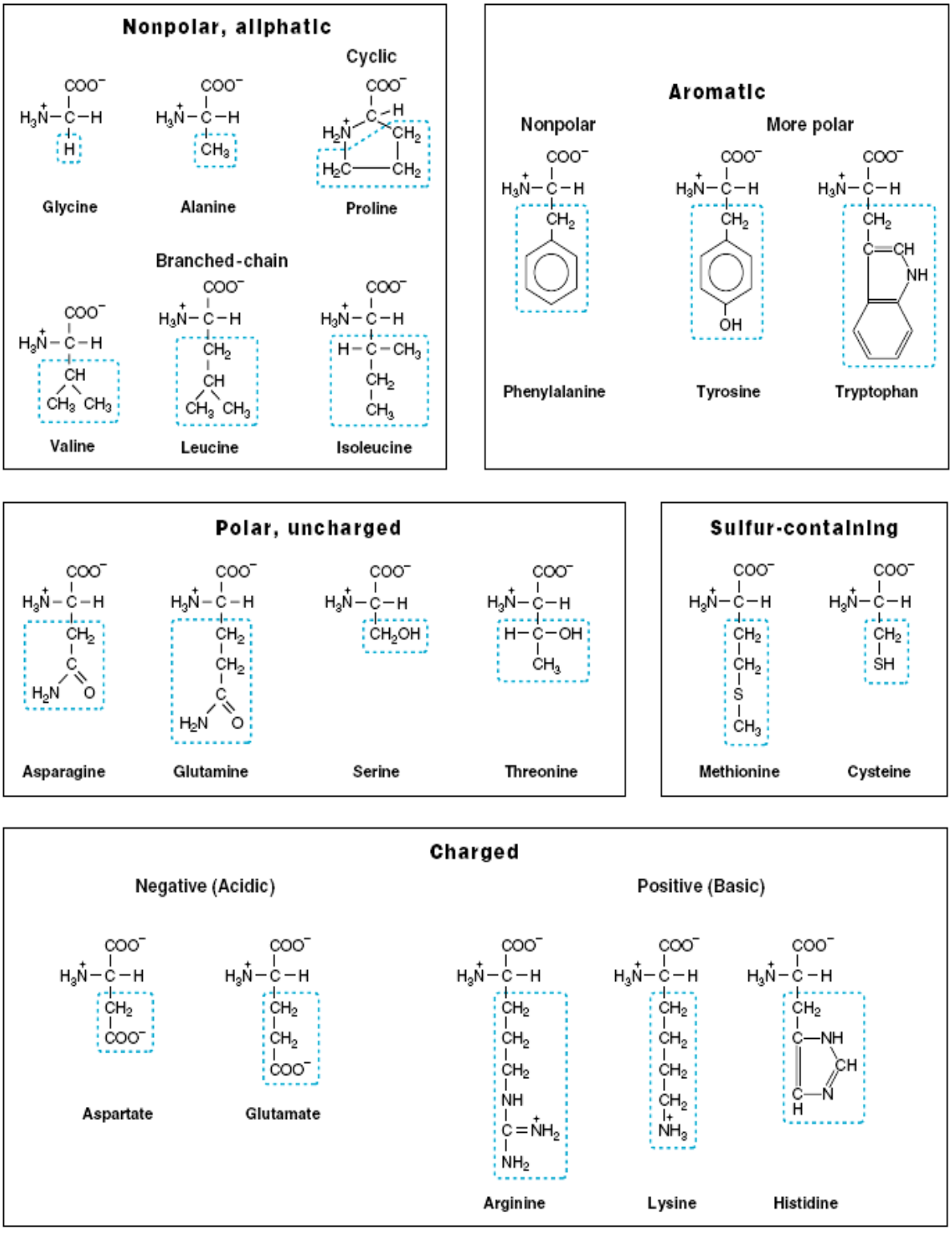



***Figure 2.1**: The structures of the twenty amino acids used in forming proteins (Smith et al., 2005*).

The chemical properties of the side chains, *R*, determine the types of bonds and interactions each amino acid in a polypeptide chain can make with other molecules. Amino acids are often grouped, using their side chains, into charged, nonpolar hydrophobic, and uncharged polar, or by structural features such as aliphatic, cyclic and aromatic. The nonpolar amino acids are alanine, valine, leucine, isoleucine, phenylalanine, and methionine. They often cluster together to exclude water because they are hydrophobic. The uncharged polar amino acids are serine, threonine, tyrosine, asparagine, and glutamine. Aspartate and glutamate are negatively charged (acidic) amino acids that form ionic bonds with positively charged (basic) amino acids such as lysine, arginine, and histidine. Methionine and cysteine are sulphur-containing amino acids. The sulphydryl group is an important component of many enzymes (Champe *et al*., 2007). The charge on an amino acid in a protein at a particular pH is determined by the $pK_a$ of each side chain that has a dissociable proton. Column 6 of Table 2.1 (page 6) shows typical side chain $pK_a$ values.

The amino acids in proteins are bonded to one another by peptide bonds formed between the carboxylic acid group of one amino acid and the amino group of the next amino acid (Equ. 1.1).

--------1.1

Mutations in the genetic code result in proteins with altered primary structure. A mutation resulting in a single amino acid substitution can affect the functioning of a protein or can confer a specific advantage to a tissue. Many proteins such as haemoglobin exist in the human population as polymorphisms (Smith *et al.*, 2005).

The haemolysates of dog (Wajeman *et al*., 1975) and donkey (Balasundaresan *et al*., 2006) contain one haemoglobin component each. The β chains of donkey and horse (*Equus caballus*) haemoglobins are identical (Smith, 1968).

## Amino acid sequences of dog haemoglobin subunits

**α- chain**

**1 10 20 30 40**

VLSPADKTNI KSTWDKIGGH AGDYGGEALD RTFQSFPTTK

**50 60 70 80**

TYFPHFDLSP GSAQVKAHGK KVADALTTAV AHLDDLPGAL

**90 100 110 120**

SALSDLHAYK LRVDPVNFKL LSH**C**LLVTLA **C**HHPTEFTPA

**130 140**

VHASLDKFFA AVSTVLTSKY R

(Antonini and Brunori, 1971)

**β- chain**

**1 10 20 30 40**

VHLTAEEKSL VSGLWGKVNV DEVGGEALGR LLIVYPWTQR

**50 60 70 80**

FFDSFGDLST PDAVMSNAKV KAHGKKVLNS FSDGLKNLDN

**90 100 110 120**

LKGTFAKLSE LH**C**DKLHVDP ENFKLLGNVL V**C**VLAHHFGK

**130 140**

EFTPQVQAAY QKVVAGVANA LAHKYH

(Brimhall *et al*., 1977)

## Amino acid sequences of donkey haemoglobin subunits

**α- chain**

**1 10 20 30 40**

VLSAADKTNV KAAWSKVGGN AGEFGAEALE RMFLGFPTTK

**50 60 70 80**

TYFPHFDLSH GSAQVKAHGK KVGDALTLAV GHLDDLPGAL

**90 100 110 120**

SNLSDLHAHK LRVDPVNFKL LSH**C**LLSTLA VHLPNDFTPA

**130 140**

VSHASLDKFL STVSTVLTSK YR

(Kilmartin and Clegg, 1967; Antonini and Brunori, 1971)

**β- chain**

**1 10 20 30 40**

VQLSGEEKAA VLALWDKVNE EEVGGEALGR LLVVYPWTQR

**50 60 70 80**

FFDSFGDLSN PGAVMGNPKV KAHGKKVLHS FGEGVHHLDN

**90 100 110 120**

LKGTFAALSE LH**C**DKLHVDP ENFRLLGNVL VVVLARHFGK

**130 140**

DFTPELQASY QKVVAGVANA LAHKYH

(Smith, 1968)

The amino acid sequences of dog and donkey haemoglobins have been presented above. Cysteines are shown in bold letters and underlined because they are the focus of interest in this study.

## 2.2 Protein secondary structure

The secondary structure of a protein describes the folding of short contiguous segments of a polypeptide chain into geometrically ordered units. (McKee and McKee, 2004). The two common secondary structures, the α-helix and the β-sheet, contain repeating elements stabilized by hydrogen bonding between atoms of the peptide bonds. In the secondary structure of haemoglobin, the polypeptide globin chains are arranged into helices, separated by non-helical turns. A complete turn of the helix contains an average of 3.6 amino acid residues and its distance per turn or pitch is 0.45nm. The stability of an α helix arises primarily from hydrogen bonds formed between the oxygen of the peptide bond carbonyl and the hydrogen atom of the peptide bond amino of the fourth residue (Fig. 2.2). The β – pleated sheet is the second form of regular secondary structure in proteins, only somewhat less common than α – helix. β – sheets consist of β – strands connected laterally by at least two or three backbone hydrogen bonds, forming a generally twisted, pleated sheet. A β – strand is a stretch of polypeptide chain typically three to ten amino acids long with backbone in an almost fully extended conformation. The higher – level association of β – sheets has been implicated in formation of the protein aggregates and fibrils observed in many human diseases, notably the amyloidoses such as Alzheimer's disease.

The X-ray crystallographic structure of haemoglobin shows that the α-chain is made up of seven helical segments while the β-chain is made up of eight helical segments. Between 70 and 80% of the residues in the protein participate in the α-helical secondary structure. The helical regions are commonly named A, B, C, ….., H, starting from the amino terminal end of the polypeptide chain. The non-helical regions are designated NA, AB, BC, CD, ....., GH, respectively. Each designation indicates the two helical regions that are joined together. The non-helical region that lies between the amino terminal end of the polypeptide chain and the A helix is designated NA; and the region between the H helix and the $COO^-$ terminal is designated HC (Shaanan, 1983; Fermi, *et al.*, 1984). The position of an amino acid residue in a polypeptide chain may be indicated by its position in a helical or non-

helical region to which it belongs. For example, CysF9β means that cysteine is the ninth residue on the F helix of the β chain, starting from the $NH_3^+$ terminal end. This is one method of identifying an amino acid in a haemoglobin chain. By convention, amino acids in a protein chain are numbered starting at the $NH_3^+$ - terminal residue and ending at the $COO^-$ - terminal residue. The position of an amino acid in a haemoglobin polypeptide chain may thus be indicated by numbering the amino acids starting from the amino terminal end of the subunit. The ninety-third amino acid residue on the β-chain of haemoglobin is a cysteine and may therefore be indicated as Cys[93]β. It is now the practice to use a combination of both methods to indicate the position of an amino acid. For example, CysF9[93]β, SerNA1[1]β, ThrA1[4]β, ArgH21[144]β, CysA11[13]α and so on (Watson and Kendrew, 1961; Perutz, 1964). The binding of haem between two specific histidine residues in the E and F helices is highly important for maintaining the secondary and tertiary structures of haemoglobin.

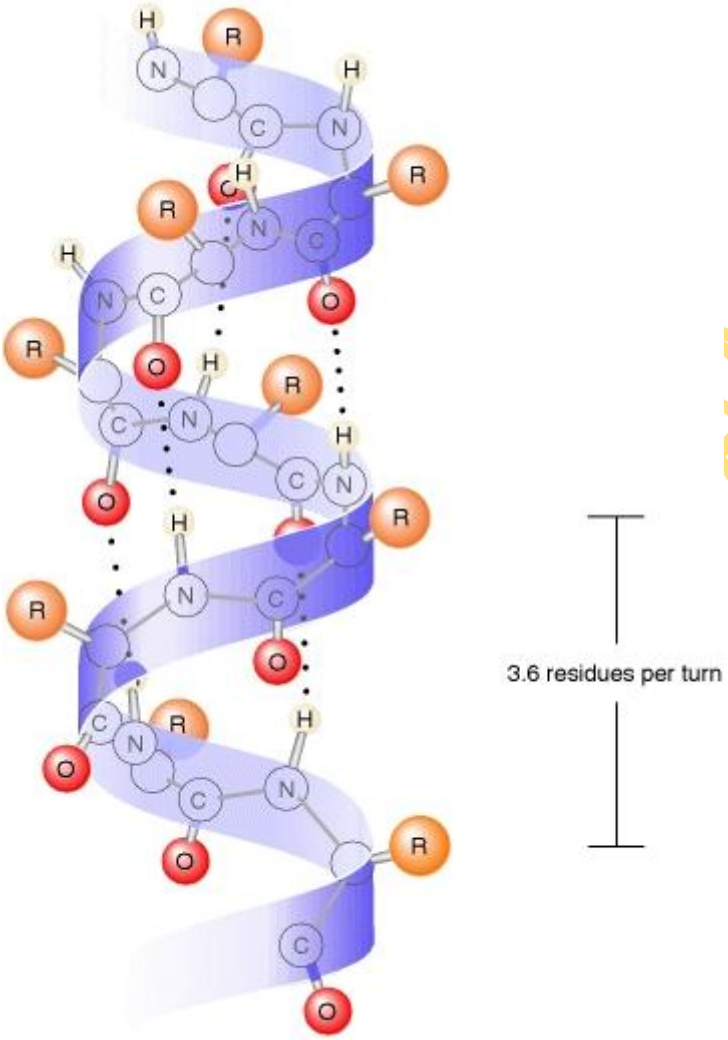



**Figure 2.2**: *The α-helix, a common basis of protein secondary structure (Lodish et al., 2003).*

## 2.3 Protein tertiary structure

The tertiary structure of a protein is the three-dimensional assembly of secondary structural units to form larger functional units (McKee and McKee, 2004). It is the arrangement of amino acid residues that gives rise to recurring three-dimensional structural patterns, the overall conformation of a polypeptide chain. Unlike the secondary structure which is stabilized by hydrogen bonds, the tertiary structure is stabilized by hydrophobic interactions between the nonpolar side chains, hydrogen bonds between polar side chains, and peptide bonds. The tertiary structure of a protein is not rigidly fixed but undergoes continual and minute fluctuations (Lodish *et al*., 2003). This variation in structure has important consequences in the function and regulation of proteins. Different ways of depicting the conformation of proteins convey different types of information (Lodish *et al*., 2003). All the levels of protein structure are interrelated as shown in Fig. 2.3.

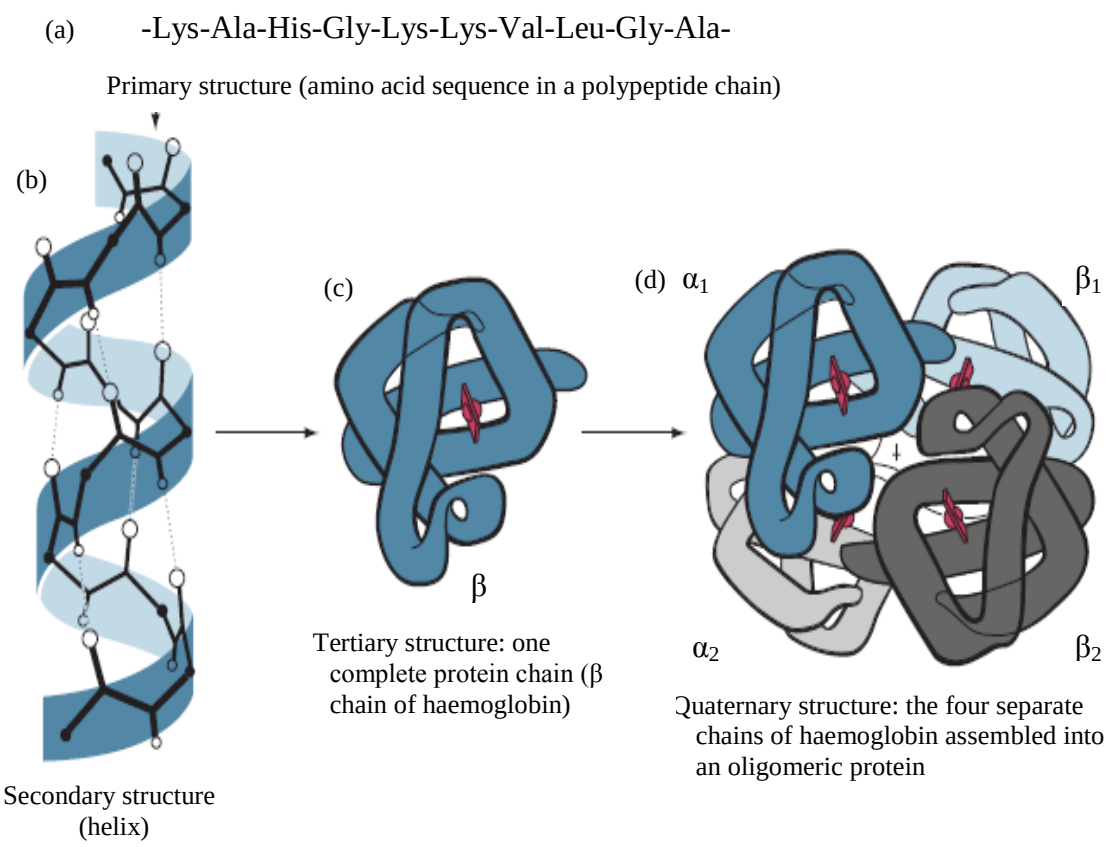



***Figure 2.3**: Levels of protein structure (Voet et al., 2006)*

## 2.4 Protein quaternary structure

The quaternary structure is important for the proper biological function of a protein. The quaternary structure reveals the number, types and spatial arrangement of polypeptide units in oligomeric proteins. In haemoglobin, it explains the relationship between the four globin chains. It is the tetrameric composition of haemoglobin that allows for a variety of functions not observable in myoglobin; a monomeric protein.

The three dimensional structure of deoxyhaemoglobin differs from that of liganded haemoglobin. X-ray crystallographic studies have shown that haemoglobin exists in two quaternary states, characterized by the presence and absence of salt bridges and by a different pattern of $\alpha_1\beta_2$ and $\alpha_2\beta_1$ interactions. The quaternary structure of deoxyhaemoglobin is denoted by the letter T (for tense or taut) while that of liganded haemoglobin is denoted by the letter R (relaxed). The T-structure of human haemoglobin is linked by salt-bridges between its four subunits, formed by the C-terminal arginine residues of the α-subunits and the C-terminal histidine residues of the β-subunits (Perutz, 1970; Baldwin and Chothia, 1979; Bettati *et al*., 1998). In the quaternary structure of deoxyhaemoglobin, there are eight electrostatic interactions (salt bridges) that are not found in liganded haemoglobin (Fig. 2.4). Binding of oxygen triggers a series of tertiary conformational changes that break salt bridges, destabilizing the T state and leading to the liganded R state (Perutz, 1970; Antonini and Brunori, 1971; Perutz, *et al*., 1998). The presence of salt bridges confers on the T form a low affinity for oxygen. The R form has a high affinity for oxygen and other ligands, such as CO and alkylisocyanides (Olson *et al.*, 1988).

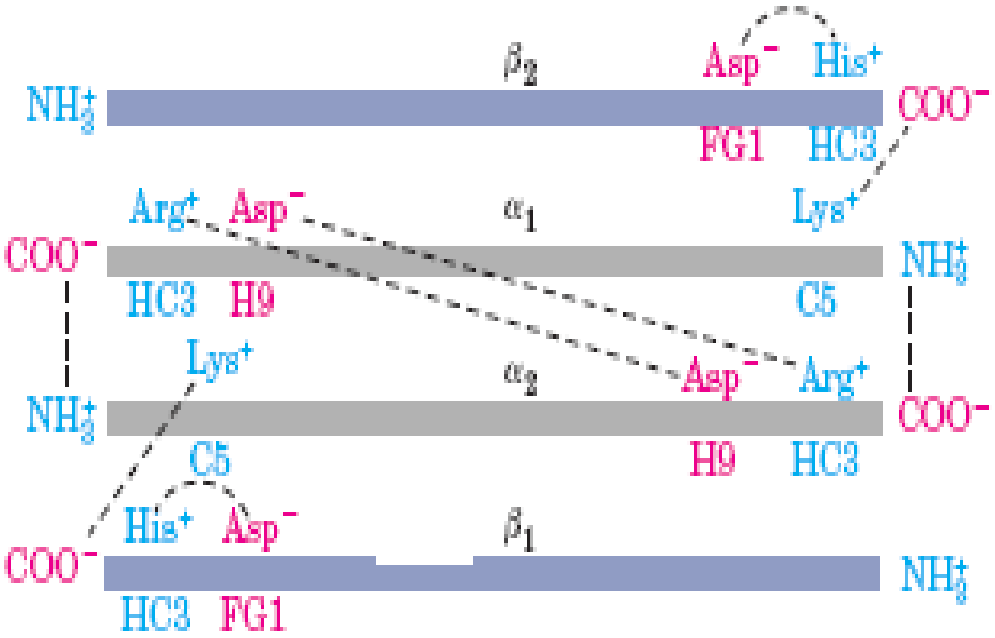



***Figure 2.4**: Some ion pairs and salt bridges that stabilize the T state of deoxyhaemoglobin.These non-covalent electrostatic cross-links are disrupted on oxygenation (Armstrong and Bennett, 1971).*

The allosteric oxygen binding properties of haemoglobin arise directly from the binding of oxygen between iron in the haem and the distal histidine (the sixth coordination site and the resultant effects of these interactions on the quaternary structure of the protein. When oxygen binds to an iron atom of deoxyhaemoglobin, it pulls the iron atom into the plane of the haem. Since the iron is directly bound to HisF8[92]β, the proximal histidine on the other side of the haem, this residue is also pulled toward the plane of the haem. The conformational change at HisF8[92]β is transmitted throughout the peptide backbone, resulting in a significant change in tertiary structure of the entire subunit and α – β couples. Conformational changes at the subunit interface lead to a new set of binding interactions between adjacent subunits. The latter changes include disruption of salt bridges and formation of new hydrogen bonds and new hydrophobic interactions, all of which contribute to the new quaternary structure (Sheriff, 2004).

## 2.5 Structure and function of haemoglobin

Haemoglobin has played a historic part in chemistry, biology and medicine. The morphology of haemoglobin crystals from different animals provided the first convincing evidence for the expression of species specificity in protein structure (Reichert and Brown, l909). Haemoglobin was named the "hydrogen atom of biology" because it provided the testing stone for the first biological applications of sophisticated new physical methods such as nanosecond kinetics, fourier transform infrared spectroscopy and high resolution nuclear magnetic resonance, and for theories of co-operative effects, such as the theory of allosteric interaction (Monod *et al*., 1965).

Haemoglobin is a tetrameric protein with the quaternary structure $\alpha_2\beta_2$ (a dimer of αβ protomers). Pairs of α and β polypeptides chains are structurally and evolutionarily related to each other and to myoglobin. Arrangement of the dimer interface provides a pathway for communication between subunits. Changes in the arrangement of subunits within the haemoglobin molecule allow it to carry oxygen from the lungs to the tissues with great efficiency. The four polypeptide chains of haemoglobin are held together by non-covalent interactions and interchain salt linkages (Berg *et al*., 2002). The reaction of haemoglobin with oxygen is cooperative, with a free energy of cooperativity of about 15 kJ $mol^{-1}$ per haem under physiological

conditions (Perutz *et al.*, 1998). When oxygen binds, electronic properties of the haem iron changes, oxygen binds to an iron atom of deoxyhaemoglobin, it pulls the iron atom into the plane of the haem, the iron which is directly bound to HisF8[92]β, the proximal histidine on the other side of the haem is also pulled toward the plane of the haem. This conformational change at HisF8[92]β is transmitted throughout the peptide backbone. The change in colour from the dark purple of oxygen-depleted venous blood to the bright red of oxygen-rich arterial blood is explained by the d – d transition that occurs as a result of the approach and binding of a ligand to the paramagnetic iron. Splitting of the energy levels into two groups ($e_g$ and $t_{2g}$) occurs which result in change in colour of iron without change in oxidation state from +2 to +3. Some small molecules, such as carbon monoxide (CO) and nitric oxide (NO), can also coordinate to haem iron even with greater affinity than oxygen. When a molecule of CO is bound to haem, oxygen is excluded; that is why CO is highly toxic to aerobic organisms. By surrounding and sequestering haem, oxygen-binding proteins regulate the access of CO and other small molecules to the haem iron (Nelson and Cox, 2004).

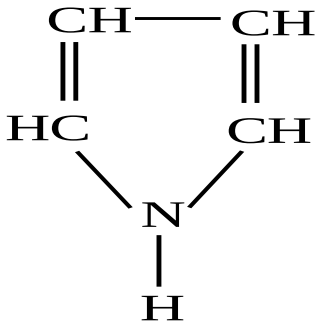
Each polypeptide chain is composed of two parts: ferroprotoporphyrin (or haem), which is responsible for the red colour of haemoglobin, and a colourless basic protein, the globin (Perutz, 1978; Berg *et al*., 2002). The haem portion is synthesised in both the mitochondria and cytosol of the immature red blood cell, while the globin (protein) portions of the molecule are synthesised by ribosomes in the cytosol. Haemoglobin consists of about six percent haem and ninety-four percent globin by weight.

## The haem

The haem is essential for oxygen transport, and the capacity of haemoglobin to bind oxygen depends on the presence of a bound prosthetic haem group (Berg *et al*., 2002). The haem group consists of an organic component, a heterocyclic ring known as porphyrin, and a central iron atom held in the ring. The basic unit of porphyrins is pyrrole (Fig. 2.5a), a ring structure consisting of four carbon atoms and a nitrogen atom. The simplest porphyrin is porphine, a cyclic tetrapyrrole in which four pyrroles are linked together by methenyl (—CH=) bridges (Fig. 2.5b) (Rao, 2006). Eight side chains serve as substituents on the porphyrin ring, two on each pyrrole. Four methyl groups, two vinyl groups and two propionate side chains are attached to porphyrin to produce protoporphyrin IX (Fig. 2.6). Porphyrins are intermediates of haem

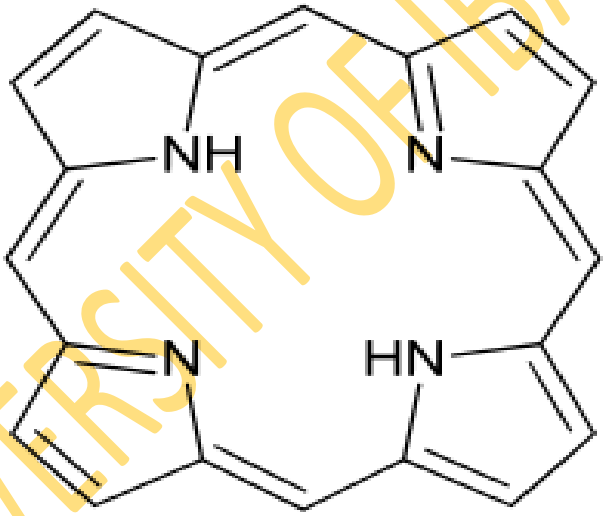
biosynthesis. Each porphyrin can exist in many isomeric forms depending on the kinds of side chains and the arrangement of these side chains. Protoporphyrin, with three types of side chains, can exist in fifteen isomeric forms, type I, II, III, and so on, depending on the arrangement of the side chains. The protoporphyrin found in haemoglobin is type IX (Sessler and Weghorn, 1997). Porphyrins are components of the haem proteins of animals and invertebrates. They are present in biological fluids like blood, bile, urine and faeces. They are also found in plants and bacteria.

**a**




Pyrrole

**b**




Porphine, the simplest porphyrin.

***Figure 2.5**: Structures of pyrrole and porphyrin (Sessler and Weghorn, 1997).*

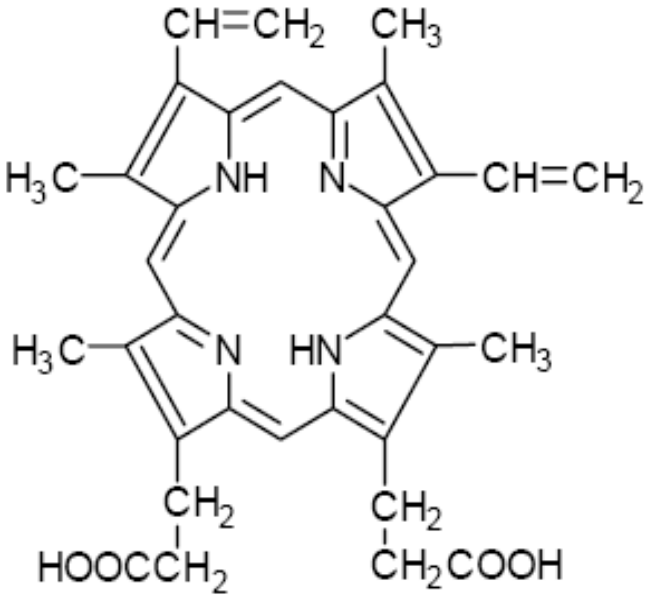



***Figure 2.6**: Protoporphyrin IX (Whitford, 2005).*

In haem, the iron atom lies at the centre of the protoporphyrin ring, bound to the four pyrrole nitrogen atoms, as shown in Fig. 2.7. The Fe atom is able to chelate six different ligands: four of the ligand positions are in a plane taken by the central nitrogen atoms in the planar porphyrin ring; two ligand positions are perpendicular to this plane. These latter ligand positions are termed the fifth and sixth co-ordination positions. The fifth co-ordination position is taken by a nitrogen atom of a histidine residue from the protein part of the haemoglobin molecule, the globin. It is this histidine that attaches the globin to the haem to form a haemoglobin subunit. This histidine, HisF8[92]β, is called the proximal histidine. The sixth co-ordination position is occupied by oxygen, as shown in Fig. 2.8. In deoxyhaemoglobin, the sixth coordination site is unoccupied and $Fe^{2+}$ is out of the haem plane towards proximal histidine.When the iron atom of the haem moves into the plane of the porphyrin, the histidine residue, HisF8[92]β bound in the fifth coordination site moves with it. The carboxyl terminal end of this helix (F8) lies in the interface between the two αβ dimers. Consequently, the structural transition at the iron atom is directly transmitted to the other subunits.

The coordinated nitrogen atoms in haem have an electron-donating character. This helps to prevent conversion of the ferrous ($Fe2^{+}$) haem iron to the ferric ($Fe^{3+}$) state. Iron in the $Fe^{2+}$ state binds oxygen reversibly but $Fe^{3+}$ does not bind oxygen. However, low levels of auto-oxidation do occur to produce methaemoglobin ($Fe^{3+}$ state). A natural occurrence of the methaemoglobin reductase enzyme helps to keep methaemoglobin at a physiologically tolerant level.

## The Globin

The protein part of haemoglobin is called globin. It is covalently attached to the haem moiety. Globin protects the haem iron from oxidation, renders the molecule soluble and causes variations in oxygen affinity between different haemoglobins. Haemoglobin has four globular polypeptide subunits. The four polypeptide chains of haemoglobin, each with one haem portion, combine to form an ellipsoidal molecule with the dimensions 64 × 55 × 50 Å (6.4 × 5.5 × 5.0 nm) and a molecular weight of about 68,000 Dalton (Cohn, 1990). Nearly all the polar amino acid residues lie on the surface of the molecule. The interior of the molecule is almost entirely non-polar (Austin and Drabkin, 1935).

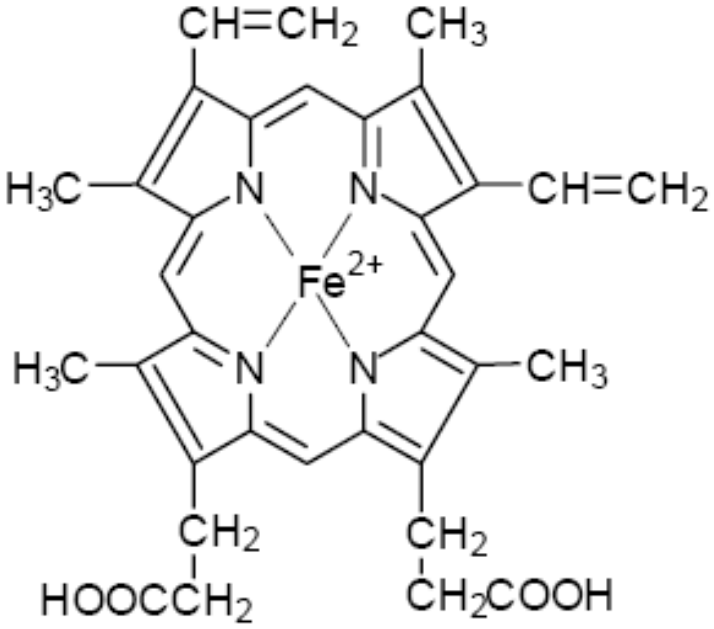



***Figure 2.7**: Haem or Fe-protoporphyrin IX (Whitford, 2005).*

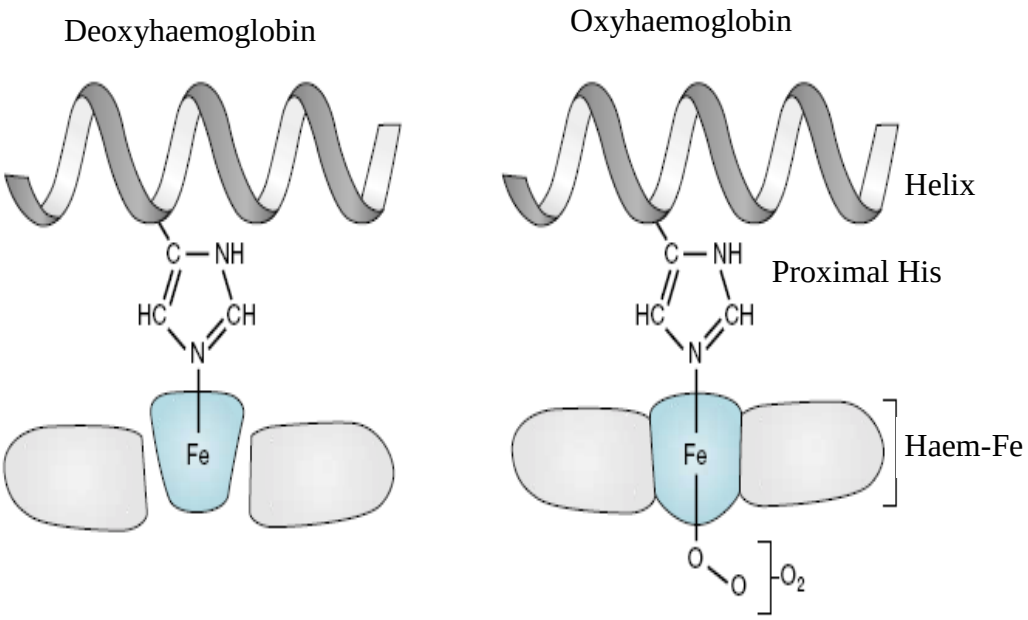



***Figure 2.8**: Oxygen binding to the $Fe^{2+}$ of haem in haemoglobin (Smith et al., 2005).*

The polypeptide chains of haemoglobin have a characteristic fold such that there are helical segments, with non-helical segments in between. This folding is stabilised by hydrogen bonds, hydrophobic interactions between alkyl groups and salt bridges between oppositely charged groups. Hydrophobic interactions are by far the most important. The common polypeptide subunits are designated α, β, γ and δ. Although the secondary and tertiary structures of various haemoglobin subunits are similar, reflecting extensive homology in amino acid composition, the variations in amino acid composition that do exist impart marked differences in the oxygen carrying properties of haemoglobin. In addition, the quaternary structure of haemoglobin leads to physiologically important allosteric interactions between the subunits, a property lacking in the monomeric myoglobin, which is otherwise very similar to the α-subunit of haemoglobin. In human adults, the major haemoglobin is identified as HbA (specifically regarded as $HbA_1$) and is a tetramer of two α and two β chains. Thus, the subunit composition of haemoglobin $A_1$ is $\alpha_2\beta_2$. The minor adult haemoglobin, identified as $HbA_2$, is a tetramer of two α chains and two δ chains ($\alpha_2\delta_2$).The overall haemoglobin composition in a normal adult is approximately 97.5% $HbA_1$, 2% $HbA_2$ and 0.5% HbF (foetal haemoglobin). The α-chain of most haemoglobins has 141 amino acid residues and the β chain has 146 amino acid residues (Tsai and Ho, 2002). The δ polypeptide chain has the same number of amino acids as the β chain, but their sequences differ at 10 positions in the sequence. The amino acid sequences of the γ and β chains differ in 39 of the 146 residues (Senozan, 2010).

## 2.6 Haemoglobin of the dog *(Canis familiaris)*

The scientific classification of the dog is as follows: Kingdom: *Animalia*, Phylum: *Chordata*, Class: *Mammalia*, Order: *Carnivora*, Family: *Canidae*, Genus: *Canis*, Species: *Canis familiaris*.

Dog haemoglobin has four sulphydryl groups per (tetramer) molecule located at the CysG18[111]α and CysF9[93]β positions of each of the two α and β chains.They are all reactive towards mercurial sulphydryl reagents. Only the two sulphydryls at the CysF9[93]β position are reactive towards nonmercurial sulphydryl reagents.Those at the CysG18[111]α positions are not (Okonjo *et al*., 1979).

Examination of the environment of the CysG18[111]α showed that this sulphydryl must be unreactive towards nonmercurials because of the presence near it

of several interacting groups: the carboxyl group of GluB5[27]α which is only 4.5Å away; the carboxyl of ValG14[107]α and the hydroxyl of TyrB5[24]α. A strong interaction has been found between CysG18[111]α and the carboxyl of GluG4[116]α, which though 12Å away, is separated from CysG18[111]α not by water but by protein which has a lower dielectric constant than water ($\delta_{H20}$ = 80; $\delta_{protein}$ = 4). All these interactions would considerably raise the $pK_a$ of the CysG18[111]α thiol. Therefore, reaction with nonmercurial sulphydryl reagents via nucleophilic attack by the thiol anion would be impossible (Okonjo *et al*., 1979).

It has been shown that the sulphydryl groups CysG11[104]α and CysG14[112]β do not react with mercurial or nonmercurial reagents. The inability of nonmercurials to react with these sulphydryl groups is due to two reasons: these groups cannot exist in the anionic form at the hydrophobic subunit interface, $\alpha_1\beta_1$ in which they are positioned, and reaction at this interface is sterically hindered. Steric hinderance is implied by the slow reaction of pMB with these groups because pMB is known to react fast with the neutral and anionic forms of sulphydryl groups (Rossi-Fanelli *et al*., 1969; Perutz, 1974).

## 2.7 Donkey haemoglobin (*Equus asinus*)

The scientific classification of the donkey is as follows: Kingdom: *Animalia*, Phylum: *Chordata*, Class: *Mammalia*, Order: *Perissodactyla* and Family: *Equidae*, Genus: *Equus*, Species: *Equus asinus*.

Donkey haemoglobin has four sulphydryl groups per (tetramer) molecule located at the CysG11[104]α and CysF9[93]β positions. The two sulphydryls at the F9[93]β position are reactive to both mercurial and nonmercurial reagents while those at the CysG11[104]α position are not reactive to either of these types of reagents.

Very close similarities have been observed in the electrophoretic behaviour of haemoglobins of the individual species of the genus *Equus*. At least two haemoglobin components (fast and slow) were distinguishable on starch gel at pH 8.6 for all species with the exception of the donkey and the Hartmann zebra, both of which contain a single slow component (Kitchen and Easley, 1969).

By the use of urea starch gel electrophoresis, the number of α and β polypeptidechain types which account for the heterogeneity of the *equine* haemoglobins has been identified. The donkey and the Hartmann zebra have been

found to have only one type of α and one type of β chain in each case. Within the haemoglobin chains of the *equine* family striking similarities have been observed in the β chains and limited differences were found to occur in the α chains, Donkey haemoglobin differs from horse haemoglobin by only two amino acids in the α-chain; HisB1[20]α to Asn and TyrB5[24]α to Phe. Although, distinct variations in the α chain structure were found among *equine* species, the differences are small when compared with the noticeable differences occurring between the α chains of the ruminant species and those of the goat, sheep and cow (Kitchen and Easley, 1969). The β chains of the *equine* haemoglobins are extremely similar; infact they have been found to be identical in horse (*Equus caballus*) and donkey (Kitchen and Easley, 1969)

## 2.8 Cooperativity

Haemoglobin, with its multiple subunits and $O_2$-binding sites, is suited for oxygen transport. The binding of oxygen by haemoglobin is cooperative and the protein cannot be considered in terms of four independent oxygen-binding subunits. Interactions among the subunits in haemoglobin cause conformational changes that progressively increase the affinity of the protein for oxygen till saturation (Pin *et al*., 1990; Ramadas and Rifkind, 1999).

Modulation of oxygen binding allows haemoglobin to respond to changes in oxygen demand by tissues. Haemoglobin binds oxygen efficiently in the lungs, where the oxygen partial pressure ($pO_2$) is about 13.3 kPa. It releases oxygen in the tissues, where the $pO_2$ is 4 kPa. Myoglobin, which is described by a hyperbolic oxygen binding curve, would be ill-suited to this function. A protein that binds oxygen with high affinity would bind it efficiently in the lungs and would not release much of it in the tissues. Haemoglobin undergoes a transition from low-affinity to a high-affinity state as more oxygen molecules are bound (Fig. 2.9a). As a result, haemoglobin has a sigmoid binding curve for oxygen, as shown in Fig. 2.9b (Berg *et al*., 2002; Nelson and Cox, 2004).

The influence of binding of one oxygen on the binding of another oxygen in the haemoglobin molecule is called a homotropic effect. This cooperative binding makes the binding curve sigmoidal rather than hyperbolic. The oxygen affinity is characterized quantitatively as the partial pressure of oxygen at which haemoglobin is half saturated ($P_{50}$). Several chemical factors, heterotropic ligands and temperature

influence the oxygen equilibrium curve. The reaction of haem iron in haemoglobin with oxygen is exothermic, so that the oxygen affinity drops with increase in temperature.

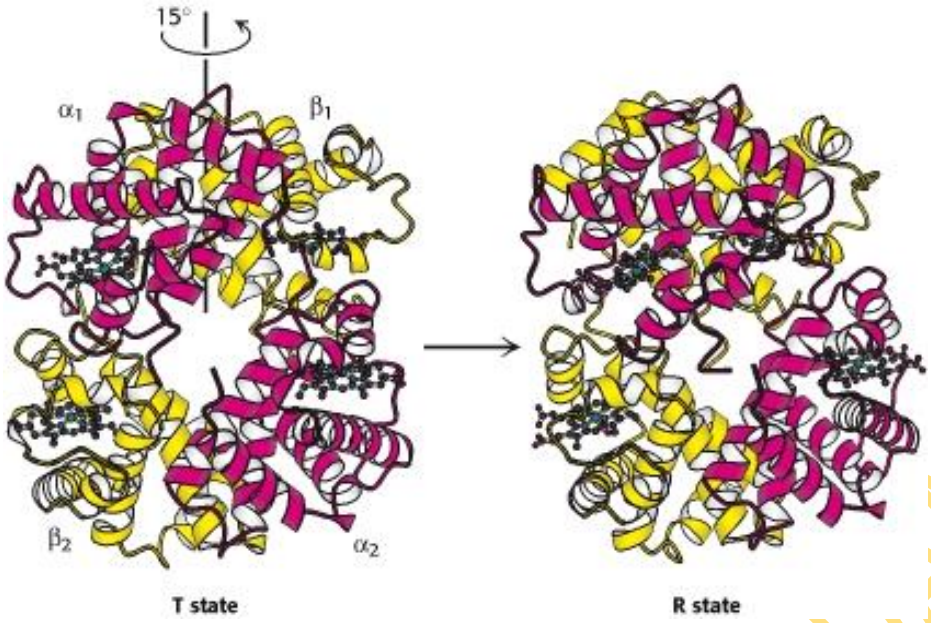



***Figure 2.9a**: Transition from T to R state in haemoglobin (Berg et al., 2002).*

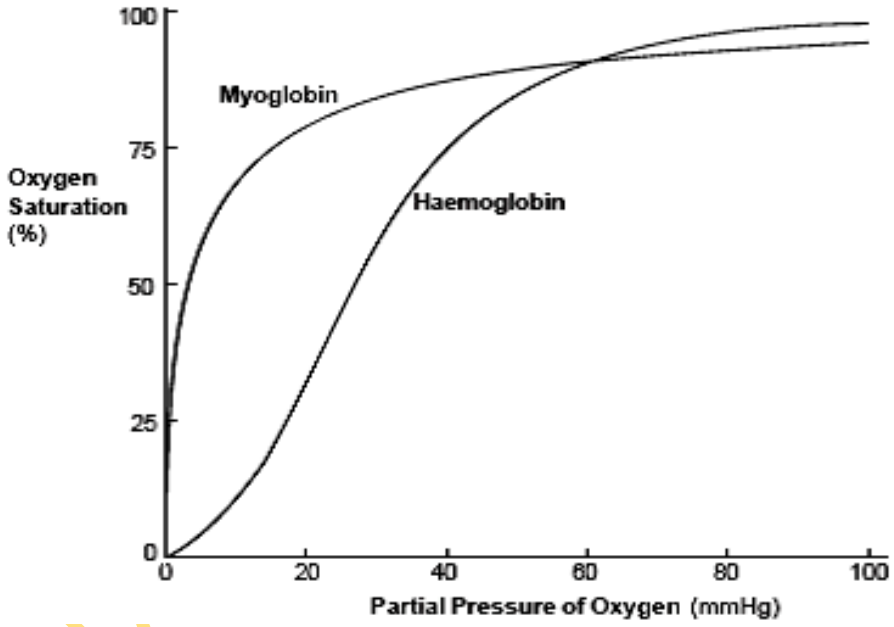



***Figure 2.9b**: Sigmoid oxygen binding curve of haemoglobin and hyperbolic oxygen binding curve of myoglobin (Mill et al., 1976).*

Haemoglobin is an allosteric protein: the binding of one ligand to the protein alters the binding affinity of other ligands at different sites on the same molecule (Monod *et al*., 1965). The binding of $O_2$ to haemoglobin and other oxygen carriers is important, but the description of these oxygenation reactions is mathematically complex. This is especially so because we must consider the effects of pH and changing concentrations of allosteric effectors on the binding equilibria. The haems are too far apart to interact directly. However, changes that occur in the structure of the globin surrounding a haem when it picks up an oxygen molecule are mechanically transmitted to other globins in the protein. These changes carry the signal that facilitates the gain or loss of an oxygen molecule by the other haems. This cooperative interaction between different binding sites makes haemoglobin a specialld designed good oxygen-transport protein and better than mere solvation of oxygen in water.

## 2.9 The Bohr Effect

The oxygen affinity of haemoglobin falls as the pH drops from 9.0 to 6.0 (Fig. 2.10) and conversely its affinity for proton rises, so that it takes up protons on release of oxygen (Fig 2.11a and Fig. 2.11b). The higher the $P_{50}$, the lower the affinity. The fall in oxygen affinity is called the alkaline Bohr effect. An increase in the $H^+$ concentration leads to an increase in oxygen ($O_2$) release from haemoglobin by the Bohr effect; increased $O_2$ release from haemoglobin, in turn, causes increased $H^+$ uptake by haemoglobin (Siggaard-Andersen and Garby, 1973; Lumb, 2000; Sherwood, 2010). Below pH 6.0, the oxygen affinity rises with falling pH and protons are taken up on oxygenation. This is the acid or reverse Bohr effect. The ability of haemoglobin to bind $H^+$, a waste product of metabolism, is an important physiological function of the macromolecule. The Bohr effect is therefore essential for the maintenance of physiological pH. For haemoglobin to play this role, the affinity of some sites for $H^+$ must be changed on oxygen binding (Moncada *et al*., 1991). The alkaline Bohr effect makes haemoglobin a two way respiratory carrier, enabling it to mop up protons released with carbon dioxide into soluble bicarbonate which can be converted in the plasma (Perutz *et al.*, 1980).

The Bohr effect results in an increase in the affinity of haemoglobin for oxygen at the lungs and a decrease in the affinity of haemoglobin for oxygen at the tissues. The Bohr effect is an allosteric effect caused by the binding of different ligands at different sites on haemoglobin (Moncada *et al*., 1991). The carboxyl-terminal histidines of the

β-chains, HisHC3[146]β, were indicated as proton binding sites by X-ray studies (Rossi-Fanelli *et al.*, 1964; Perutz, 1970). Other residues that contribute significantly to alkaline Bohr effect of human haemoglobin include ValNA1[1]α, HisH5[122]α, ValNA1[1]β, HisNA2[2]β and HisH21[143]β (Perutz *et al.*, 1980). The contribution of ValNA1[1]α, HisNA2[2]β and HisHC3[146]β have been determined quantitatively by measurements of their $pK_a$ values and the changes in their $pK_a$ values have been explained on a structural basis (Perutz *et al.*, 1980). It is possible to infer the contribution of ValNA1[1]β from the structure of haemoglobin. A low $pO_2$ in peripheral tissues promotes the synthesis of 2,3-BPG in erythrocytes from the glycolytic intermediate 1,3 – BPG. However, the space between the H helices of the chains lining the cavity is sufficiently wide to accommodate BPG only when haemoglobin is in the T (tensed) state. BPG forms salt bridges with the terminal amino groups of both chains via ValNA1[1]β with LysEF6[82]β and with Histidine. BPG therefore stabilizes deoxygenated (T state) haemoglobin by forming additional salt bridges that must be broken prior to conversion to the R state.

The contribution of HisHC3[146]β is independent of the concentration of inorganic phosphate, organic phosphate or chloride ion. The close proximity of the negatively charged AspFG1[94]β to HisHC3[146]β in deoxyhaemoglobin raises its $pK_a$ (Kilmartin, 1976) from a nominal value of 5.27 to about 6.1 in haemoglobin. The Bohr effect may be determined direcly in terms of the amount of protons ($\Delta h^+$) released per mole of $O_2$ bound to haemoglobin (Fig. 2.11). Between pH 6 and 9 protons are released on $O_2$ binding. This is the alkaline Bohr effect. Below pH 6 protons are taken up from solution as oxygen is bound. This is the acid Bohr effect.

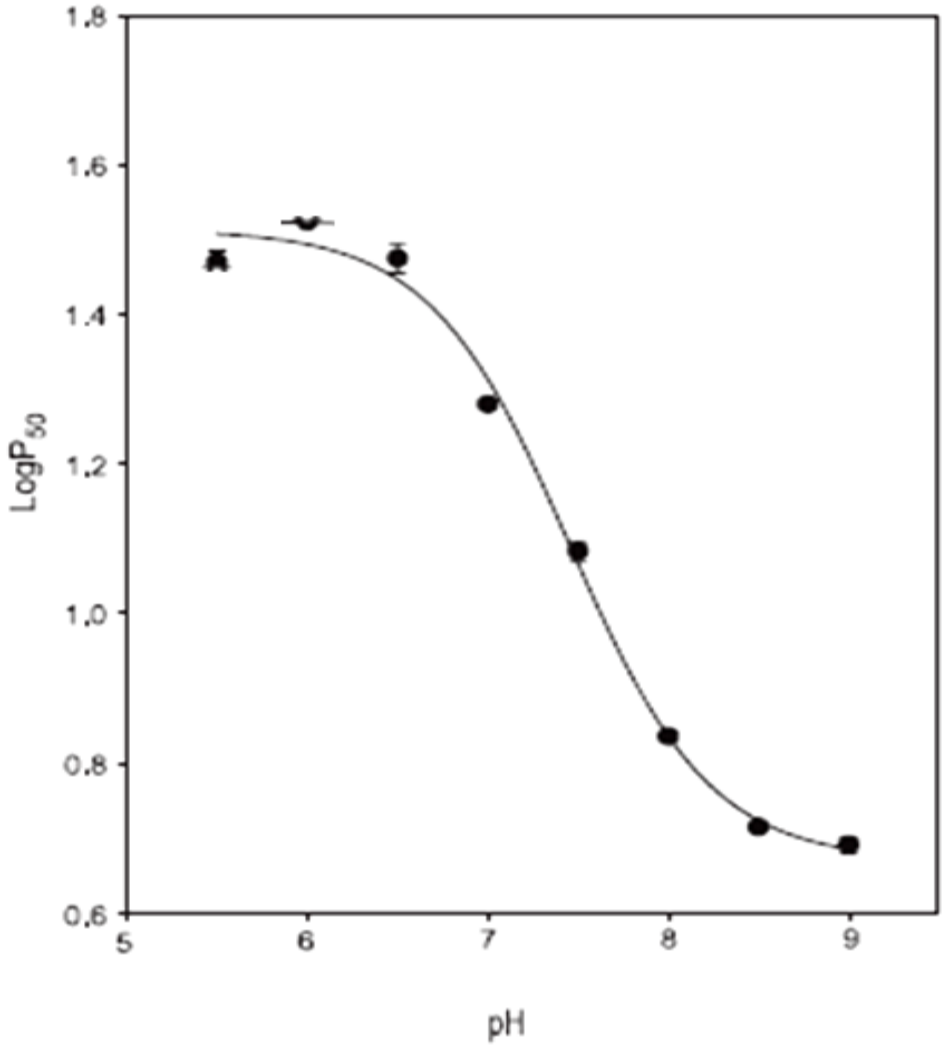



***Figure 2.10**: The Bohr Effect of HbA (Jia et al., 2004).*

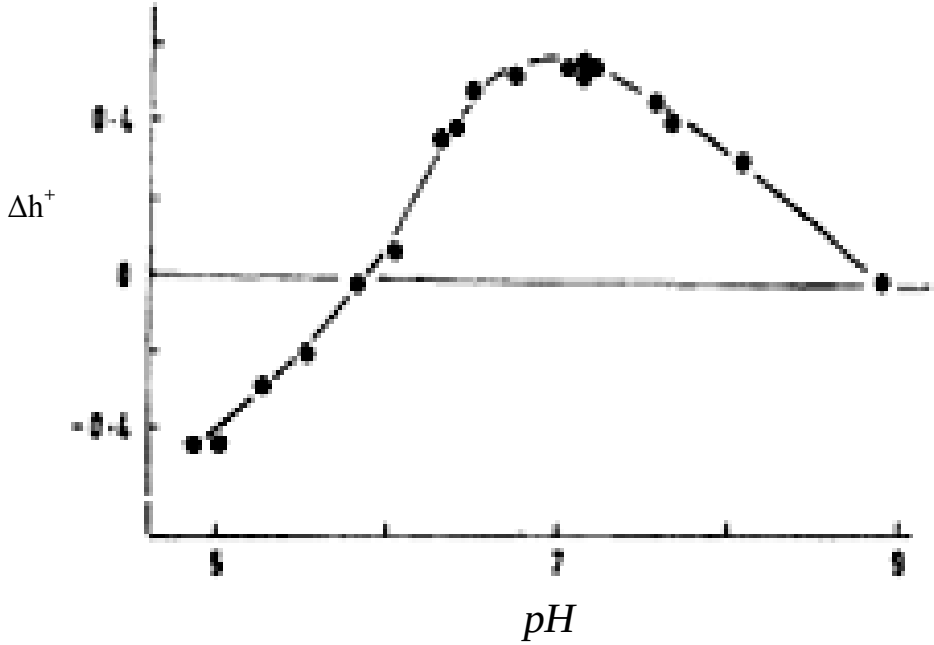



***Figure 2.11a**: A curve showing a plot of Δh+(proton uptake) against pH for the reaction of human haemoglobin with oxygen (Bailey et al., 1970).*

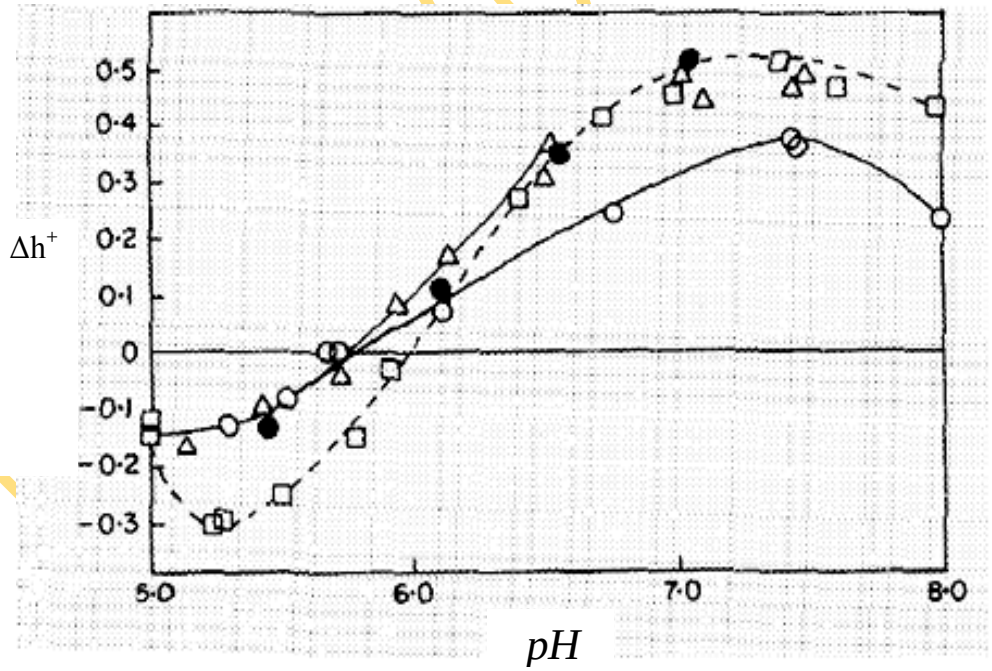



***Figure 2.11b**: Δh+proton released or taken up on oxygenation by solutions of different haemoglobins (Perutz et al., 1980).*

## 2.10 The interaction of organic phosphates with haemoglobins

The affinity of human haemoglobin for oxygen is regulated by 2,3-bisphosphoglycerate (2,3-BPG), a small molecule with a high density of negative charge (Benesch, *et al*., 1969; Arnone, 1972). 2,3-BPG is produced in erythrocytes during glycolysis and is a potent allosteric effector of the oxygen binding properties of haemoglobin. The effector regulating the conformational equilibrium in human red cells is 2,3-BPG (Benesch *et al*., 1968). The structures of some of the organic phosphates, that act as allosteric effectors in haemoglobin are shown in Fig. 2.12.

Inositol pentakisphosphate acts as allosteric effector for avian haemoglobins, inositol hexakisphosphate, 2,3-Bisphosphoglycerate, adenosine triphosphate, adenosine diphosphate and adenosine monophosphate act as allosteric effectors for human haemoglobins.

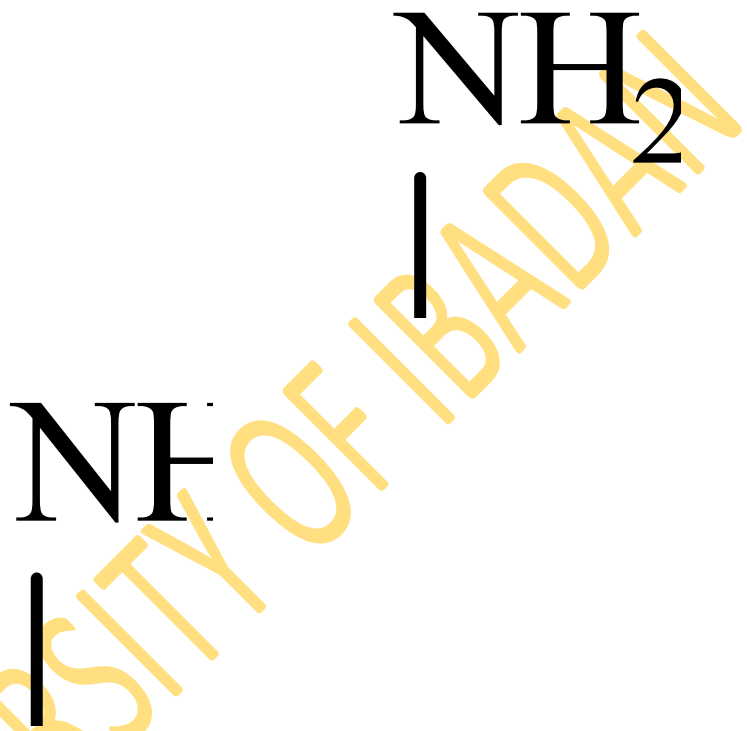


***Figure 2.12**: Structures of some organic phosphates*

There is an inverse relationship between the binding of $O_2$ and the binding of organic phosphates like 2,3-BPG and inositol-$P_6$. They bind at sites distant from the oxygen-binding site but regulate the oxygen affinity of haemoglobin in relation to the $pO_2$ in the lungs (Castilho *et al*., 2003; Nelson and Cox, 2004). Fig. 2.13a shows the effect of 2,3-BPG on the oxygen binding of haemoglobin. It is clear from the illustration that 2,3-BPG decreases the affinity of haemoglobin for oxygen. 2,3-BPG can bind to ligated and unligated haemoglobin in a process that is proton driven and involves proton uptake. The most striking functional changes occur when allosteric effectors are fully bound to ligated haemoglobin: the oxygen affinity decreases dramatically, the Bohr effect is enhanced, and cooperativity of oxygen ligation almost disappears (de Bruin *et al*., 1973). Foetal haemoglobin has a higher affinity for oxygen than adult haemoglobin because it binds less 2,3-BPG (Benesch *et al*., 1969; Tyuma and Shimizu, 1970). The values of $P_{50}$ for HbA and HbF are 26 and 20 mm Hg respectively. HbF has high affinity for $O_2$, indictating that it can deliver less $O_2$ to the tissues. The benefit of HbF having a higher oxygen affinity than HbA is that when both are in equilibration in the placenta, HbA discharges to HbF. Note that tissue/metabolic reqirement for oxygen in foetus is less than in the mother – on size comparison. However, the gradient of oxygen flow in the placenta runs to the advantage of the baby. The subunit composition of haemoglobin tetramers undergoes complex changes during development. The human foetus initially synthesises a $\zeta_2\varepsilon_2$ tetramer. By the end of the first trimester, ζ and ε subunits have been replaced by α and γ subunits, forming HbF ($\alpha_2\gamma_2$), the haemoglobin of late foetal life. While syntheses of β subunits begin in the third trimester, β subunits do not actually completely replace γ subunits to yield adult HbA ($\alpha_2\beta_2$) until some weeks postpartum (Ganong, 2000).

ValNA1[1]β, HisNA2[2]β, LysEF6[82]β and HisH21[143]β have been implicated in the binding of 2,3-BPG to human haemoglobin (Arnone, 1972). Fig. 2.13b shows the binding site of 2,3-BPG to human deoxyhaemoglobin. The same residues have been reported to bind inositol hexakisphosphate (inositol-$P_6$) (Arnone and Perutz, 1974). In most mammals, the molar concentration of 2,3-BPG in the erythrocyte is equal to or greater than that of haemoglobin. In the red blood cells of cats and most ruminants, this concentration of 2,3- BPG seems not to be high enough to have any effect on oxygen transport (Bartlet, 1980).

There is, however, more 2,3-BPG in the red cells of cats and ruminants than in the red cells of non-mammalian vertebrates, with a few exceptions (Bartlett, 1980).

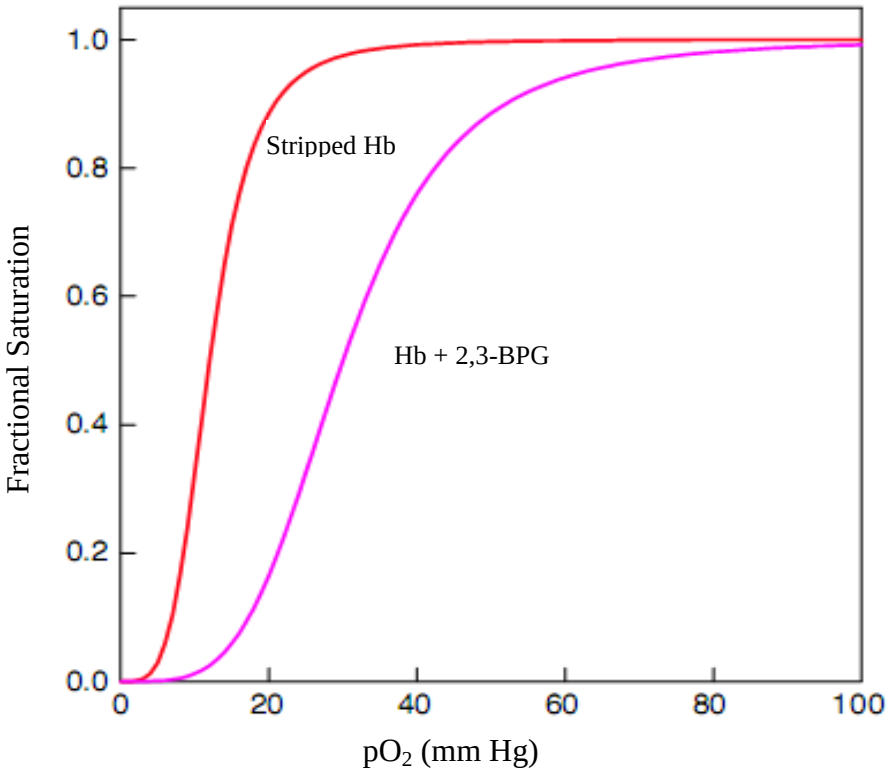



***Figure 2.13a**: Effect of 2,3-BPG on the oxygen binding of haemoglobin (Whitford, 2005).*

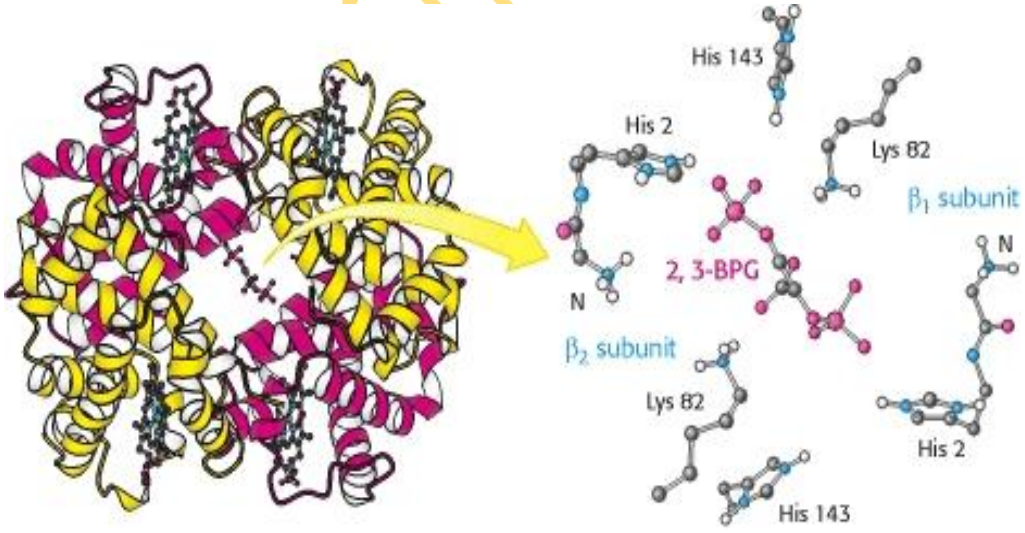



***Figure 2.13b**: Mode of binding of 2,3-BPG to human deoxyhaemoglobin (Arnone, 1972).*

Organic phosphates are responsible for the adaptation of erythrocytes to various physiological and pathological conditions (Benesch and Benesch, 1969). In the presence of sufficiently high concentrations of other salts, the effect of organic phosphates tends to disappear. At a sufficiently high concentration of NaCl (500 mmol $dm^{-3}$), the effect of 2,3-BPG on the oxygen affinity of human haemoglobin disappears. Conversely, 0.25 mmol $dm^{-3}$ of 2,3-BPG is sufficient to make the oxygen binding curve essentially independent of salt concentration. This suggests that different salts have qualitatively similar effects (Benesch *et al*., 1969; Pomponi *et al*., 2000). The affinity of haemoglobin for 2,3-BPG decreases with increasing pH as the cationic groups at the organic phosphate binding site ionize and become neutral. In deoxygenated haemoglobin (T conformation), a cavity capable of binding 2,3-BPG forms at the centre of the molecule. 2,3-BPG can occupy this cavity, thus stabilizing the T (deoxy) state. Deoxyhaemoglobin can be converted to oxyhaemoglobin more readily when 2,3-BPG is not bound at the central cavity. Thus, increasing the 2,3-BPG concentration favours conversion of R form of haemoglobin to the T form and decreases the amount of oxygen bound by haemoglobin at any given oxygen partial pressure.

Among all the common organic phosphates, inositol-$P_6$ has the highest overall negative charge. More than any other organic phosphate, it is able to neutralize the charges on the cationic residues at the organic phosphate binding site in haemoglobin. This makes inositol-$P_6$ the most potent organic phosphate in decreasing the oxygen affinity of haemoglobin. Besides, it is commercially available and can be stored as the sodium salt at room temperature for a long time. Inositol-$P_6$ is the most widely used organic phosphate in studies of organic phosphate effects on haemoglobin reactivity and function (Salhany *et al*., 1975).

## 2.11 Sulphydryl groups of haemoglobin

Haemoglobin sulphydryl groups are some of the most studied amino acid residues. This is due to the relationship between the reactivity of the sulphydryl group and the tertiary and quaternary structures of haemoglobin (Antonini and Brunori, 1971; Okonjo *et al.*, 1995; Okonjo *et al.*, 1996; Okonjo *et al.*, 2007).

The numbers of sulphydryl groups in the haemoglobins of various organisms have been determined by spectroscopic titration with sulphydryl sensitive reagents.

This has been found to be generally less than the total number of cysteines in the molecule, as determined by amino acid sequencing. This is because some thiols are buried (masked). The number of titratable thiol groups found in haemoglobin depends on the thiol reagent used (Okonjo *et al.,* 1979). Organic mercurials titrate all the free thiol groups in haemoglobin, while non-mercurial reagents titrate only those thiols that can form the thiolate anion. Haemoglobin contains masked sulphydryl groups. The masked sulphydryl groups are at positions CysG14[112]β and CysG11[104]α (Okonjo and Okia,1993; Okonjo *et al.,* 1995; Okonjo *et al.,* 1996; Okonjo *et al.,* 2007). These masked sulphydryl groups are in the region of the subunit contact between the $\alpha_1$ and $\beta_1$ subunits and cannot be detected with any sulphydryl reagent. The total number of sulphydryl groups per molecule and the number of sulphydryl groups that are reactive towards pMB and DTNB are presented in Table 2.2 for various haemoglobins.

***Table 2.2**: Titratable sulphydryl groups and their positions in the haemoglobin molecule of various animal species.*

| Haemoglobin of | Sulphydryls titratable with DTNB | Sulphydryls titratable with pMB | Total sulphydryls | Location |
|---|---|---|---|---|
| Rabbit | 2 | 2 | 4 | [a]F9[93]β, [c]G11[104]α |
| Human foetal | 2 | 2 | 4 | [a]F9[93]β, [c]G11[104]α |
| Human Adult | 2 | 2 | 6 | [a]F9[93]β, [c]G14[112]β, [c]G11[104]α |
| Horse | 2 | 2 | 4 | [a]F9[93]β, [c]G11[104]α |
| Guinea pig | 4 | 4 | 6 | [a]F9[93]β, [a]H3[125]β, [c]G11[104]α |
| Japanese Quail Major | 4 | 8 | 10 | [a]F9[93]β, [a]B5[23]β, [b]H4[126]β, [c]G11[104]α, [b]H13[130]α, |
| Japanese Quail Minor | 4 | 6 | 8 | [a]F9[93]β, [a]B5(23)β, [b]H4[126]β, [c]G11[104]α |
| Dog | 2 | 4 | 8 | [a]F9[93]β, [b]G18[111]α, [c]G11[104]α, [c]G14[112]β |
| Chicken minor | 4 | 6 | 8 | [a]F9[93]β, [a]B5[23]β, [b]H4[126]β, [c]G11[104]α |
| Chicken major | 4 | 8 | 10 | [a]F9[93]β, [a]B5[23]β, [b]H4[126]β, [b]H13[130]α, [c]G11[104]α |
| Duck major | 4 | 6 | 10 | [a]F9[93]β, [a]B5[23]β, [b]H4[126]β, [b]H13[130]α, [c]G11[104]α |
| Pigeon | 4 | 8 | 10 | [a]F9[93]β, [a]B5[23]β, [b]H4[126]β, [b]H13[130]α, [c]G11[104]α |

[a]Sulphydryl groups titratable with all sulphydryl reagents. [b]Sulphydryl groups titratable with only organic mercurials. [c]Masked sulphydryl groups (Okonjo *et al*., 1979; Okonjo and Adejoro, 1993; Okonjo and Okia, 1993; Okonjo and Nwozo, 1997; Okonjo *et al*., 2008).

## 2.12 Sulphydryl reagents

Reagents that react with sulphydryl groups are called sulphydryl reagents. These reagents react in different ways, such as found in alkylation, formation of metal derivatives and thiol-disulphide exchange. The sulphydryl groups of haemoglobin are reactive with most alkylating and arylating agents, combine with many heavy-metal ions, and are easily oxidized by even very mild oxidizing agents. They react readily with most acylating agents, but the resulting thiol esters are quite unstable in an aqueous environment and are usually hydrolysed.

The most sensitive technique for assaying thiol groups is by direct titration and spectrophotometry.

Thiols form strong complexes with mercury ions. Reaction of a given thiol with mercury chloride gives a number of such complexes as $Hg(SR)_2$, $Hg_2(SR)_2$ and $Hg_3(SR)_2$. The equilibrium constants for the dissociation (Eqn. 2.2) are in the range, $10^{-40}$ to $10^{-44}$ (Boyer, 1954; Kajihara-Kano, 1997).

$$Hg_x(SR)_y \rightleftharpoons xHg^{+} + yRS^{-} \quad \text{.....2.2}$$

These complexes, including the soluble ones formed with aminothiols, are stable over the entire pH range. At low pH, $Hg^{2+}$ is the most specific of all thiol-combining agents. Several complexes form organic mercurials of the type $RHg^{+}$, which are univalent and have a more dependable stoichiometry. The difficulty of insufficiently defined stoichiometries has been eliminated by the use of univalent organic mercurials such as p-chloromercuri(II)benzoate (pCMB) or p-hydroxymercuri(II)benzoate (pMB) (Boyer, 1954). These reagents are more specific since, unlike free mercury ion, they do not hydrolyse thioesters (Jocelyn, 1972) or cleave disulphides except under very drastic conditions. The structures of pMB and pCMB are shown in Fig. 2.14.

***Figure 2.14**: Structures of pCMB and pMB*

A simple technique for the determination of the number of sulphydryl groups in haemoglobin with organic mercurials is by spectrophotometric measurement of the amount of mercurial-thiol complex formed (Boyer, 1954). The reaction of mercuribenzoate with protein (PSH) thiols at pH 4.5 - 7.0 (Eqn. 2.3) is accompanied by a big increase in the absorption of the mercurial at 250 nm.

.....2.3

The thiol is titrated by increasing the amount of the mercuribenzoate until further addition produces no change in the absorbance of the complex formed. The disadvantages of this method are that organic mercurials have low solubilities, their light absorptions are affected by the anionic composition of the medium and they are not very stable in aqueous solutions (Johannesson, 2002).

The kinetics of the reaction of pMB and of pCMB with simple thiols have been studied (Hasinoff *et al*., 1971). The rates of reaction of organic mercurials with protein sulphydryl groups is very fast and is best monitored by stopped-flow techniques (Karim *et al*., 1998). For example, the reaction of pMB with CysF9[93]β of human oxyhaemoglobin has a rate as high as 5.8 x $10^5$ mol $dm^{-3}$ $s^{-1}$ (Jocelyn, 1972). Proteins absorb in the same wavelength region as organic mercurials, so that for protein sulphydryl group assays, the initial absorption is high (Boyer, 1954).

Two aromatic disulphides that are also commonly used for assaying thiols are 2,2′-dithiobispyridine and 4,4′-dithiobispyridine. Their corresponding thiones absorb light at 343 nm and 324 nm, respectively ($\varepsilon_{max}$ 7060 and 19800) (Grassetti and Murray, Jr., 1966; Taketa *et al*., 1980). Fig. 2.15b shows the structures of 2,2′-dithiobispyridine and 4,4′-dithiobispyridine. Eqn. 2.5 shows the reaction of 2,2'-dithiopyridine with a sulphydryl group in its thiol anion form.

.....2.5

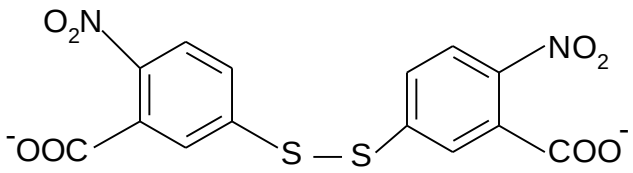
5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) is available commercially as a salt. It is fairly water-soluble and can be used for quantitating free sulphydryl groups in solution. It is very stable in neutral solution if protected from light. DTNB is very useful as a sulphydryl assay reagent because of its specificity for sulphydryl groups (Ellman, 1959).

When DTNB is added to a solution containing a non-protein thiol, colour development is complete within 2 - 10 minutes in the pH range 6 - 8. An extinction coefficient of 13.6 x $10^3$ $mol^{-1}$ $dm^3$ $cm^{-1}$ at a wavelength of 412 nm can be used to calculate the concentration of sulphydryl groups reacting. The colour fades slowly due to autoxidation but this is delayed by the inclusion of EDTA in the medium. DTNB reacts with a free sulphydryl group to yield a mixed disulphide and 2-nitro-5-thiobenzoate. If $PS^-$ represents the thiolate anion form of a protein or non-protein thiol, the reaction of DTNB with the thiolate anion occurs as shown in Eqn. 2.4.

...….2.4

The rate of this reaction is dependent on several factors, such as the pH of the reaction medium, the $pK_a$ of the sulphydryl group, steric and electrostatic effects. An important advantage of DTNB over most of the other sulphydryl reagents is that it can be used to study the electrostatic environments of sulphydryl groups in haemoglobins because of its charge (Okonjo and Aboluwoye, 1992; Okonjo and Nwozo, 1997). This method, with various modifications, is currently the most used for the assay of protein and non-protein thiols (Jocelyn, 1972). At higher concentrations, DTNB has been used to assay protein thiols either directly or after precipitation (Laragoine *et al.*, 2003). Under certain conditions, mixed disulphides can be obtained with protein sulphydryl groups. The structure of DTNB is shown in Fig. 2.15a.

DTNB was used as reagent of choice in this study for the following reasons: 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) is available commercially as a salt, it is fairly water-soluble and can be used for quantitating free sulphydryl groups in solution. It is very stable in neutral solution if protected from light, can be studied within the visible range and in seconds to minutes, can be used to study the electrostatic environments of sulphydryl groups in haemoglobins because of its charge (Okonjo and Aboluwoye, 1992; Okonjo and Nwozo, 1997). DTNB is very useful as a sulphydryl assay reagent because of its specificity for sulphydryl groups (Ellman, 1959). There are four wavelengths at which DTNB can be studied; 412nm, 430nm, 450nm and 470nm (Britto *et al*., 2005) whereas 2,2-DTP and 4,4-DTP can only be studied within the ultraviolet range.




5,5' -dithiobis(2 -nitrobenzoate) (DTNB)

***Figure 2.15a**: Structure of 5,5'-dithiobis(2-nitrobenzoate) DTNB*

***Figure 2.15b**: Structures of 2,2'-dithiobispyridine and 4,4'-dithiobispyridine.*

## 2.13 Dependence of the reactivities of haemoglobin sulphydryl groups on pH

Considerable interest has been generated by the CysF9[93]β sulphydryl group of haemoglobin for more than four decades because its reactivity depends on tertiary or quaternary structure (Guidotti *et al.*, 1965; Antonini and Brunori, 1969; Gibson, 1973; Perutz *et al.*, 1974; Hensley *et al.*, 1975; Baldwin, 1980; Shaanan, 1983; Okonjo *et al.*, 1989). The reactivities of the CysF9(93)β sulphydryl group of human oxy, carbonmonoxy, aquomet and deoxyhaemoglobin have been studied at a single pH (7.6) with various sulphydryl reagents, including iodoacetamide, iodoacetic acid, N-ethylmaleimide, 2,2'-dithiobispyridine, 4,4'-dithiobispyridine and 5,5'-dithiobis (2-nitrobenzoate) (Guidotti, 1965). The rates of the reaction of liganded haemoglobins were shown to be four to thirty-two times faster than those of deoxyhaemoglobin. This is not surprising since the reactivity of this sulphydryl in deoxyhaemoglobin is reduced owing to steric hindrance by TyrHC2(145)β which becomes fixed in a pocket between helices F and H of the β chains. Analysis carried out based on the finding that there is a Bohr effect in R-state haemoglobin involving HisHC3[146]β (Kwiatkowsky and Noble, 1982), proved that this histidine forms a salt bridge with AspFG1[94]β, resulting in CysF9[93]β being sterically hindered by TyrHC2[145]β. When the histidine ionizes, the salt bridge is broken and the hindrance to access of the sulphydryl reagent to CysF9[93]β is removed. Consequently, the reactivity of this sulphydryl group should increase as the histidine ionizes with increasing pH.

pH-dependence studies of the reactivities of haemoglobin sulphydryl groups have been useful in determining the number and the nature of the amino acid residues influencing sulphydryl group reactivity (Okonjo and Okia, 1993; Okonjo *et al*., 1996; Okonjo and Nwozo, 1997). The environment of the reacting sulphydryl group determines the nature of the pH dependence profile.

Using 5,5′-dithiobis(2-nitrobenzoate), DTNB, as sulphydryl reagent, it was found that at an ionic strength of 50 mmol $dm^{-3}$ the apparent forward second order rate constant, $k_F$, for the reaction with CysF9[93]β of human haemoglobins A and S varies with pH in a complex manner (Okonjo and Okia, 1993; Okonjo *et al*., 1995; Okonjo *et al*., 1996; Okonjo and Nwozo, 1997). At an ionic strength of 200 mmol $dm^{-3}$ the complex profile became simple, resembling the titration curve of a diprotic acid which is biphasic. Similarly, at an ionic strength of 50 mmol $dm^{-3}$ in the presence of the

organic phosphate, inositol hexakisphosphate, the complex profile also became simple showing that there were other groups ionizing within the vicinity of the sulphydryl groups. These findings led to the proposition that at ionic strength of 50 mmol $dm^{-3}$, CysF9[93]β of human haemoglobin is electrostatically linked to ValNA1[1]β, HisNA2[2]β and HisH21[143]β, the positively charged ionisable groups at the organic phosphate binding site (Arnone, 1972; Arnone and Perutz, 1974). This linkage gives rise to the observed complex pH-dependence of the apparent second order forward rate constant (Okonjo *et al*., 1995; Okonjo *et al*., 1996).

This indicated that electrostatic interactions between charged amino acid residues on the protein and the reagent may have a complicating effect on the observations. The positive charges of the groups in the vicity of the negatively charged sulphydryl may interact with the negatively charged reagent to give a complex pH – depence profile.

In order to exclude such interactions, the uncharged reagent 2,2'-dithiobispyridine (2-DTP) was selected. The profiles obtained were much simpler than that of DTNB. Similarly simple profiles were obtained for the reaction of 2-DTP with dog and rabbit aquomethaemoglobins (Okonjo and Aboluwoye, 1992).

These simple profiles obtained for the DTNB reaction with haemoglobin sulphydryl groups at 200 mmol $dm^{-3}$ ionic strength, those obtained in the presence of inositol-$P_6$ and those of 2-DTP reacting with haemoglobin sulphydryl groups were quantitatively analysed with a two-term equation (Okonjo and Aboluwoye, 1992; Okonjo *et al*., 1996; Okonjo and Nwozo, 1997).

$$k_F = k_1 \frac{Q_1}{Q_1 + [H^+]} + k_2 \frac{Q_2}{Q_2 + [H^+]} \quad \text{----------2.7}$$

Where $k_F$ is the apparent forward second order rate constant, $k_1$ is the limiting apparent second-order rate constant at high pH for the reaction of the sulphydryl reagent when the reactivity of the sulphydryl group is linked to the ionization of HisHC3[146]β, with ionization constant $Q_1$ and $k_2$ is the limiting apparent second-order rate constant at high pH when the sulphydryl reactivity is linked to the ionization of the sulphydryl group itself, with ionization constant $Q_2$. The first fractional term is the fraction of the neutral form of the histidine, while the second fractional term is the fraction of the thiol anion form of the reacting sulphydryl group. This analysis showed that at least two ionisable groups are coupled to the reaction of the sulphydryl group, HisHC3[146]β and CysF9[93]β with $pK_a$ of 6.1 and 8.7 respectively.

The pH dependence profile of $k_{F,}$ the apparent forward second order rate constant may also be analysed with the one term equation.

$$k_F = k\frac{Q}{Q+\left[H^+\right]} + c \quad \text{.....2.8}$$

Where $k$ is the limiting apparent second-order rate constant for the reaction when the reactivity of the sulphydryl group is linked to the ionization of CysF9[93]β; $Q$ is the ionization constant of CysF9[93]β; and c is a constant whose value equals the base line of the computer fit of the data.

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 PREPARATIONS OF BUFFER SOLUTIONS

### 3.1.1 Preparation of standard buffer solutions

**(a) Standard buffer pH 4.000 ± 0.020**: Standard buffer pH 4.000 ± 0.020 was prepared by dissolving one tablet of the standard buffer in a 100 $cm^3$ flask and then making up to the mark with distilled water. The tablets were products of British Drug Houses Limited, Poole, England. This standard solution was used to standardize the pH meter at $25^0$C.

**(b) Standard buffer pH 9.220 ± 0.020**: Standard buffer pH 9.220 ± 0.020 was prepared by dissolving one tablet of the standard buffer in a 100 $cm^3$ flask and then making up to the mark with distilled water. The tablets were products of British Drug Houses Limited, Poole, England. This standard solution was used to standardize the pH meter at $25^0$C.

### 3.1.2 Preparation of phosphate buffer solutions: pH 5.6 – 8.0

Phosphate buffer solutions were prepared with Analar grade chemicals from British Drug Houses (BDH). A stock solution of 0.4 mol $dm^{-3}$ sodium hydroxide, NaOH (molecular weight, 40 g $mol^{-1}$), was prepared by weighing 16.00 g $mol^{-1}$ of sodium hydroxide pellets and dissolving in distilled water in a 1000 $cm^3$ volumetric flask and then making up to the mark with distilled water.

0.4 mol $dm^{-3}$ of sodium dihydrogen phosphate, $NaH_2PO_4.2H_20$ (molecular weight, 156.1 g $mol^{-1}$) solution was prepared using the same procedure by weighing 62.4 g $mol^{-1}$ of $NaH_2PO_4$ and dissolving in distilled water in a 1000 $cm^3$ volumetric flask and then making up to the mark with distilled water.

Phosphate buffer solutions at specific pH values were prepared by mixing specified amounts of 0.4 mol $dm^{-3}$ sodium hydroxide and 0.4 mol $dm^{-3}$ sodium dihydrogen phosphate solutions (Haemoglobin Laboratory Procedures, J.G. Beetlestone). The ionic strength of each buffer solution was made up to 50 mmol $dm^{-3}$ by adding the required amount of sodium chloride (molecular weight, 58.5 g $mol^{-1}$).

Each buffer solution was made up to the mark with distilled water in a 1000 $cm^3$ standard flask. The pH of each buffer solution was checked using a Radiometer PHM 85 Precision Research pH meter, which had earlier been standardized with standard buffer solutions of pH 4.000 ± 0.020 and 9.220 ± 0.020. When necessary the buffer solutions prepared were adjusted to the desired pH with a buffer solution of a higher or lower pH. The amounts of 0.4 mol $dm^{-3}$ NaOH solution, 0.4 mol $dm^{-3}$ $NaH_2PO_4.2H_2O$ solution and NaCl required for the preparation of each buffer are shown in Table 3.1.

***Table 3.1**: The amounts of 0.4 mol $dm^{-3}$ NaOH solution, 0.4 mol $dm^{-3}$ $NaH_2PO_4$ solution and NaCl required for phosphate buffers pH 5.6 – 8.0, I = 50 mmol $dm^{-3}$, total volume; 1000 $cm^3$.*

| pH | Volume of 0.4 mol $dm^{-3}$ NaOH ($cm^3$) | Volume of 0.4 mol $dm^{-3}$ $NaH_2PO_4$ ($cm^3$) | Mass of NaCl added in grams |
|---|---|---|---|
| 5.60 | 1.00 | 25.00 | 2.29 |
| 5.80 | 1.83 | 25.00 | 2.26 |
| 6.00 | 2.82 | 25.00 | 2.21 |
| 6.20 | 4.28 | 25.00 | 2.14 |
| 6.40 | 6.30 | 25.00 | 2.04 |
| 6.60 | 8.87 | 25.00 | 1.93 |
| 6.80 | 11.80 | 25.00 | 1.79 |
| 7.00 | 14.80 | 25.00 | 1.65 |
| 7.20 | 17.50 | 25.00 | 1.52 |
| 7.40 | 19.70 | 25.00 | 1.42 |
| 7.60 | 21.40 | 25.00 | 1.35 |
| 7.80 | 22.60 | 25.00 | 1.29 |
| 8.00 | 23.30 | 25.00 | 1.24 |

### 3.1.3 Preparation of borate buffer solutions: pH 8.0 – 9.0

A stock solution of 0.3 mol $dm^{-3}$ sodium hydroxide, NaOH, was prepared by weighing 12.00 g of sodium hydroxide pellets and dissolving in distilled water in a 1000 $cm^3$ standard flask and then making up to the mark with distilled water. 0.3 mol $dm^{-3}$ boric acid, $H_3BO_3$, (molecular weight, 61.83 g $mol^{-1}$), solution was also prepared by weighing and dissolving 18.55 g of boric acid in distilled water in a 1 $dm^3$ standard flask and then making up to the mark with distilled water.

Borate buffer solutions at specific pH values were prepared by mixing specified amounts of 0.3 mol $dm^{-3}$ NaOH and 0.3 mol $dm^{-3}$ $H_3BO_3$ solutions. The ionic strength of each buffer solution was made up to 50 mmol $dm^{-3}$ by adding the required amount of sodium chloride, NaCl. Each buffer solution was made up to the mark with distilled water in a 1000 $cm^3$ standard flask. The pH of each buffer solution was checked using a Radiometer PHM 85 Precision Research pH meter, which had earlier been standardized with standard buffer solutions pH 4.000 ± 0.020 and 9.220 ± 0.020. The pH was adjusted when necessary by titrating the buffer solutions with a buffer solution that had a higher or lower pH, depending on whether the measured pH was higher or lower than the calculated pH. The amounts of 0.3 mol $dm^{-3}$ NaOH solution, 0.3 mol $dm^{-3}$ $H_3BO_3$ solution and NaCl required for the preparation of each buffer are shown in Table 3.2.

***Table 3.2**: The amounts of 0.3 mol $dm^{-3}$ NaOH solution, 0.3 mol $dm^{-3}$ $H_3BO_3$ solution and NaCl required for borate buffers pH 8.0 – 9.0, I = 50 mmol $dm^{-3}$, total volume; 1000 $cm^3$.*

| pH | Volume of 0.3 mol $dm^{-3}$ NaOH ($cm^3$) | Volume of 0.3 mol $dm^{-3}H_3BO_3(cm^3)$ | Mass of NaCl added in grams |
|---|---|---|---|
| 8.00 | 20.00 | 250 | 2.57 |
| 8.20 | 29.50 | 250 | 2.40 |
| 8.40 | 42.70 | 250 | 2.17 |
| 8.60 | 60.00 | 250 | 1.82 |
| 8.80 | 82.00 | 250 | 1.48 |
| 9.00 | 107.00 | 250 | 1.05 |

## 3.2 PREPARATION OF REAGENTS AND SAMPLES

### 3.2.1 Preparation of ACD anticoagulant

An acid-citrate-dextrose (ACD) anticoagulant solution was prepared by weighing and dissolving 5.10 g of trisodium citrate dihydrate, $Na_3C_6H_5O_7.2H_2O$ (molecular weight, 294.1 g $mol^{-1}$) 1.60 g of citric acid monohydrate, $C_3H_4(OH)(COOH)_3.H_2O$ (molecular weight, 210.14 g $mol^{-1}$) and 2.40 g of anhydrous dextrose, D-$(CH\text{-}CH)_4CH\text{-}CH_6OH$ (molecular weight, 152.0 g $mol^{-1}$) in a 200 $cm^3$ volumetric flask with distilled water. The solution was made up to the mark with distilled water (Haemoglobin Laboratory Procedures, J.G. Beetlestone).

### 3.2.2 Preparation of saline solution

Normal saline solution for erythrocytes is 9.50 g of sodium chloride per 1000 $cm^3$ of solution in water. Therefore 9.50 g of sodium chloride was weighed and dissolved in a 1000 $cm^3$ volumetric flask, which was then made up to the mark with distilled water. The solution was stored at 5°C.

### 3.2.3 Preparation of dialysis solutions

Dialysis solutions were prepared by addition of 0.29 g of sodium hydroxide, 10 $cm^3$ of 0.4 mol $dm^{-3}$ sodium dihydrogen phosphate and 5 $cm^3$ of 0.4 mol $dm^{-3}$ disodium hydrogen phosphate (molecular weight, 141.96 g $mol^{-1}$) to 5000 $cm^3$ of distilled water (Haemoglobin Laboratory Procedure, J.G. Beetlestone). It was ensured that the pH of the final solution was between pH 6.5 and 7.5. The solutions were stored at 5°C in the cold room.

### 3.2.4 Preparation of Drabkin's solution

Drabkin's solution was prepared by weighing and dissolving 0.05 g of potassium ferricyanide (molecular weight, 329.26 g $mol^{-1}$), 0.125 g of potassium cyanide (molecular weight, 65.12 g $mol^{-1}$) and 2.50 g of sodium hydrogen carbonate (molecular weight, 84.0 g $mol^{-1}$) in a 250 $cm^3$ volumetric flask with distilled water. The solution was stored in a dark bottle to protect it from light. The solution was used within one month of preparation (Haemoglobin Laboratory Procedures, J.G. Beetlestone).

### 3.2.5 Preparation of inositol hexakisphosphate (inositol–$P_6$) solution (0.01M)

Reagent grade inositol hexakisphosphate, $C_6H_6O_{24}Na_2$ (molecular weight, 948 g $mol^{-1}$), supplied as the disodium salt of the acid, was purchased from Sigma Chemical Company and was used without further treatment. A 0.01 mol $dm^{-3}$ solution of inositol-$P_6$ was prepared by dissolving 0.237 g of the salt in phosphate buffer (pH 6.0) in a 25 $cm^3$ volumetric flask. The resulting solution was titrated to pH 6.7 from 7.8 using 1.0 mol $dm^{-3}$ hydrochloric acid.

### 3.2.6 Preparation of p-hydroxymercuri(II)benzoate solution

The sodium salt of p-hydroxymercuri(II)benzoic acid, pMB [$C_5H_4(C)(Hg)(OH)COONa$], was obtained from Aldrich Chemical Company and was used without any further treatment. 0.1894g pMB (360.7 g $mol^{-1}$) was accurately weighed, dissolved in a 25 $cm^3$ volumetric flask using 0.1 mol $dm^{-3}$ NaOH and then made up to the mark using the same solution. This gave a 0.021 mol $dm^{-3}$ solution. 1 $cm^3$ of this was pipetted into a 25 $cm^3$ standard flask and then made up to the mark with phosphate buffer pH 7.6, ionic strength 50 mmol $dm^{-3}$. This produced a solution of p-hydroxymercuri(II)benzoate of 840 µmol $dm^{-3}$ concentration. This solution was used for the sulphydryl titrations.

### 3.2.7 Preparation of 5,5'-dithiobis(2-nitrobenzoate), DTNB, solution

DTNB, $C_{14}H_8N_2O_8S_2$ (molecular weight, 396.3 g $mol^{-1}$) was purchased from Sigma Chemical Company, St Louis, USA. A 50 mmol $dm^{-3}$ solution of DTNB was prepared by weighing 0.4954 g of the reagent and dissolving it in 95% ethanol in a 25 $cm^3$ volumetric flask. This was then made up to the mark with 95% ethanol.

25 $cm^3$ of 0.2 mol $dm^{-3}$ sodium dihydrogen phosphate was prepared by dissolving 0.78005 g of the salt ($NaH_2PO_4.2H_2O$, 156.01 g $mol^{-1}$) in distilled water in a 25 $cm^3$ volumetric flask and then making up to the mark. 50 $cm^3$ of 0.2 mol $dm^{-3}$ disodium hydrogen phosphate was also prepared by dissolving 1.4196 g of disodium hydrogen phosphate, $Na_2HPO_4$ (141.96 g $mol^{-1}$) in distilled water in a 50 $cm^3$ volumetric flask and then making up to the mark. The 50 $cm^3$ 0.2 mol $dm^{-3}$ disodium hydrogen phosphate solution was then titrated with the sodium dihydrogen phosphate

solution to pH 8.00 ± 0.01. The volume of dihydrogen phosphate used was about 1.5 $cm^3$.

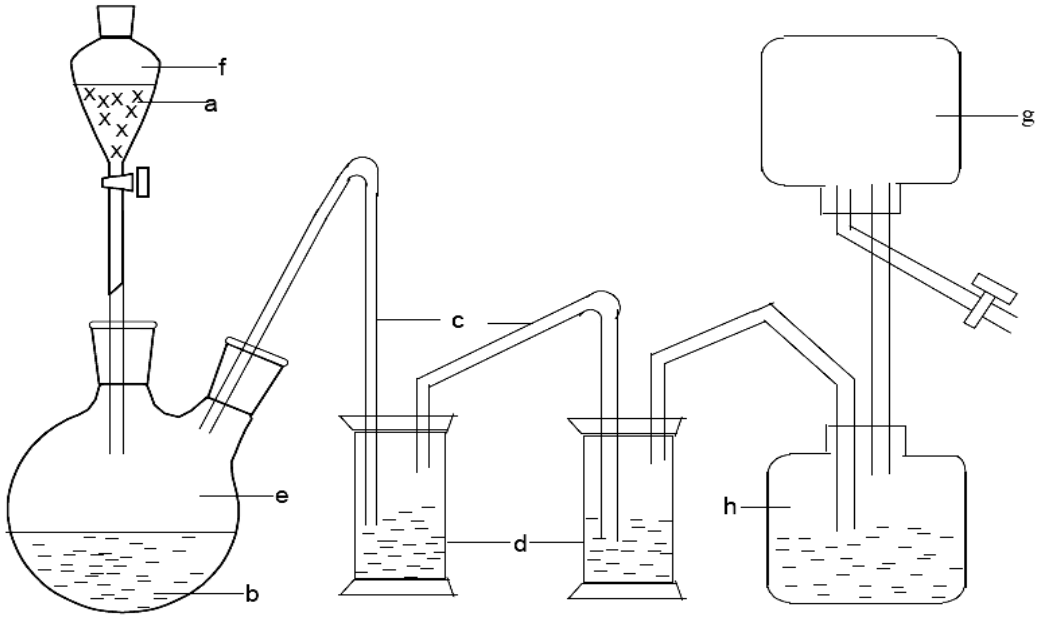
18 $cm^3$ of the 0.2 mol $dm^{-3}$ phosphate buffer pH 8.00 ± 0.01 was added, with stirring, to 25 $cm^3$ of the 50 mmol $dm^{-3}$ DTNB solution in ethanol (prepared above) to give a solution of pH 6.5. The concentration of this DTNB solution was 29.07 mmol $dm^{-3}$. This solution was used for the kinetics experiments and for equilibrium constant determinations.

### 3.2.8 Preparation of carbon monoxide

Carbon monoxide gas (CO) was prepared by reacting concentrated tetraoxosulphate(VI) acid, $H_2SO_4$, with sodium methanoate, HCOONa.

$$H_2SO_{4(aq)} + HCOONa_{(s)} \rightarrow NaHSO_{4(aq)} + CO_{(g)} + H_2O_{(l)}$$

The carbon monoxide evolved was passed through two wash bottles, containing distilled water. Carbon monoxide is not soluble in distilled water, so it merely removes the acid vapour that might have been released along with the carbon monoxide gas. The carbon monoxide was stored in a gas trap bottle for later use. This preparation was carried out in a fume cupboard. Fig. 3.1 shows the setup for the preparation of carbon monoxide.




a: Concentrated tetraoxosulphate(VI) acid; b: Sodium methanoate; c: Delivery tube; d: Water to remove fumes of conc. $H_2SO_4$; e: Two-necked round bottom flask; f: Separating funnel; g: Gas trap; h: Gas collected over water.

***Figure 3.1:*** *Set up for the preparation of carbon monoxide.*

### 3.2.9 Preparation of haemolysates

Blood was obtained by anaesthesizing and venal drawing of blood from one dog (*Canis familiaris*). The blood was collected into freshly prepared acid-citrate-dextrose anticoagulant. Haemoglobin was prepared using standard laboratory procedures (Haemoglobin Laboratory Procedures, J.G. Beetlestone). The blood sample was centrifuged at a speed of 15,000 rpm for 20 minutes at 5°C using a refrigerated MSE High Speed 18 centrifuge and the supernatant was discarded. The red blood cells were washed three times with isotonic saline solution (9.50 g NaCl/$dm^3$). After each washing the resulting mixture was centrifuged at 10,000 rpm for 15 minutes. The sediment (erythrocytes) was lysed by shaking it vigorously with an equal volume of ice-cold distilled water to yield a mixture of haemolysate and red cell debris.

The mixture was centrifuged at 15,000 rpm for 20 minutes to remove all stromal impurities. The haemolysate was decanted from the cake of cell debris, after which sodium chloride (5% w/v) was added. The mixture was left for 20 minutes at 5°C in a refrigerator to precipitate non-haem proteins. The haemolysate was further centrifuged at a speed of 15,000 rpm for 20 minutes. Low molecular weight impurities contained in the haemoglobin were then removed by dialysing it in a 5000 $cm^3$ flask against 10 mmol $dm^{-3}$ phosphate buffer (pH ≈ 6.8) at 5°C for three hours. Polyvinyl chloride dialysis tubing with 14.3 mm diameter and molecular weight, 12,000 – 14,000 Daltons was used for the dialysis. The dialysis tubing was manufactured by Medicell International Limited, London. The dialysis was repeated two times.

Donkey blood was obtained by venal drawing from one donkey at the University of Ibadan Veterinary Teaching Hospital or from a local abattoir at Tattara Village in Nasarawa State.

The procedure used above for the preparation of dog haemolysate was used to prepare the donkey haemolysate.

### 3.2.10 Preparation of carbonmonoxyhaemoglobin

The oxyhaemoglobin prepared above was converted to the carbonmonoxy derivative by bubbling carbon monoxide through it for about 20 minutes. The formation of carbonmonoxyhaemoglobin was indicated by the appearance of a bright pink colour at a maximum wavelength of 426 - 430 nm. (Antonini and Brunori, 1971). Most haemoglobins are stable in the carbonmonoxy form, and are best stored as carbonmonoxyhaemoglobin to prevent conversion to aquomethaemoglobin. Carbonmonoxyhaemoglobin can be readily reconverted to oxyhaemoglobin when necessary.

### 3.2.11 Conversion of carbonmonoxyhaemoglobin to oxyhaemoglobin

Carbonmonoxyhaemoglobin was converted to oxyhaemoglobin by placing it in an ice bath and directing light from a reading lamp on it. The haemoglobin was stirred using a magnetic stirrer as light shone on it for one hour. Carbon monoxide was photolysed off to give oxyhaemoglobin.

### 3.2.12 Preparation of aquomethaemoglobin

Aquomethaemoglobin was prepared by the addition of a two-fold excess of potassium ferricyanide, $K_3Fe(CN)_6$, to oxyhaemoglobin of a known concentration. An approximately 1 mol $dm^{-3}$ $K_3Fe(CN)_6$ solution was prepared by dissolving 0.33 g of the reagent in 1 $cm^3$ of distilled water. The volume of the $K_3Fe(CN)_6$ , $v$ $cm^3$, added to $V$ $cm^3$ of haemoglobin to give a two-fold excess is related to the concentration of the haemoglobin solution by Eqn. 3.1.

$v = 2MV$ ……3.1

$v$ = volume of 1 mol $dm^{-3}$ $K_3Fe(CN)_6$ in $cm^3$

$M$ = concentration of oxyhaemoglobin solution in moles of haem $dm^{-3}$

$V$ = volume of oxyhaemoglobin solution in $cm^3$

The aquomethaemoglobin obtained was dark brown. It was passed through a Dintzis column to remove excess potassium ferricyanide and used within three days of preparation (Haemoglobin Laboratory Procedures, J.G. Beetlestone).

## 3.3 DETERMINATION OF HAEMOGLOBIN CONCENTRATION

### 3.3.1 Determination of oxyhaemoglobin concentration

Oxyhaemoglobin was passed through a Dintzis column to remove extraneous ions. Concentration of oxyhaemoglobin was determined at 540 nm by routinely taking the absorbance of the cyano-methaemoglobin complex formed after reaction with Drabkin's solution. The molar extinction coefficient (per haem) at 540 nm was 1.09 x $10^4$ $mol^{-1}$ $dm^3$ $cm^{-1}$ (Austin and Drabkin, 1935; Drabkin and Austin, 1935; Wiedermann and Olson, 1975). 10 $cm^3$ of Drabkin's solution was added to 0.1 $cm^3$ of oxyhaemoglobin and the absorbance at 540 nm was measured using Drabkin's solution as reference. The concentration of oxyhaemoglobin was calculated using Eqn. 3.2.

$$C = \frac{A_{540} \times (10 + v) \times 10^{-4}}{1.09 \times l \times v} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.2$$

$A_{540}$ = absorbance of mixture at 540 nm

$C$ = concentration of oxyhaemoglobin in moles of haem/$dm^3$

$l$ = path length of cuvette in cm

$v$ = volume of oxyhaemoglobin used

### 3.3.2 Determination of carbonmonoxyhaemoglobin concentration

The carbonmonoxyhaemoglobin was first passed through a Dintzis column to remove extraneous ions. The absorbance of a solution of 0.1 $cm^3$ of carbonmonoxyhaemoglobin mixed with 10 $cm^3$ distilled water was measured at 537.5 nm using distilled water as reference. The concentration was determined in moles haem $dm^{-3}$ using 1.4 x $10^4$ $mol^{-1}$ $dm^3$ $cm^{-1}$ as the molar extinction coefficient (Zuiderweg *et al.*, 1981). Eqn. 3.3 was used to calculate the carbonmonoxyhaemoglobin concentration.

$$C = \frac{A_{537.5} \times (10 + v) \times 10^{-4}}{1.4 \times l \times v} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.3$$

$A_{537.5}$ = absorbance of carbonmonoxyhaemoglobin at 537.5 nm

$C$ = concentration of carbonmonoxyhaemoglobin in moles of haem/$dm^3$

$l$ = path length of cuvette in cm

$v$ = volume of carbonmonoxyhaemoglobin used

### 3.3.3 Determination of aquomethaemoglobin concentration

0.1 $cm^3$ of the aquomethaemoglobin was added to 10 $cm^3$ of distilled water. A few crystals of recrystallized potassium cyanide were then added. The absorbance of the resulting solution was then taken at 540 nm. The molar extinction coefficient (per haem) at 540 nm was 1.09 x $10^4$ $mol^{-1}$ $dm^3$ $cm^{-1}$ (Austin and Drabkin, 1935; Drabkin and Austin, 1935; Wiedermann and Olson, 1975). The absorbance measured was used to calculate the haemoglobin concentration using Eqn. 3.2 as with oxyhaemoglobin since the wavelength of measurement was 540 nm.

## 3.4 PREPARATION OF RESINS AND PACKING OF DINTZIS COLUMN

The Dintzis column is a bed of ion exchange resins used for deionising haemoglobin.

### 3.4.1 Preparation of resins

All resins used in this study were obtained from British Drug Houses Limited, Poole, England.

**(a) Hydrogen form**

Amberlite resin IR 120 was packed into a 50 $cm^3$ burette and about 10 times its volume of 3 mol $dm^{-3}$ HCl was passed slowly through it for two to three hours. The resin was then washed with distilled water until the effluent was acid free, i.e., neutral to blue litmus paper.

**(b) Acetate form**

Amberlite resin IRA 400 was packed into a 50 $cm^3$ burette and about 10 times its volume of 3 mol $dm^{-3}$ hydrochloric acid was passed slowly through it for two to three hours. The resin was then washed until the effluent was acid free (neutral to blue litmus). About 10 times its volume of 3 mol $dm^{-3}$ sodium acetate was then passed slowly through the resin in the column. The resin was again washed with distilled water until the effluent gave no precipitate with a 2 M silver nitrate solution. Formation of a precipitate (AgCl) would indicate the presence of $Cl^-$ ions

**(c) Ammonium form**

100 $cm^3$ of 3 mol $dm^{-3}$ sodium chloride was passed slowly for about two hours through a packed column of Amberlite IR 120 resin. The resin was washed with

distilled water until the effluent gave no precipitate with a 2 M silver nitrate solution. Thereafter ten times its volume of 3 mol $dm^{-3}$ ammonium chloride was slowly passed through the column for two to three hours. The resin was then washed with distilled water until the effluent from the column gave no precipitate with a 2 M silver nitrate solution.

**(d) Mixed form and mixed indicator form**

Each of these two forms of the resins was washed with distilled water, without further treatment.

### 3.4.2 Packing of Dintzis column for deionising haemoglobins

A 50 $cm^3$ burette, with one end plugged with glass wool, served as the column. About 7 cm length of the generated hydrogen form of the resin was packed into the burette containing distilled water. (The tap was kept running while the column was being packed, and care was taken to ensure that there was always distilled water over the resin throughout the packing process). About 3 cm length of the mixed indicator form was added followed by 3 cm length of the mixed form, 7 cm length of the acetate form and, finally, 7 cm length of the ammonium form of the resin were added to the column (Fig. 3.2). The column was then washed thoroughly with distilled water (Dintzis, 1952). The Dintzis column was discarded when the indicator resins showed a change of colour or when heavy discolouration was noticed in either the acetate or the ammonium layer.

The preparation and packing of the Dintzis column all together took between 3 to 5 days.

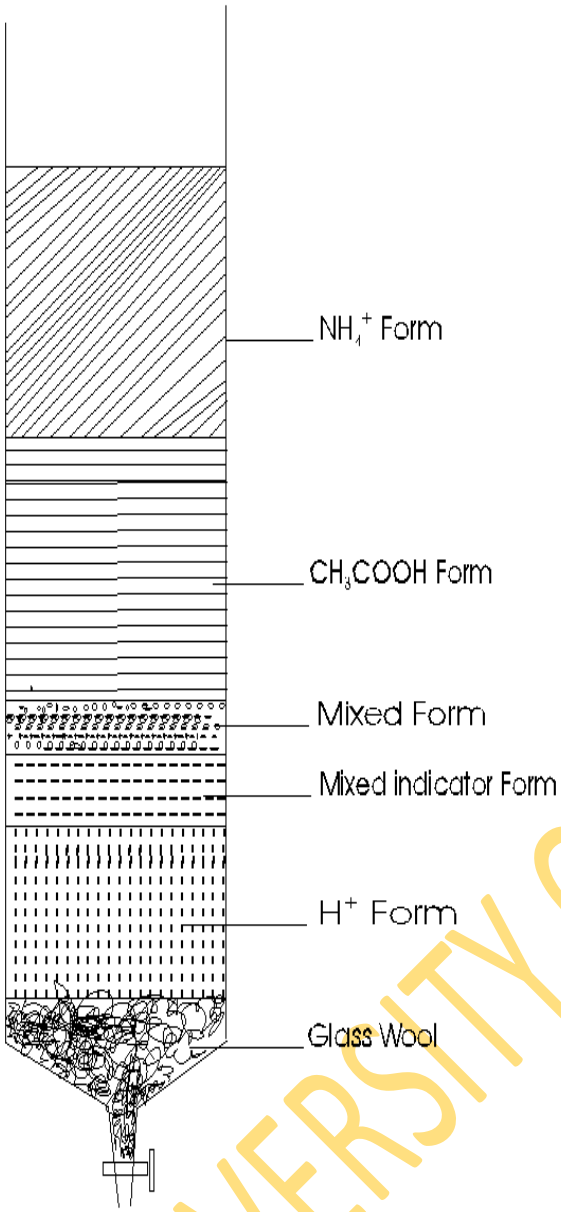



***Figure 3.2**: Dintzis column*

## 3.5 EXPERIMENTAL PROCEDURES

### 3.5.1. Titration of donkey carbonmonoxyhaemoglobin sulphydryl groups with p-hydroxymercuri(II)benzoate, pMB

The sulphydryl titration of donkey carbonmonoxyhaemoglobin was done according to Boyer's method (Boyer, 1954), as previously reported for dog haemoglobin (Okonjo *et al*., 1979; Okonjo and Adejoro, 1993). 3 $cm^3$ of carbonmonoxyhaemoglobin solution (10 μmol $dm^{-3}$ in haem) in phosphate buffer, pH 7.6 and ionic strength 50 mmol $dm^{-3}$, was pipetted into a cuvette and the absorbance at 250 nm was read on a Zeiss PMQ II UV-Visible spectrophotometer, using the pH 7.6 buffer as the reference. 10 $mm^3$ of p-hydroxymercuri(II)benzoate, pMB (840 μmol $dm^{-3}$), in the same buffer was added to the solution in the cuvette with a Gilson Pipettman micropipette. The solution was stirred and the absorbance was read on the spectrophotometer. This procedure was repeated for subsequent additions of 10 $mm^3$ of pMB. This was continued until the increase in the absorbance could be accounted for in terms of the absorbance of pMB alone. A similar titration was done with the pH 7.6 buffer alone (no haemoglobin) to obtain the absorbance of pMB as a function of the total volume. The absorbances of pMB at various total volumes were subtracted from the absorbances of the complex formed between pMB and haemoglobin. The absorbance readings were corrected for dilution to obtain $\Delta A_{corr}$. (Table1; Appendix I; page 181) shows a typical set of readings.

A graph of $\Delta A_{corr.}$ against the volume of pMB added was plotted and the maximum change in absorbance, $\Delta A_{max}$, was read at the part of the plot where there was a break showing that all free sulphydryl groups had reacted (Fig. 4.1). The number of reactive thiol groups was calculated assuming an absorption coefficient of 7,600 $mol^{-1}$ $dm^3$ $cm^{-1}$ for the complex formed between pMB and Hb (Boyer, 1954). This was then related to the haemoglobin concentration to obtain the number of sulphydryl groups per tetramer. The ratio of [p-HbSMB], the concentration of the product of the reaction of haemoglobin with pMB, to the concentration of haemoglobin tetramer at various volumes of pMB was then plotted against the volume of pMB (Fig. 4.2). This experiment was repeated at least three times and the mean of the titratable sulphydryl groups was calculated.

### 3.5.2 Sulphydryl titration of donkey carbonmonoxyhaemoglobin with 5,5'-dithiobis(2-nitrobenzoate), DTNB

DTNB is a useful reagent for the spectrophotometric determination of sulphydryl groups in biological materials (Ellman, 1959; Grassetti and Murray, 1966). DTNB is not useful under very acidic conditions (< pH 4) for two reasons: the TNB product is only chromophoric in the dianionic form (> 98% above pH 7) (Ellman, 1959; Gargouri, *et al*., 1988), and DTNB has been shown not to react with cysteine at pH 3.3 (Grassetti and Murray, 1966). Since the reaction of DTNB with sulphydryl groups is not as fast as that of pMB, the following procedure was used: 10 $cm^3$ portions of a 10 µmol (haem) $dm^{-3}$ carbonmonoxyhaemoglobin solution in phosphate buffer pH 7.6, ionic strength, 50 mmol $dm^{-3}$, were accurately pipetted into 18 clean, dry test tubes. Increasing volumes (0.5 - 10 $mm^3$) of stock 29.07 mmol $dm^{-3}$ DTNB were added to the different test tubes. These mixtures were stirred and left to equilibrate for at least 3 hours. The absorbance of the solution in each tube was read at 412 nm on a Zeiss PMQ II UV-visible spectrophotometer. A haemoglobin solution containing no DTNB was used as reference.

The absorbance of each haemoglobin/DTNB mixture was corrected for dilution and for the absorbance of DTNB alone. To obtain the absorbance of DTNB alone, the absorbances of increasing volumes of stock 29.07 mmol $dm^{-3}$ DTNB in 10 $cm^3$ of phosphate buffer (pH 7.6) were read on the same spectrophotometer. The absorbances of DTNB alone were subtracted from the absorbances of the haemoglobin/DTNB mixtures and corrected for dilution to obtain $\Delta A_{corr}$.

A plot of $\Delta A_{corr.}$ against the volume of DTNB was made and $\Delta A_{max}$ was read off (see Fig. 4.3). The concentration of 5-thio-2-nitrobenzoate ($TNB^-$) produced was calculated from the change in absorbance, assuming a molar absorption coefficient of 14,000 $mol^{-1}$ $dm^3$ $cm^{-1}$ for TNB.

The amount of TNB produced per haemoglobin tetramer, $[TNB^-]/Hb_{4,}$ was plotted against the volume of DTNB (see Fig. 4.4). The graph levels up only when all the reactive sulphydryl groups have been titrated. This experiment was repeated at least three times and the mean of the titratable sulphydryl groups was calculated.

### 3.5.3 Kinetics of the reaction of DTNB with CysF9[93]β

The kinetics of the reaction of pMB with haemoglobin sulphydryl groups are so fast that they can only be monitored by the stopped-flow technique. This machine was not available. On the other hand, the kinetics of the reaction of DTNB occur in the seconds to minutes time range and can be monitored on a UV-visible spectrophotometer. DTNB has an added advantage over pMB: it is very sensitive to the electrostatic environment of sulphydryl groups (Okonjo *et al*., 1995). Therefore, DTNB was used as the sulphydryl reagent of choice.

The procedure used for the kinetics was the same for dog and donkey haemoglobins. DTNB contains a disulphide bond, the experiments were therefore restricted to pH ≤ 9.0 because it is known that above pH 9.0 complications arise on account of increased rate of hydrolysis of disulphide bonds (Robyt *et al.*, 1971). The kinetics of the reaction of DTNB with haemoglobin were monitored at 412 nm on a Cecil BioQuest UV-visible spectrophotometer, under pseudo-first order conditions. This was achieved by reacting haemoglobin samples with at least a sixty-fold excess of DTNB over the sulphydryl groups. 50 $cm^3$ of a 10 μmol (haem) $dm^{-3}$ (5 μmol $dm^{-3}$ in reactive sulphydryl groups) solution of a haemoglobin derivative in a chosen buffer was allowed to temperature equilibrate at 25°C in a thermostated water bath. A 3 $cm^3$ aliquot of this haemoglobin solution was then transferred to a 1 cm x 1 cm cuvette. The cuvette was placed in the cell compartment of the spectrophotometer, thermostated at 25°C. A calculated volume (31 – 62 $mm^3$) of a 29.07 mmol $dm^{-3}$ solution of DTNB in 95% ethanol, which would give a final DTNB concentration of between 300 and 600 μmol $dm^{-3}$, was reacted with the haemoglobin by mixing in the cuvette, using a glass rod as stirrer.

The kinetic traces (transmittance as a function of time) were obtained from the computer that was coupled to the spectrophotometer. Each kinetic run was repeated three times under identical experimental conditions. The data were analyzed with a 1990 update of DISCRETE, a computer program for the analysis of multiple exponential signals (Provencher, 1976). The analyses gave one kinetic phase under pseudo-first order conditions. The pseudo-first order rate constants, $k_{obs}$, were plotted against the DTNB concentration.

### 3.5.4. Equilibrium constant determination for the reaction of DTNB with CysF9[93]β

The same procedure was employed for dog and donkey haemoglobins. 10 $cm^3$ aliquots of a 50 μmol (haem) $dm^{-3}$ (25 μmol $dm^{-3}$ in reactive sulphydryl groups) solution of a haemoglobin derivative in buffer at a specific pH were accurately measured into eighteen (18) clean, dry test tubes. Increasing volumes (10.0 – 100.0 $mm^3$) of stock 29.07 mmol $dm^{-3}$ DTNB were added to the tubes. The mixtures were stirred and left to equilibrate for about 6 hours at 25°C in a thermostated waterbath. The absorbance of each solution was read at 412 nm on a Zeiss PMQ II UV-visible spectrophotometer using haemoglobin in buffer without DTNB as a reference.The absorbances ($A_{412}$) were corrected for dilution by DTNB. The concentration of 5-thio-2-nitrobenzoate, $TNB^-$, produced was calculated from the change in absorbance, assuming a molar absorption coefficient of 14,000 $mol^{-1}$ $dm^3$ $cm^{-1}$ for $TNB^-$.

The equilibrium constant, $K_{equ}$, was calculated for the content of each test tube using a program written on the MicroMath Scientist software (Salt Lake City, Utah). The mean values of $K_{equ}$ so calculated had a standard error of *ca* 20%. The pH of the mixture of the contents of the tubes was measured at the end of the incubation period using a Radiometer PHM 85 Precision Research pH meter previously standardized with standard buffers pH 4.0 and 9.22. This procedure was repeated in phosphate buffers pH 5.6 – 7.8 and borate buffers pH 8.0 – 9.0, each of which had an ionic strength of 50 mmol $dm^{-3}$. The negative logarithms to base ten of the equilibrium constants ($-\log_{10}K_{equ}$) were then plotted against the pH. The results for stripped dog haemoglobin are reported in Figs. 4.29 – 4.31; those for donkey haemoglobin are shown in Figs. 4.35- 4.37.

# CHAPTER FOUR

# RESULTS

## 4.1 Number of sulphydryl groups in dog haemoglobin

The dog has only one haemoglobin type (Wajeman *et al*., 1975). Dog haemoglobin has a total of eight sulphydryl groups per tetramer, according to its amino acid sequence; these are located at the CysG11[104]α, CysG14[112]β, CysG18[111]α and CysF9[93]β sites (Brimhall *et al*., 1977). Titrations with p-hydroxymercuri(II)benzoate, pMB, and 5,5′-dithiobis(2-nitrobenzoate), DTNB, to determine the number of sulphydryl groups in dog haemoglobin have already been carried out (Okonjo *et al*., 1979; 1993). The sulphydryl groups located at the CysG11[104]α and CysG14[112]β positions are at the $\alpha_1\beta_1$ subunit interface and therefore do not react with any sulphydryl reagent (Antonini and Brunori, 1971). The four sulphydryl groups located at the CysG18[111]α and CysF9[93]β positions are reactive towards mercurial sulphydryl reagents. However, only two sulphydryls are reactive towards nonmercurial sulphydryl reagents (Okonjo *et al*., 1979; 1993).

At all pH values studied, the reaction of dog haemoglobin with DTNB was found to be kinetically monophasic, indicating that only one of the sulphydryls (CysG18[111]α or CysF9[93]β) is reactive towards DTNB.

In an attempt to determine whether CysG18[111]α reacts with DTNB, Okonjo *et al*. (1979; 1993) examined the environment of this sulphydryl group and showed that this sulphydryl must be unreactive towards nonmercurials due to its negatively charged environment and dielectric constants of proteins less than water. All these interactions would considerably raise the $pK_a$ of the CysG18[111]α thiol. Therefore, reaction with nonmercurial sulphydryl reagents via nucleophilic attack by the thiol anion of the CysG18[111]α sulphydryl group would be impossible (Okonjo *et al*., 1979). These observations led to the conclusion that only CysF9[93]β reacts with nonmercurial reagents in dog haemoglobin (Okonjo *et al*., 1979).

## 4.2 Number of sulphydryl groups in donkey haemoglobin

The haemolysate of the donkey has only one haemoglobin type (Balasundaresan *et al*., 2006). The amino acid sequences of the α and β chains of donkey haemoglobin are very similar to those of horse (*Equus caballus*), with only a few replacements in their α-chains (Kilmartin and Clegg, 1967). The amino acid sequences of their β subunits are identical (Kitchen and Easley, 1969; Balasundaresan *et al*., 2006).

Donkey haemoglobin differs from horse haemoglobin by only two amino acids in the α-chain; HisB1[20]α to Asn and TyrB5[24]α to Phe. These substitutions do not significantly change the secondary structural features of donkey haemoglobin: the haem group region and subunit contacts closely resemble those of horse methaemoglobin (Balasundaresan *et al*., 2006).

Donkey haemoglobin has four sulphydryl groups located at positions CysG11[104]α and CysF9[93]β, whereas horse haemoglobin has two located at CysF9[93]β (Balasundaresan *et al*., 2006). Two sulphydryl groups located at position CysF9[93]β react with all reagents and two sulphydryl groups located at CysG11[104]β at the αβ interface and do not react with any reagent (Antonini and Brunori, 1971).

### 4.2.1 Titration of the sulphydryl groups of donkey haemoglobin with p-hydroxymercuri(II)benzoate, pMB

The results of the titration of donkey carbonmonoxyhaemoglobin with p-hydroxymercuri(II)benzoate, pMB, are shown in Figs. 4.1- 4.2. Fig. 4.1 is a plot of the corrected change in absorbance ($\Delta A_{corr.}$) against the volume of pMB added. Fig. 4.2 is a plot of the concentration of the complex formed with pMB per haemoglobin tetramer against the volume of pMB added. Fig. 4.2 shows that only two sulphydryl groups are reactive towards pMB.

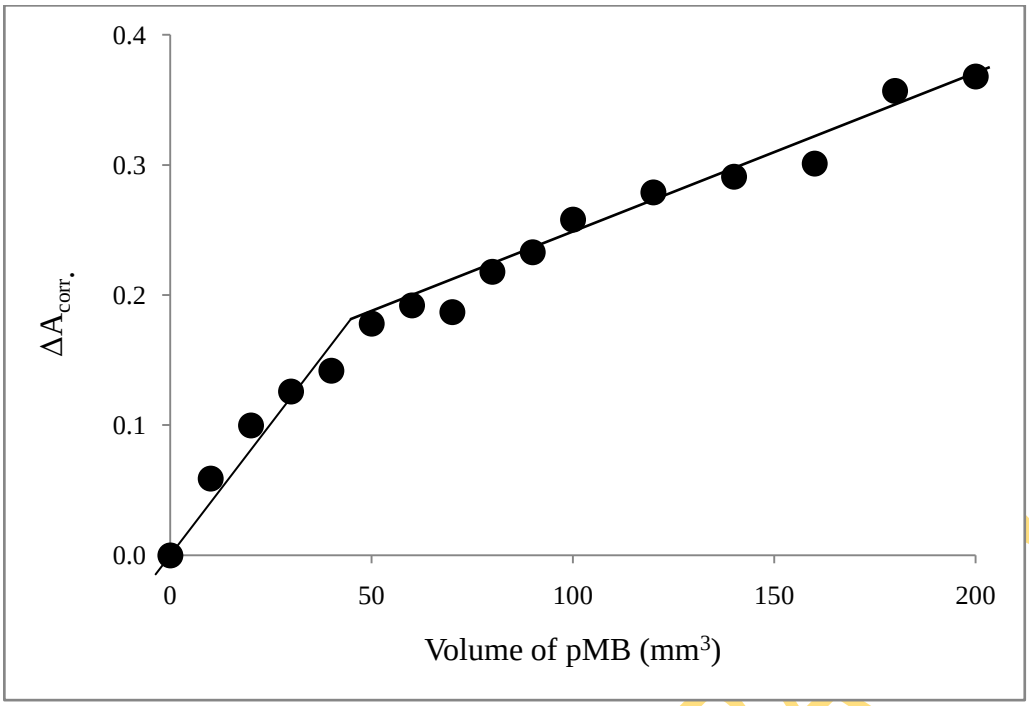



***Figure 4.1**: Titration of the **carbonmonoxy** derivative of **donkey** haemoglobin with pMB at 250 nm: plot of corrected absorbance change as a function of the volume of pMB mixed with 3 $cm^3$ of haemoglobin. The absorbance was corrected for dilution and for the absorbance due to pMB.*

*Conditions: haemoglobin concentration = 60 µmol (haem) $dm^{-3}$; stock concentration of pMB = 840 µmol $dm^{-3}$; phosphate buffer pH 7.6, (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl).* The software used to plot the graph was Microsoft Excel.

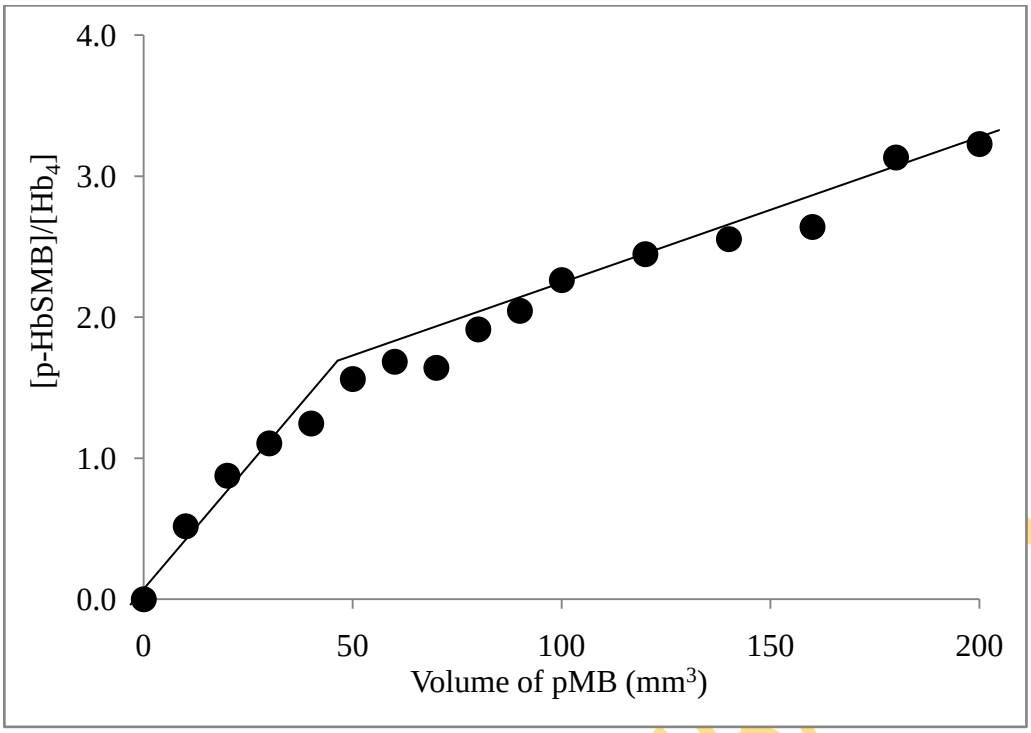



***Figure 4.2**: Titration of the **carbonmonoxy** derivative of **donkey** haemoglobin with pMB at 250 nm: plot of [p-HbSMB]/[Hb$_4$] as a function of the volume of pMB mixed with 3 cm$^3$ of haemoglobin. The absorbance was corrected for dilution and for the absorbance due to pMB.*

*Conditions: haemoglobin concentration = 60 µmol (haem) dm$^{-3}$; stock concentration of pMB = 840 µmol dm$^{-3}$; phosphate buffer pH 7.6, (ionic strength 50 mmol dm$^{-3}$; added salt, NaCl), Absorption coefficient, 7,600 mol$^{-1}$ dm$^3$ cm$^{-1}$. An absorption coefficient of 7,600 mol$^{-1}$ dm$^3$ cm$^{-1}$ was assumed for the haemoglobin-pMB complex.*

The software used to plot the graph was Microsoft Excel.

### 4.2.2 Titration of the sulphydryl groups of donkey haemoglobin with DTNB

Typical results for the titration of donkey carbonmonoxyhaemoglobin with DTNB are shown in Figs. 4.3 and 4.4. Fig. 4.3 is a plot of the corrected change in absorbance ($\Delta A_{corr.}$) against the volume of DTNB added. Fig. 4.4 is a plot of the concentration of the complex formed with DTNB per haemoglobin tetramer against the volume of DTNB added. Fig. 4.4 shows that only two sulphydryl groups are reactive towards DTNB.

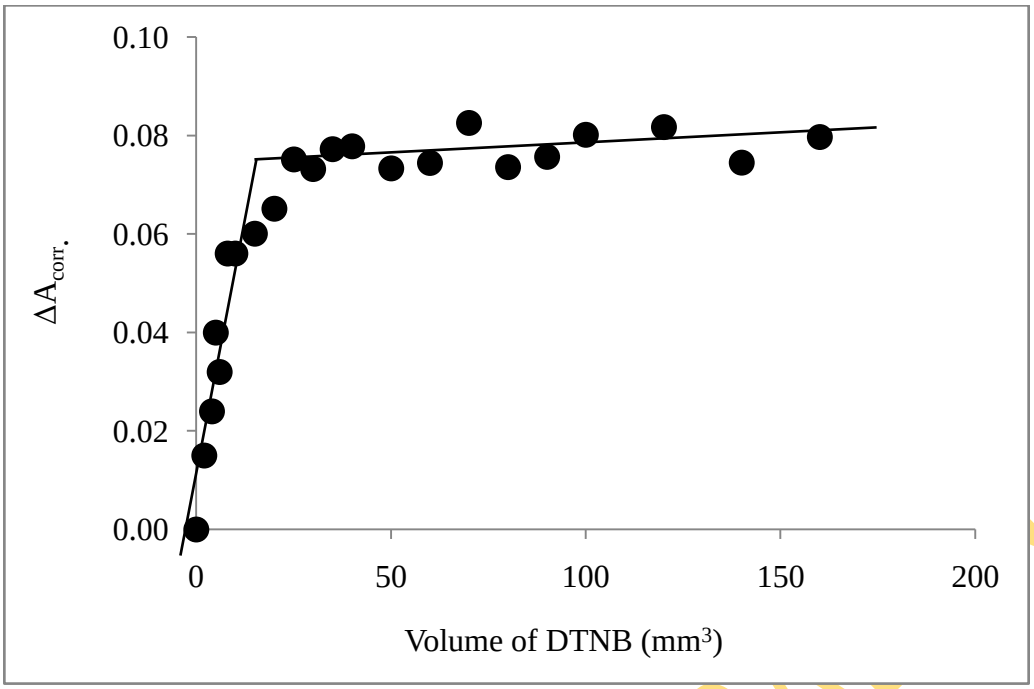



***Figure 4.3**: Titration of **donkey carbonmonoxyhaemoglobin** with DTNB at 412 nm: plot of corrected absorbance change ($\Delta A_{corr.}$) as a function of the volume of DTNB mixed with 10 $cm^3$ of haemoglobin.*

*Conditions: haemoglobin concentration, 10 µmol (haem) $dm^{-3}$; stock concentration of DTNB, 29.07 mmol $dm^{-3}$; phosphate buffer pH 7.6, (ionic strength, 50 mmol $dm^{-3}$; added salt, NaCl), Absorption coefficient, 14,000 $mol^{-1}$ $dm^3$ $cm^{-1}$ was assumed for TNB, the product of the DTNB reaction with haemoglobin.*

The software used to plot the graph was the Microsoft Excel.

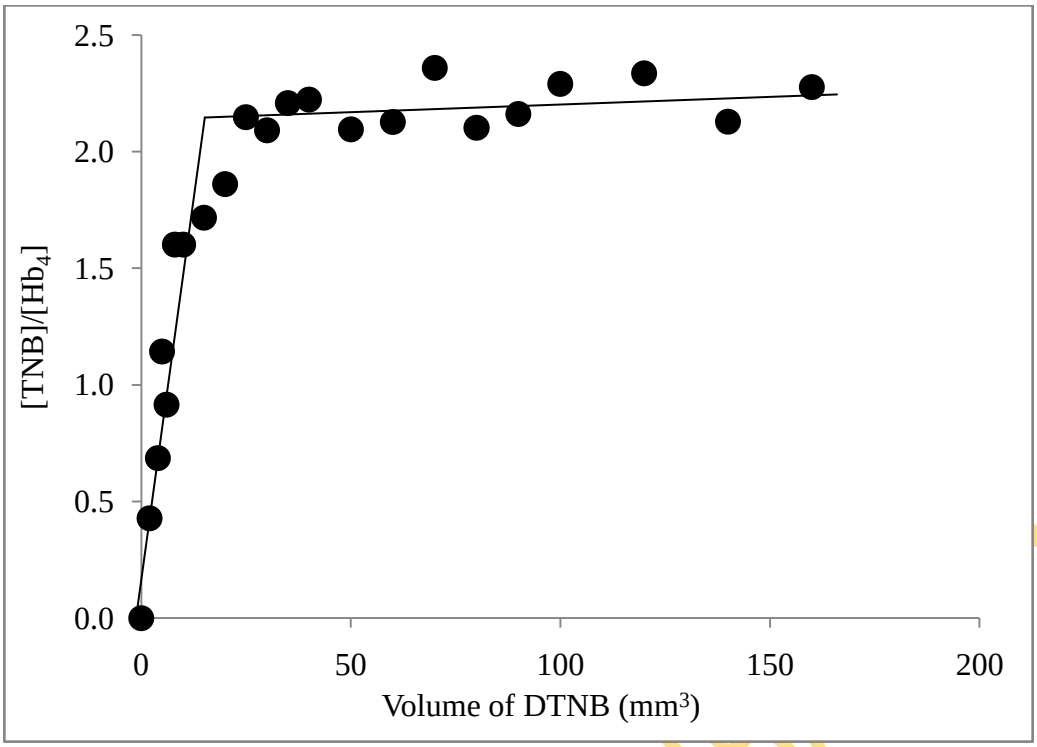



***Figure 4.4**: Titration of the **carbonmonoxy** derivative of **donkey** haemoglobin with DTNB at 412 nm: plot of [TNB]/[$Hb_4$] as a function of the volume of DTNB mixed with 10 $cm^3$ of haemoglobin.*

*Conditions: haemoglobin concentration, 10 µmol (haem) $dm^{-3}$; stock concentration of DTNB, 29.07 mmol $dm^{-3}$; phosphate buffer pH 7.6, (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl), An absorption coefficient of 14,000 $mol^{-1}$ $dm^3$ $cm^{-1}$was assumed for TNB, the product of the DTNB reaction with haemoglobin.* The software used to plot the graph was Microsoft Excel.

## 4.3 KINETICS

### 4.3.1 Theory of kinetics

The complete reaction of haemoglobin with DTNB can be depicted as

.....4.1

Where:

PSH is haemoglobin with its CyF9[93]β sulphydryl group protonated;

$PS^-$ is haemoglobin with its CyF9[93]β sulphydryl group deprotonated;

PS.ST is the mixed disulphide formed by haemoglobin with DTNB; and

$TNB^-$ is the chromophoric (anionic) product of the reaction of DTNB with CyF9[93]β of haemoglobin; and TNBH is the protonated form of $TNB^-$

$Q_{SH}$ is the ionisation constant of CysF9[93]β

$Q_{TNB}$ is the ionisation constant of TNBH, the protonated fprm of $TNB^-$

$k_F$ is the forward second order rate constant

$k_R$ is the second order reverse rate constant

If a = the initial concentration of $PS^-$

b = the initial concentration of DTNB

$x$ = the concentration of each of PS.ST and ($TNB^-$ + TNBH) formed at time t, and $k_F$ and $k_R$ are the second order forward and reverse rate constants, respectively, the rate of reaction is given by (Okonjo and Fodeke, 2005; modified):

$$-\frac{d(a-x)}{dt} = k_F(a-x)(b-x) - k_R(x)^2 \quad \text{.....4.2}$$

$$-\frac{d(a)}{dt} + \frac{dx}{dt} = k_F ab - k_F(a+b)x + k_F x^2 - k_R x^2 \quad \text{.....4.3}$$

But $-\frac{d(a)}{dt} = k_F ab$ .....4.4

$$\therefore \frac{dx}{dt} = -k_F(a+b)x + (k_F - k_R)x^2 \quad \text{.....4.5}$$

Under pseudo-first order conditions, when b >> a, Eqn.4.5 becomes

$$\frac{dx}{dt} = -k_F bx + (k_F - k_R)x^2 \quad \text{.....4.6}$$

$$\frac{dx}{dt} = -k_F \left\{ bx - \left(1 - \frac{1}{K}\right)x^2 \right\} \quad \text{.....4.7}$$

Here, $K = \frac{k_F}{k_R}$ is the equilibrium constant for the DTNB reaction step.

$$\frac{dx}{dt} = -k_F \left\{ bx + \left(\frac{1}{K} - 1\right)x^2 \right\} \quad \text{.....4.8}$$

If $bx >> (1/K - 1)x^2$, then Eqn. 4.8 becomes;

$$\frac{dx}{dt} = -k_F bx \quad \text{.....4.9}$$

Eqn. 4.9, on integration, becomes

$$-\frac{1}{b}\int \frac{dx}{x} = k_F \int dt \quad \text{.....4.10}$$

$$-\frac{1}{b}\ln x = k_F t + c \quad \text{.....4.11}$$

where $c$ is a constant

$$-\ln x = k_F bt + c' \quad \text{.....4.12}$$

where $c' = bc$ *and* $b = [DTNB]_{t=0}$

A plot of $-ln\ x$ against time $t$ should give a straight line of slope $k_F b$ which is the pseudo-first order rate constant, represented by $k_{obs}$.

Let $A_0$ be the absorbance at time t = 0; $A_t$ the absorbance at any time $t$ after the reaction has started and $A_{equ}$ the absorbance at equilibrium. Let $x$ be the concentration of the species whose absorbance is monitored ($TNB^-$) in the course of the reaction. At time t, Eqn. 4.12 can be written as:

$$-\ln(A_{equ} - A_t) = k_F bt + c' \quad \text{.....4.13}$$

When t = 0, $-\ln(A_{equ} - A_0) = c'$ .....4.14

Then $-\ln(A_{equ} - A_t) = k_F bt - \ln(A_{equ} - A_0)$ .....4.15

Hence a plot of $-ln(A_{equ} - A_t)$ versus $t$ should give a straight line of slope $k_F b$ which represents $k_{obs}$.

However, if in Eqn. 4.8 $\left(\frac{1}{K} - 1\right)x^2$ is not negligible compared to $bx$, then a semilog plot according to Eqn. 4.12 and 4.15 should not give a straight line, as has been found for cat haemoglobins at pH values above 8.6 (Okonjo and Fodeke, 2005).

### 4.3.2 Forward kinetics

#### 4.3.2.1 Kinetics of DTNB reaction with dog haemoglobin.

Figs. 4.5 and 4.6 show the variation of transmittance with time for a typical kinetic run for the reaction of DTNB with the *stripped* carbonmonoxy derivative of dog haemoglobin at various pH values. Similar runs were obtained for the three derivatives of dog haemoglobin (- CO, oxy and aquomet) with and without inositol-$P_6$ over the pH range $5.6 \leq pH \leq 9.0$. The percentage transmittance readings (T) were converted to decimal fractions and then substituted into the following equation to obtain the absorbance, A:

$$A = \log_{10} 1/T$$

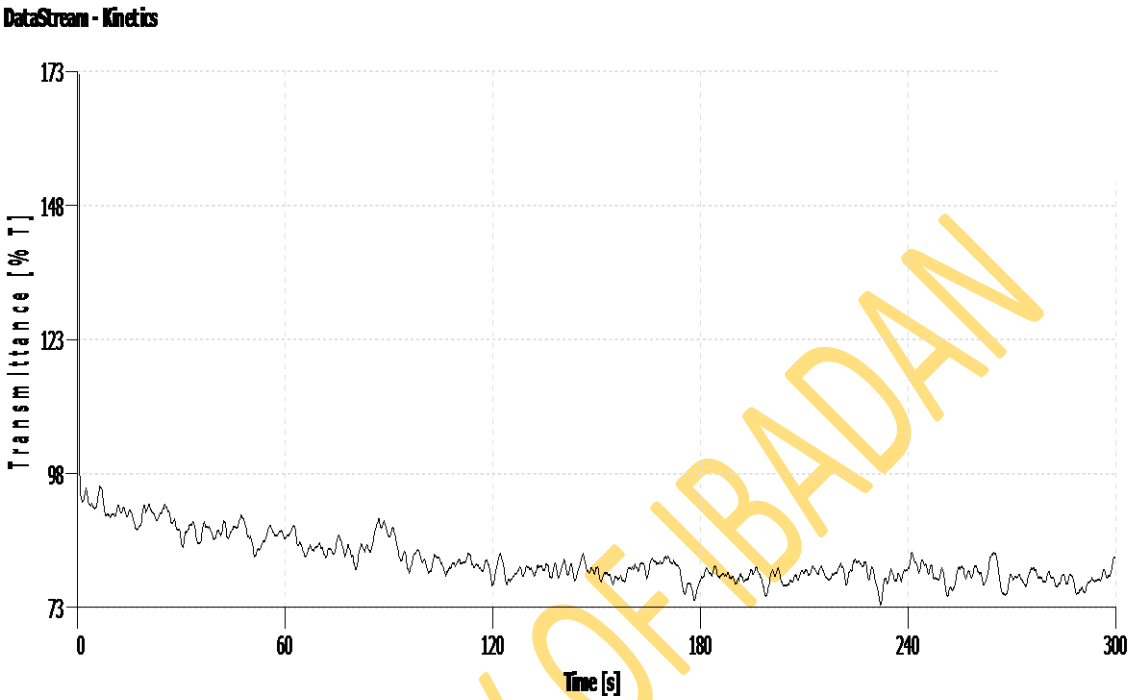



***Figure 4.5:*** *A typical kinetic run for the reaction of 5,5'-dithiobis(2-nitrobenzoate) with* ***stripped dog carbonmonoxyhaemoglobin*** *sulphydryl groups at pH* ***5.570****, Conditions: temperature, 25$^{o}$C; phosphate buffer (ionic strength, 50 mmol dm$^{-3}$; added salt, NaCl); [HbCO] = 10 µmol (haem) dm$^{-3}$ (5 µmol dm$^{-3}$ in reactive sulphydryl groups); [DTNB] = 300 µmol dm$^{-3}$.*

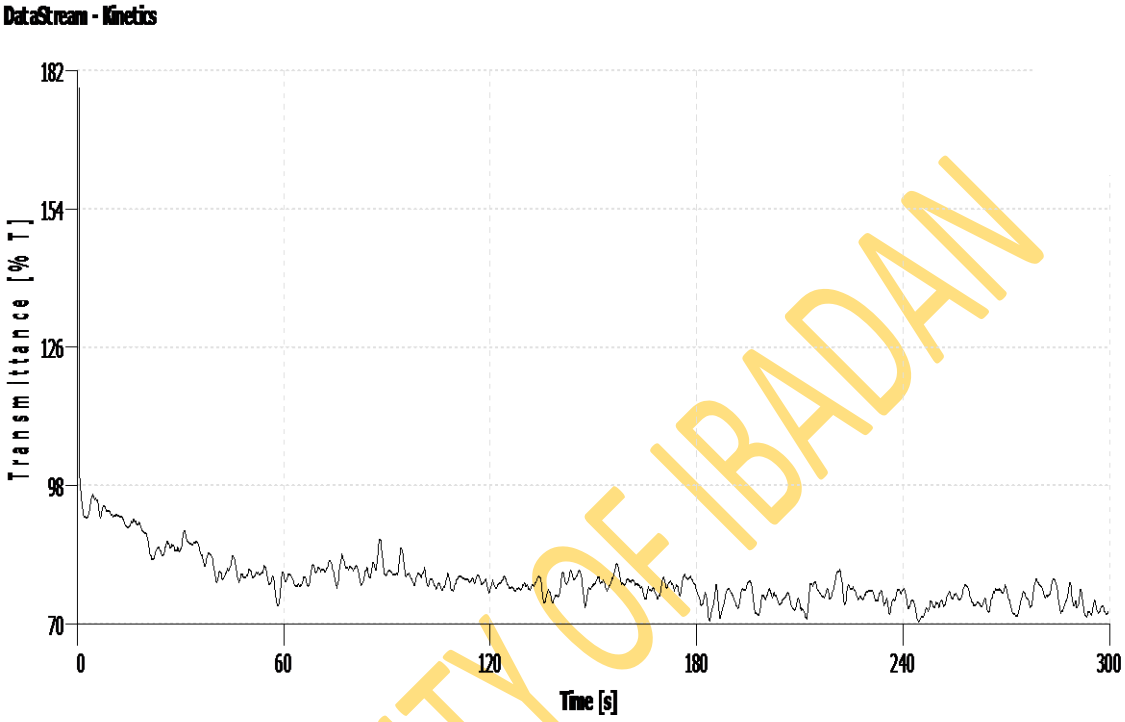



***Figure 4.6:*** *A typical kinetic run for the reaction of 5,5'-dithiobis(2-nitrobenzoate) with* ***stripped dog carbonmonoxyhaemoglobin*** *sulphydryl groups at pH* ***9.110****, Conditions: temperature, 25°C; borate buffer (ionic strength, 50 mmol* $dm^{-3}$*; added salt,NaCl); [HbCO] = 10 µmol (haem)* $dm^{-3}$ *(5 µmol* $dm^{-3}$ *in reactive sulphydryl groups); [DTNB] = 300 µmol* $dm^{-3}$*.*

Figs. 4.7 and 4.8 show the semi - logarithmic plots of the time courses for the reaction of DTNB with *stripped* dog carbonmonoxyhaemoglobin for the traces shown in Figs. 4.5 and 4.6. $A_{equ}$ is the absorbance when the reaction is at equilibrium and $A_t$ is the absorbance at any other time t. A straight line is obtained in each case, in conformity with Eqn. 4.15. Similar results were obtained for all the other haemoglobin derivatives, *stripped* and with inositol-$P_{6,}$ under the same experimental conditions. This proves that the use of Eqn. 4.15 is valid and that $(1/K -1)x^2$ is much less than bx for dog haemoglobin.

The slope of each semi–log plot gives $k_{obs}$, the pseudo-first order rate constant.

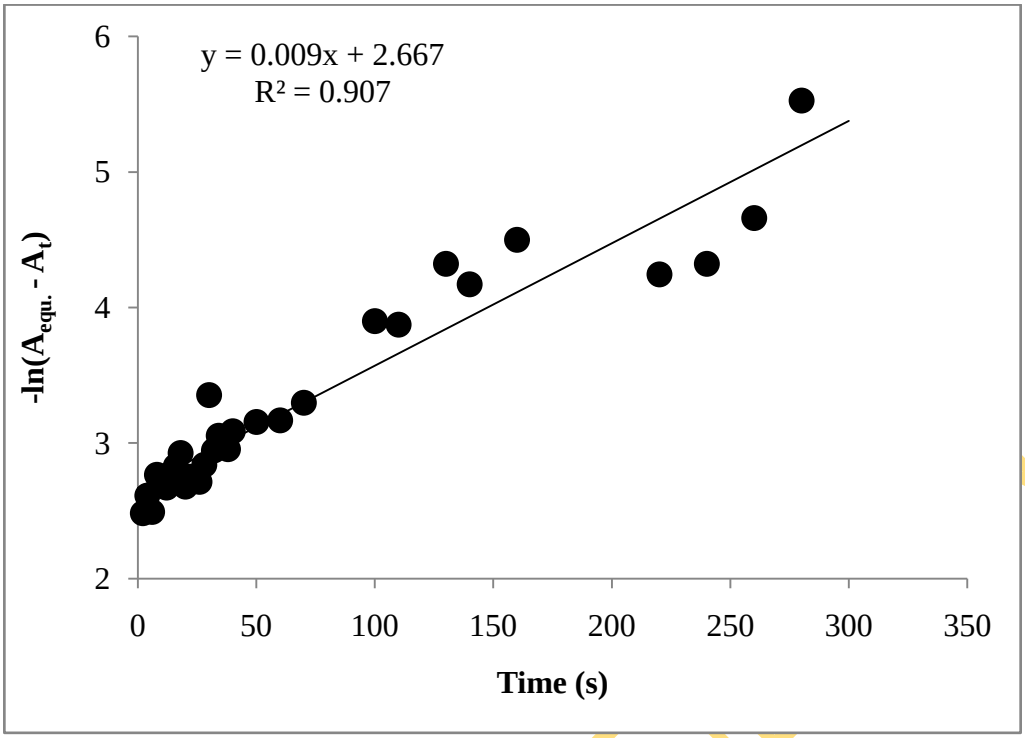



***Figure 4.7:*** *Semi-logarithmic plot of the time course for the reaction of 5,5'-dithiobis(2-nitrobenzoate) with the sulphydryl groups of* ***dog carbonmonoxyhaemoglobin*** *at pH* ***5.570****. Conditions: temperature, 25°C; phosphate buffer (ionic strength, 50 mmol* $dm^{-3}$*; added salt, NaCl); [HbCO] = 10 µmol (haem)* $dm^{-3}$ *(5 µmol* $dm^{-3}$ *in reactive sulphydryl groups); [DTNB] = 300 µmol* $dm^{-3}$*;* $k_{obs}$ *=0.016072 ± 0.000653* $s^{-1}$*. Data from Fig. 4.5.*

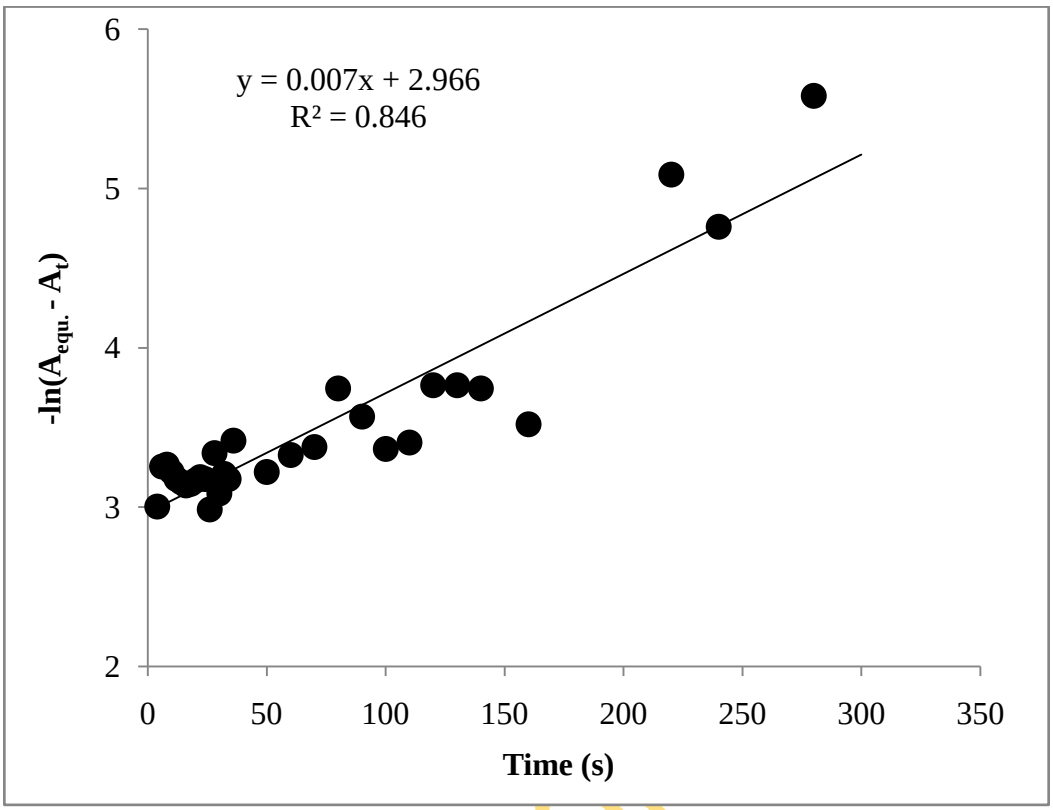



***Figure 4.8:*** *Semi-logarithmic plot of the time course for the reaction of 5,5'-dithiobis(2-nitrobenzoate) with the sulphydryl groups of* ***dog carbonmonoxyhaemoglobin*** *at pH* ***9.110****. Conditions: temperature, 25°C; borate buffer (ionic strength, 50 mmol* $dm^{-3}$*; added salt, NaCl); [HbCO] = 10 µmol (haem)* $dm^{-3}$ *(5 µmol* $dm^{-3}$ *in reactive sulphydryl groups); [DTNB] = 300 µmol* $dm^{-3}$*;* $k_{obs}$ *=0.015731 ± 0.000603* $s^{-1}$*. Data from Fig. 4.6.*

The monophasic nature of the kinetics is in agreement with the fact that there are two identical reactive sulphydryl groups per dog haemoglobin tetramer (Okonjo *et al*., 1979; Okonjo and Adejoro, 1993).

### 4.3.2.2 Kinetics of DTNB reaction with donkey haemoglobin.

Figs. 4.9 and 4.10 show the variation of the absorbance with time for a typical kinetic run for the reaction of DTNB with *stripped* donkey carbonmonoxyhaemoglobin at two pH values. Similar runs were obtained for the oxy and aquomet derivatives in the absence and presence of inositol-$P_6$ over the pH range $5.6 \leq pH \leq 9.0$.

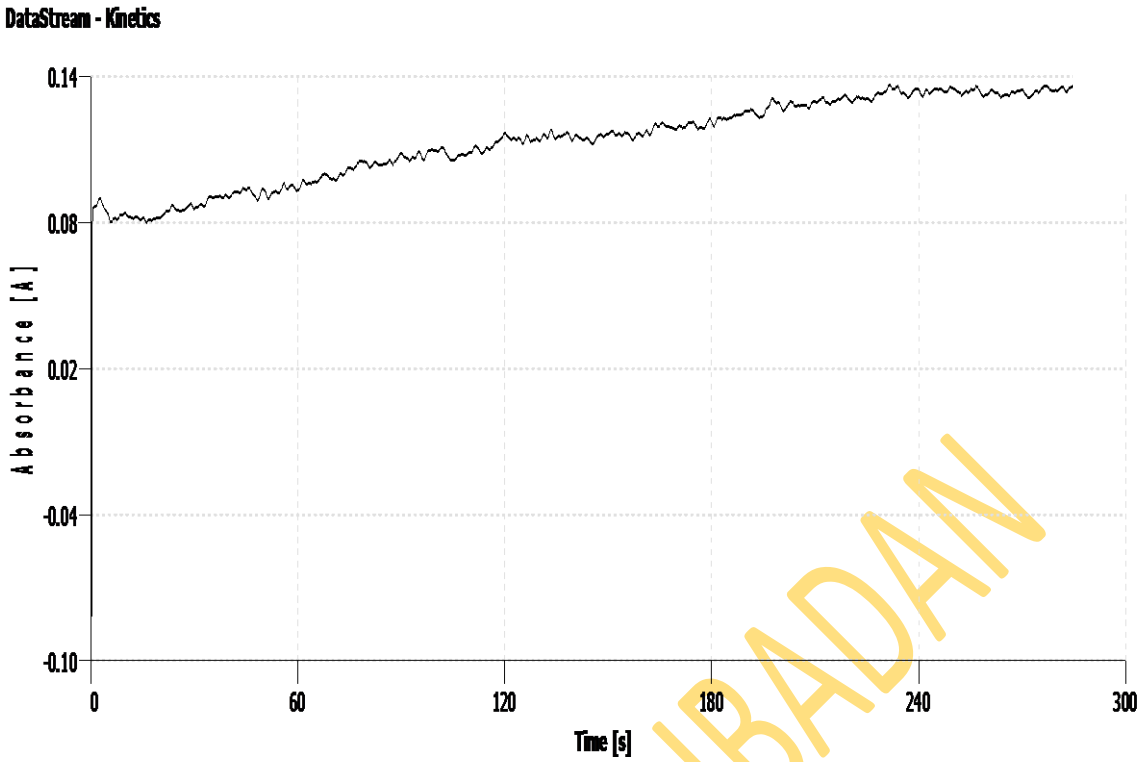



***Figure 4.9:*** *A typical kinetic run for the reaction of 5,5'-dithiobis(2-nitrobenzoate) with* ***stripped donkey carbonmonoxyhaemoglobin*** *sulphydryl groups at pH* ***5.645****. Conditions: temperature, 25°C; phosphate buffer (ionic strength, 50 mmol* $dm^{-3}$*; added salt, NaCl); [HbCO] = 10 µmol (haem)* $dm^{-3}$ *(5 µmol* $dm^{-3}$ *in reactive sulphydryl groups); [DTNB] = 300 µmol* $dm^{-3}$*.*

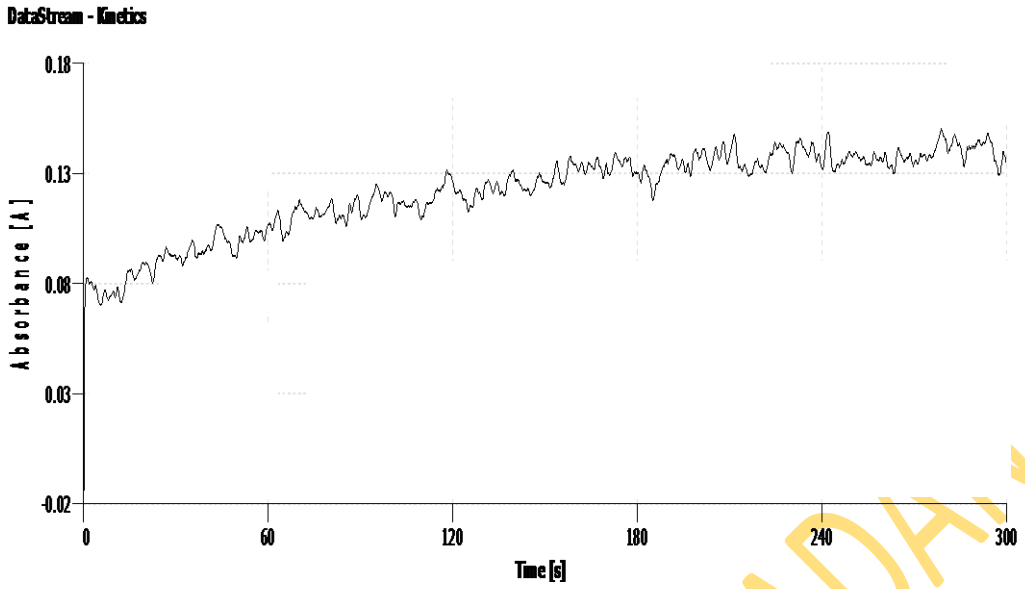



***Figure 4.10:*** *A typical kinetic run for the reaction of 5,5'-dithiobis(2-nitrobenzoate) with* ***stripped donkey carbonmonoxyhaemoglobin*** *sulphydryl groups at pH* ***8.956****. Conditions: temperature, 25°C; borate buffer (ionic strength, 50 mmol* $dm^{-3}$*; added salt, NaCl); [HbCO] = 10 µmol (haem)* $dm^{-3}$ *(5 µmol* $dm^{-3}$ *in reactive sulphydryl groups); [DTNB] = 300 µmol* $dm^{-3}$*.*

Figs. 4.11 and 4.12 show the semi - logarithmic plots of the time courses for the reaction of DTNB with *stripped* donkey carbonmonoxyhaemoglobin whose traces are shown in Figs. 4.9 and 4.10. A straight line is obtained in each case, in conformity with Eqn. 4.15. Similar results were obtained for all other derivatives, with and without inositol-$P_6$ under the same experimental conditions. This proves that the use of Eqn. 4.15 is valid and that $(1/K - 1)x^2$ is much less than bx for donkey haemoglobin. The monophasic nature of the kinetics is in agreement with the result of the DTNB titration of donkey carbonmonoxyhaemoglobin sulphydryl groups (Fig. 4.3). This indicates that there are two identical sulphydryl groups reactive with DTNB per donkey haemoglobin (tetramer) molecule.

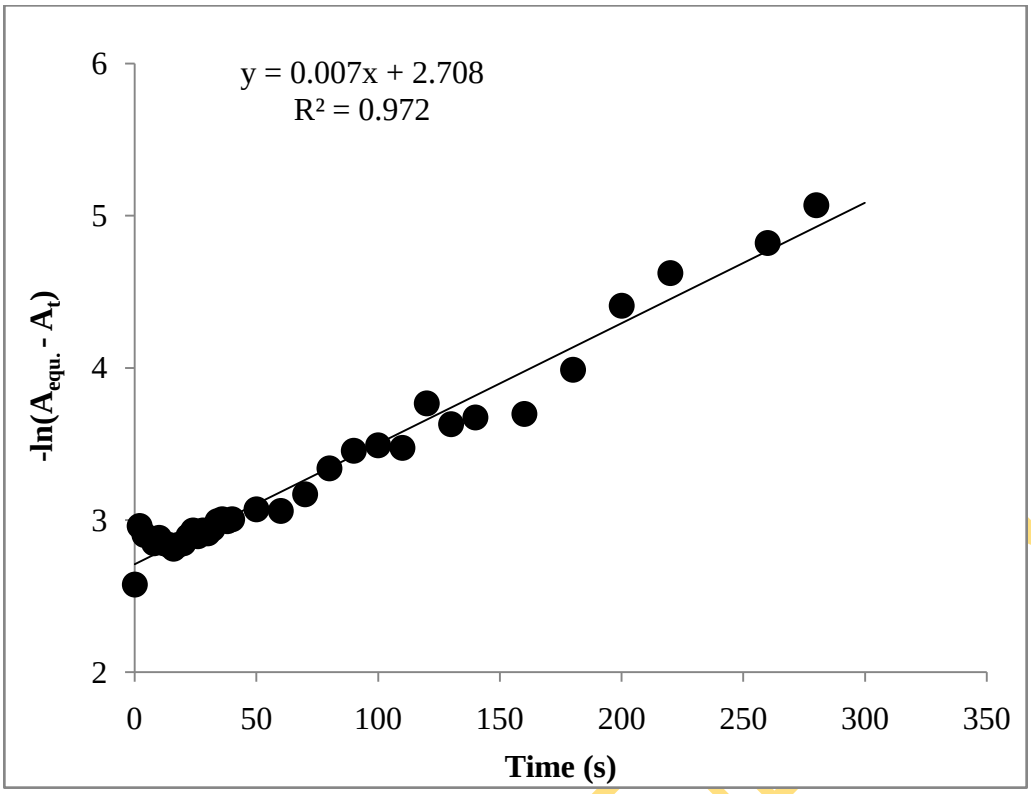



***Figure 4.11:*** *Semi-logarithmic plot of the time course for the reaction of 5,5'-dithiobis(2-nitrobenzoate) with* ***stripped donkey carbonmonoxyhaemoglobin*** *sulphydryl groups at pH* ***5.645****. Conditions: temperature, 25°C; phosphate buffer (ionic strength, 50 mmol* $dm^{-3}$*; added salt, NaCl); [HbCO] = 10 µmol (haem)* $dm^{-3}$*(5 µmol* $dm^{-3}$ *in reactive sulphydryl groups); [DTNB] = 300 µmol* $dm^{-3}$*;* $k_{obs}$ *=0.014278 ± 0.000682* $s^{-1}$*. Data from Fig.4.9.*

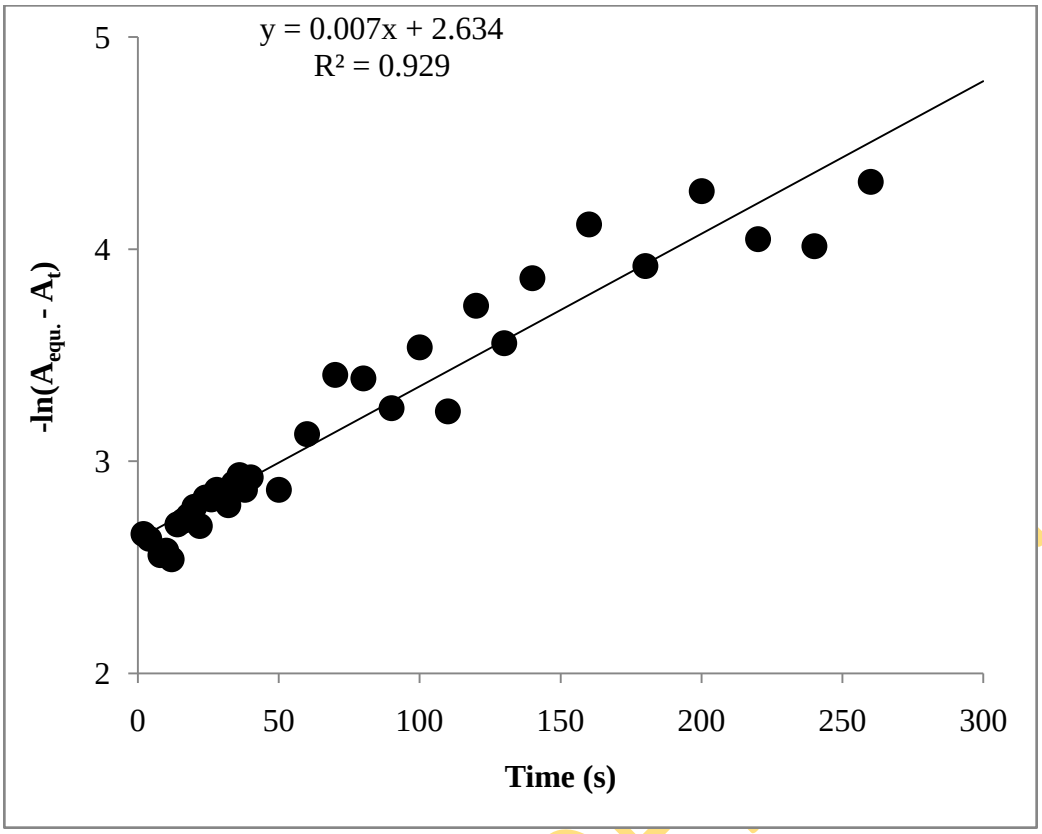



***Figure 4.12:*** *Semi-logarithmic plot of the time course for the reaction of 5,5'-dithiobis(2-nitrobenzoate) with* ***stripped donkey carbonmonoxyhaemoglobin*** *sulphydryl groups at pH* ***8.956****. Conditions: temperature, 25°C; borate buffer (ionic strength, 50 mmol* $dm^{-3}$*; added salt, NaCl); [HbCO] = 10 µmol (haem)* $dm^{-3}$*(5 µmol* $dm^{-3}$ *in reactive sulphydryl groups); [DTNB] = 300 µmol* $dm^{-3}$*;* $k_{obs}$ *= 0.015620 ± 0.000192* $s^{-1}$*. Data from Fig. 4.10.*

### 4.3.2.3 Determination of the apparent forward second order rate constant, $k_F$

(i) Dog haemoglobin

The kinetics of the reaction of a 10 µmol (haem) $dm^{-3}$ dog haemoglobin solution were studied at different DTNB concentrations (300 - 600 µmol $dm^{-3}$) in the pH range $5.6 \leq pH \leq 9.0$. The aim was to test whether the reaction is reversible or not. The pseudo-first order rate constant, $k_{obs}$ was plotted against various concentrations of DTNB. The plots obtained are linear and have significant intercepts, as shown in Figs. 4.13 and 4.14. This indicates that the reaction of DTNB with dog haemoglobin is reversible. Similar results were obtained at all pH values in the range $5.6 \leq pH \leq 9.0$ for the three derivatives of dog haemoglobin in the absence or presence of inositol-$P_6$. The values of the apparent forward second order rate constant, $k_F$, were calculated from the least squares slopes of the linear plots.

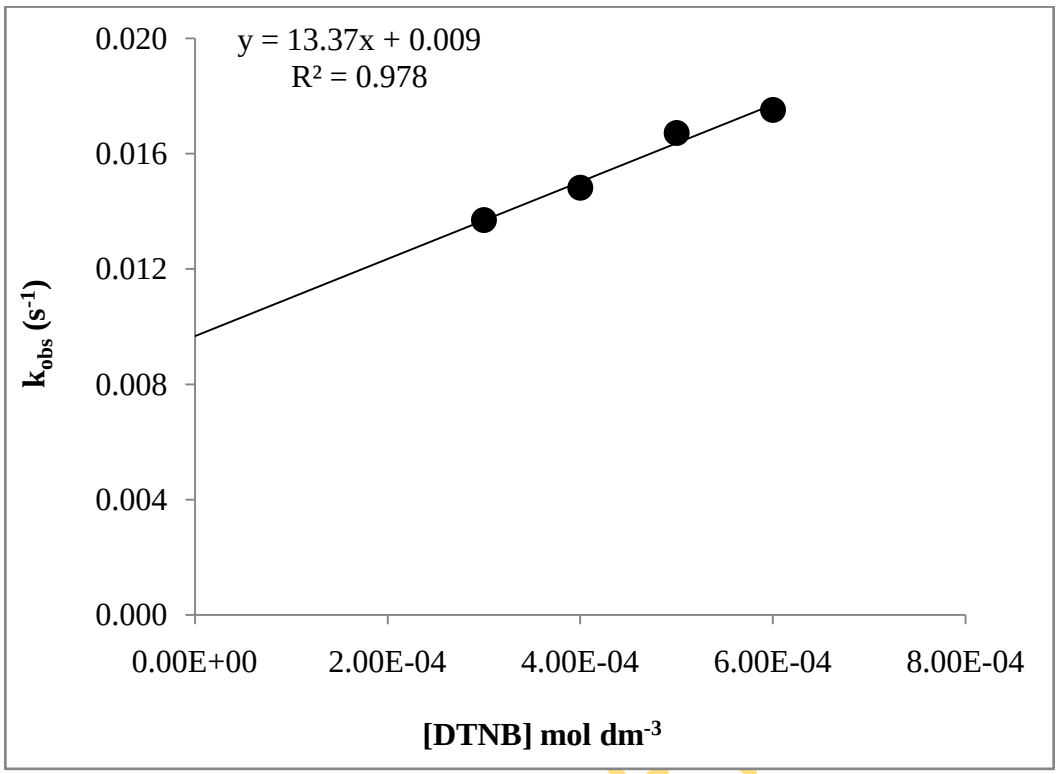



***Figure 4.13:*** *The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with* ***stripped dog carbonmonoxyhaemoglobin*** *at pH* ***6.076.*** *Conditions: temperature, 25°C; phosphate buffer (ionic strength, 50 mmol $dm^{-3}$; added salt, NaCl); [HbCO] = 10 µmol (haem) $dm^{-3}$ (5 µmol $dm^{-3}$ in reactive sulphydryl groups); wavelength, λ = 412 nm. Each experimental point is the mean of at least three determinations and is subject to a standard error of about ± 0.07 x $10^{-2}$.* *(See Table 47, Appendix I, page 212)*

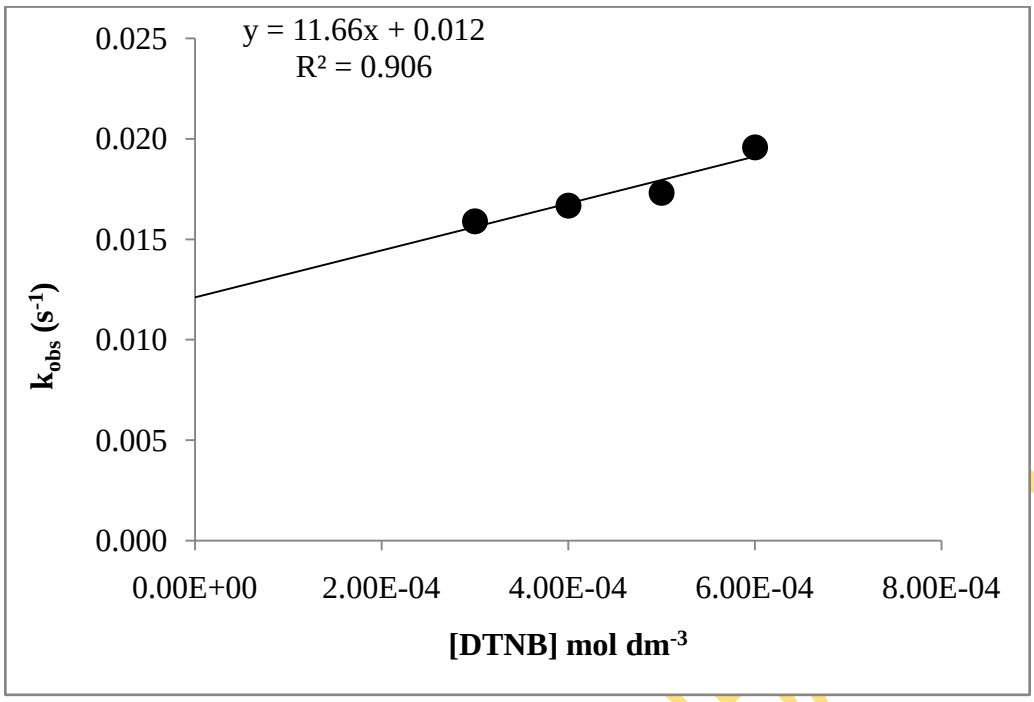



***Figure 4.14:*** *The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with* ***stripped dog carbonmonoxyhaemoglobin*** *at pH* ***8.604.*** *Conditions: temperature, 25°C; borate buffer (ionic strength, 50 mmol $dm^{-3}$; added salt, NaCl); [HbCO] = 10 µmol (haem) $dm^{-3}$ (5 µmol $dm^{-3}$ in reactive sulphydryl groups); wavelength, λ = 412 nm. Each experimental point is the mean of at least three determinations and is subject to a standard error about ± 0.06 x $10^{-2}$.*

*(See Table 60, Appendix I, page 217)*

## (ii) Donkey haemoglobin

The plots obtained for the reaction of DTNB with each of the three derivatives of donkey haemoglobin are linear and have significant intercepts, as shown for carbonmonoxyhaemoglobin in Figs. 4.15 and 4.16. This indicates that the reaction of DTNB with donkey haemoglobin is reversible. Similar results were obtained at all pH values in the range $5.6 \leq pH \leq 9.0$ for the three derivatives of donkey haemoglobin in the absence or presence of inositol-$P_6$. The values of the apparent forward second order rate constant, $k_F$, were calculated from the least squares slopes of the linear plots.

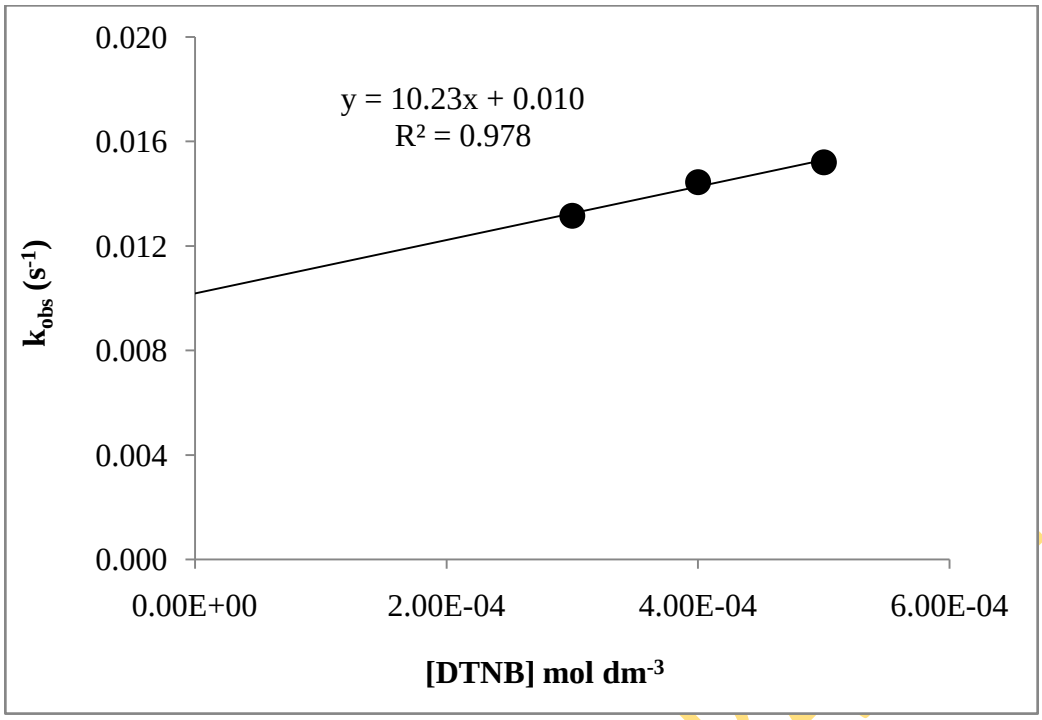



***Figure 4.15:*** *The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with* ***stripped donkey carbonmonoxyhaemoglobin*** *at pH* ***5.704.*** *Conditions: temperature = 25°C; phosphate buffer (ionic strength, 50 mmol $dm^{-3}$; added salt, NaCl); [HbCO] = 10 µmol (haem) $dm^{-3}$ (5 µmol $dm^{-3}$ in reactive sulphydryl groups); wavelength, λ = 412 nm. Each experimental point is the mean of at least three determinations and is subject to a standard error about ± 0.11 x $10^{-2}$. (see Table 152, Appendix 1, page 247).*

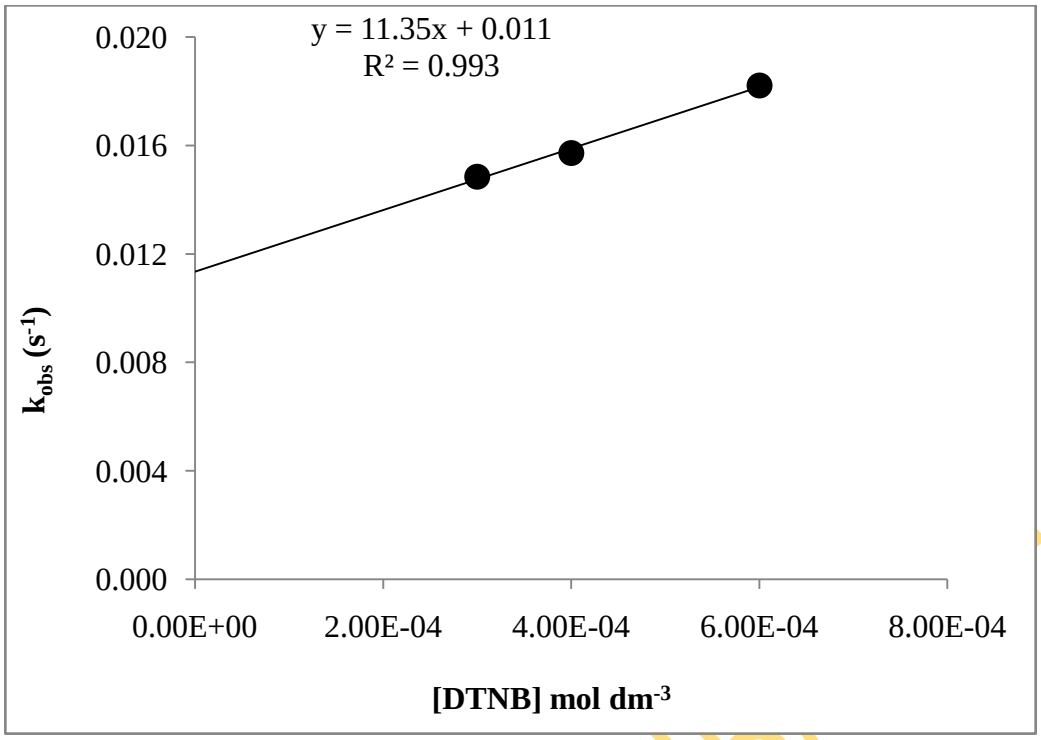



***Figure 4.16:*** *The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with* ***stripped donkey carbonmonoxyhaemoglobin*** *at pH* ***8.780.*** *Conditions: temperature = 25°C; borate buffer (ionic strength, 50 mmol $dm^{-3}$; added salt, NaCl); [HbCO] = 10 µmol (haem) $dm^{-3}$ (5 µmol $dm^{-3}$ in reactive sulphydryl groups); wavelength, λ = 412 nm. Each experimental point is the mean of at least three determinations and is subject to a standard error about ± 0.113 x $10^{-2}$. (see Table 168, Appendix 1, page 253).*

### 4.3.2.4 Dependence of the apparent forward second order rate constant, $k_F$ on pH : Dog haemoglobin

Fig. 4.17 shows the dependence of $k_F$ on pH for *stripped* dog oxyhaemoglobin. The curve is skewed - bell shaped. Values of $k_F$ remain essentially constant up to pH 7.4, $k_F$ increases as pH increases up to 8.2. A sharp decrease is seen from pH 8.4.

Data for the carbonmonoxy derivative are shown in Fig. 4.18. The curve is bowl shaped. $k_F$ is seen to decrease up to pH 7.2 and increases gradually up to 9.0.

The corresponding data for aquomet show that $k_F$ decreases fairly rapidly up to pH 6.6 and remains essentially constant through the higher pH (Fig. 4.19).

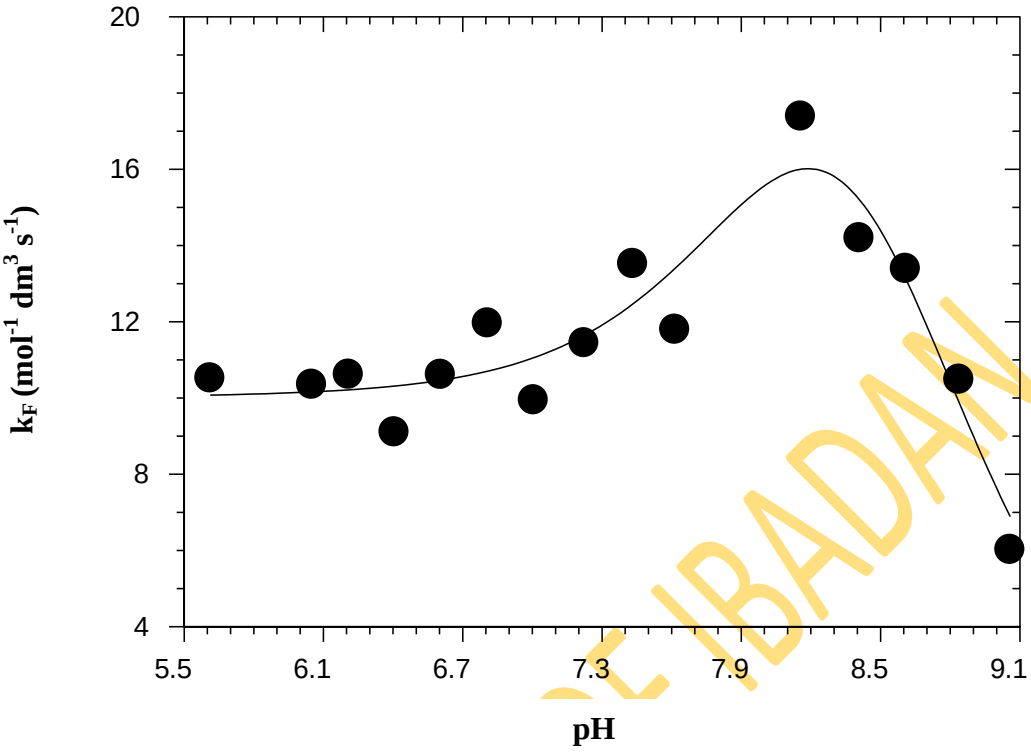



***Figure 4.17**: Dependence of $k_F$ on pH for the reaction of 5,5'-dithiobis(2-nitrobenzoate) with CysF9[93]ß of **stripped dog oxyhaemoglobin**. Conditions: phosphate buffers pH 5.6 – 7.8; borate buffers pH 8.0 – 9.0 (ionic strength, 50 mmol dm$^{-3}$; added salt, NaCl); [$HbO_2$] = 10 µmol (haem) dm$^{-3}$ (5µmol dm$^{-3}$ in reactive sulphydryl groups); observation wavelength, λ = 412 nm. Each data point is subject to a standard error of about ± 0.70*

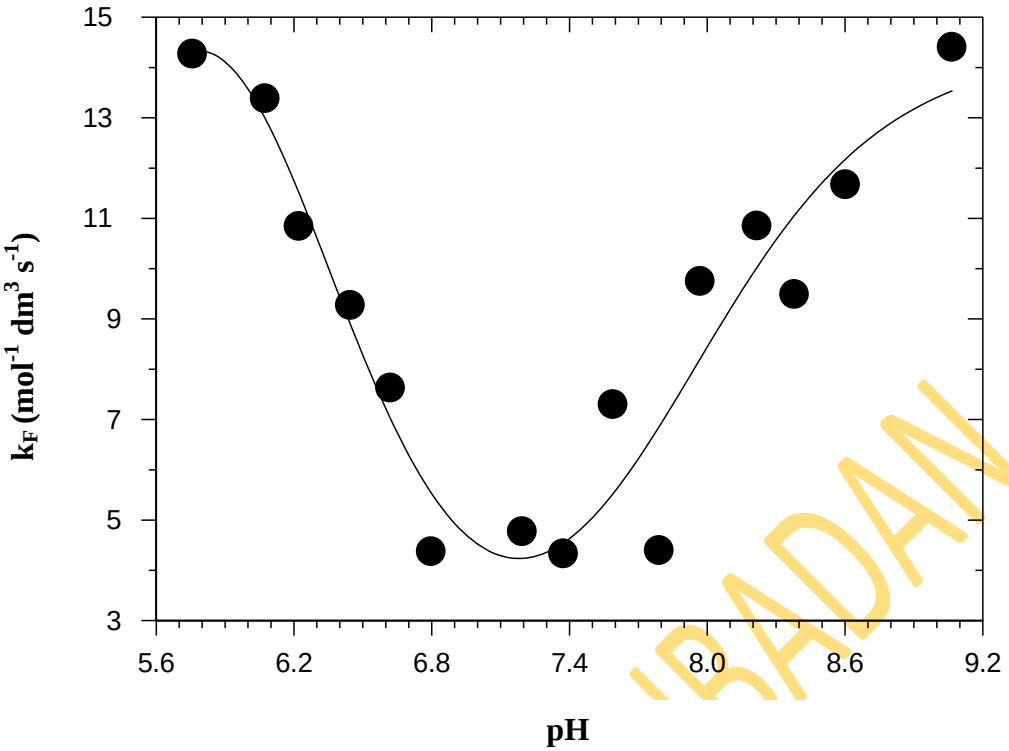



***Figure 4.18**: Dependence of $k_F$ on pH for the reaction of 5,5'-dithiobis(2-nitrobenzoate) with CysF9[93]ß of **stripped dog carbonmonoxyhaemoglobin**.*

*Conditions: phosphate buffers pH 5.6 – 7.8; borate buffers pH 8.0 – 9.0 (ionic strength, 50 mmol $dm^{-3}$; added salt, NaCl); [HbCO] = 10 µmol (haem) $dm^{-3}$ (5µmol $dm^{-3}$ in reactive sulphydryl groups); observation wavelength, λ = 412 nm. Each data point is subject to a standard error of about ± 0.60*

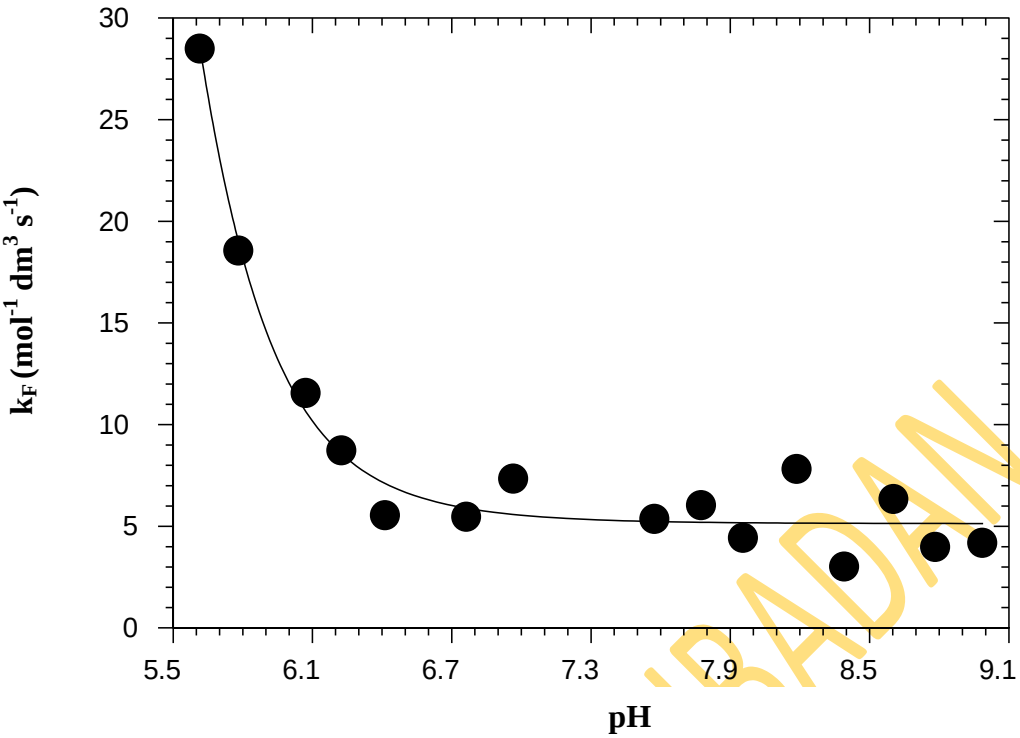



***Figure 4.19**: Dependence of $k_F$ on pH for the reaction of 5,5'-dithiobis(2-nitrobenzoate) with CysF9[93]ß of **stripped dog aquomethaemoglobin**.*

*Conditions: phosphate buffers pH 5.6 – 7.8; borate buffers pH 8.0 – 9.0 (ionic strength, 50 mmol dm$^{-3}$; added salt, NaCl); [Hbmet] = 10 µmol (haem) dm$^{-3}$ (5µmol dm$^{-3}$ in reactive sulphydryl groups); observation wavelength, λ = 412 nm. Each data point is subject to a standard error of about ± 0.30*

### 4.3.2.5 Dependence of the apparent forward second order rate constant, $k_F$ on pH : Dog haemoglobin plus inositol-$P_6$

The pH dependences of $k_F$ in the presence of inositol-$P_6$ for the oxy, carbonmonoxy and aquomet derivatives of haemoglobin are shown in Figs. 4.20 – 4.22 respectively.

Fig. 4.20 shows a bell-shaped profile for oxyhaemoglobin in the presence of inositol-$P_6$. It is seen that $k_F$ decreases gradually up to pH 7.4 and increases from 7.4 – 9.0.

For carbonmonoxyhaemoglobin (Fig 4.21), $k_F$ decreases up to pH 7.2 and remains essentially constant at higher pH. It shows a bell – shaped profile.

For aquomethaemoglobin (Fig 4.22), $k_F$ shows a bell – shaped profile. $k_F$ increases up to pH 7.0 and remains essentially constant at higher pH. This seems to be a mirror image of the profile observed in the carbonmonoxy derivative.

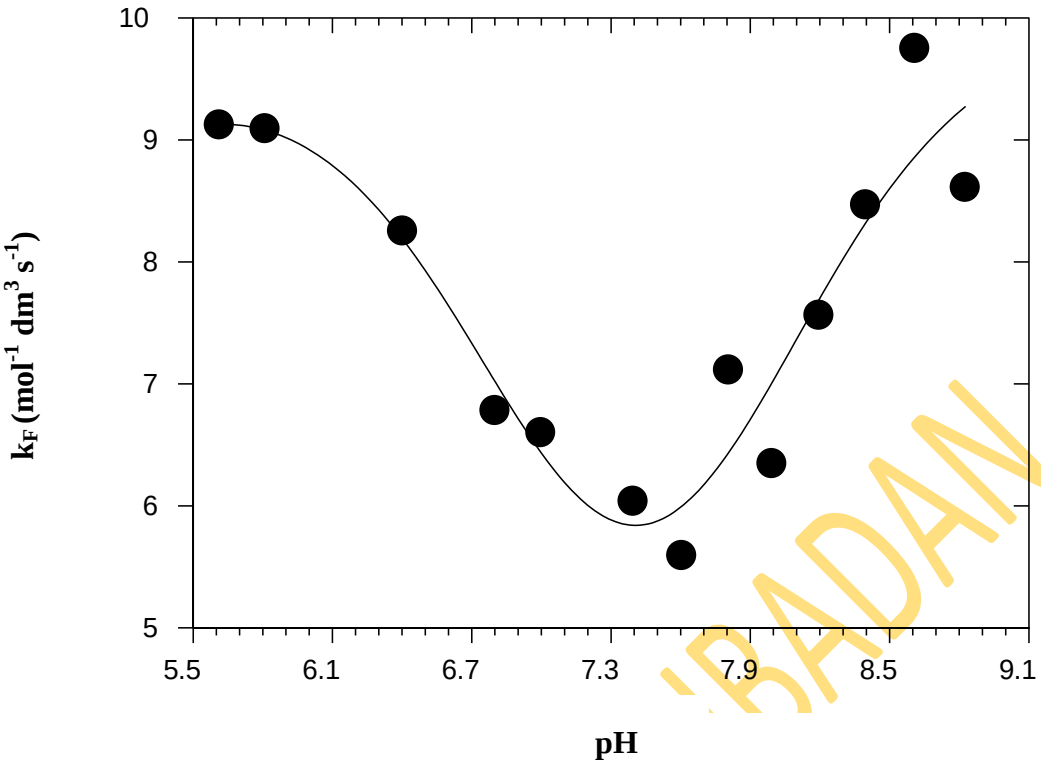



***Figure 4.20**: Dependence of $k_F$ on pH for the reaction of 5,5'-dithiobis(2-nitrobenzoate) with CysF9[93]ß of **dog oxyhaemoglobin in the presence of inositol-$P_6$**.*

*Conditions: phosphate buffers pH 5.6 – 7.8; borate buffers pH 8.0 – 9.0 (ionic strength, 50 mmol dm$^{-3}$; added salt, NaCl); [$HbO_2$] = 10 µmol (haem) dm$^{-3}$ (5 µmol dm$^{-3}$ in reactive sulphydryl groups); [inositol-$P_6$] = 10 µmol dm$^{-3}$; observation wavelength, λ = 412 nm. Each data point is subject to a standard error of about ± 0.50*

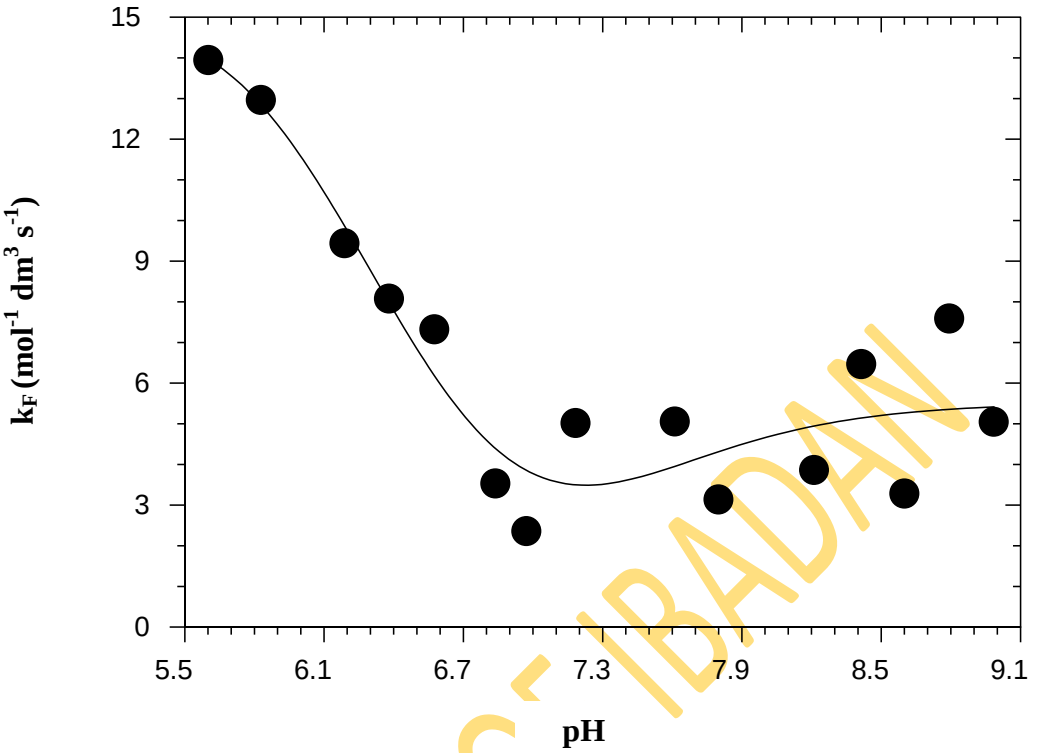



***Figure 4.21**: Dependence of $k_F$ on pH for the reaction of 5,5'-dithiobis (2-nitrobenzoate) with CysF9[93]ß of **dog carbonmonooxyhaemoglobin in the presence of inositol-$P_6$**.*

*Conditions: phosphate buffers pH 5.6 – 7.8; borate buffers pH 8.0 – 9.0 (ionic strength, 50 mmol dm$^{-3}$; added salt, NaCl); [HbCO] = 10 µmol (haem) dm$^{-3}$ (5 µmol dm$^{-3}$ in reactive sulphydryl groups); [inositol-$P_6$] = 10 µmol dm$^{-3}$; observation wavelength, λ = 412 nm. Each data point is subject to a standard error of about ± 0.40*

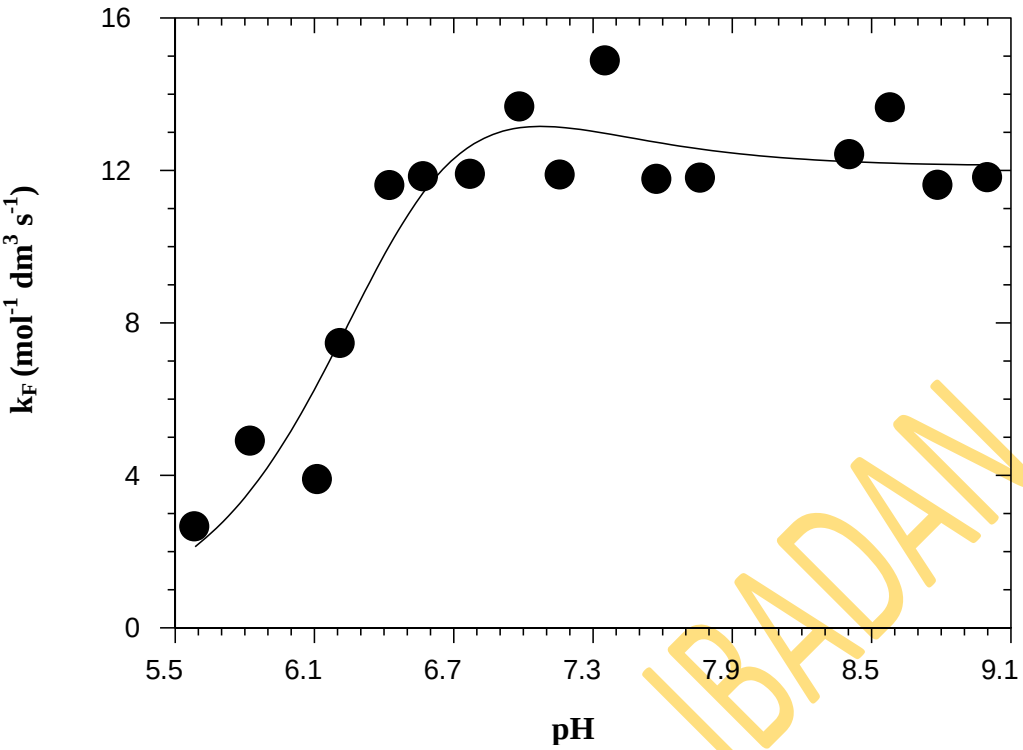



***Figure 4.22**: Dependence of $k_F$ on pH for the reaction of 5,5'-dithiobis(2-nitrobenzoate) with CysF9[93]ß of* **dog aquomethaemoglobin in the presence of inositol-$P_6$.**

*Conditions: phosphate buffers pH 5.6 – 7.8; borate buffers pH 8.0 – 9.0 (ionic strength, 50 mmol dm$^{-3}$; added salt, NaCl); [Hbmet] = 10 µmol (haem) dm$^{-3}$ (5 µmol dm$^{-3}$ in reactive sulphydryl groups); [inositol-$P_6$] = 10 µmol dm$^{-3}$; observation wavelength, λ = 412 nm. Each data point is subject to a standard error of about ± 0.70*

#### 4.3.2.6 Comparison of the pH dependence of $k_F$ for *stripped* haemoglobin and haemoglobin in the presence of inositol-$P_6$: Dog haemoglobin

The pH dependences of $k_F$ are compared in Fig. 4.23 for *stripped* dog oxyhaemoglobin and oxyhaemoglobin in the presence of inositol-$P_6$. It is seen that $k_F$ is decreased by inositol-$P_6$ throughout the experimental pH range. In addition, inositol-$P_6$ changes the profile from bell- to bowl-shaped.

The pH dependence of $k_F$ for the carbonmonoxy derivative is reported in Fig. 4.24, where it is seen that inositol-$P_6$ does not change the profile and has only a minor effect at pH < 7.8. However, above this pH a noticeable lowering of $k_F$ is observed as the pH increases.

The most interesting result for dog haemoglobin is seen in Fig. 4.25, where it is seen that inositol-$P_6$ converts the pH dependence of $k_F$ for stripped haemoglobin into a mirror image of itself. Surprisingly, inositol-$P_6$ *increases* the values of $k_F$ 2-fold above pH 6.2. This is a most unusual result. Previous results on human haemoglobin show that inositol-$P_6$ lowers the value of $k_F$.

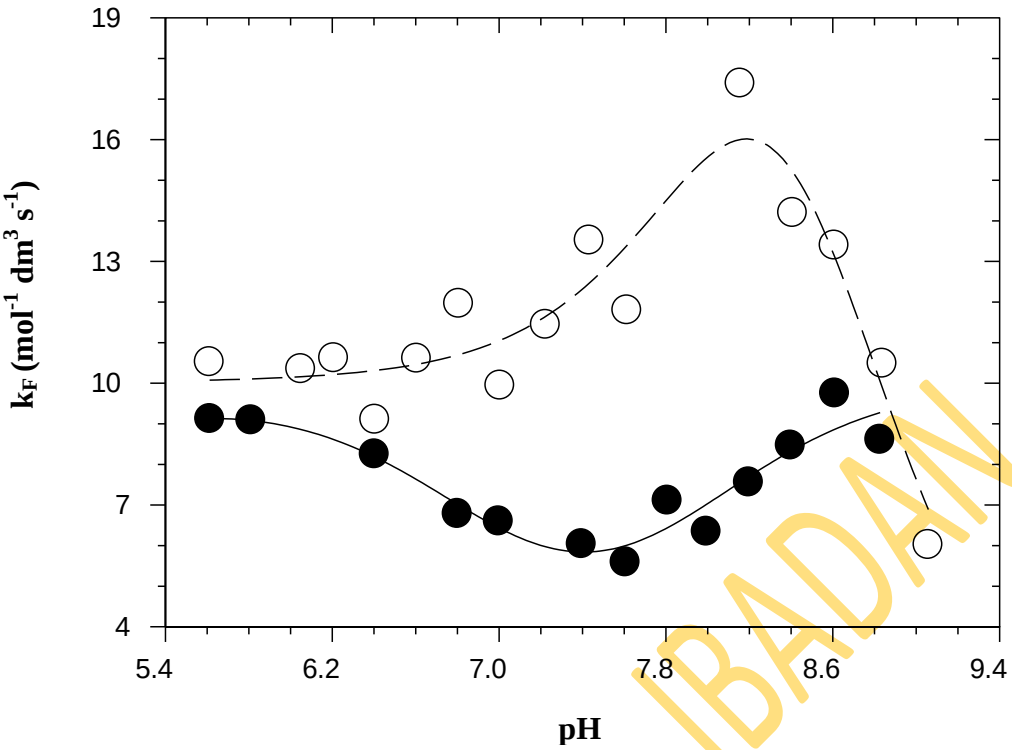



***Figure 4.23**: Dependence of $k_F$ on pH for the reaction of 5,5'-dithiobis(2-nitrobenzoate) with CysF9[93]ß of **dog oxyhaemoglobin (open circles, stripped haemoglobin; filled circles, haemoglobin in the presence of inositol-$P_6$)**. Conditions: phosphate buffers pH 5.6 – 7.8; borate buffers pH 8.0 – 9.0 (ionic strength, 50 mmol dm$^{-3}$; added salt, NaCl); [$HbO_2$] = 10 µmol (haem) dm$^{-3}$ (5 µmol dm$^{-3}$ in reactive sulphydryl groups); [inositol-$P_6$] = 10 µmol dm$^{-3}$; observation wavelength, λ = 412 nm. Each data point is subject to a standard error of about ± 0.70*

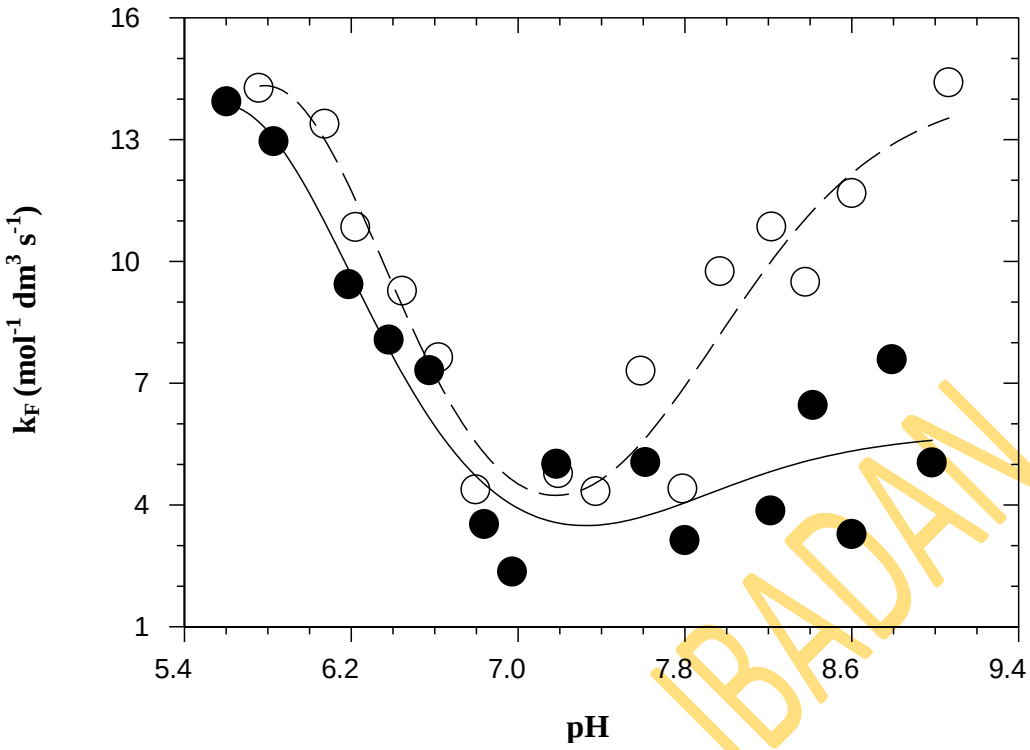



***Figure 4.24**: Dependence of $k_F$ on pH for the reaction of 5,5'-dithiobis(2-nitrobenzoate) with CysF9[93]ß of **dog carbonmonoxyhaemoglobin (open circles, stripped haemoglobin; filled circles, haemoglobin in the presence of inositol-$P_6$).** Conditions: phosphate buffers pH 5.6 – 7.8; borate buffers pata H 8.0 – 9.0 (ionic strength, 50 mmol dm$^{-3}$; added salt, NaCl); [HbCO] = 10 µmol (haem) dm$^{-3}$ (5 µmol dm$^{-3}$in reactive sulphydryl groups); [inositol-$P_6$]=10 µmol dm$^{-3}$; observation wavelength, λ = 412 nm. Each dpoint is subject to a standard error of about ± 0.50*

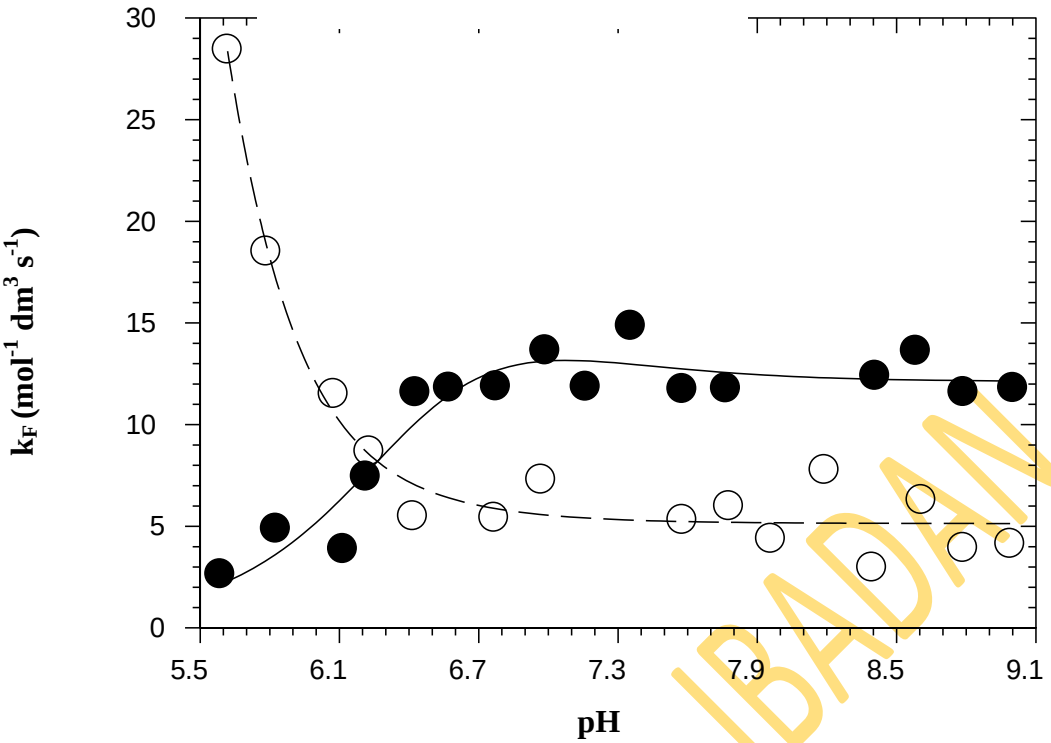



***Figure 4.25**: Dependence of $k_F$ on pH for the reaction of 5,5'-dithiobis(2-nitrobenzoate) with CysF9[93]ß of **dog aquomethaemoglobin (open circles, stripped haemoglobin; filled circles, haemoglobin in the presence of inositol-$P_6$).** Conditions: phosphate buffers pH 5.6 – 7.8; borate buffers pH 8.0 – 9.0 (ionic strength, 50 mmol dm$^{-3}$; added salt, NaCl); [Hbmet] = 10 µmol (haem) dm$^{-3}$ (5 µmol dm$^{-3}$ in reactive sulphydryl groups); [inositol-$P_6$] =10 µmol dm$^{-3}$, observation wavelength, λ = 412 nm. Each data point is subject to a standard error of about ± 0.53*

### 4.3.2.7 Dependence of the apparent forward second order rate constant, $k_F$, on pH: Donkey haemoglobin

Fig. 4.26 shows the dependence of $k_F$ on pH for *stripped* oxyhaemoglobin. It has a skewed bell shape. It is seen that $k_F$ increases with pH up to pH 6.8. Above this pH $k_F$ remains essentially constant.

Fig 4.27 reports the dependence of $k_F$ on pH for *stripped* carbonmonoxyhaemoglobin. The profile has a crooked bell-shape: $k_F$ increases fairly rapidly up to pH 6.8 and decreases gradually at higher pH values.

The profile for the aquomet derivative (Fig 4.28) is bell-shaped between pH 5.6 and 8.4, with a peak at pH 7. Above pH 8.4, $k_F$ increases as the pH increases.

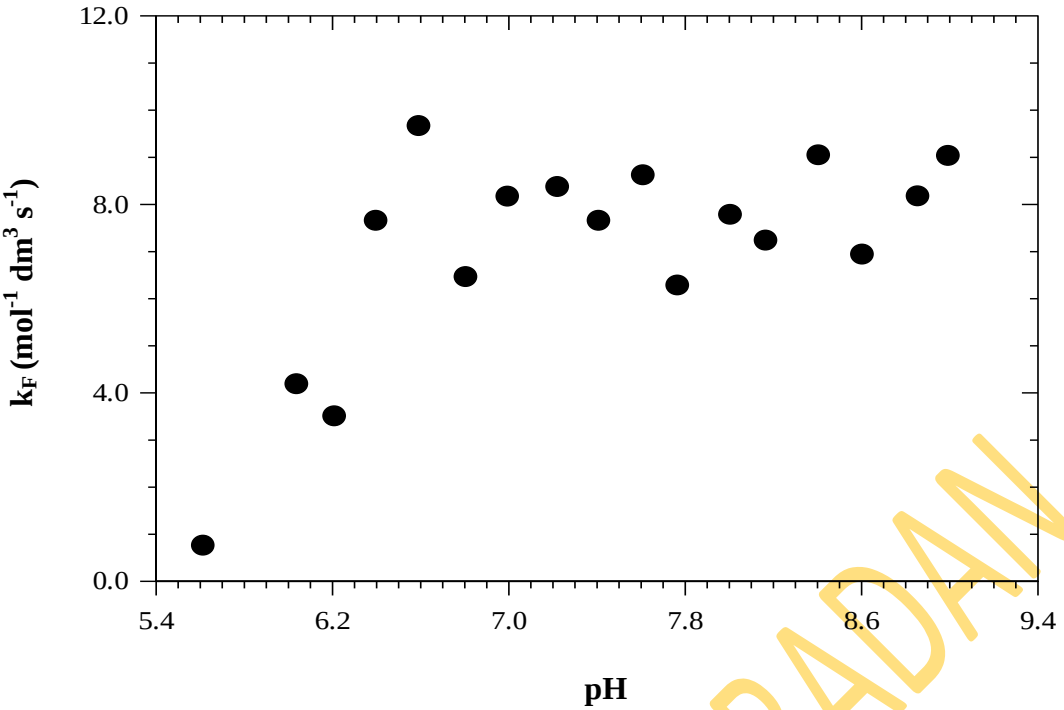



***Figure 4.26**: Dependence of $k_F$ on pH for the reaction o f 5,5'-dithiobis(2-nitrobenzoate) with CysF9[93]ß of **stripped donkey oxyhaemoglobin**.*

*Conditions: phosphate buffers pH 5.6 – 7.8; borate buffers pH 8.0 – 9.0 (ionic strength, 50 mmol dm$^{-3}$; added salt, NaCl); [$HbO_2$] = 10 µmol (haem) dm$^{-3}$ (5 µmol dm$^{-3}$ in reactive sulphydryl groups); observation wavelength, λ = 412 nm. Each data point is subject to a standard error of about ± 0.48*

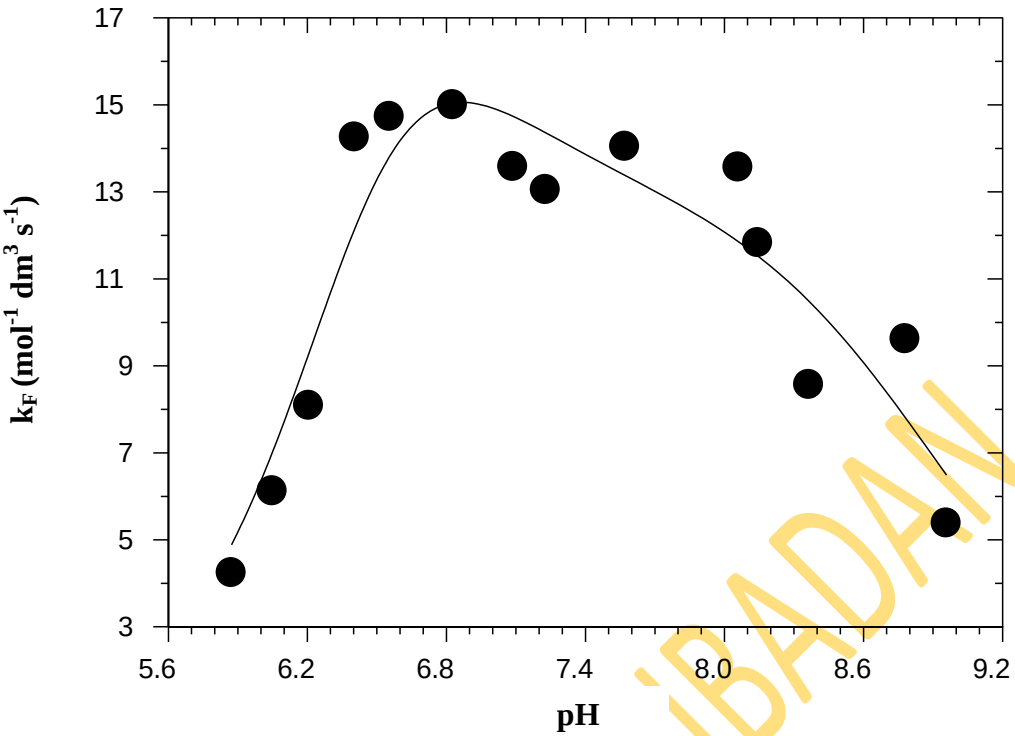



***Figure 4.27**: Dependence of $k_F$ on pH for the reaction of 5,5'-dithiobis(2-nitrobenzoate) with CysF9[93]ß of **stripped donkey carbonmonoxyhaemoglobin**. Conditions: phosphate buffers pH 5.6 – 7.8; borate buffers pH 8.0 – 9.0 (ionic strength, 50 mmol dm$^{-3}$; added salt, NaCl); [HbCO] = 10 µmol (haem) dm$^{-3}$ (5 µmol dm$^{-3}$ in reactive sulphydryl groups); observation wavelength, λ = 412 nm. Each data point is subject to a standard error of about ± 0.45*

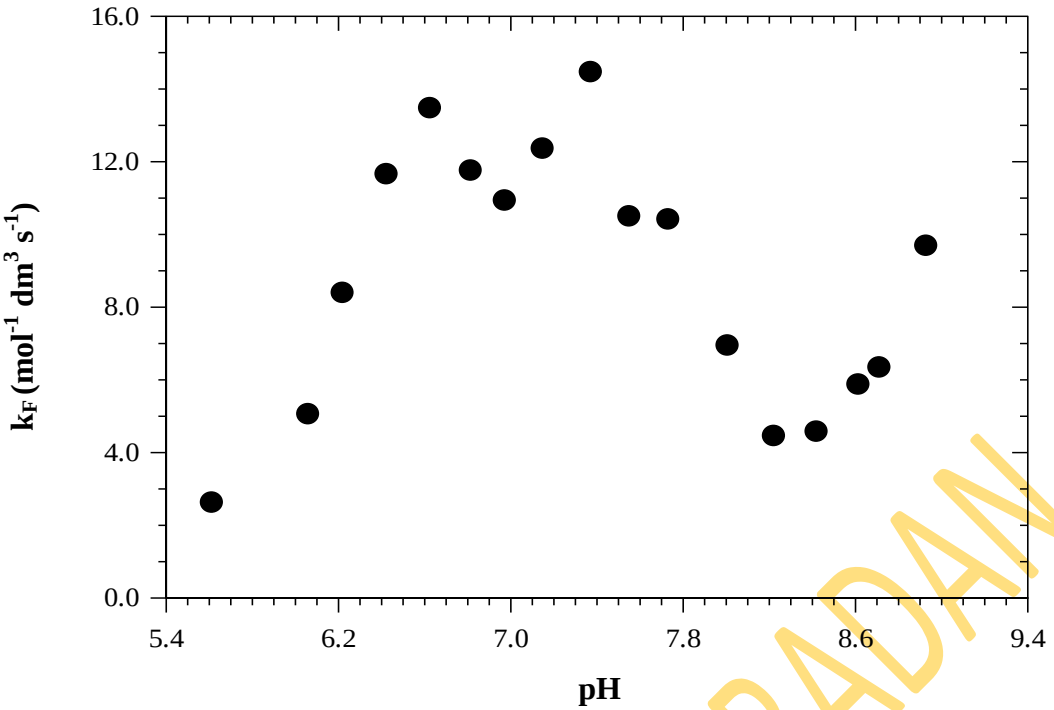



***Figure 4.28**: Dependence of $k_F$ on pH for the reaction of 5,5'-dithiobis(2-nitrobenzoate) with CysF9[93]ß of **stripped donkey aquomethaemoglobin**. Conditions: phosphate buffers pH 5.6 – 7.8; borate buffers pH 8.0 – 9.0 (ionic strength, 50 mmol $dm^{-3}$; added salt, NaCl); [Hbmet] = 10 µmol (haem) $dm^{-3}$ (5 µmol $dm^{-3}$ in reactive sulphydryl groups); observation wavelength, λ = 412 nm. Each data point is subject to a standard error of about ± 0.50*

### 4.3.2.8 Dependence of the apparent forward second order rate constant, $k_F$, on pH: Donkey haemoglobin plus inositol-$P_6$

The pH dependences of $k_F$ for the oxy, carbonmonoxy and aquomet derivatives of donkey haemoglobin in the presence of inositol-$P_6$ are shown in Figs. 4.29 – 4.31. Each data point was obtained from the slope of a plot of $k_{obs}$ against [DTNB] at a fixed pH, as described in Section 4.3.3. It is seen that each profile shows a fairly strong pH dependence for $k_F$.

For the oxy derivative, $k_F$ decreases as the pH increases up to pH 7.8; thereafter it increases with increasing pH. The overall profile is a skewed bowl shape. For the carbonmonoxy derivative, the profile has a skewed bell shape with a peak at pH 7.7. For the aquomet derivative, the dependence of $k_F$ on pH is also bell-shaped (Fig. 4.31), with a peak at pH 7.0.

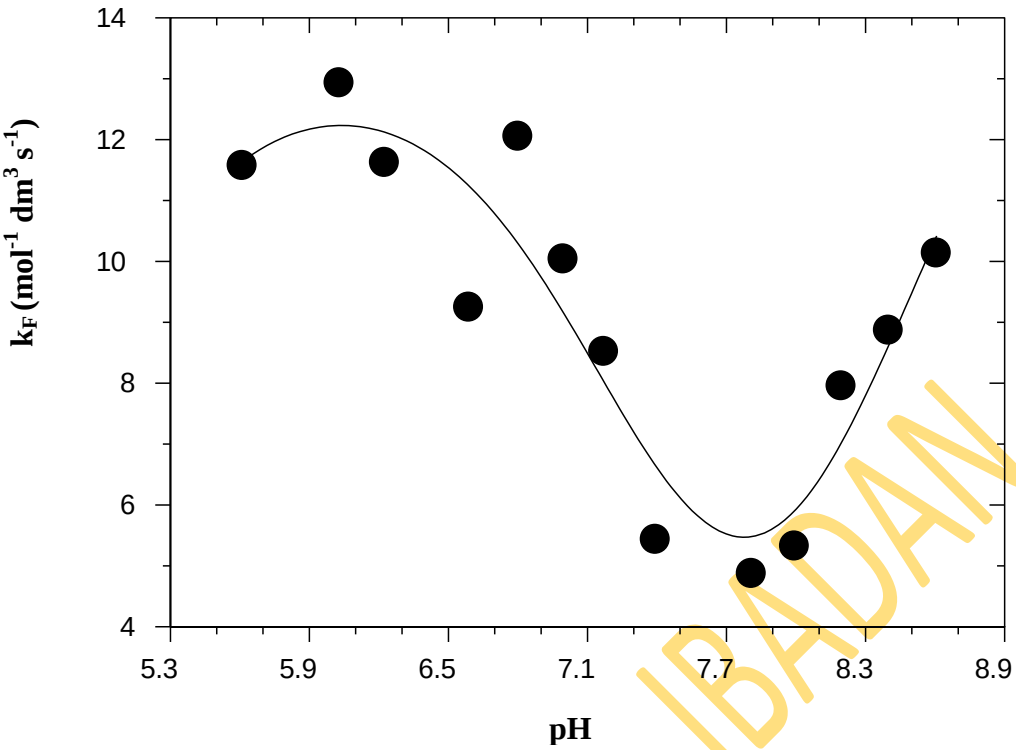



***Figure 4.29**: Dependence of $k_F$ on pH for the reaction of 5,5'-dithiobis(2-nitrobenzoate) with CysF9[93]ß of* ***donkey oxyhaemoglobin in the presence of inositol-$P_6$.***

*Conditions: phosphate buffers pH 5.6 – 7.8; borate buffers pH 8.0 – 9.0 (ionic strength, 50 mmol dm$^{-3}$; added salt, NaCl); [$HbO_2$] = 10 µmol (haem) dm$^{-3}$ (5 µmol dm$^{-3}$ in reactive sulphydryl groups); [inositol-$P_6$] = 10 µmol dm$^{-3}$; observation wavelength, λ = 412 nm. Each data point is subject to a standard error of about ± 0.15*

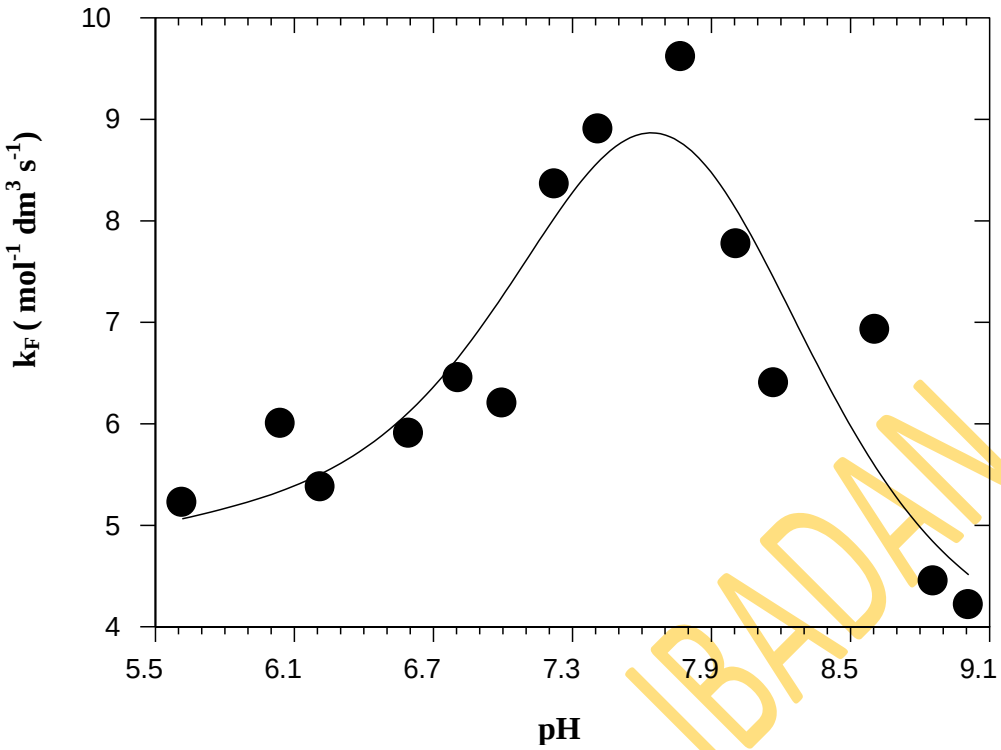



***Figure 4.30**: Dependence of $k_F$ on pH for the reaction of 5,5'-dithiobis(2-nitrobenzoate) with CysF9[93]ß of* ***donkey carbonmonoxyhaemoglobin in the presence of inositol-$P_6$.***

*Conditions: phosphate buffers pH 5.6 – 7.8; borate buffers pH 8.0 – 9.0 (ionic strength, 50 mmol $dm^{-3}$; added salt, NaCl); [HbCO] = 10 µmol (haem) $dm^{-3}$ (5 µmol $dm^{-3}$ in reactive sulphydryl groups); [inositol-$P_6$] = 10 µmol $dm^{-3}$; observation wavelength, λ = 412 nm. Each data point is subject to a standard error of about ± 0.20*

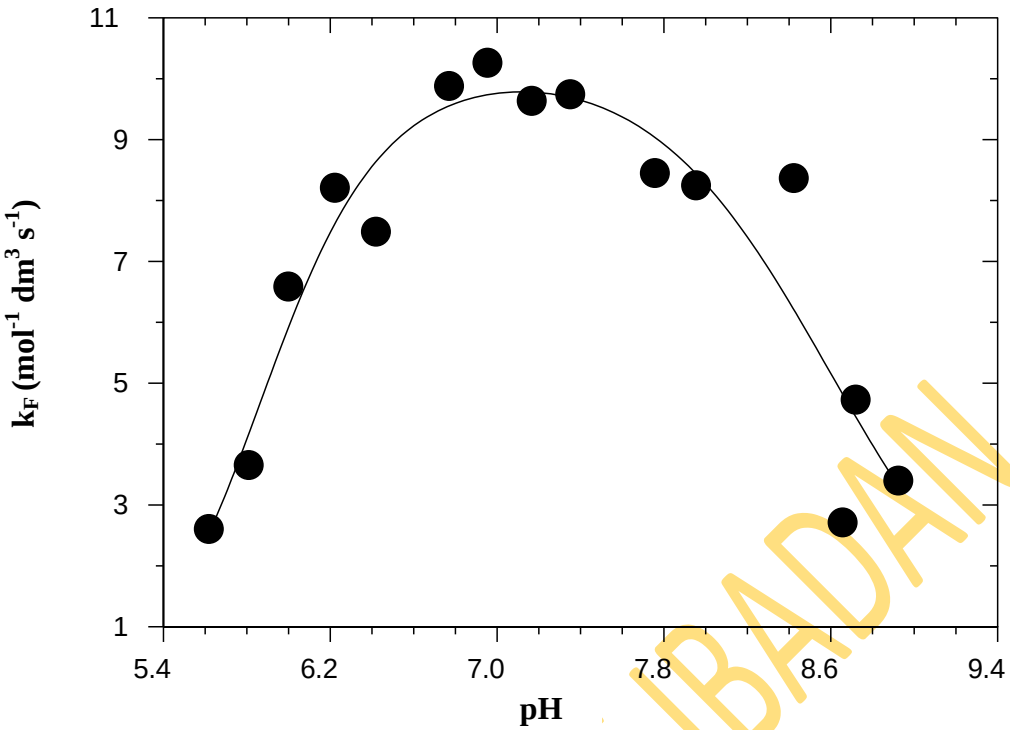



***Figure 4.31**: Dependence of $k_F$ on pH for the reaction of 5,5'-dithiobis(2-nitrobenzoate) with CysF9[93]ß of **donkey aquomethaemoglobin in the presence of inositol-$P_6$**.*

*Conditions: phosphate buffers pH 5.6 – 7.8; borate buffers pH 8.0 – 9.0 (ionic strength, 50 mmol dm$^{-3}$; added salt, NaCl); [Hbmet] = 10 µmol (haem) dm$^{-3}$ (5 µmol dm$^{-3}$ in reactive sulphydryl groups); [inositol-$P_6$] = 10 µmol dm$^{-3}$; observation wavelength, λ = 412 nm. Each data point is subject to a standard error of about ± 0.20*

### 4.3.2.9 Comparison of the pH dependence of $k_F$ for *stripped* haemoglobin and for haemoglobin in the presence of inositol-$P_6$: Donkey haemoglobin

The pH dependences of $k_F$ for the derivatives of donkey haemoglobin with and without inositol-$P_6$ are compared in Figs. 4.32 – 4.34 for the oxy, carbonmonoxy and aquomet derivatives respectively. In each case the organic phosphate changes the nature of the pH dependence. It should be recalled that each data point was obtained from the slope of a plot of $k_{obs}$ against [DTNB] at a fixed pH, as described in Section 4.3.3.

Inositol-$P_6$ is seen to increase $k_F$ for oxyhaemoglobin below pH 7.1. Above this pH the difference in $k_F$ caused by inositol-$P_6$ is no longer very significant (Fig. 4.32). For the carbonmonoxy derivative, inositol-$P_6$ decreases $k_F$ through most of the experimental pH (5.6 to 9) range. In aquomethaemoglobin, $k_F$ is not significantly affected, except over a narrow pH range centred around pH 7.0.

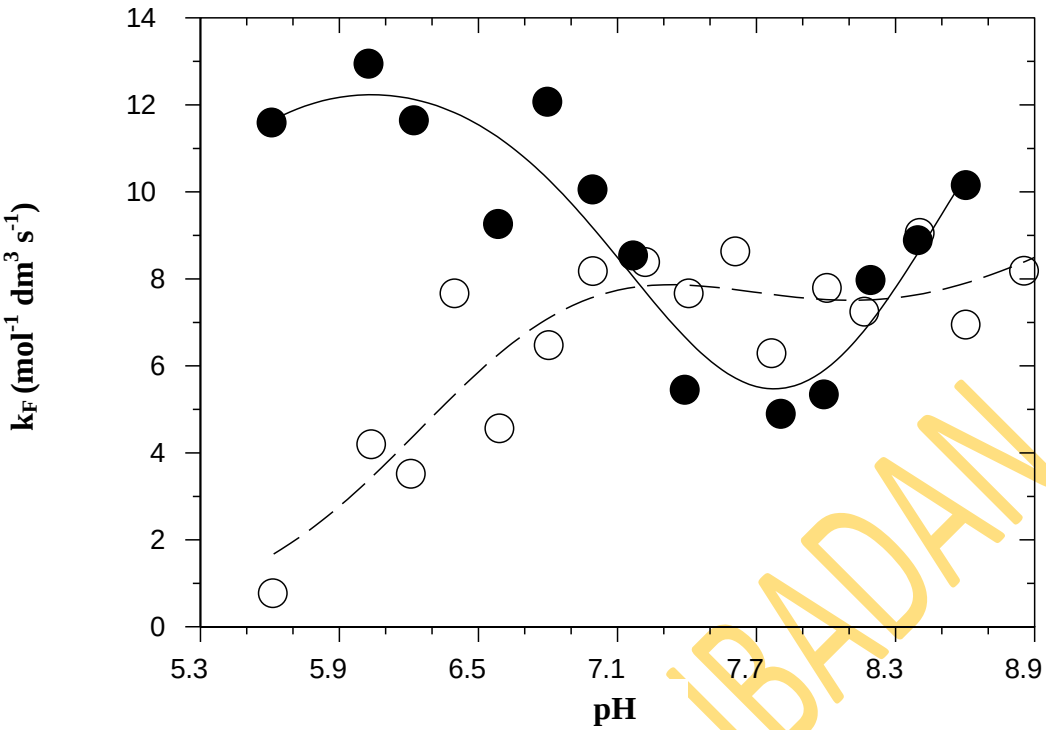



***Figure 4.32**: Dependence of $k_F$ on pH for the reaction of 5,5'-dithiobis(2-nitrobenzoate) with CysF9[93]ß of **donkey oxyhaemoglobin (open circles, stripped haemogloin; filled circles, haemoglobin in the presence of inositol-$P_6$).** Conditions: phosphate buffers pH 5.6 – 7.8; borate buffers pH 8.0 – 9.0 (ionic strength, 50 mmol dm$^{-3}$; added salt, NaCl); [$HbO_2$], 10 µmol (haem) dm$^{-3}$ (5 µmol dm$^{-3}$in reactive sulphydryl groups); [inositol-$P_6$], 10 µmol dm$^{-3}$, observation wavelength, $\lambda$ = 412 nm. Each data point is subject to a standard error of about ± 0.30*

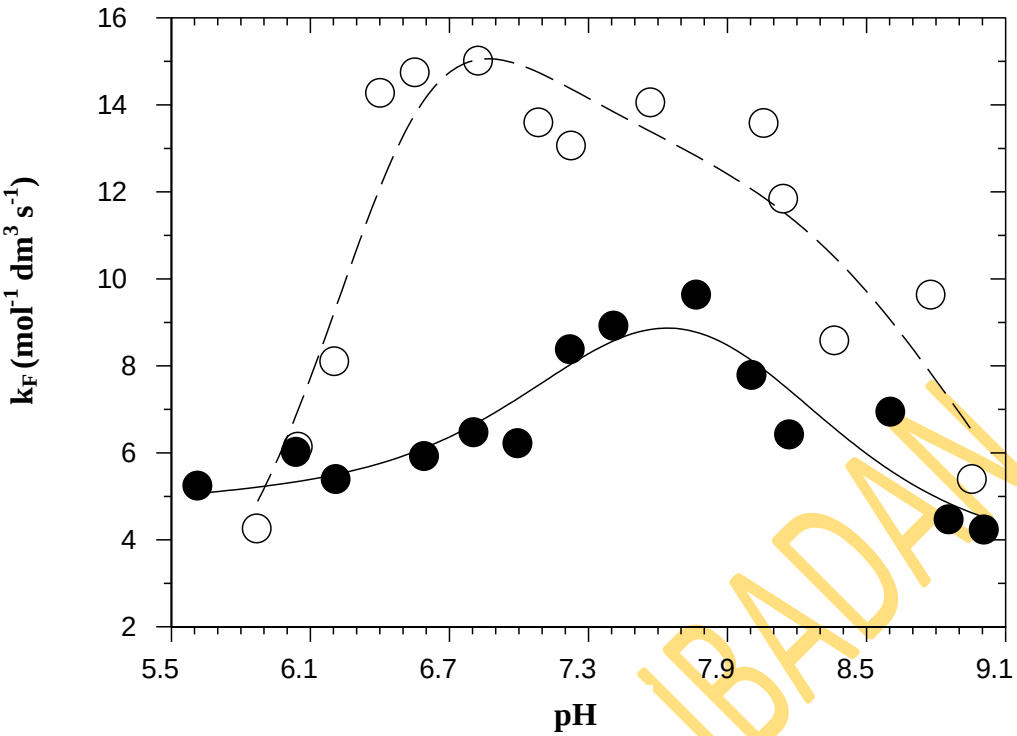



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***Figure 4.33**: Dependence of $k_F$ on pH for the reaction of 5,5'-dithiobis(2-nitrobenzoate) with CysF9[93]ß of **donkey carbonmonoxyhaemoglobin (open circles, stripped haemogloin; filled circles, haemoglobin in the presence of inositol-$P_6$).** Conditions: phosphate buffers pH 5.6 – 7.8; borate buffers pH 8.0 – 9.0 (ionic strength, 50 mmol dm$^{-3}$; added salt, NaCl); [HbCO] = 10 µmol (haem) dm$^{-3}$ (5 µmol dm$^{-3}$ in reactive sulphydryl groups); [inositol-$P_6$], 10 µmol dm$^{-3}$; observation wavelength, $\lambda$ = 412 nm. Each data point is subject to a standard error of about ± 0.40*

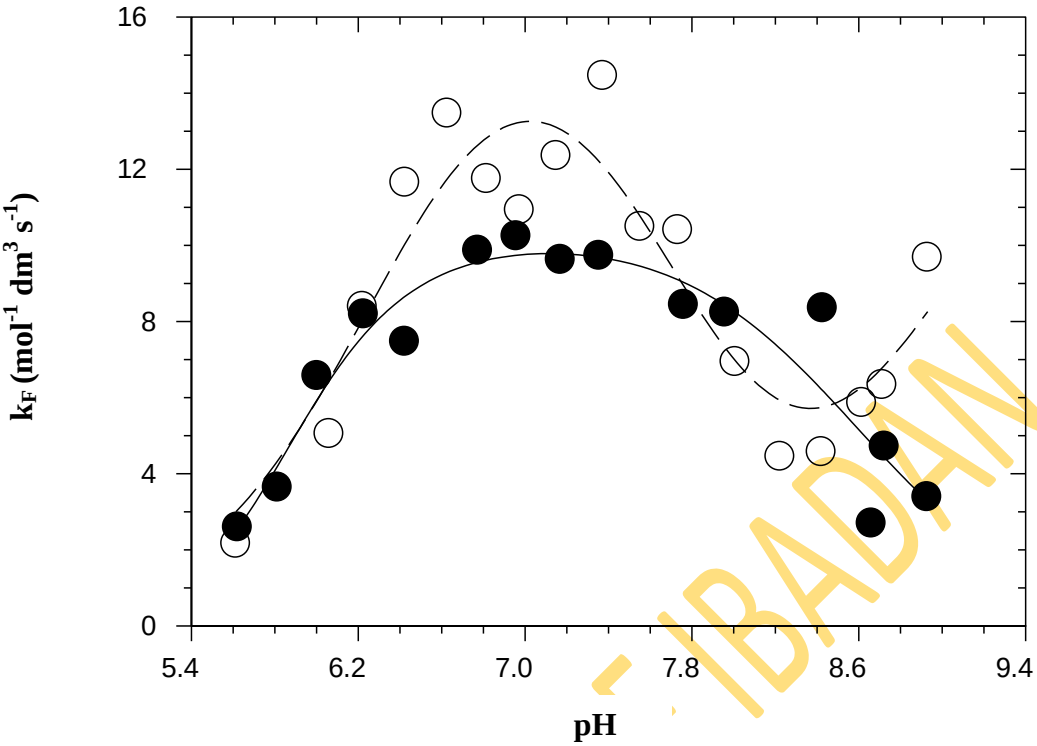
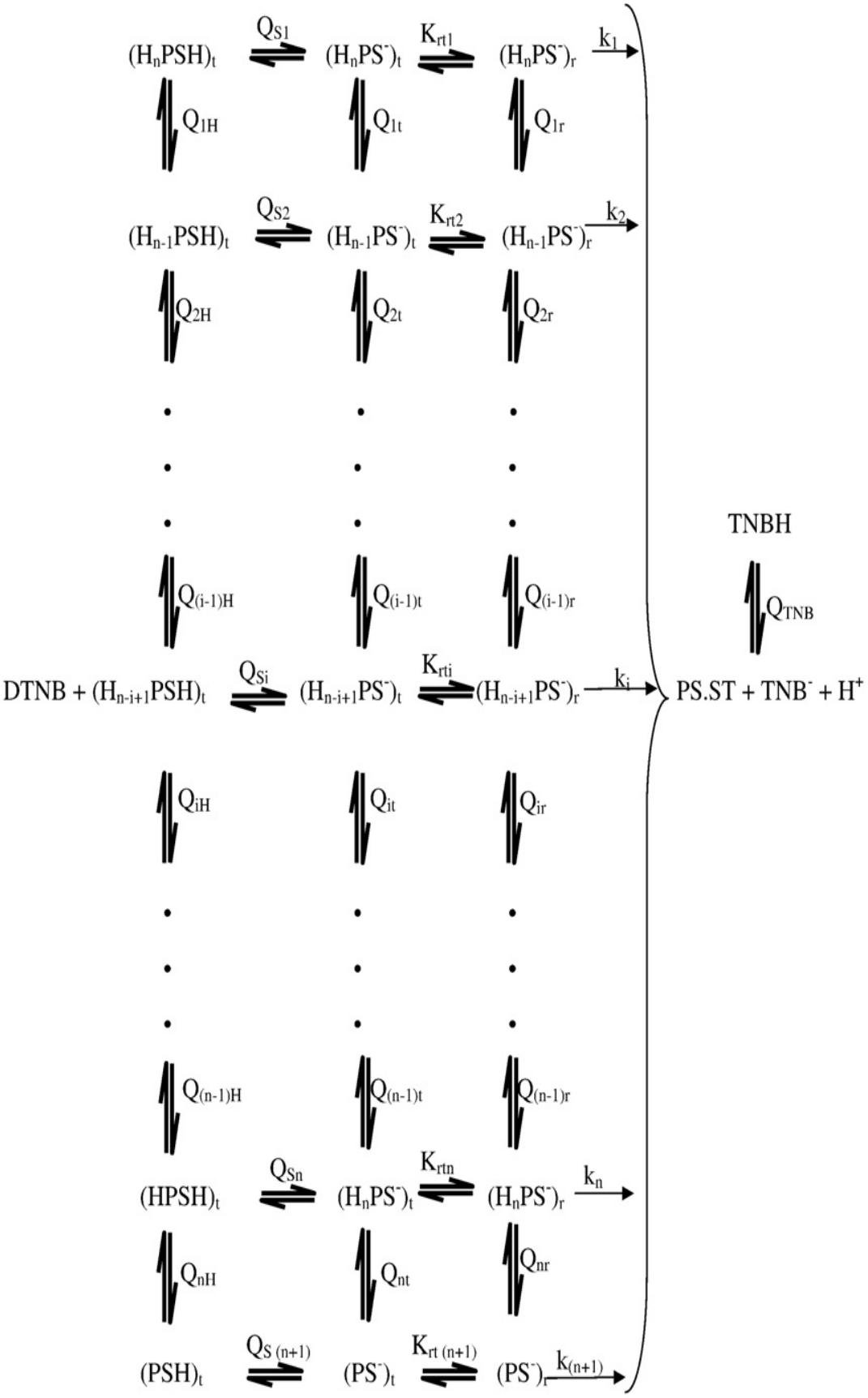



***Figure 4.34**: Dependence of $k_F$ on pH for the reaction of 5,5'-dithiobis(2-nitrobenzoate) with CysF9[93]ß of the **donkey aquomethaemoglobin (open circles, stripped haemogloin; filled circles, haemoglobin in the presence of inositol-$P_6$).** Conditions: phosphate buffers pH 5.6 – 7.8; borate buffers pH 8.0 – 9.0 (ionic strength, 50 mmol dm$^{-3}$; added salt, NaCl); [Hbmet] = 10 µmol (haem) dm$^{-3}$ (5 µmol dm$^{-3}$in reactive sulphydryl groups); [inositol-$P_6$], 10 µmol dm$^{-3}$; observation wavelength, λ = 412 nm. Each data point is subject to a standard error of about ± 0.40*

#### 4.3.2.10 Quantitative analysis of the pH dependence of the apparent second order forward rate constant, $k_F$

Studies of the pH dependence of the reactivities of sulphydryl groups are useful in the determination of the nature and number of amino acid residues influencing such reactivities (Okonjo and Aboluwoye, 1992; Okonjo and Okia, 1993; Okonjo and Adejoro, 1993; Okonjo *et al.*, 1996). This information is obtained from quantitative analyses of the pH dependence profiles of $k_F$. The dependence of the apparent second order forward rate constant, $k_F$, on pH was analysed for each haemoglobin derivative using Scheme 4.1.


$(H_nPSH)_t$
$Q_{S1}$
$(H_nPS^-)_t$
$K_{rt1}$
$(H_nPS^-)_r$
$k_1$
$Q_{1H}$
$Q_{1t}$
$Q_{1r}$
$(H_{n-1}PSH)_t$
$Q_{S2}$
$(H_{n-1}PS^-)_t$
$K_{rt2}$
$(H_{n-1}PS^-)_r$
$k_2$
$Q_{2H}$
$Q_{2t}$
$Q_{2r}$
TNBH
$Q_{(i-1)H}$
$Q_{(i-1)t}$
$Q_{(i-1)r}$
$Q_{TNB}$
DTNB + $(H_{n-i+1}PSH)_t$
$Q_{Si}$
$(H_{n-i+1}PS^-)_t$
$K_{rti}$
$(H_{n-i+1}PS^-)_r$
$k_i$
PS.ST + $TNB^-$ + $H^+$
$Q_{iH}$
$Q_{it}$
$Q_{ir}$
$Q_{(n-1)H}$
$Q_{(n-1)t}$
$Q_{(n-1)r}$
$(HPSH)_t$
$Q_{Sn}$
$(H_nPS^-)_t$
$K_{rtn}$
$(H_nPS^-)_r$
$k_n$
$Q_{nH}$
$Q_{nt}$
$Q_{nr}$
$(PSH)_t$
$Q_{S\,(n+1)}$
$(PS^-)_t$
$K_{rt\,(n+1)}$
$(PS^-)_r$
$k_{(n+1)}$

***Scheme 4.1***

In Scheme 4.1, n is the number of ionisable groups that are electrostatically linked to the CysF9[93]β site. $H_nPSH$, $H_{n-1}PSH$, ..., $H_{n-i+1}PSH$, ..., HPSH and PSH are haemoglobin species having n, (n-1),…, (n-i+1), ...,1 and 0 protons bound to the electrostatically (thiol) linked ionisable groups. Each of these species has its thiol group protonated. $H_nPS^-$, $H_{n-1}PS^-$…, $H_{n-i+1}PS^-$, ...., $HPS^-$ and $PS^-$ are the corresponding thiolate anion forms of the various species. (The $Q_i$ terms in Scheme 4.1 signify ionization constants). Since only the thiolate anion forms are reactive towards DTNB (Okonjo *et al.*, 1979; Hallaway *et al.*, 1980), the relationship between $k_F$ and the parameters in Scheme 4.1 is given by Eqn. 4.16 (Okonjo *et al*., 2010).

$$k_F = \frac{k_{n+1} + \sum_{i=1}^{n} k_i \left[H^+\right]^{n-i+1} \left(\prod_{j=i}^{n} Q_{jr}\right)^{-1}}{1 + \sum_{i=1}^{n} \left[H^+\right]^{n-i+1} \left(\prod_{j=i}^{n} Q_{jr}\right)^{-1} + K_{rt(n+1)} \left[1 + \left[H^+\right]^{n-i+1} \left(\prod_{j=i}^{n} Q_{jt}\right)^{-1} + \frac{\left(H^+\right)}{Q_{s(n+1)}} \left\{1 + \sum_{i=1}^{n} \left[H^+\right]^{n-i+1} \left(\prod_{j=i}^{n} Q_{jH}\right)^{-1}\right\}\right]} \quad \text{.....4.16}$$

The dependences of $k_F$ on pH for the oxy, carbonmonoxy and aquomet derivatives of dog and donkey haemoglobins were analyzed using Eqn. 4.16 for n = 1 and n = 2. The curve fitting was done with the aid of computer programmes written on a MicroMath SCIENTIST software. Equation 4.16 did not give good fits for n = 1 but gave good fits for n = 2. A listing of the programme for n = 2 is presented in Appendix II. The lines through the experimental points in Figs. 4.17 - 4.22 and 4.26 – 4.31 are theoretical lines drawn with Eqn. 4.16 using the best-fit parameters reported in Tables 4.1 – 4.4.

**Table 4.1**: Reaction of DTNB with CysF9[93]β of ***stripped*** **dog haemoglobin**. Best-fit parameters used to fit the data in Figs. 4.17– 4.19. Compare with Scheme 4.1 and Eqn. 4.16 for n = 2.

| Parameters | Oxy | Carbonmonoxy | Aquomet | Mean |
|---|---|---|---|---|
| $k_1$ ($mol^{-1}dm^3s^{-1}$) | 57.352 | 83.508 | 3093.80 | |
| $k_2$($mol^{-1}dm^3s^{-1}$) | 37.123 | 0.000 | 31.893 | |
| $k_3$($mol^{-1}dm^3s^{-1}$) | 0.000 | 14.876 | 5.129 | |
| $pQ_{1r}$ | 7.625 | 5.956 | 4.004 | 6.218 ± 1.21 |
| $pQ_{2r}$ | 8.423 | 7.580 | 5.162 | 7.055 ± 1.09 |
| $pQ_{1t}$ | 9.124 | 5.956 | 5.072 | 6.717 ± 1.35 |
| $pQ_{2t}$ | 9.936 | 8.972 | 5.622 | 8.177 ± 1.44 |
| $pQ_{1H}$ | 6.914 | 5.800 | 4.457 | 5.724 ± 0.82 |
| $pQ_{2H}$ | 7.079 | 7.028 | 5.571 | 6.559 ± 0.50 |
| $pQ_{S3}$ | 8.000 | 8.078 | 8.844 | 8.306 ± 0.28 |
| $K_{rt3}$ | 0.005 | 0.030 | 0.001 | 0.012 ± 0.01 |

It was also possible to fit all the kinetic pH dependence data for all the derivatives of dog haemoglobin in the presence of inositol-$P_6$ using Eqn. 4.16. The data were all fitted with n = 2 since they could not be fitted with n = 1. The best-fit lines are those drawn through the data points in Figs. 4.20 – 4.22. The fitting parameters are reported in Table 4.2. n = 2 indicates that there are two ionisable groups involved in the ionisation of CysF9[93]β as shown in Scheme 4.1. For n = 2, Scheme 4.1 becomes;

***Table 4.2**: Reaction of DTNB with CysF9[93]β of **dog haemoglobin in the presence of inositol-$P_6$**. Best-fit parameters used to fit the data in Figs. 4.20 – 4.22. Compare with Scheme 4.1 and Eqn. 4.16 for n = 2.*

| Parameters | Oxy | Carbonmonoxy | Aquomet | Mean |
|---|---|---|---|---|
| $k_1(mol^{-1}dm^3s^{-1})$ | 281.430 | 1570.700 | 4879.300 | |
| $k_2(mol^{-1}dm^3s^{-1})$ | 6.806 | 10.388 | 203.89 | |
| $k_3(mol^{-1}dm^3s^{-1})$ | 11.714 | 7.665 | 15.656 | |
| $pQ_{1r}$ | 5.762 | 5.001 | 4.931 | 5.231± 0.28 |
| $pQ_{2r}$ | 7.720 | 7.007 | 6.059 | 6.929 ± 0.55 |
| $pQ_{1t}$ | 7.359 | 6.215 | 5.962 | 6.512 ± 0.47 |
| $pQ_{2t}$ | 8.300 | 7.813 | 6.945 | 7.686 ± 0.45 |
| $pQ_{1H}$ | 5.614 | 5.407 | 6.409 | 5.810 ± 0.33 |
| $pQ_{2H}$ | 6.191 | 5.978 | 6.443 | 6.204 ± 0.16 |
| $pQ_{S3}$ | 8.046 | 8.049 | 7.620 | 7.905 ± 0.14 |
| $K_{rt3}$ | 0.166 | 0.321 | 0.293 | 0.260 ± 0.01 |

It was also possible to fit the pH dependence profiles of all the derivatives of stripped donkey haemoglobin with Scheme 4.1 and Eqn. 4.16 for n = 2 (Fig. 4.26 - 4.28). The parameters are reported in Table 4.3.

**Table 4.3**: Reaction of DTNB with CysF9[93]β of ***stripped*** **donkey haemoglobin**. Best-fit parameters used to fit the data in Figs. 4.26– 4.28. Compare with Scheme 4.1 and Eqn. 4.16 for n = 2.

| Parameters | Oxy | Carbonmonoxy | Aquomet | Mean |
|---|---|---|---|---|
| $k_1(mol^{-1}dm^3s^{-1})$ | 21.508 | 123.270 | 36.049 | |
| $k_2(mol^{-1}dm^3s^{-1})$ | 14.444 | 27.987 | 0.000 | |
| $k_3(mol^{-1}dm^3s^{-1})$ | 25.116 | 0.499 | 28.786 | |
| $pQ_{1r}$ | 7.493 | 5.956 | 8.024 | 7.158 ± 0.69 |
| $pQ_{2r}$ | 9.062 | 8.831 | 8.577 | 8.823 ± 0.16 |
| $pQ_{1t}$ | 7.364 | 5.956 | 6.941 | 6.753 ± 0.47 |
| $pQ_{2t}$ | 8.934 | 9.002 | 9.035 | 8.990 ± 0.03 |
| $pQ_{1H}$ | 6.864 | 7.178 | 6.754 | 6.932 ± 0.14 |
| $pQ_{2H}$ | 7.956 | 7.220 | 8.276 | 7.817 ± 0.35 |
| $pQ_{S3}$ | 8.267 | 7.802 | 8.245 | 8.105 ± 0.16 |
| $K_{rt3}$ | 1.291 | 0.718 | 0.877 | 0.962 ± 0.19 |

It was also possible to fit all the kinetic pH dependence data for all derivatives of donkey haemoglobin in the presence of inositol-$P_6$ using Eqn. 4.16. The best-fit lines are those drawn through the data points in Figs. 4.29 – 4.31. The fitting parameters are reported in Table 4.4. These data were all fitted with n = 2.

***Table 4.4**: Reaction of DTNB with CysF9[93]β of **donkey haemoglobin in the presence of inositol-$P_6$.** Best-fit parameters used to fit the data in Figs.4.29 – 4.31. Compare with Scheme 4.1 and Eqn.4.16 for n = 2.*

| Parameters | Oxy | Carbonmonoxy | Aquomet | Mean |
|---|---|---|---|---|
| $k_1(mol^{-1}dm^3s^{-1})$ | 162.33 | 12.616 | 0.000 | |
| $k_2(mol^{-1}dm^3s^{-1})$ | 0.000 | 25.197 | 23.216 | |
| $k_3(mol^{-1}dm^3s^{-1})$ | 21.371 | 5.783 | 0.000 | |
| $pQ_{1r}$ | 6.650 | 7.287 | 5.521 | 6.486 ± 0.59 |
| $pQ_{2r}$ | 8.073 | 7.932 | 8.210 | 8.072 ± 0.09 |
| $pQ_{1t}$ | 7.342 | 7.572 | 5.327 | 6.747 ± 0.75 |
| $pQ_{2t}$ | 8.791 | 8.045 | 8.445 | 8.427 ± 0.25 |
| $pQ_{1H}$ | 5.302 | 5.928 | 5.927 | 5.719 ± 0.21 |
| $pQ_{2H}$ | 8.111 | 6.637 | 6.030 | 6.926 ± 0.69 |
| $pQ_{S3}$ | 7.691 | 7.217 | 8.317 | 7.742 ± 0.37 |
| $K_{rt3}$ | 0.291 | 0.592 | 0.394 | 0.426 ± 0.10 |

## 4.4 EQUILIBRIUM STUDIES

### 4.4.1 Equilibrium constant for the reaction of CysF9[93]β of haemoglobin with DTNB

DTNB reacts with only the thiolate anion form of a sulphydryl group (Okonjo *et al.*, 1979; Okonjo *et al.*, 1995; Okonjo and Nwozo, 1997). We have already demonstrated that the reactions of DTNB with dog and donkey haemoglobins are reversible processes. (Figs. 4.13 – 4.16).

The reversible reaction of DTNB with a haemoglobin sulphydryl group can be written as:

----------4.17

where, PSH is haemoglobin with the CysF9[93]β sulphydryl in its protonated, unreacting form; $PS^-$ is the corresponding reacting anion form; $TNB^-$; 5'-thio-2-nitrobenzoate, the anionic, chromophoric product of the reaction; TNBH is the protonated form of $TNB^-$; $Q_{SH}$ and $Q_{TNB}$ are the ionization constants of CysF9[93]β and TNBH, respectively; $K_{equ}$ is the equilibrium constant for the formation of the mixed disulphide, that is, the DTNB reaction step.

The definitions of some of the terms that appear in Eqn. 4.17 are given below (Okonjo *et al.*, 2005):

$$Q_{SH} = \frac{[H^+][PS^-][DTNB]_f}{[PSH][DTNB]_f} = \frac{[H^+][PS^-]}{[PSH]} \quad \text{.....4.18}$$

$$K_{equ} = \frac{[H^+][PS.ST][TNB^-]}{[H^+][PS^-]_f[DTNB]_f} = \frac{[PS.ST][TNB^-]}{[PS^-]_f[DTNB]_f} \quad \text{.....4.19}$$

$$Q_{TNB} = \frac{[H^+][PS.ST][TNB^-]}{[PS.ST][TNBH]} = \frac{[H^+][TNB^-]}{[TNBH]} \quad \text{..... 4.20}$$

The subscript *f* denotes the unreacted species. It is clear from Eqn. 4.20 that

$$[TNBH] = \frac{[H^+][TNB^-]}{Q_{TNB}} \quad \text{.....4.21}$$

From the stoichiometry of Eqn. 4.17;

$$[PS.ST] = [TNB^-] + [TNBH] \quad \text{.....4.22}$$

Substituting for [TNBH] (Eqn. 4.21) into Eqn. 4.22;

$$[PS.ST]=[TNB^-]\left\{1+\frac{[H^+]}{Q_{TNB}}\right\} \quad \text{.....4.23}$$

The total concentration of haemoglobin in terms of reacting sulphydryl groups, $[P]_{\text{total}}$ is given by:

$$[P]_{total}=[PSH]+[PS^-]_f+[PS.ST] \quad \text{.....4.24}$$

Substituting into Eqn. 4.24 for *[PSH]* and *[PS.ST]* from Eqns. 4.18 and 4.23 respectively, then

$$[P]_{total}=[PS^-]_f\left\{1+\frac{[H^+]}{Q_{SH}}\right\}+[TNB^-]_f\left\{1+\frac{[H^+]}{Q_{TNB}}\right\} \quad \text{.....4.25}$$

Therefore,

$$[PS^-]_f=\frac{[P]_{total}-[TNB^-]_f\left\{1+\frac{[H^+]}{Q_{TNB}}\right\}}{\left\{1+\frac{[H^+]}{Q_{TNB}}\right\}} \quad \text{.....4.26}$$

$[\text{DTNB}]_{\text{total}}$, the total concentration of DTNB, is given by:

$$[DTNB]_{total}=[DTNB]_f+\frac{1}{2}\{[PS.ST]+[TNB^-]+[TNBH]\} \quad \text{.....4.27}$$

$$[DTNB]_f=[DTNB]_{total}-\frac{1}{2}\{[PS.ST]+[TNB^-]+[TNBH]\} \quad \text{.....4.28}$$

$$[DTNB]_f=[DTNB]_{total}-[TNB^-]\left\{1+\frac{[H^+]}{Q_{TNB}}\right\} \quad \text{.....4.29}$$

Substitution into Eqn. 4.19 for [PS.ST] from Eqn. 4.23, for $[PS^-]_f$ from Eqn. 4.26 and for $[DTNB]_f$ from Eqn. 4.28 gives

$$K_{equ}=\frac{[TNB^-]^2\left\{1+\frac{[H^+]}{Q_{TNB}}\right\}}{\left\{\frac{[P]_{total}-[TNB^-]\left\{1+\frac{[H^+]}{Q_{TNB}}\right\}}{\left\{1+\frac{[H^+]}{Q_{SH}}\right\}}\right\}\left\{\{[DTNB]_{total}-[TNB^-]\}\left\{1+\frac{[H^+]}{Q_{TNB}}\right\}\right\}} \quad \text{.....4.30}$$

$$K_{equ} = \frac{\left[TNB^{-}\right]^{2}\left\{1+\frac{\left[H^{+}\right]}{Q_{TNB}}\right\}\left\{1+\frac{\left[H^{+}\right]}{Q_{SH}}\right\}}{\left\{\left[P\right]_{total}-\left[TNB^{-}\right]\left\{1+\frac{\left[H^{+}\right]}{Q_{TNB}}\right\}\right\}\left\{\left\{\left[DTNB\right]_{total}-\left[TNB^{-}\right]\right\}\left\{1+\frac{\left[H^{+}\right]}{Q_{TNB}}\right\}\right\}}$$

……4.31

If $[P]_{total}$, $[DTNB]_{total}$ as well as $[TNB^{-}]$ formed at equilibrium are known,then $K_{equ}$ can be determined, provided that $Q_{TNB}$ and $Q_{SH}$ are known. The $pQ_{SH}$ of the CysF9[93]β sulphydryl group ranges between 8 and 8.6 (Okonjo and Okia 1993; Okonjo *et al.*,1996). Therefore, in calculating $K_{equ}$. a value of 8.3 was assumed for $pQ_{SH}$, while 5.27 was assumed for $pQ_{TNB}$ (Nwosu, 2004; Okonjo *et al*., 2006). An absorption coefficient of 14,000 $mol^{-1}$ $dm^{3}$ $cm^{-1}$ at 412 nm was assumed for $TNB^{-}$. The standard error in the determination of $K_{equ}$ was about 20%.

A data set for the determination of the equilibrium constant, $K_{equ}$ for the reaction of DTNB with *stripped* dog oxyhaemoglobin at pH 8.56 is shown in Table 4.5

***Table 4.5**: Reaction of DTNB with CysF9[93]β of **stripped dog oxyhaemoglobin**: raw data for the determination of $K_{equ}$. Conditions: [Hb] = 50 µmol (haem) $dm^{-3}$; pH **8.56**; temp = 25°C; stock [DTNB] = 29 mmol $dm^{-3}$*

| DTNB Vol. ($mm^3$) | $A_{412}$ | [TNB] in µmol $dm^{-3}$ | $K_{equ}$ |
|---|---|---|---|
| 10 | 0.20 | 14.30 | 0.36 |
| 20 | 0.21 | 14.90 | 0.19 |
| 30 | 0.24 | 17.00 | 0.21 |
| 40 | 0.21 | 15.10 | 0.10 |
| 50 | 0.21 | 15.30 | 0.08 |
| 60 | 0.19 | 13.80 | 0.05 |
| 70 | 0.20 | 14.50 | 0.05 |
| 80 | 0.23 | 16.30 | 0.06 |
| 90 | 0.31 | 21.80 | 0.28 |
| 100 | 0.23 | 16.40 | 0.05 |
| | | | Mean = 0.14 ± 0.03 |

### 4.4.2 pH dependence of the equilibrium constant, $K_{equ}$, for the DTNB reaction: *stripped* dog haemoglobin

Tables 3 – 5 (pages 183 – 185) of Appendix I show typical raw data for the determination of $K_{equ}$, the equilibrium constant for the reaction of DTNB with the oxy, carbonmonoxy and aquomet derivatives of dog haemoglobin.

In Figs. 4.35 – 4.37, we show the dependence of $-\log_{10}K_{equ}$ on pH for the oxy, carbonmonoxy and aquomet derivatives of *stripped* dog haemoglobin. Each data point is the mean of at least 10 values and is subject to a standard error of about 20%. It is seen that the equilibrium constant shows strong pH dependence. For each derivative, the value of $K_{equ}$ decreases by almost three orders of magnitude over the range $5.6 \leq pH \leq 9.0$. The strong pH dependences seen in these figures imply that the DTNB reaction (Eqn. 4.17) is electrostatically coupled to the ionizations of groups on the haemoglobin molecule.

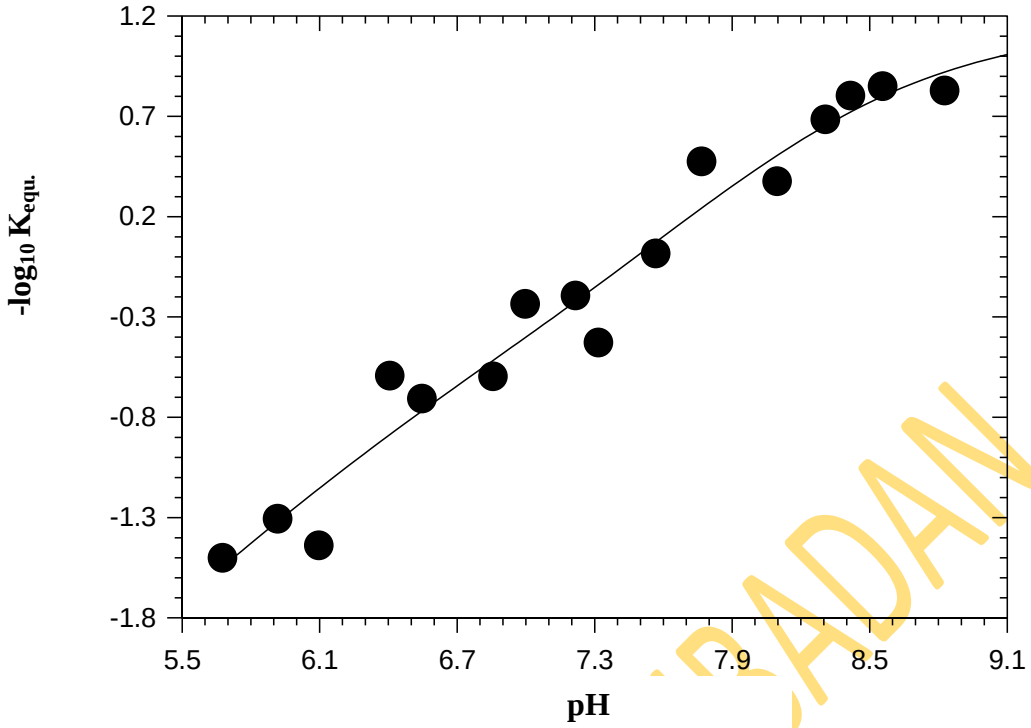



***Figure 4.35**: Dependence of -log$_{10}$K$_{equ}$ on pH for the reaction of DTNB with CysF9[93]β of **stripped dog oxyhaemoglobin**. Conditions: phosphate buffers, pH 5.6 – 7.8; borate buffers, pH 8.0 – 9.0 (ionic strength, 50 mmol dm$^{-3}$; added salt, NaCl); haemoglobin concentration, 50 µmol (haem) dm$^{-3}$ (25 µmol dm$^{-3}$ in reactive sulphydryl groups). Each data point is subject to a standard error of ± 0.10 in the log$_{10}$ of K$_{equ}$.*

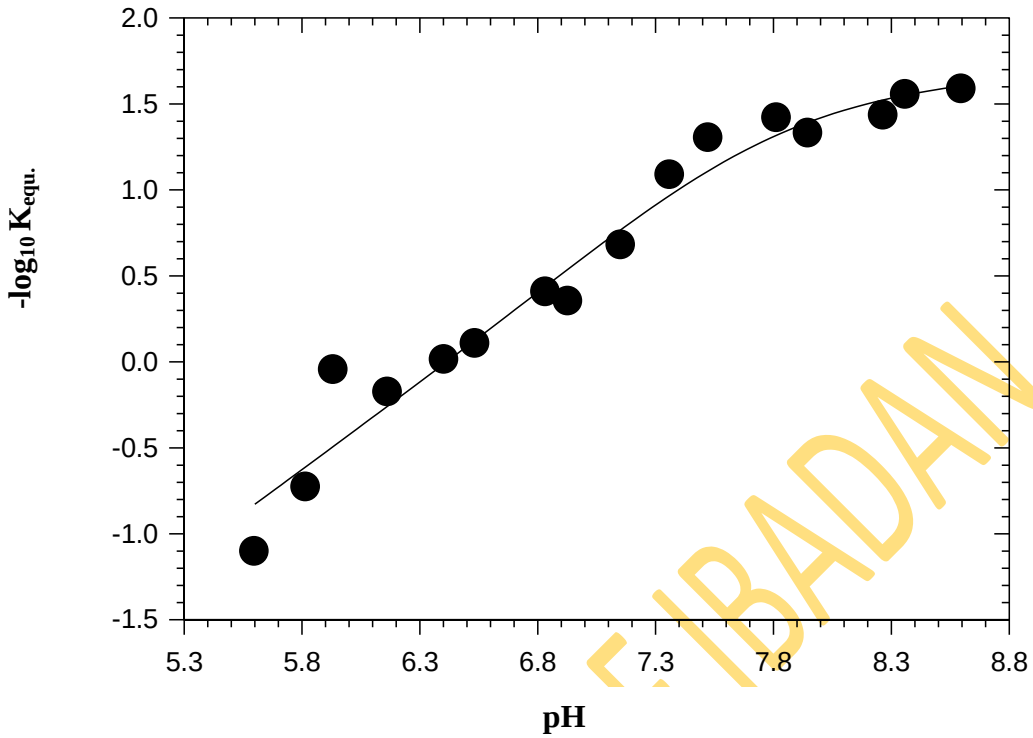



***Figure 4.36**: Dependence of -log$_{10}$K$_{equ}$ on pH for the reaction of DTNB with CysF9[93]ß of **stripped dog carbonmonoxyhaemoglobin**.Conditions: phosphate buffers, pH 5.6 – 7.8; borate buffers, pH 8.0 – 9.0 (ionic strength, 50 mmol dm$^{-3}$; added salt, NaCl); haemoglobin concentration, 50 µmol (haem) dm$^{-3}$ (25 µmol dm$^{-3}$ in reactive sulphydryl groups). Each data point is subject to an error of ± 0.10 in the log$_{10}$ of K$_{equ}$.*

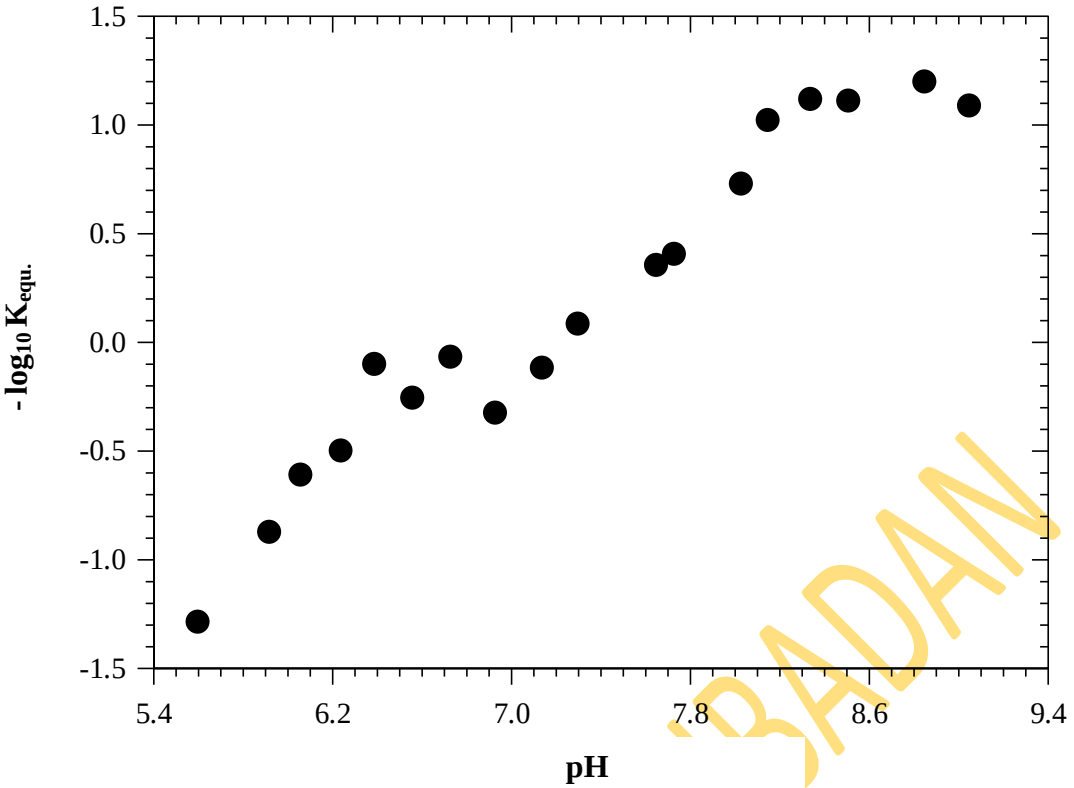



***Figure 4.37**: Dependence of -log$_{10}$K$_{equ}$ on pH for the reaction of DTNB with CysF9[93]ß of **stripped dog aquomethaemoglobin**. Conditions: phosphate buffers, pH 5.6 – 7.8; borate buffers, pH 8.0 – 9.0 (ionic strength, 50 mmol dm$^{-3}$; added salt, NaCl); haemoglobin concentration, 50 µmol (haem) dm$^{-3}$ (25 µmol dm$^{-3}$ in reactive sulphydryl groups). Each data point is subject to an error of ± 0.09 in the log$_{10}$ of K$_{equ}$.*

### 4.4.3 pH dependence of the equilibrium constant, $K_{equ}$, for the DTNB reaction: dog haemoglobin plus inositol-$P_6$

Figs. 4.38 – 4.40 and Tables 14 -16 (pages 193 – 195 of Appendix I) report the pH dependence of the equilibrium constant, $K_{equ}$, for the reaction of DTNB with CysF9[93]β of dog haemoglobin in the presence of inositol-$P_6$. Each data point is the mean of at least 10 values and is subject to a standard error of about 0.02 in the $\log_{10}$ of $K_{equ}$.

For each derivative — oxy (Fig. 4.38), carbonmonoxy (Fig. 4.39) and aquomet (Fig. 4.40) — it is seen that $K_{equ}$ is strongly dependent on pH and decreases by about two to three orders of magnitude over the range $5.6 \leq pH \leq 9.0$. The shapes of the pH dependence profiles are similar, in contrast to the corresponding profiles for $k_F$, the second order forward rate constant.

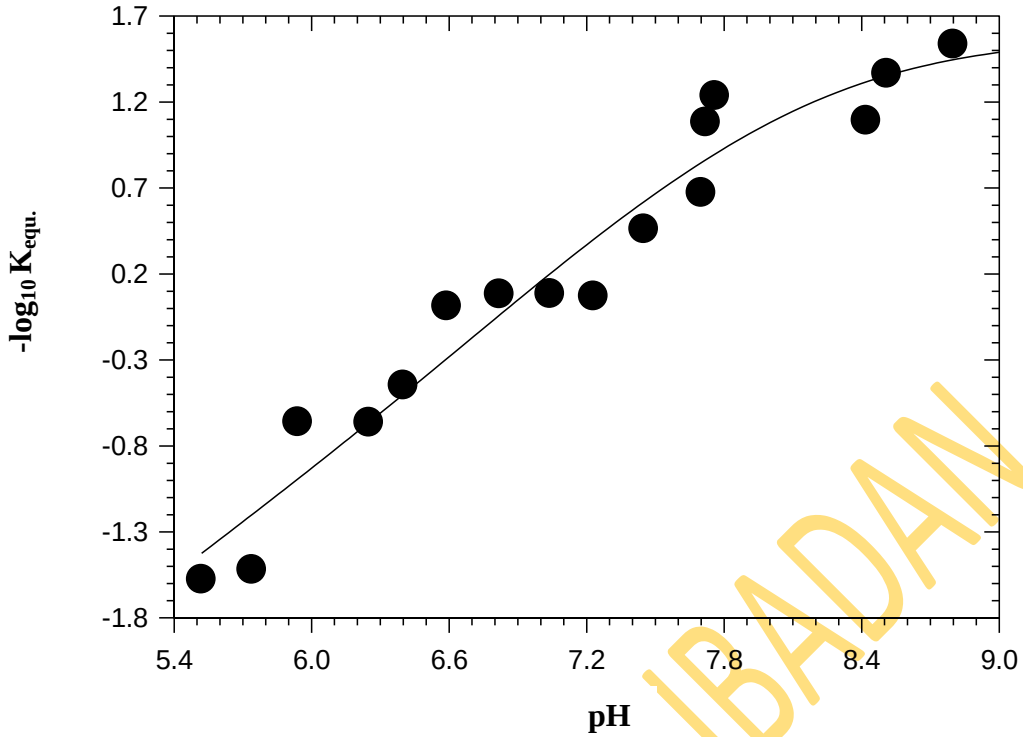



***Figure 4.38**: Dependence of -log$_{10}$K$_{equ}$ on pH for the reaction of DTNB with CysF9[93]ß of **dog oxyhaemoglobin in the presence of inositol-P$_6$**. Conditions: phosphate buffers, pH 5.6 – 7.8; borate buffers, pH 8.0 – 9.0 (ionic strength, 50 mmol dm$^{-3}$; added salt, NaCl); haemoglobin concentration, 50 µmol (haem) dm$^{-3}$ (25 µmol dm$^{-3}$ in reactive sulphydryl groups); [inositol-P$_6$], 50 µmol dm$^{-3}$. Each data point is subject to an error of ± 0.05 in the log$_{10}$ of K$_{equ}$.*

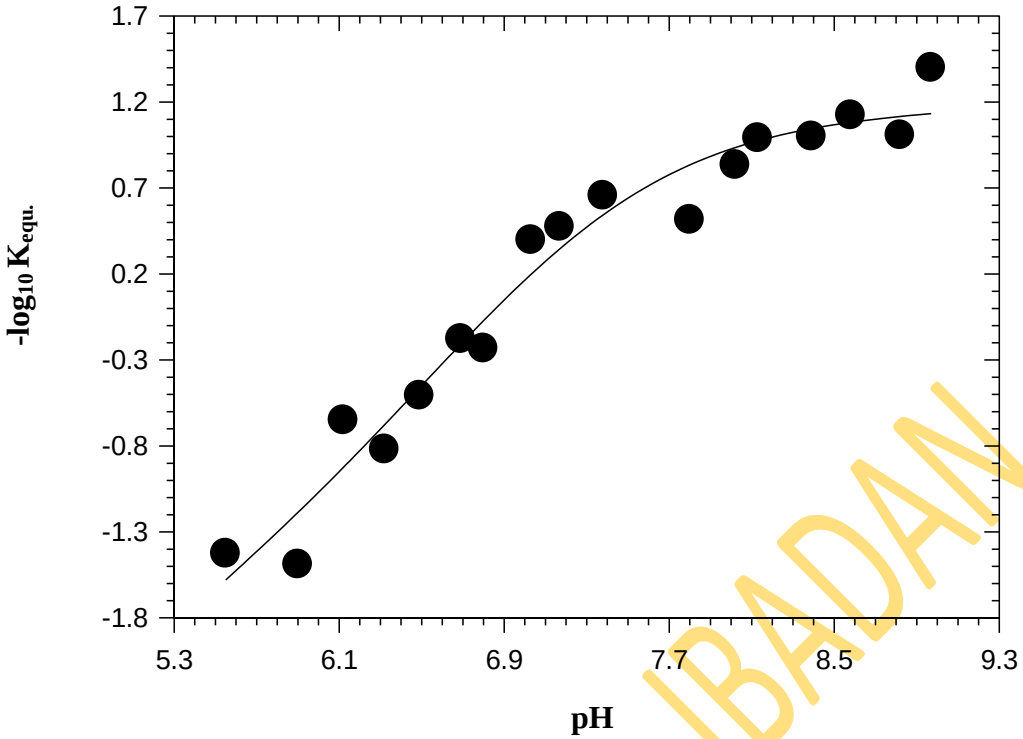



***Figure 4.39**: Dependence of -log$_{10}$K$_{equ}$ on pH for the reaction of DTNB with CysF9[93]ß of **dog carbonmonoxyhaemoglobin in the presence of inositol-P$_6$**). Conditions: phosphate buffers, pH 5.6 – 7.8; borate buffers, pH 8.0 – 9.0 (ionic strength, 50 mmol dm$^{-3}$; added salt, NaCl); haemoglobin concentration, 50 µmol (haem) dm$^{-3}$ (25 µmol dm$^{-3}$ in reactive sulphydryl groups); [inositol-P$_6$], 50 µmol dm$^{-3}$. Each data point is subject to an error of ± 0.10 in the log$_{10}$ of K$_{equ}$.*

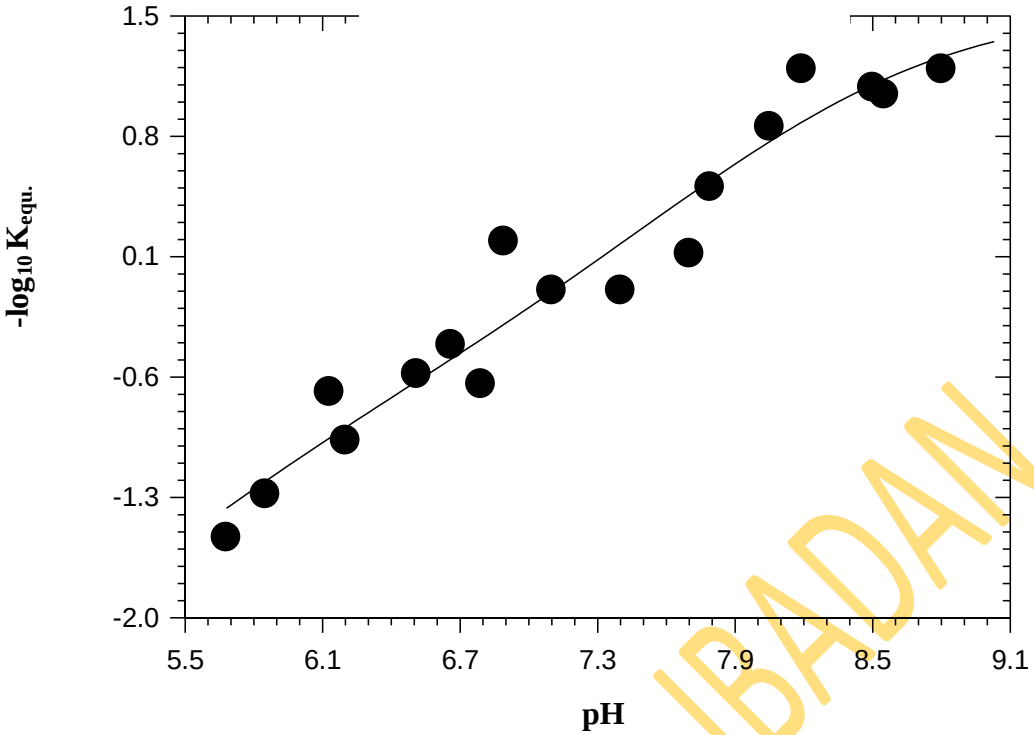



***Figure 4.40**: Dependence of -log$_{10}$K$_{equ}$ on pH for the reaction of DTNB with CysF9[93]ß of **dog aquomethaemoglobin in the presence of inositol-P$_6$**. Conditions: phosphate buffers, pH 5.6 – 7.8; borate buffers, pH 8.0 – 9.0 (ionic strength, 50 mmol dm$^{-3}$; added salt, NaCl); haemoglobin concentration, 50 µmol (haem) dm$^{-3}$ (25 µmol dm$^{-3}$ in reactive sulphydryl groups); [inositol-P$_6$], 50 µmol dm$^{-3}$. Each data point is subject to an error of ± 0.09 in the log$_{10}$ of K$_{equ}$.*

### 4.4.4 Comparison of the pH dependence of the equilibrium constant, $K_{equ}$, for *stripped* dog haemoglobin and dog haemoglobin in the presence of inositol-$P_6$

Figs. 4.41 – 4.43 report comparisons of the pH dependence of the equilibrium constant, $K_{equ,}$ for the reaction of DTNB with CysF9[93]β of stripped dog haemoglobin and for dog haemoglobin in the presence of inositol-$P_6$. It is seen that, apart from a slight decrease in affinity caused by inositol-$P_6$ in the oxy derivative, the organic phosphate appears to have no effect on the equilibrium constant. This is in sharp contrast to its effect on $k_F$, the second order forward rate constant.

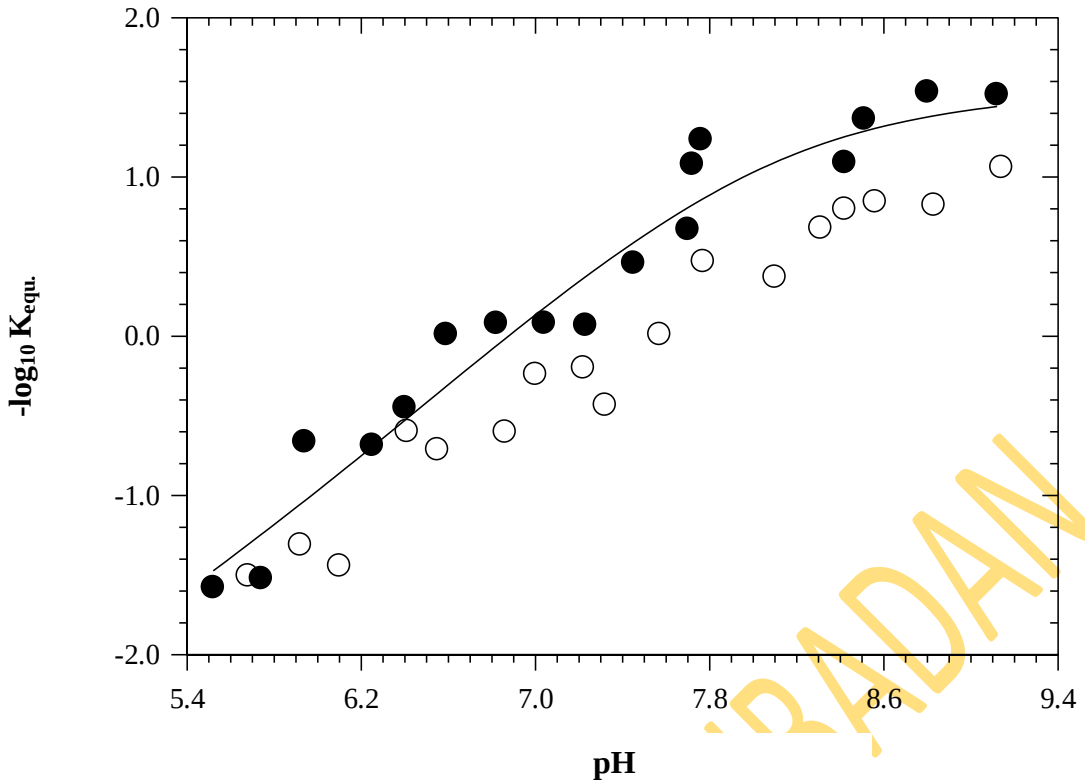



***Figure 4.41**: Dependence of -log$_{10}$K$_{equ}$ on pH for the reaction of DTNB with CysF9[93]ß of **dog oxyhaemoglobin (open circles, stripped haemoglobin; filled circles, haemoglobin in the presence of inositol-P$_6$)**. Conditions: phosphate buffers, pH 5.6 – 7.8; borate buffers, pH 8.0 – 9.0 (ionic strength, 50 mmol dm$^{-3}$; added salt, NaCl); haemoglobin concentration, 50 µmol (haem) dm$^{-3}$ (25 µmol dm$^{-3}$ in reactive sulphydryl groups); [inositol-P$_6$], 50 µmol dm$^{-3}$. Each data point is subject to an error of ± 0.05 in the log$_{10}$ of K$_{equ}$.*

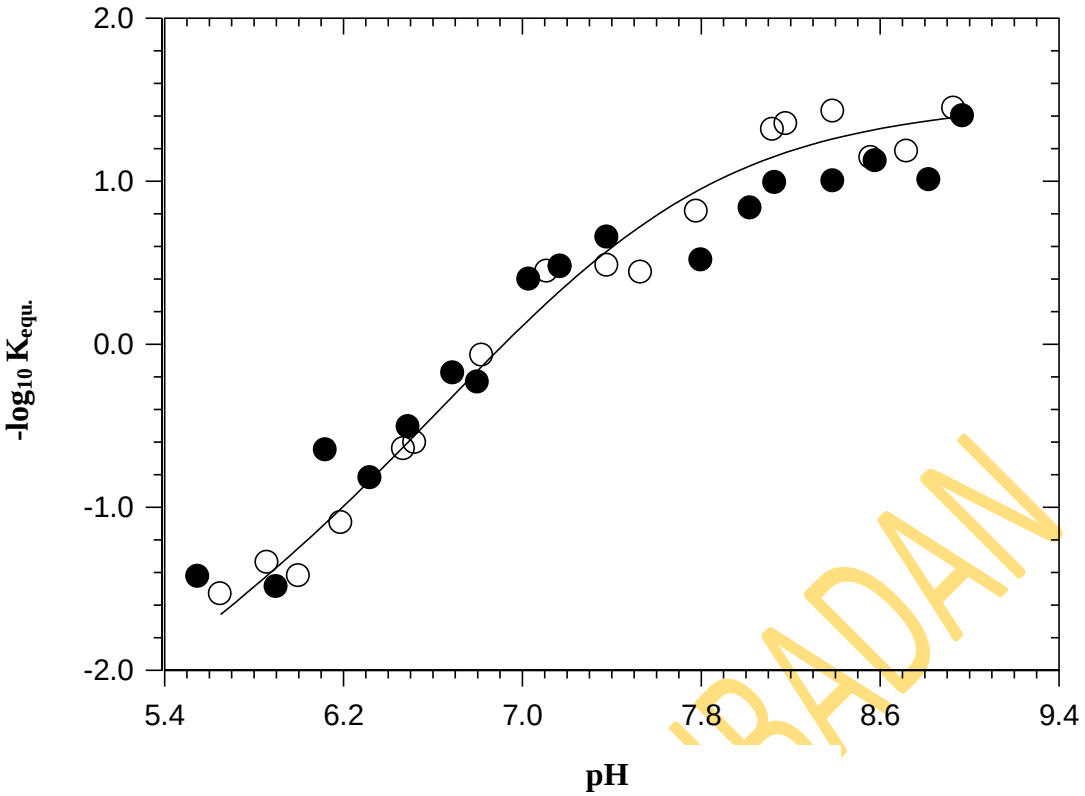



***Figure 4.42**: Dependence of -log$_{10}$K$_{equ}$ on pH for the reaction of DTNB with CysF9[93]ß of **dog carbonmonoxyhaemoglobin (open circles, stripped haemoglobin; filled circles, haemoglobin in the presence of inositol-P$_6$).** Conditions: phosphate buffers, pH 5.6 – 7.8; borate buffers, pH 8.0 – 9.0 (ionic strength, 50 mmol dm$^{-3}$; added salt, NaCl); haemoglobin concentration, 50 µmol (haem) dm$^{-3}$ (25 µmol dm$^{-3}$ in reactive sulphydryl groups); [inositol-P$_6$], 50 µmol dm$^{-3}$. Each data point is subject to an error of ± 0.10 in the log$_{10}$ of K$_{equ}$.*

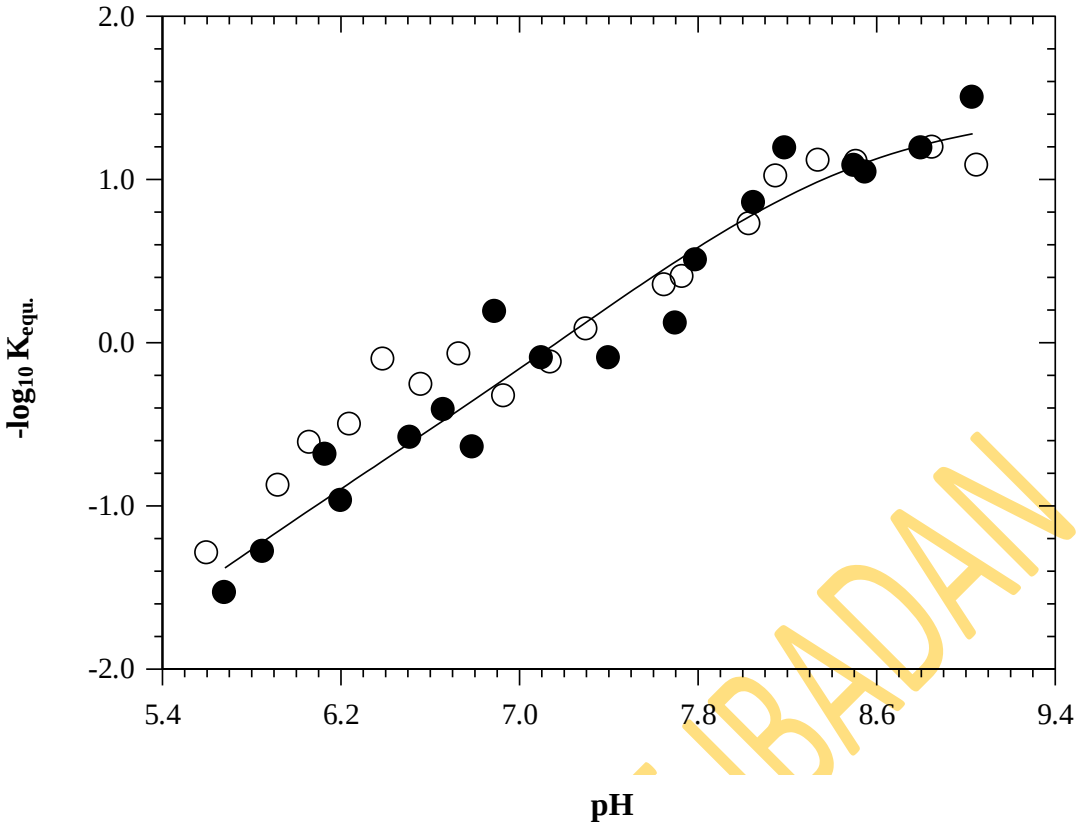



***Figure 4.43**: Dependence of -$log_{10}K_{equ}$ on pH for the reaction of DTNB with CysF9[93]ß of **dog aquomethaemoglobin (open circles, stripped haemoglobin; filled circles, haemoglobin in the presence of inositol-$P_6$)**. Conditions: phosphate buffers, pH 5.6 – 7.8; borate buffers, pH 8.0 – 9.0 (ionic strength, 50 mmol $dm^{-3}$; added salt, NaCl); haemoglobin concentration, 50 µmol (haem) $dm^{-3}$ (25 µmol $dm^{-3}$ in reactive sulphydryl groups); [inositol-$P_6$], 50 µmol $dm^{-3}$. Each data point is subject to an error of ± 0.09 in the $log_{10}$ of $K_{equ}$.*

### 4.4.5 pH dependence of the equilibrium constant, $K_{equ}$, for the DTNB reaction: stripped donkey haemoglobin

Figs. 4.44 – 4.46 show the dependence of $-\log_{10}K_{equ}$ on pH for the oxy, carbonmonoxy and aquomet derivatives of *stripped* donkey haemoglobin. Each data point is the mean of at least 10 values and is subject to a standard error of about 0.10 in the $\log_{10}$ of $K_{equ}$. For each haemoglobin derivative, the equilibrium constant shows a strong pH dependence. The values of $K_{equ}$ decrease by about two to three orders of magnitude for all the derivatives over the range $5.6 \leq pH \leq 9.0$. The strong pH dependence seen in these figures imply that the DTNB reaction is coupled to the ionizations of groups on the haemoglobin molecule.

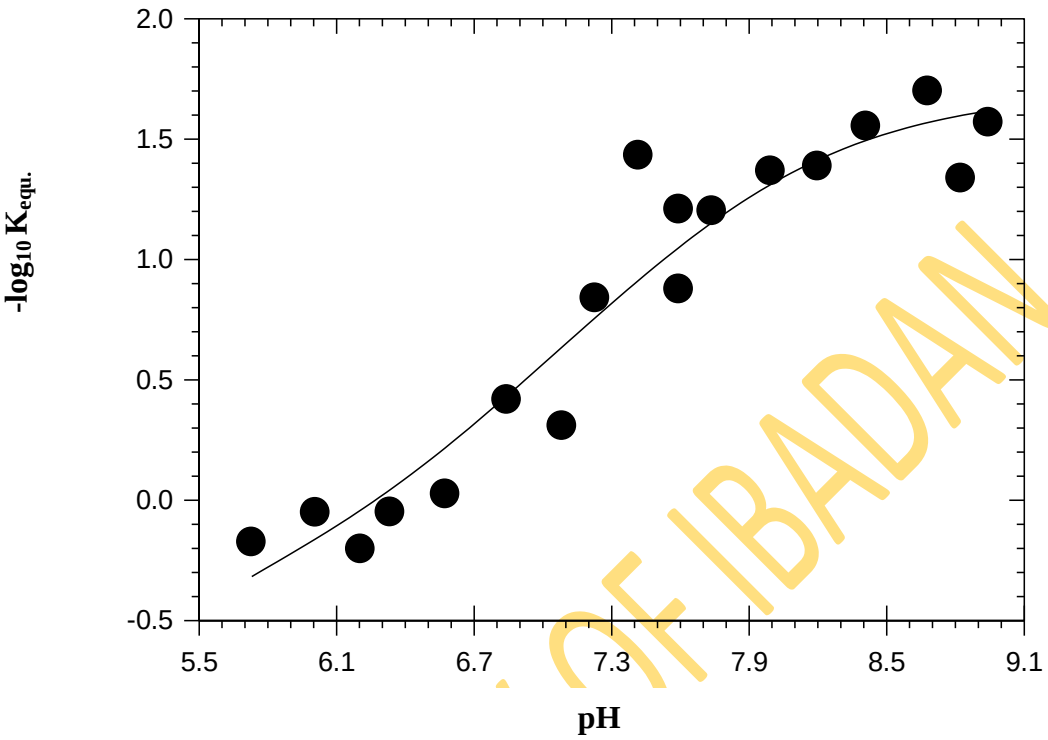



***Figure 4.44**: Dependence of -log$_{10}$K$_{equ}$ on pH for the reaction of DTNB with CysF9[93]ß of **stripped donkey oxyhaemoglobin**. Conditions: phosphate buffers, pH 5.6 – 7.8; borate buffers, pH 8.0 – 9.0 (ionic strength, 50 mmol dm$^{-3}$; added salt, NaCl); haemoglobin concentration, 50 µmol (haem) dm$^{-3}$ (25 µmol dm$^{-3}$ in reactive sulphydryl groups). Each data point is subject to an error of about ± 0.09 in the log$_{10}$ of K$_{equ}$.*

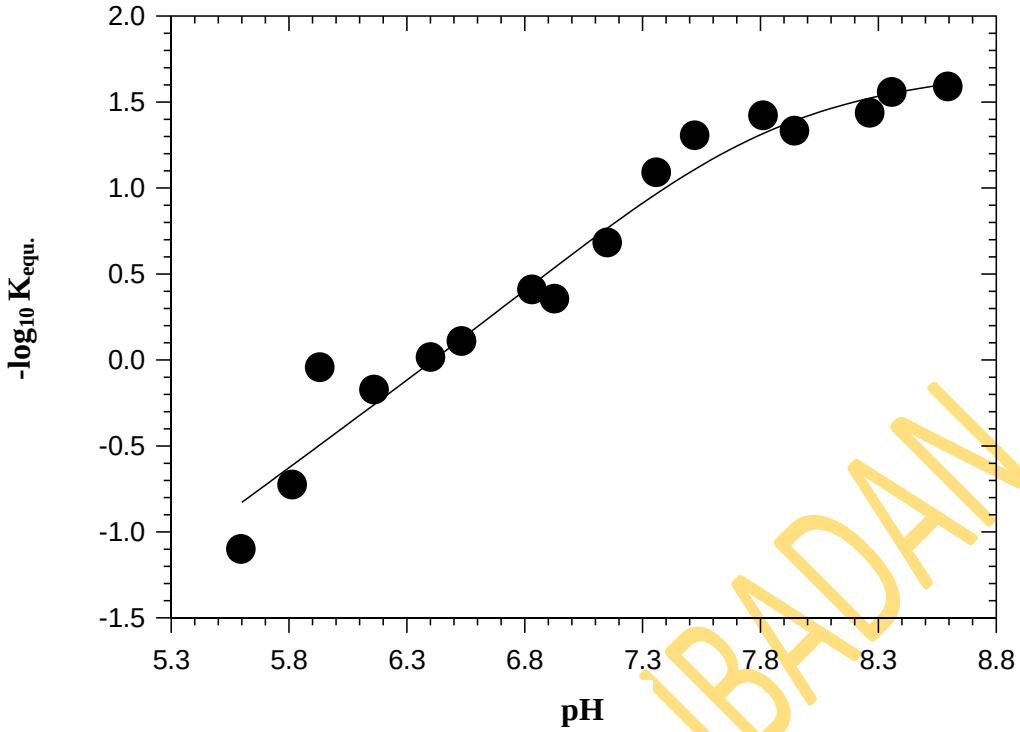



***Figure 4.45**: Dependence of -log$_{10}$K$_{equ}$ on pH for the reaction of DTNB with CysF9[93]ß of **stripped donkey carbonmonoxyhaemoglobin**. Conditions: phosphate buffers, pH 5.6 – 7.8; borate buffers, pH 8.0 – 9.0 (ionic strength, 50 mmol dm$^{-3}$; added salt, NaCl); haemoglobin concentration, 50 µmol (haem) dm$^{-3}$ (25 µmol dm$^{-3}$ in reactive sulphydryl groups). Each data point is subject to an error of about ± 0.03 in the log$_{10}$ of K$_{equ}$.*

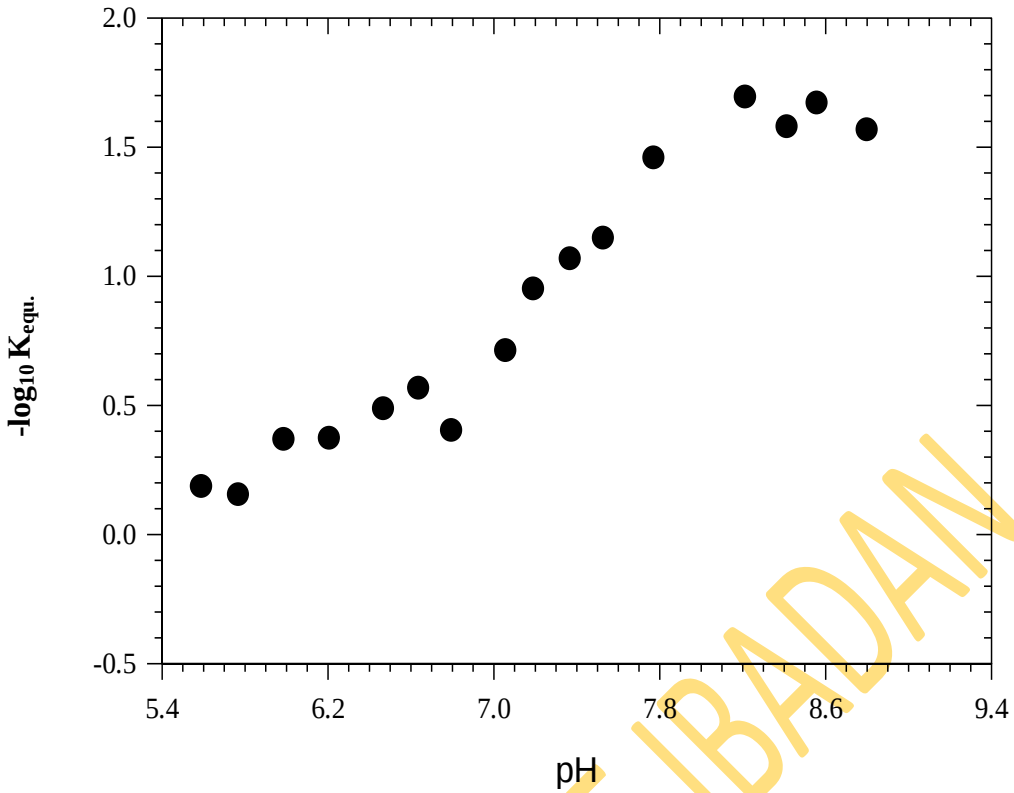



***Figure 4.46**: Dependence of -log$_{10}$K$_{equ}$ on pH for the reaction of DTNB with CysF9[93]ß of **stripped donkey aquomethaemoglobin**. Conditions: phosphate buffers, pH 5.6 – 7.8; borate buffers, pH 8.0 – 9.0 (ionic strength, 50 mmol dm$^{-3}$; added salt, NaCl); [Hbmet], 50 µmol (haem) dm$^{-3}$ (25 µmol dm$^{-3}$ in reactive sulphydryl groups). Each data point is subject to an error of about ± 0.10 in the log$_{10}$ of K$_{equ}$.*

### 4.4.6 pH dependence of the equilibrium constant, $K_{equ}$, for the DTNB reaction: donkey haemoglobin plus inositol-$P_6$

Data for the dependence of $K_{equ}$ on pH for the reaction of DTNB with CysF9[93]β of donkey haemoglobin in the presence of inositol-$P_6$ are shown in Figs. 4.47, 4.48 and 4.49 for the oxy, carbonmonoxy and aquomet derivatives, respectively. Each data point is the average of at least 10 values and is subject to a standard error of about 0.10 in the $\log_{10}$ of $K_{equ}$. The values of $K_{equ}$ decrease by about two to three orders of magnitude for each haemoglobin derivative as the pH increases from 5.6 to 9.0.

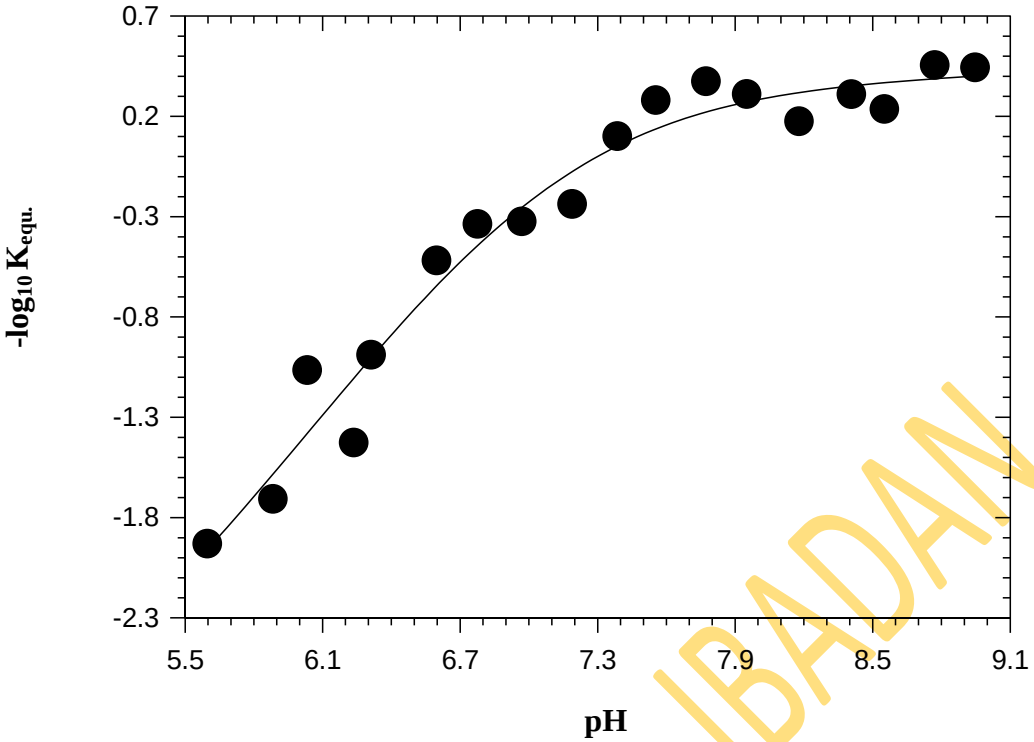



***Figure 4.47**: Dependence of -log$_{10}$K$_{equ}$ on pH for the reaction of DTNB with CysF9[93]ß of **donkey oxyhaemoglobin in the presence of inositol-P$_6$**. Conditions: phosphate buffers, pH 5.6 – 7.8; borate buffers, pH 8.0 – 9.0 (ionic strength, 50 mmol dm$^{-3}$; added salt, NaCl); haemoglobin concentration, 50 µmol (haem) dm$^{-3}$ (25 µmol dm$^{-3}$ in reactive sulphydryl groups); [inositol-P$_6$], 50 µmol dm$^{-3}$. Each data point is subject to an error of about ± 0.08 in the log$_{10}$ of K$_{equ}$.*

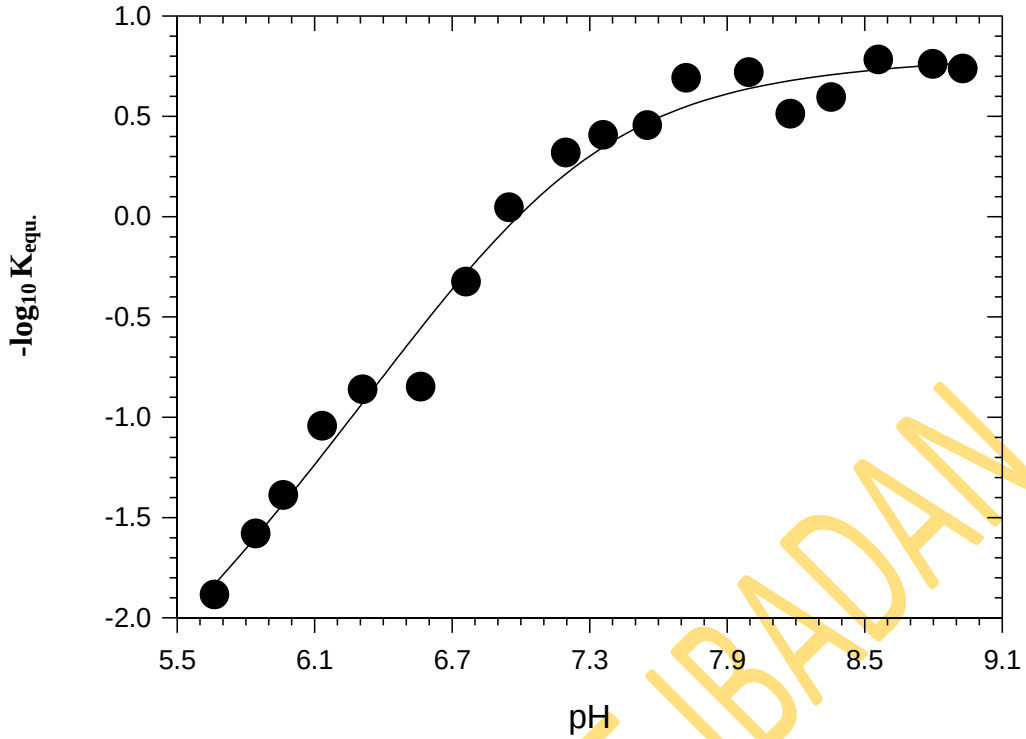



***Figure 4.48**: Dependence of -$log_{10}K_{equ}$ on pH for the reaction of DTNB with CysF9[93]ß of **donkey carbonmonoxyhaemoglobin in the presence of inositol-$P_6$**. Conditions: phosphate buffers, pH 5.6 – 7.8; borate buffers, pH 8.0 – 9.0 (ionic strength, 50 mmol $dm^{-3}$; added salt, NaCl); haemoglobin concentration, 50 µmol (haem) $dm^{-3}$ (25 µmol $dm^{-3}$ in reactive sulphydryl groups); [inositol-$P_6$], 50 µmol $dm^{-3}$. Each data point is subject to an error of about ± 0.04 in the $log_{10}$ of $K_{equ}$.*

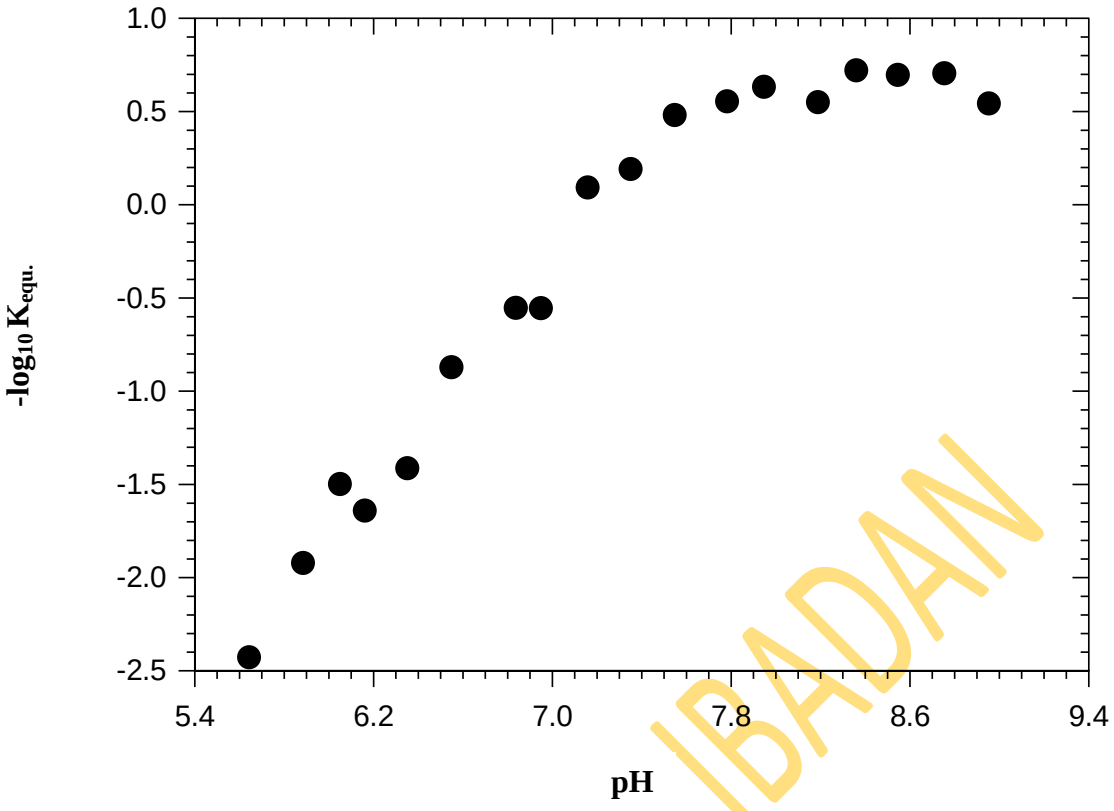



***Figure 4.49**: Dependence of -$log_{10}K_{equ}$ on pH for the reaction of DTNB with CysF9[93]ß of **donkey aquomethaemoglobin in the presence of inositol-$P_6$**. Conditions: phosphate buffers, pH 5.6 – 7.8; borate buffers, pH 8.0 – 9.0 (ionic strength, 50 mmol $dm^{-3}$; added salt, NaCl); haemoglobin concentration, 50 µmol (haem) $dm^{-3}$ (25 µmol $dm^{-3}$ in reactive sulphydryl groups); [inositol-$P_6$], 50 µmol $dm^{-3}$. Each data point is subject to an error of about ± 0.10 in the $log_{10}$ of $K_{equ}$.*

### 4.4.7 Comparison of the pH dependence of the equilibrium constant, $K_{equ}$ for *stripped* haemoglobin and haemoglobin in the presence of inositol-$P_6$: Donkey haemoglobin

Data for the dependence of $K_{equ}$ on pH for the reaction of DTNB with CysF9[93]β of donkey haemoglobin in the presence of inositol-$P_6$ are compared with the *stripped* haemoglobin data in Figs. 4.50, 4.51and 4.52 for the oxy, carbonmonoxy and aquomet derivatives, respectively. In contrast to the lack of any appreciable inositol-$P_6$ effect on dog haemoglobin (Fig. 4.41 – 4.43), clear-cut increases in $K_{equ}$ of between one and two orders of magnitude are caused by the organic phosphate in donkey haemoglobin (Fig. 4.50 – 4.52).

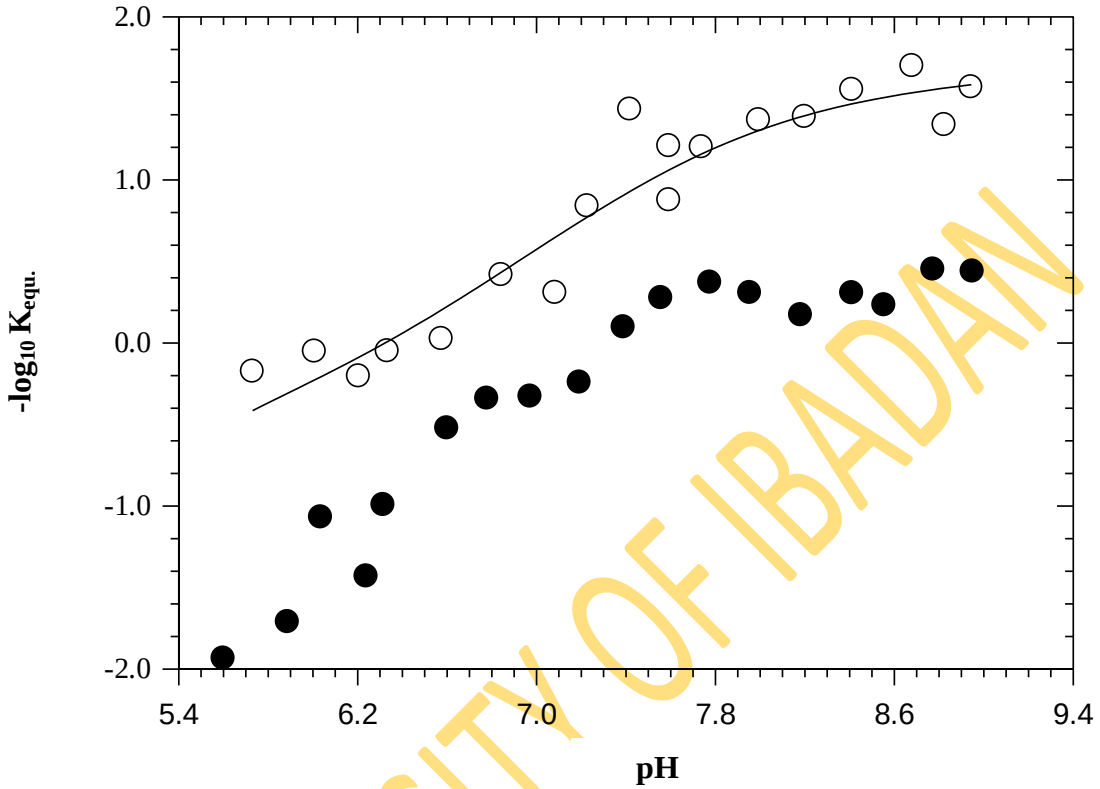



***Figure 4.50**: Dependence of -log$_{10}$K$_{equ}$ on pH for the reaction of DTNB with CysF9[93]ß of **donkey oxyhaemoglobin (open circles, stripped haemoglobin; filled circles, haemoglobin in the presence of inositol-P$_6$)**. Conditions: phosphate buffers, pH 5.6 – 7.8; borate buffers, pH 8.0 – 9.0 (ionic strength, 50 mmol dm$^{-3}$; added salt, NaCl); [HbO$_2$], 50 µmol (haem) dm$^{-3}$ (25 µmol dm$^{-3}$ in reactive sulphydryl groups); [inositol-P$_6$], 50 µmol dm$^{-3}$.*

*Each data point is subject to an error of about ± 0.10 in the log$_{10}$ of K$_{equ}$.*

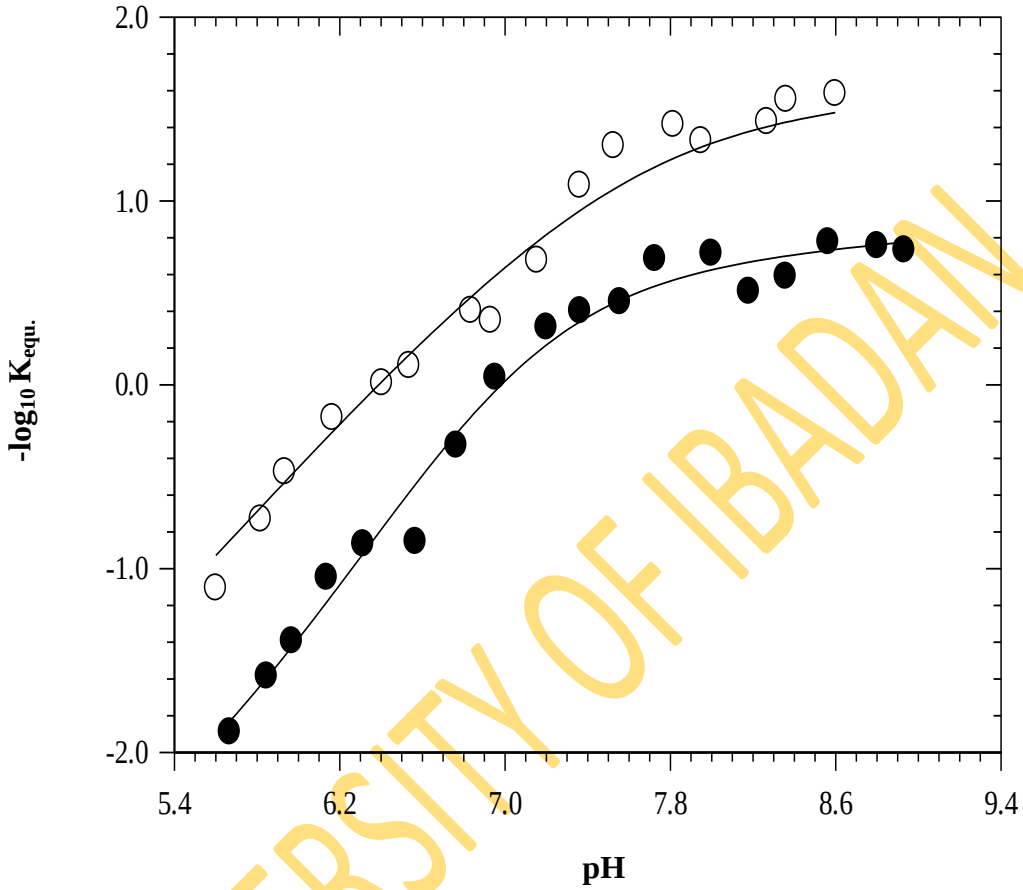



***Figure 4.51**: Dependence of -log$_{10}$K$_{equ}$ on pH for the reaction of DTNB with CysF9[93]ß of **donkey carbonmonoxyhaemoglobin (open circles, stripped haemoglobin; filled circles, haemoglobin in the presence of inositol-P$_6$)**. Conditions: phosphate buffers, pH 5.6 – 7.8; borate buffers, pH 8.0 – 9.0 (ionic strength, 50 mmol dm$^{-3}$; added salt, NaCl); haemoglobin concentration, 50 µmol (haem) dm$^{-3}$ (25 µmol dm$^{-3}$ in reactive sulphydryl groups); [inositol-P$_6$], 50 µmol dm$^{-3}$. Each data point is subject to a standard error of about ± 0.10 in the log$_{10}$ K$_{equ.}$*

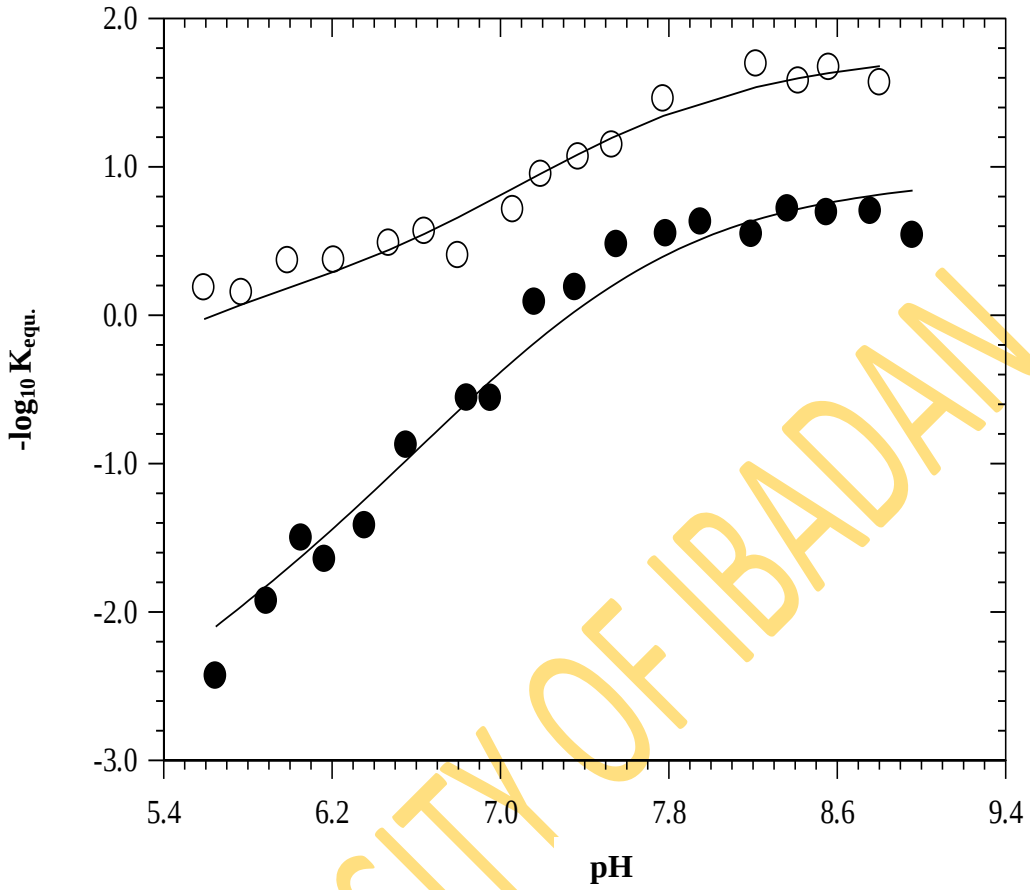
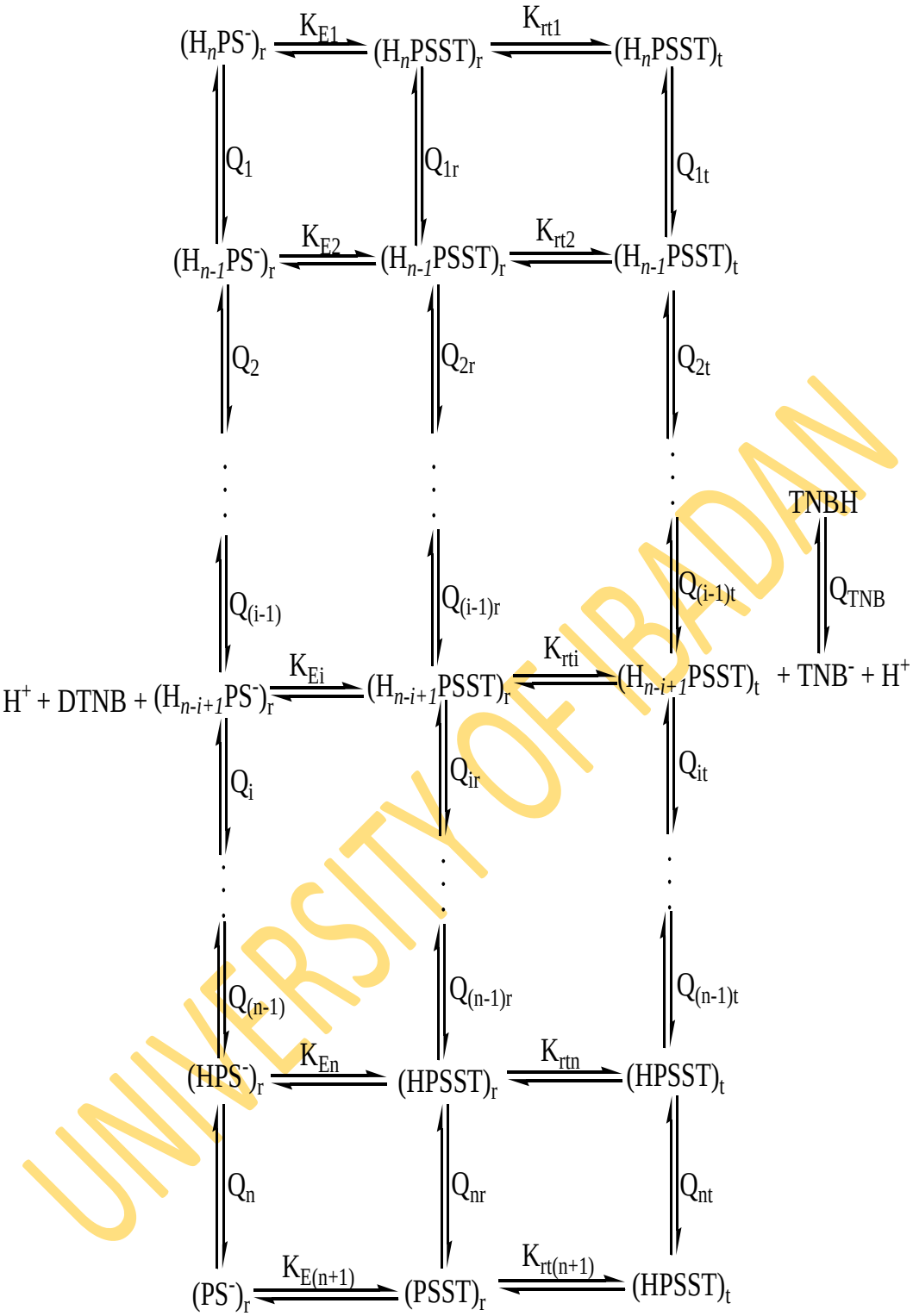





***Figure 4.52**: Dependence of -log$_{10}$K$_{equ}$ on pH for the reaction of DTNB with CysF9[93]ß of **donkey aquomethaemoglobin (open circles, stripped haemoglobin; filled circles, haemoglobin in the presence of inositol-P$_6$).** Conditions: phosphate buffers, pH 5.6 – 7.8; borate buffers, pH 8.0 – 9.0 (ionic strength, 50 mmol dm$^{-3}$; added salt, NaCl); haemoglobin concentration, 50 µmol (haem) dm$^{-3}$ (25 µmol dm$^{-3}$ in reactive sulphydryl groups); [inositol-P$_6$], 50 µmol dm$^{-3}$. Each data point is subject to a standard error of about ± 0.10 in the log$_{10}$ K$_{equ}$*

### 4.4.8 Analysis of the pH dependence of $K_{equ}$

The strong pH dependences seen in Figs. 4.35 – 4.40 and 4.44 – 4.49 imply that the DTNB reaction (Eqn. 4.17; page131) is coupled to the ionizations of groups on the haemoglobin molecule. The dependences of $K_{equ}$ on pH were quantitatively analysed with the aid of Scheme 4.2 (page 160) and Eqn. 4.31 (page 133) (Okonjo *et al*., 2010) for all the dog and donkey haemoglobin derivatives. Scheme 4.2 is based on the experimental finding that the CysF9[93]β sulphydryl group exists in two conformations coupled to two tertiary isomeric forms (***r*** and ***t***) of haemoglobin in dynamic equilibrium (Shaanan, 1983; Okonjo *et al.*, 1989). Studies of the pH dependence of the equilibrium constant, $K_{equ}$, for the reaction of DTNB with the sulphydryl groups are useful in determining the *pQs* of thiol-linked amino acid residues, the transition constant, $K_{rt,}$ for the tertiary conformational transition and other parameters influencing the affinity of such sulphydryl groups for DTNB (Okonjo *et al.,* 2008; 2009).




***Scheme 4.2***

In Scheme 4.2, n ionisable groups are electrostatically linked to the CysF9[93]β site. $H_nPS^-$, $H_{n-1}PS^-$,.., $H_{n-i+1}PS^-$,.., $HPS^-$ and $PS^-$ are haemoglobin species in the thiolate anion forms of the various species having n, (n-1),…, n-i+1,…, 1 and 0 protons bound to the electrostatically thiol-linked ionisable groups. $H_{n-i+1}PS^-$ (i = 1,2,3, …, n+1) are the species that react with DTNB. ($H_{n-i+1}PS.ST$) (i = 1, 2, 3,..., n+1) are the mixed disulphide species formed after the reaction of the sulphydryl with DTNB. The $H^+$ ions produced in the various ionization steps have been omitted from Scheme 4.2 for clarity. The species marked with subscripts ***r*** and ***t*** are tertiary isomeric forms of haemoglobin.The various proton ionization constants are represented as $Q_{ir}$ and $Q_{it}$ (i = 1,2,3, …, n) to differentiate them from the equilibrium constants $K_{Ei}$ (i = 1,2,3, …, n+1) for the reaction of DTNB. $K_{rti}$ (i = 1,2,3…, n+1) are pH dependent equilibrium constants for the **r** ⇋ **t** isomerisation. From Scheme 4.2, the relationship between the equilibrium constant and the parameters of Scheme 4.2 may be written as: (Okonjo *et al.*, 2010)

$$K_{equ} = \frac{K_{E(n+1)}\left\{1+\sum_{i=1}^{n}\left[H^+\right]^{n-i+1}\left(\prod_{j=i}^{n}Q_{jr}\right)^{-1}+K_{rt(n+1)}\left\{1+\sum_{i=1}^{n}\left[H^+\right]^{n-i+1}\left(\prod_{j=i}^{n}Q_{jt}\right)^{-1}\right\}\right\}}{\left\{1+K_{E(n+1)}\left\{\sum_{i=1}^{n}\left[H^+\right]^{n-i+1}\left(\prod_{j=i}^{n}Q_{jr}\right)^{-1}K_{Ei}^{-1}\right\}\right\}} \quad \text{……..4.32}$$

The theoretical lines through the experimental points reported in Figs. 4.35 – 4.40 and 4.44 – 4.49 were calculated with Eqn. 4.32 for n = 2 for all derivatives of dog and donkey haemoglobins. It was not possible to fit the data using n = 1.

The dependence of the equilibrium constant, $K_{equ}$, on pH for the oxy, carbonmonoxy and aquomet derivatives of dog and donkey haemoglobins, both in the absence (*stripped*) and presence of inositol-$P_6$, were analysed using Eqn. 4.32. A computer program was written based on Eqn. 4.32 to fit the $K_{equ}$ data. The lines in Figs. 4.35 – 4.40 and 4.44 – 4.49 are the best-fit lines drawn through the data for the dog and donkey haemoglobin derivatives. The fitting parameters obtained from these calculations are reported in Tables 4.6 – 4.9

***Table 4.6**: Reaction of 5,5'-dithiobis(2-nitrobenzoate) with the CysF9[93]β sulphydryl group of the oxy, carbonmonoxy and aquomet derivatives of **stripped dog haemoglobin**. Best-fit parameters employed to fit the equilibrium data reported in Figs. 4.35 – 4.37 using Scheme 4.2 and Eqn. 4.32 for n = 2*

| Parameters | Haemoglobin Derivatives | | | Mean |
|---|---|---|---|---|
| | Oxy | Carbonmonoxy | Aquomet | |
| $pQ_{1r}$ | 5.716 | 5.044 | 5.661 | 5.47 ± 0.22 |
| $pQ_{1t}$ | 6.737 | 7.364 | 6.449 | 6.85 ± 0.31 |
| $pQ_{2r}$ | 7.564 | 7.341 | 7.742 | 7.55 ± 0.13 |
| $pQ_{2t}$ | 8.956 | 8.102 | 8.715 | 8.59 ± 0.28 |
| $K_{E3}/K_{E2}$ | 0.615 | 0.207 | 0.408 | 0.41 ± 0.14 |
| $K_{E3}/K_{E1}$ | 0.015 | 0.021 | 0.027 | 0.02 ± 0.01 |
| $-log_{10}K_{E3}$ | 0.844 | 1.417 | 1.130 | 1.13± 0.19 |
| $K_{rt3}$ | 0.500 | 0.875 | 0.861 | 0.75 ± 0.13 |

***Table 4.7**: Reaction of 5,5'-dithiobis(2-nitrobenzoate) with the CysF9[93]β sulphydryl group of the oxy, carbonmonoxy and aquomet derivatives of **dog haemoglobin in the presence of inositol-$P_6$**. Best-fit parameters employed to fit the equilibrium data reported in Figs. 4.38 – 4.40 using Scheme 4.2 and Eqn.4.32 for n = 2.*

| Parameters | Haemoglobin Derivatives | | | Mean |
|---|---|---|---|---|
| | Oxy | Carbonmonoxy | Aquomet | |
| $pQ_{1r}$ | 5.416 | 5.114 | 5.176 | 5.24 ± 0.10 |
| $pQ_{1t}$ | 6.921 | 6.855 | 6.538 | 6.77 ± 0.13 |
| $pQ_{2r}$ | 7.479 | 6.721 | 7.740 | 7.31± 0.34 |
| $pQ_{2t}$ | 8.895 | 7.989 | 8.870 | 8.58± 0.30 |
| $K_{E3}/K_{E2}$ | 0.199 | 0.669 | 0.307 | 0.39± 0.16 |
| $K_{E3}/K_{E1}$ | 0.007 | 0.006 | 0.144 | 0.05 ± 0.05 |
| $-log_{10}K_{E3}$ | 1.481 | 1.087 | 1.151 | 0.91 ± 0.11 |
| $K_{rt3}$ | 0.248 | 0.905 | 0.956 | 0.70 ± 0.24 |

***Table 4.8**: Reaction of 5,5'-dithiobis(2-nitrobenzoate) with the CysF9[93]β sulphydryl group of the oxy, carbonmonoxy and aquomet derivatives of **stripped donkey haemoglobin.** Best-fit parameters employed to fit the equilibrium data reported in Figs.4.41, 4.42 and 4.43 using Scheme 4.2 and Eqn. 4.32 for n = 2.*

| Parameters | Haemoglobin Derivatives | | | Mean |
|---|---|---|---|---|
| | Oxy | Carbonmonoxy | Aquomet | |
| $pQ_{1r}$ | 4.966 | 5.956 | 5.004 | 5.32 ± 0.33 |
| $pQ_{1t}$ | 4.929 | 7.423 | 6.723 | 6.51 ± 0.83 |
| $pQ_{2r}$ | 6.644 | 7.478 | 4.929 | 6.52 ± 0.85 |
| $pQ_{2t}$ | 8.515 | 8.250 | 8.353 | 8.36 ± 0.88 |
| $K_{E3}/K_{E2}$ | 0.615 | 0.653 | 0.645 | 0.49 ± 0.13 |
| $K_{E3}/K_{E1}$ | 0.006 | 0.003 | 0.003 | 0.03 ± 0.001 |
| $-log_{10}K_{E3}$ | 1.599 | 1.584 | 1.700 | 1.62 ± 0.39 |
| $K_{rt3}$ | 0.657 | 0.425 | 0.479 | 0.46 ± 0.77 |

***Table 4.9**: Reaction of 5,5'-dithiobis(2-nitrobenzoate) with the CysF9[93]β sulphydryl group of the oxy, carbonmonoxy and aquomet derivatives of **donkey haemoglobinin the presence of inositol-$P_6$**. Best-fit parameters employed to fit the equilibrium data reported in Figs. 4.44 – 4.46 using Scheme 4.2 and Eqn. 4.32 for n = 2.*

| **Parameters** | **Haemoglobin Derivatives** | | | **Mean** |
|---|---|---|---|---|
| | **Oxy** | **Carbonmonoxy** | **Aquomet** | |
| $pQ_{1r}$ | 5.059 | 5.223 | 5.013 | 5.10 ± 0.07 |
| $pQ_{1t}$ | 6.535 | 7.032 | 6.847 | 6.80 ± 0.17 |
| $pQ_{2r}$ | 6.972 | 7.084 | 7.098 | 7.05 ± 0.04 |
| $pQ_{2t}$ | 7.513 | 7.551 | 7.556 | 7.54 ± 0.01 |
| $K_{E3}/K_{E2}$ | 0.090 | 0.112 | 0.086 | 0.10 ± 0.01 |
| $K_{E3}/K_{E1}$ | 0.009 | 0.009 | 0.005 | 0.01 ± 0.01 |
| $-log_{10}K_{E3}$ | 0.455 | 0.746 | 0.453 | 0.55 ± 0.10 |
| $K_{rt3}$ | 1.094 | 0.857 | 0.541 | 0.83 ± 0.18 |

# CHAPTER FIVE

# DISCUSSION

## 5.1 Kinetics of the reaction of DTNB with haemoglobins

The kinetics of the reaction of stripped dog haemoglobin with DTNB has been reported previously (Okonjo and Adejoro, 1993). These authors carried out this work on the assumption that the DTNB reaction is an irreversible process. We have demonstrated in this thesis that this assumption is incorrect because plots of the pseudo-first order rate constant ($k_{obs}$) against the DTNB concentration were linear and gave positive intercepts for dog (see Fig. 4.13 and 4.14; p. 92 and 93) and for donkey haemoglobin (see Fig. 4.15, p. 95 and 4.16, p. 96). This finding made it possible to embark on equilibrium studies for the two haemoglobins.

In view of the reversible nature of the DTNB reaction, it is important to compare the present kinetic data for stripped dog haemoglobin with those of Okonjo and Adejoro (1993), for which non-reversibility was assumed. Okonjo and Adejoro (1993) found that $k_F$ increased monotonically with pH for various stripped dog haemoglobin derivatives. In contrast, the present study demonstrates that the pH dependence profiles are bowl- or bell-shaped (Fig. 4.13 and 4.14). Furthermore, the present $k_F$ values are at least one order of magnitude lower than the previous results.

Inositol-$P_6$ caused appreciable changes in $k_F$ for dog haemoglobin: a reduction for oxyhaemoglobin (Fig. 4.23; p. 106); a reduction for carbonmonoxyhaemoglobin that becomes significant only above pH 7.8 (Fig. 4.24; p. 107); and an *increase* for aquomethaemoglobin that is significant above pH 6.4 (Fig. 4.25; p. 108). These results are not readily rationalised on the basis of the mean $K_{rt3}$ values of 0.012 for stripped haemoglobin (Table 4.1, p. 128) and 0.260 for inositol-$P_6$ bound haemoglobin (Table 4.2, p. 130). .

Inositol-$P_6$ also had considerable effects on $k_F$ for donkey haemoglobin: an increase for oxyhaemoglobin between pH 5.6 and 7.1 (Fig. 4.32, p. 118); a decrease throughout the experimental pH range for carbonmonoxyhaemoglobin (Fig. 4.33, p. 119); and only a slight decrease over a narrow pH range centred around pH 7.0. Again, these results are not readily rationalised on the basis of the mean $K_{rt3}$ values of

0.963 for stripped haemoglobin (Table 4.3, p. 128) and 0.426 for inositol-$P_6$ bound haemoglobin (Table 4.4, p. 130).

It should be noted from the kinetic scheme employed for analysing the $k_F$ data (Scheme 4.1, p. 122) that the the parameters estimated from the dog and donkey kinetic data (Tables 4.1 – 4.4) are for species that have not bound DTNB.

### 5.1.1 Effect of inositol-$P_6$ on the kinetic parameters

**(a) Dog haemoglobin**

Tables 4.1 and 4.2 report the parameters employed to fit the dog kinetic data for stripped and for inositol-$P_6$ bound haemoglobin. A comparison of the parameters in this table reveals that the organic phosphate has the following effects on the mean parameters: (i) It lowers $pQ_{1r}$ by 1, $pQ_{2t}$ by 0.5, and $pQ_{S3}$ by 0.4 of a $pK_a$ unit, but increases $pQ_{2H}$ by 0.4. (ii) It increases all the model kinetic parameters, $k_i$ (i = 1-3). (iii) It also increased the mean $K_{rt3}$ value from 0.012 for stripped to 0.26 for inositol-$P_6$ bound haemoglobin. These represent an increase of the **t** isomer population from 1.2 to 20.6%

**(b) Donkey haemoglobin**

In Tables 4.3 and 4.4 we report the parameters employed to fit the kinetic data for donkey haemoglobin, stripped and with inositol-$P_6$ respectively. It is seen that the organic phosphate lowers each of the $pQ_i$ values. No clear-cut effect is seen regarding the model kinetic parameters, $k_i$., but the mean $K_{rt3}$ value is lowered from 0.962 for stripped to 0.426 for inositol-$P_6$ bound haemoglobin. These $K_{rt3}$ values represent a lowering by inositol-$P_6$ of the **t** isomer population from 49% to 29.9%.

It is clear from the above discussion that the effect of inositol-$P_6$ on either the dog or donkey kinetic data is complex and cannot be attributed solely to changes in the relative populations of the **r** and **t** tertiary isomers, as was done for the sheep haemoglobins (Okonjo *et al.,* 2010). For the dog data, inositol-$P_6$ increased the **t** isomer population, whereas it lowered it for the donkey data.

## 5.2 Equilibrium studies of the reaction of DTNB with haemoglobins

**(a) Dog haemoglobin**

A remarkable aspect of the equilibrium data for dog haemoglobin is that inositol – $P_6$ to have had any effect on the equilibrium constant (Fig.4.41 –

4.43; p. 144 - 146). This is reflected in the parameters used to fit these data with Scheme 4.2. The organic phosphate has no effect on any of the $pQ_i$ values; neither does it have any effect on the mean $K_{rt3}$ values: 0.75 for stripped haemoglobin and 0.7 for inositol – $P_6$ bound haemoglobin. These numbers represent **t** isomer proportions of 42.9 and 41.2%.

**(b) Donkey haemoglobin**

Inositol – $P_6$ increased the equilibrium constant for each donkey haemoglobin derivative across the whole experimental pH range of 5.6 – 9.0 (Fig.4.50 – 4.52). The effects of the organic phosphate on the mean values of the parameters used to fit the $K_{equ.}$ Data with Scheme 4.2 are as follows: (i) $pQ_{1r}$ and $pQ_{2t}$ are decreased by 0.2 and 0.8 $pK_a$ units respectively, and $pQ_{1t}$ and $pQ_{2r}$ are increased by 0.3 and 0.5 respectively. These changes roughly cancel out. (ii) $K_{rt3}$ , the tertiary transition constant is increased from 0.46 for stripped haemoglobin to 0.83. These numbers represent **t** isomer populations of 31.5 and 45.4% respectively. These results are qualitatively similar to those reported for the sheep haemoglobins (Okonjo *et al*., 2009). The increased of the sheep haemoglobins for DTNB caused by inositol - $P_6$ were attributed to a shift in the **r** ⇋ **t** tertiary equilibrium in favour of the **t** isomer. Following this, we attribute the affinity increases seen in Fig. 4.50 – 4.52 to an increase in the **t** isomer population from 31.5 to 45.4%.

# CHAPTER SIX

# CONCLUSION AND RECOMMENDATION

## 6.1 CONCLUSION

It is established from the kinetic data reported in this work that the reaction of DTNB with CysF9[93]β of both dog and donkey haemoglobins are reversible processes: plots of $k_{obs}$ against [DTNB] are linear, with positive significant slopes and non-zero intercepts.

The pH dependence profiles are bowl- or bell-shaped, which contrasts with the monotonical increase of $k_F$ with pH in the previous work on dog haemoglobin by Okonjo and Adejoro (1993). Furthermore, the present $k_F$ values are at least one order of magnitude lower than the previous results.

Inositol-$P_6$ has considerable effects on $k_F$ for dog haemoglobin. It also has considerable effects on $k_F$ for donkey haemoglobin. These results are not readily rationalised on the basis of the mean $K_{rt3}$ value: from 0.012 for stripped to 0.26 for inositol-$P_6$ bound haemoglobin in dog, representing an increase of the **t** isomer population from 1.2 to 20.6% and $K_{rt3}$ values of 0.963 for stripped haemoglobin and 0.426 for inositol-$P_6$ bound haemoglobin in donkey, representing a decrease of the **t** isomer population from 49% to 29.9%

The effect of inositol-$P_6$ on either the dog or donkey kinetic data is therefore complex and cannot be attributed solely to changes in the relative populations of the **r** and **t** tertiary isomers, as was done for the sheep haemoglobins (Okonjo *et al.*, 2010). For the dog data, inositol-$P_6$ increased the **t** isomer population, whereas it lowered it for the donkey data.

Inositol-$P_6$ has no effect on the affinity of dog haemoglobin for DTNB since it appears to have hardly any effect on the equilibrium constant, but increases the equilibrium constant for each donkey haemoglobin derivative across the entire experimental pH range of 5.6 – 9.0. $K_{rt3}$, the tertiary structure transition constant, is increased from 0.46 for stripped haemoglobin to 0.83. These numbers represent **t** isomer populations of 31.5 and 45.4% respectively. This affinity increase therefore may be attributed to an increase in the **t** isomer population.

## 6.2 RECOMMENDATION

Sheep and donkey are ruminants; they feed on plants. Dog is carnivorous and feed on flesh. There is a lot of phosphate in plants/vegetables. Combined with the amount of organic phosphates already present in the blood, it may be assumed that an increase in phosphate level due to the nutrition of the ruminants, could result in an increase in affinity for DTNB and other similar reagents. The physiology of the significance of this behaviour would be worth exploring as further work.

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## Appendix I

***Table 1**: Titration of **stripped donkey carbonmonoxyhaemoglobin** with **p-hydroxymercuri(II)benzoate (pMB)** at 25°C.*

*Conditions: [HbCO] = **60 µmol (haem) $dm^{-3}$**; volume of haemoglobin used = 3 $cm^3$; stock [pMB] = 840 µmol $dm^{-3}$; phosphate buffer pH 7.6, (ionic strength, 50 mmol $dm^{-3}$; added salt, NaCl; wavelength, $\lambda$ = 250 nm; molar absorptivity, $\varepsilon$ = 7,600 $mol^{-1}$ $dm^3$ $cm^{-1}$. The complex concentration was calculated from the change in absorbance, $\Delta A_{corr}$. corrected for dilution.*

| p-MB volume ($mm^3$) | $A_{250}$ | $A_{corr.}$ | $\Delta A$ | APMB | $\Delta A_{corr.}$ | p-[HbSMB]/[$Hb_4$] |
|---|---|---|---|---|---|---|
| 0 | 1.030 | 1.030 | 0.000 | 0.000 | 0.000 | 0.000 |
| 10 | 1.110 | 1.114 | 0.084 | 0.025 | 0.059 | 0.518 |
| 20 | 1.180 | 1.188 | 0.158 | 0.058 | 0.100 | 0.877 |
| 30 | 1.230 | 1.242 | 0.212 | 0.086 | 0.126 | 1.105 |
| 40 | 1.270 | 1.287 | 0.257 | 0.115 | 0.142 | 1.246 |
| 50 | 1.310 | 1.332 | 0.302 | 0.124 | 0.178 | 1.561 |
| 60 | 1.340 | 1.367 | 0.337 | 0.145 | 0.192 | 1.684 |
| 70 | 1.360 | 1.392 | 0.362 | 0.175 | 0.187 | 1.640 |
| 80 | 1.400 | 1.437 | 0.407 | 0.189 | 0.218 | 1.912 |
| 90 | 1.425 | 1.468 | 0.438 | 0.205 | 0.233 | 2.044 |
| 100 | 1.450 | 1.498 | 0.468 | 0.210 | 0.258 | 2.263 |
| 120 | 1.480 | 1.539 | 0.509 | 0.230 | 0.279 | 2.447 |
| 140 | 1.500 | 1.570 | 0.540 | 0.249 | 0.291 | 2.553 |
| 160 | 1.520 | 1.601 | 0.571 | 0.270 | 0.301 | 2.640 |
| 180 | 1.580 | 1.675 | 0.645 | 0.288 | 0.357 | 3.132 |
| 200 | 1.600 | 1.707 | 0.677 | 0.309 | 0.368 | 3.228 |
| 220 | 1.610 | 1.728 | 0.698 | 0.320 | 0.378 | 3.316 |
| 240 | 1.650 | 1.782 | 0.752 | 0.355 | 0.397 | 3.482 |
| 280 | 1.700 | 1.859 | 0.829 | 0.382 | 0.447 | 3.921 |
| 300 | 1.700 | 1.870 | 0.840 | 0.410 | 0.430 | 3.772 |
| 320 | 1.710 | 1.892 | 0.862 | 0.435 | 0.427 | 3.746 |
| 340 | 1.750 | 1.948 | 0.918 | 0.440 | 0.478 | 4.193 |
| 360 | 1.770 | 1.982 | 0.952 | 0.510 | 0.442 | 3.877 |
| 380 | 1.790 | 2.017 | 0.987 | 0.540 | 0.447 | 3.921 |
| 400 | 1.810 | 2.051 | 1.021 | 0.560 | 0.461 | 4.044 |

***Table 2**: Titration of **stripped donkey carbonmonoxyhaemoglobin** with **5,5'-dithiobis(2-nitrobenzoate) (DTNB)** at 25°C.*

*Conditions: [HbCO] = 10 µmol (haem) $dm^{-3}$; volume of haemoglobin used = 10 $cm^3$; stock [DTNB] = 29 mmol $dm^{-3}$; phosphate buffer pH 7.6, (ionic strength, 50 mmol $dm^{-3}$; added salt, NaCl; wavelength, $\lambda$ = 412 nm; molar absorptivity, $\varepsilon$ = 14,000 $mol^{-1}$ $dm^3$ $cm^{-1}$. The complex concentration was calculated from the change in absorbance, $\Delta A_{corr}$. corrected for dilution.*

| DTNB vol. ($mm^3$) | $A_{412}$ | $A_{DTNB}$ | $\Delta A$ | $\Delta A_{corr}$ | [TNB]/[$Hb_4$] |
|---|---|---|---|---|---|
| 0 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 2 | 0.016 | 0.001 | 0.015 | 0.015 | 0.429 |
| 4 | 0.026 | 0.002 | 0.024 | 0.024 | 0.686 |
| 5 | 0.042 | 0.002 | 0.040 | 0.040 | 1.143 |
| 6 | 0.036 | 0.004 | 0.032 | 0.032 | 0.915 |
| 8 | 0.062 | 0.006 | 0.056 | 0.056 | 1.601 |
| 10 | 0.058 | 0.002 | 0.056 | 0.056 | 1.602 |
| 15 | 0.074 | 0.014 | 0.060 | 0.060 | 1.717 |
| 20 | 0.076 | 0.011 | 0.065 | 0.065 | 1.861 |
| 25 | 0.086 | 0.011 | 0.075 | 0.075 | 2.148 |
| 30 | 0.085 | 0.012 | 0.073 | 0.073 | 2.092 |
| 35 | 0.091 | 0.014 | 0.077 | 0.077 | 2.208 |
| 40 | 0.0935 | 0.016 | 0.078 | 0.078 | 2.223 |
| 50 | 0.089 | 0.016 | 0.073 | 0.073 | 2.096 |
| 60 | 0.095 | 0.021 | 0.074 | 0.074 | 2.127 |
| 70 | 0.094 | 0.012 | 0.082 | 0.083 | 2.359 |
| 80 | 0.096 | 0.023 | 0.073 | 0.074 | 2.102 |
| 90 | 0.092 | 0.017 | 0.075 | 0.076 | 2.162 |
| 100 | 0.094 | 0.015 | 0.079 | 0.080 | 2.291 |
| 120 | 0.096 | 0.025 | 0.071 | 0.072 | 2.047 |
| 140 | 0.096 | 0.026 | 0.070 | 0.071 | 2.042 |
| 160 | 0.105 | 0.027 | 0.078 | 0.080 | 2.277 |

***Table 3**: Reaction of DTNB with CysF9[93]β of **stripped dog oxyhaemoglobin**: raw data for the determination of $K_{equ}$. Conditions: [Hb] = 50 µmol (haem) $dm^{-3}$; pH **6.24**; temp = 25°C; Stock [DTNB] = 29 mmol $dm^{-3}$*

| DTNB Vol. ($mm^3$) | $A_{412}$ | [TNB] in µmol $dm^{-3}$ | $K_{equ}$ |
|---|---|---|---|
| 10 | 0.035 | 2.500 | 0.385 |
| 20 | 0.036 | 2.570 | 0.202 |
| 30 | 0.045 | 3.210 | 0.218 |
| 40 | 0.043 | 3.070 | 0.148 |
| 50 | 0.049 | 3.500 | 0.158 |
| 60 | 0.047 | 3.360 | 0.120 |
| 70 | 0.049 | 3.500 | 0.113 |
| 80 | 0.049 | 3.500 | 0.099 |
| 90 | 0.049 | 3.500 | 0.088 |
| 100 | 0.051 | 3.640 | 0.087 |
| | | | Mean = 0.162 ± 0.03 |

***Table 4**: Reaction of DTNB with CysF9[93]β of **stripped dog oxyhaemoglobin**: raw data for the determination of $K_{equ}$. Conditions: [Hb] = 50 µmol (haem) $dm^{-3}$; pH **8.56**; temp = 25°C; Stock [DTNB] = 29 mmol $dm^{-3}$*

| DTNB Vol. ($mm^3$) | $A_{412}$ | [TNB] in µmol $dm^{-3}$ | $K_{equ}$ |
|---|---|---|---|
| 10 | 0.20 | 14.30 | 0.36 |
| 20 | 0.21 | 14.90 | 0.19 |
| 30 | 0.24 | 17.00 | 0.21 |
| 40 | 0.21 | 15.10 | 0.10 |
| 50 | 0.21 | 15.30 | 0.08 |
| 60 | 0.19 | 13.80 | 0.05 |
| 70 | 0.20 | 14.50 | 0.05 |
| 80 | 0.23 | 16.30 | 0.06 |
| 90 | 0.31 | 21.80 | 0.28 |
| 100 | 0.23 | 16.40 | 0.05 |
| | | | Mean = 0.14 ± 0.03 |

***Table 5**: Reaction of DTNB with CysF9[93]β of **dog oxyhaemoglobin in the presence of inositol-$P_6$**: raw data for the determination of $K_{equ}$.*
*Conditions: [Hb] = 50 µmol (haem) $dm^{-3}$; pH **6.40**; temp = 25°C; Stock [DTNB] = 29 mmol $dm^{-3}$;* [inositol-$P_6$] : [$Hb_4$] = 4:1.

| DTNB Vol. ($mm^3$) | $A_{412}$ | [TNB] in µmol $dm^{-3}$ | $K_{equ}$ |
|---|---|---|---|
| 20 | 0.170 | 12.100 | 5.945 |
| 30 | 0.198 | 14.100 | 6.471 |
| 40 | 0.196 | 14.000 | 4.632 |
| 50 | 0.189 | 13.500 | 3.250 |
| 60 | 0.144 | 10.300 | 1.174 |
| 70 | 0.163 | 11.600 | 1.444 |
| 80 | 0.138 | 9.860 | 0.784 |
| 90 | 0.136 | 9.710 | 0.670 |
| 100 | 0.154 | 11.000 | 0.858 |
| | | | Mean = 2.803±0.64 |

***Table 6**: Reaction of DTNB with CysF9[93]β of **dog oxyhaemoglobin in the presence of inositol-$P_6$**: raw data for the determination of $K_{equ}$.*

*Conditions: [Hb] = 50 µmol (haem) $dm^{-3}$; pH **8.51**; temp = 25°C; Stock [DTNB] = 29 mmol $dm^{-3}$;* [inositol-$P_6$] : [$Hb_4$] = 4:1.

| DTNB Vol. ($mm^3$) | $A_{412}$ | [TNB] in µmol $dm^{-3}$ | $K_{equ}$ |
|---|---|---|---|
| 10 | 0.180 | 12.900 | 0.2639 |
| 20 | 0.138 | 9.860 | 0.0570 |
| 30 | 0.182 | 13.000 | 0.0832 |
| 40 | 0.166 | 11.900 | 0.0468 |
| 50 | 0.162 | 11.600 | 0.0348 |
| 60 | 0.154 | 11.000 | 0.0251 |
| 70 | 0.189 | 13.500 | 0.0396 |
| 80 | 0.194 | 13.900 | 0.0377 |
| 90 | 0.206 | 14.700 | 0.0411 |
| 100 | 0.179 | 12.800 | 0.0235 |
| | | | Mean = 0.043±0.01 |

***Table 7**: Reaction of DTNB with CysF9[93]β of **stripped donkey carbonmonoxyhaemoglobin**: raw data for the determination of $K_{equ}$. Conditions: [Hb] = 50 µmol (haem) $dm^{-3}$; pH **5.934**; temp = 25°C; Stock [DTNB] = 29 mmol $dm^{-3}$*

| DTNB Vol. ($mm^3$) | $A_{412}$ | [TNB] in µmol $dm^{-3}$ | $K_{equ}$ |
|---|---|---|---|
| 5 | 0.062 | 2.21E-06 | 5.2744 |
| 7 | 0.099 | 3.54E-06 | 10.6960 |
| 10 | 0.112 | 4.00E-06 | 9.3328 |
| 15 | 0.098 | 3.50E-06 | 4.2682 |
| 20 | 0.072 | 2.57E-06 | 1.5634 |
| 25 | 0.126 | 4.50E-06 | 4.3926 |
| 30 | 0.108 | 3.86E-06 | 2.5282 |
| 35 | 0.107 | 3.82E-06 | 2.1055 |
| 40 | 0.166 | 5.93E-06 | 5.1637 |
| 45 | 0.072 | 2.57E-06 | 0.6752 |
| 50 | 0.084 | 3.00E-06 | 0.8488 |
| 55 | 0.074 | 2.64E-06 | 0.5842 |
| 60 | 0.101 | 3.61E-06 | 1.0604 |
| 70 | 0.092 | 3.29E-06 | 0.7366 |
| 80 | 0.114 | 4.07E-06 | 1.0396 |
| 90 | 0.123 | 4.39E-06 | 1.0973 |
| 100 | 0.143 | 5.11E-06 | 1.3992 |
| 110 | 0.125 | 4.46E-06 | 0.9299 |
| | | | Mean = 2.971 ± 0.57 |

***Table 8**: Reaction of DTNB with CysF9[93]β of **stripped donkey carbonmonoxyhaemoglobin**: raw data for the determination of $K_{equ}$. Conditions: [Hb] = 50 µmol (haem) $dm^{-3}$; pH **8.598**; temp = 25°C; Stock [DTNB] = 29 mmol $dm^{-3}$*

| DTNB Vol. ($mm^3$) | $A_{412}$ | [TNB] in µmol $dm^{-3}$ | $K_{equ}$ |
|---|---|---|---|
| 15 | 0.122 | 4.36E-06 | 0.0354 |
| 20 | 0.084 | 3.00E-06 | 0.0112 |
| 25 | 0.105 | 3.75E-06 | 0.0145 |
| 30 | 0.143 | 5.11E-06 | 0.0242 |
| 35 | 0.164 | 5.86E-06 | 0.0283 |
| 40 | 0.144 | 5.14E-06 | 0.0182 |
| 45 | 0.180 | 6.43E-06 | 0.0271 |
| 50 | 0.206 | 7.36E-06 | 0.0337 |
| 55 | 0.215 | 7.68E-06 | 0.0339 |
| 70 | 0.198 | 7.07E-06 | 0.0216 |
| 80 | 0.236 | 8.43E-06 | 0.0291 |
| 90 | 0.255 | 9.11E-06 | 0.0315 |
| 100 | 0.251 | 8.96E-06 | 0.0271 |
| 110 | 0.282 | 1.01E-05 | 0.0335 |
| | | | Mean = 0.026 ± 0.001 |

***Table 9**: Reaction of DTNB with CysF9[93]β of **donkey oxyhaemoglobin in the presence of inositol-$P_6$**: raw data for the determination of $K_{equ}$.*

*Conditions: [Hb] = 50 µmol (haem) $dm^{-3}$; pH **6.315**; temp = 25°C; Stock [DTNB] = 29 mmol $dm^{-3}$*; [inositol-$P_6$] : [$Hb_4$] = 4:1.

| DTNB Vol. ($mm^3$) | $A_{412}$ | [TNB] in µmol $dm^{-3}$ | $K_{equ}$ |
|---|---|---|---|
| 2 | 0.082 | 5.86E-06 | 15.1442 |
| 4 | 0.107 | 7.64E-06 | 12.3012 |
| 8 | 0.125 | 8.93E-06 | 8.2362 |
| 10 | 0.135 | 9.64E-06 | 7.9480 |
| 12 | 0.165 | 1.18E-05 | 11.8296 |
| 14 | 0.175 | 1.25E-05 | 12.0578 |
| 16 | 0.155 | 1.11E-05 | 7.1065 |
| 18 | 0.16 | 1.14E-05 | 6.8963 |
| 20 | 0.18 | 1.29E-05 | 8.9828 |
| 22 | 0.155 | 1.11E-05 | 5.0618 |
| 24 | 0.2 | 1.43E-05 | 10.7189 |
| 26 | 0.205 | 1.46E-05 | 10.8079 |
| | | | Mean = 9.832 ± 0.84 |

***Table 10**: Reaction of DTNB with CysF9[93]β of **donkey oxyhaemoglobin in the presence of inositol-$P_6$**: raw data for the determination of $K_{equ}$.*

*Conditions: [Hb] = 50 µmol (haem) $dm^{-3}$; pH **8.411**; temp = 25°C; Stock [DTNB] = 29 mmol $dm^{-3}$;* [inositol-$P_6$]:[$Hb_4$] = 4:1.

| DTNB Vol. ($mm^3$) | $A_{412}$ | [TNB] in µmol $dm^{-3}$ | $K_{equ}$ |
|---|---|---|---|
| 8 | 0.14 | 0.00001 | 0.1765 |
| 10 | 0.155 | 1.11E-05 | 0.1834 |
| 12 | 0.162 | 1.16E-05 | 0.1704 |
| 14 | 0.152 | 1.09E-05 | 0.1196 |
| 16 | 0.246 | 1.76E-05 | 0.5426 |
| 18 | 0.273 | 1.95E-05 | 0.8021 |
| 20 | 0.288 | 2.06E-05 | 0.9929 |
| 24 | 0.282 | 2.01E-05 | 0.7085 |
| 28 | 0.283 | 2.02E-05 | 0.6134 |
| 30 | 0.261 | 1.86E-05 | 0.3624 |
| | | | Mean = 0.494 ± 0.09 |

***Table 11:*** *Dependence of $-\log_{10}K_{equ}$ on pH for the reaction of CysF9[93]β of* ***stripped dog oxyhaemoglobin*** *with 5,5'-dithiobis(2-nitrobenzoate) at 25°C.*

*Conditions: phosphate buffers pH 5.6 – 7.8 and borate buffers pH 8.0 – 9.0 (ionic strength, 50 mmol $dm^{-3}$; added salt, NaCl); [stock DTNB] = 29 mmol $dm^{-3}$; [$HbO_2$] = 50 µmol (haem) $dm^{-3}$ (25 µmol $dm^{-3}$ in reactive sulphydryl groups); volume of haemoglobin used = 10 $cm^3$; wavelength, λ = 412 nm. A molar extinction of 14,000 $mol^{-1}$ $dm^3$ $cm^{-1}$ was assumed for 5-thio-2-nitrobenzoate ($TNB^-$) formed; $pK_{SH}$ = 8.3; $pK_{TNB}$ = 5.267.*

| pH | $K_{equ}$ | $-\log_{10}K_{equ}$ |
|---|---|---|
| 5.680 | 31.952 ± 10.85 | -1.505 |
| 5.920 | 20.431 ± 9.07 | -1.310 |
| 6.100 | 27.701 ± 4.55 | -1.443 |
| 6.240 | 0.162 ± 0.03* | 0.791 |
| 6.410 | 3.950 ± 1.08 | -0.597 |
| 6.550 | 5.148 ± 0.79 | -0.712 |
| 6.860 | 3.989 ± 0.82 | -0.601 |
| 7.000 | 1.734 ± 0.31 | -0.239 |
| 7.220 | 1.577 ± 0.30 | -0.198 |
| 7.320 | 2.703 ± 0.62 | -0.432 |
| 7.570 | 0.972 ± 0.23 | 0.012 |
| 7.770 | 0.339 ± 0.09 | 0.470 |
| 8.100 | 0.425 ± 0.07 | 0.372 |
| 8.310 | 0.209 ± 0.04 | 0.680 |
| 8.420 | 0.159 ± 0.06 | 0.799 |
| 8.560 | 0.143 ± 0.03 | 0.845 |
| 8.830 | 0.150 ± 0.03 | 0.824 |
| 9.140 | 0.087 ± 0.02* | 1.061 |

***Table 12:*** *Dependence of $-\log_{10}K_{equ}$ on pH for the reaction of CysF9[93]β of* ***stripped dog carbonmonoxyhaemoglobin*** *with 5,5'-dithiobis(2-nitrobenzoate) at 25°C.*

*Conditions: phosphate buffers pH 5.6 – 7.8 and borate buffers pH 8.0 – 9.0 (ionic strength, 50 mmol $dm^{-3}$; added salt, NaCl); [stock DTNB] = 29 mmol $dm^{-3}$; [$HbO_2$] = 50 µmol (haem) $dm^{-3}$ (25 µmol $dm^{-3}$ in reactive sulphydryl groups); volume of haemoglobin used = 10 $cm^3$; wavelength, λ = 412 nm. A molar extinction of 14,000 $mol^{-1}$ $dm^3$ $cm^{-1}$ was assumed for 5-thio-2-nitrobenzoate ($TNB^-$) formed; $pK_{SH}$ = 8.3; $pK_{TNB}$ = 5.267.*

| pH | $K_{equ}$ | $-\log_{10}K_{equ}$ |
|---|---|---|
| 5.650 | 34.125 ± 3.81 | -1.533 |
| 5.860 | 21.891 ± 3.61 | -1.340 |
| 6.000 | 26.441 ± 6.09 | -1.422 |
| 6.190 | 12.469 ± 2.63 | -1.096 |
| 6.470 | 4.394 ± 0.98 | -0.643 |
| 6.520 | 4.029 ± 0.87 | -0.605 |
| 6.820 | 1.172 ± 0.21 | -0.069 |
| 7.110 | 0.358 ± 0.05 | 0.446 |
| 7.170 | 0.334 ± 0.06 | 0.477 |
| 7.380 | 0.330 ± 0.06 | 0.482 |
| 7.530 | 0.363 ± 0.07 | 0.440 |
| 7.780 | 0.153 ± 0.04 | 0.814 |
| 8.120 | 0.048 ± 0.01 | 1.316 |
| 8.180 | 0.045 ± 0.01 | 1.351 |
| 8.390 | 0.037 ± 0.01 | 1.428 |
| 8.560 | 0.072 ± 0.01 | 1.143 |
| 8.720 | 0.066 ± 0.01 | 1.182 |
| 8.930 | 0.036 ± 0.01 | 1.446 |

***Table 13:*** *Dependence of $-\log_{10}K_{equ}$ on pH for the reaction of CysF9[93]β of* ***stripped dog aquomethaemoglobin*** *with 5,5'-dithiobis(2-nitrobenzoate) at 25°C.*

*Conditions: phosphate buffers pH 5.6 – 7.8 and borate buffers pH 8.0 – 9.0 (ionic strength, 50 mmol $dm^{-3}$; added salt, NaCl); [stock DTNB] = 29 mmol $dm^{-3}$; [$HbO_2$] = 50 µmol (haem) $dm^{-3}$ (25 µmol $dm^{-3}$ in reactive sulphydryl groups); volume of haemoglobin used = 10 $cm^3$; wavelength, λ = 412 nm. A molar extinction of 14,000 $mol^{-1}$ $dm^3$ $cm^{-1}$ was assumed for 5-thio-2-nitrobenzoate ($TNB^-$) formed; $pK_{SH}$ = 8.3; $pK_{TNB}$ = 5.267.*

| pH | $K_{equ}$ | $-\log_{10}K_{equ}$ |
|---|---|---|
| 5.600 | 19.476 ± 3.65 | -1.290 |
| 5.920 | 7.513 ± 2.05 | -0.876 |
| 6.060 | 4.098 ± 0.94 | -0.613 |
| 6.240 | 3.175 ± 0.66 | -0.502 |
| 6.390 | 1.268 ± 0.10 | -0.103 |
| 6.560 | 1.813 ± 0.33 | -0.259 |
| 6.730 | 1.177 ± 0.26 | -0.071 |
| 6.930 | 2.129 ± 0.33 | -0.328 |
| 7.140 | 1.321 ± 0.28 | -0.121 |
| 7.300 | 0.829 ± 0.14 | 0.082 |
| 7.650 | 0.445 ± 0.15 | 0.352 |
| 7.730 | 0.396 ± 0.04 | 0.403 |
| 8.030 | 0.188 ± 0.03 | 0.726 |
| 8.150 | 0.096 ± 0.02 | 1.019 |
| 8.340 | 0.077 ± 0.01 | 1.115 |
| 8.510 | 0.078 ± 0.01 | 1.108 |
| 8.850 | 0.064 ± 0.01 | 1.195 |
| 9.050 | 0.082 ± 0.01 | 1.085 |

***Table 14:*** *Dependence of $-\log_{10}K_{equ}$ on pH for the reaction of CysF9[93]β of* ***dog oxyhaemoglobin in the presence of inositol-$P_6$*** *with 5,5'-dithiobis(2-nitrobenzoate) at 25°C.*

*Conditions: phosphate buffers pH 5.6 – 7.8 and borate buffers pH 8.0 – 9.0 (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [stock DTNB] = 29 mmol $dm^{-3}$; [$HbO_2$] = 50 µmol (haem) $dm^{-3}$ (25 µmol $dm^{-3}$ in reactive sulphydryl groups); volume of haemoglobin used = 3 $cm^3$; [inositol-$P_6$] = 50 µmol $dm^{-3}$; wavelength, λ = 412 nm. A molar extinction of 14,000 $mol^{-1}$ $dm^3$ $cm^{-1}$ was assumed for 5-thio-2-nitrobenzoate ($TNB^-$) formed; $pK_{SH}$ = 8.3; $pK_{TNB}$ = 5.267.*

| pH | $K_{equ}$ | $-\log_{10}K_{equ}$ |
|---|---|---|
| 5.520 | 37.809 ± 10.99 | -1.578 |
| 5.740 | 33.212 ± 6.05 | -1.521 |
| 5.940 | 4.590 ± 1.09 | -0.662 |
| 6.250 | 4.825 ± 0.46 | -0.684 |
| 6.400 | 2.803 ± 0.64 | -0.448 |
| 6.590 | 0.974 ± 0.16 | 0.012 |
| 6.820 | 0.828 ± 0.18 | 0.082 |
| 7.040 | 0.826 ± 0.16 | 0.083 |
| 7.230 | 0.851 ± 0.10 | 0.070 |
| 7.450 | 0.346 ± 0.11 | 0.460 |
| 7.700 | 0.213 ± 0.10 | 0.672 |
| 7.720 | 0.083 ± 0.01 | 1.081 |
| 7.760 | 0.058 ± 0.01 | 1.235 |
| 8.280 | 0.002 ± 0.01* | 2.767 |
| 8.420 | 0.081 ± 0.01 | 1.092 |
| 8.510 | 0.043 ± 0.01 | 1.365 |
| 8.800 | 0.029 ± 0.01 | 1.535 |
| 9.120 | 0.030 ± 0.02 | 1.518 |

***Table 15:*** *Dependence of $-\log_{10}K_{equ}$ on pH for the reaction of CysF9[93]β of* ***dog carbonmonoxyhaemoglobin in the presence of inositol-$P_6$*** *with 5,5'-dithiobis(2-nitrobenzoate) at 25°C.*

*Conditions: phosphate buffers pH 5.6 – 7.8 and borate buffers pH 8.0 – 9.0 (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [stock DTNB] = 29 mmol $dm^{-3}$; [$HbO_2$] = 50 µmol (haem) $dm^{-3}$ (25 µmol $dm^{-3}$ in reactive sulphydryl groups); volume of haemoglobin used = 3 $cm^3$; [inositol-$P_6$] = 50 µmol $dm^{-3}$; wavelength, λ = 412 nm. A molar extinction of 14,000 $mol^{-1}$ $dm^3$ $cm^{-1}$ was assumed for 5-thio-2-nitrobenzoate ($TNB^-$) formed; $pK_{SH}$ = 8.3; $pK_{TNB}$ = 5.267.*

| pH | $K_{equ}$ | $-\log_{10}K_{equ}$ |
|---|---|---|
| 5.550 | 26.669 ± 3.05 | -1.426 |
| 5.900 | 30.825 ± 3.64 | -1.489 |
| 6.120 | 4.462 ± 0.60 | -0.650 |
| 6.320 | 6.622 ± 1.04 | -0.821 |
| 6.490 | 3.222 ± 0.80 | -0.508 |
| 6.690 | 1.506 ± 0.30 | -0.178 |
| 6.800 | 1.712 ± 0.35 | -0.234 |
| 7.030 | 0.401 ± 0.10 | 0.397 |
| 7.170 | 0.335 ± 0.08 | 0.474 |
| 7.380 | 0.221 ± 0.06 | 0.655 |
| 7.500 | 0.010 ± 0.01* | 1.994 |
| 7.800 | 0.306 ± 0.13 | 0.515 |
| 8.020 | 0.147 ± 0.02 | 0.834 |
| 8.130 | 0.102 ± 0.04 | 0.990 |
| 8.390 | 0.100 ± 0.03 | 1.000 |
| 8.580 | 0.075 ± 0.01 | 1.123 |
| 8.820 | 0.098 ± 0.02 | 1.007 |
| 8.970 | 0.040 ± 0.01 | 1.399 |

***Table 16:*** *Dependence of $-\log_{10}K_{equ}$ on pH for the reaction of CysF9[93]β of* ***dog aquomethaemoglobin in the presence of inositol-$P_6$*** *with 5,5'-dithiobis(2-nitrobenzoate) at 25°C.*

*Conditions: phosphate buffers pH 5.6 – 7.8 and borate buffers pH 8.0 – 9.0 (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [stock DTNB] = 29 mmol $dm^{-3}$; [$HbO_2$] = 50 µmol (haem) $dm^{-3}$ (25 µmol $dm^{-3}$ in reactive sulphydryl groups); volume of haemoglobin used = 3 $cm^3$; [inositol-$P_6$] = 50 µmol $dm^{-3}$; wavelength, λ = 412 nm. A molar extinction of 14,000 $mol^{-1}$ $dm^3$ $cm^{-1}$ was assumed for 5-thio-2-nitrobenzoate ($TNB^-$) formed; $pK_{SH}$ = 8.3; $pK_{TNB}$ = 5.267.*

| pH | $K_{equ}$ | $-\log_{10}K_{equ}$ |
|---|---|---|
| 5.680 | 34.143 ± 6.67 | -1.533 |
| 5.850 | 19.103 ± 2.68 | -1.281 |
| 6.130 | 4.852 ± 1.12 | -0.686 |
| 6.200 | 9.315 ± 2.16 | -0.969 |
| 6.510 | 3.821 ± 0.83 | -0.582 |
| 6.660 | 2.581 ± 0.41 | -0.412 |
| 6.790 | 4.374 ± 1.25 | -0.641 |
| 6.890 | 0.647 ± 0.18 | 0.189 |
| 7.100 | 1.244 ± 0.20 | -0.095 |
| 7.400 | 1.244 ± 0.12 | -0.095 |
| 7.700 | 0.761 ± 0.15 | 0.118 |
| 7.790 | 0.313 ± 0.04 | 0.505 |
| 8.050 | 0.139 ± 0.03 | 0.856 |
| 8.190 | 0.064 ± 0.01 | 1.191 |
| 8.500 | 0.082 ± 0.01 | 1.084 |
| 8.550 | 0.091 ± 0.02 | 1.043 |
| 8.800 | 0.064 ± 0.01 | 1.191 |
| 9.030 | 0.032 ± 0.01 | 1.501 |

***Table 17:*** *Dependence of $-\log_{10}K_{equ}$ on pH for the reaction of CysF9[93]β of* ***stripped donkey oxyhaemoglobin*** *with 5,5'-dithiobis(2-nitrobenzoate) at 25°C.*

*Conditions: phosphate buffers pH 5.6 – 7.8 and borate buffers pH 8.0 – 9.0 (ionic strength, 50 mmol $dm^{-3}$; added salt, NaCl); [stock DTNB] = 29 mmol $dm^{-3}$; [$HbO_2$] = 50 µmol (haem) $dm^{-3}$ (25 µmol $dm^{-3}$ in reactive sulphydryl groups); volume of haemoglobin used = 10 $cm^3$; wavelength, λ = 412 nm. A molar extinction of 14,000 $mol^{-1}$ $dm^3$ $cm^{-1}$ was assumed for 5-thio-2-nitrobenzoate ($TNB^-$) formed; $pK_{SH}$ = 8.3; $pK_{TNB}$ = 5.267*

| pH | $K_{equ}$ | $-\log_{10}K_{equ}$ |
|---|---|---|
| 5.730 | 1.497 ± 0.06 | -0.175 |
| 6.008 | 1.126 ± 0.14 | -0.052 |
| 6.205 | 1.601 ± 0.12 | -0.204 |
| 6.334 | 1.121 ± 0.16 | -0.050 |
| 6.575 | 0.945 ± 0.08 | 0.025 |
| 6.843 | 0.383 ± 0.04 | 0.417 |
| 7.084 | 0.492 ± 0.04 | 0.308 |
| 7.228 | 0.145 ± 0.02 | 0.839 |
| 7.418 | 0.037 ± 0.01 | 1.432 |
| 7.593 | 0.133 ± 0.02 | 0.876 |
| 7.593 | 0.062 ± 0.01 | 1.208 |
| 7.738 | 0.063 ±0.01 | 1.201 |
| 7.994 | 0.043 ± 0.002 | 1.367 |
| 8.199 | 0.041 ±001 | 1.387 |
| 8.411 | 0.028 ± 0.003 | 1.553 |
| 8.680 | 0.020 ±0.002 | 1.699 |
| 8.824 | 0.046 ± 0.01 | 1.337 |
| 8.944 | 0.027 ±0.003 | 1.569 |

***Table 18:*** *Dependence of $-\log_{10}K_{equ}$ on pH for the reaction of CysF9[93]β of* ***stripped donkey carbonmonoxyhaemoglobin*** *with 5,5'-dithiobis(2-nitrobenzoate) at 25°C. Conditions: phosphate buffers pH 5.6 – 7.8 and borate buffers pH 8.0 – 9.0 (ionic strength, 50 mmol $dm^{-3}$; added salt, NaCl); [stock DTNB] = 29 mmol $dm^{-3}$; [$HbO_2$] = 50 µmol (haem) $dm^{-3}$ (25 µmol $dm^{-3}$ in reactive sulphydryl groups); volume of haemoglobin used = 10 $cm^3$; wavelength, λ = 412 nm. A molar extinction of 14,000 $mol^{-1}$ $dm^3$ $cm^{-1}$ was assumed for 5-thio-2-nitrobenzoate ($TNB^-$) formed; $pK_{SH}$ = 8.3; $pK_{TNB}$ = 5.267*

| pH | $K_{equ}$ | $-\log_{10}K_{equ}$ |
|---|---|---|
| 5.600 | 12.734 ± 0.23 | -1.105 |
| 5.817 | 5.369 ± 0.71 | -0.730 |
| 5.934 | 2.971 ± 0.57 | -0.473 |
| 6.164 | 1.507 ± 0.18 | -0.178 |
| 6.404 | 0.974 ± 0.11 | 0.011 |
| 6.535 | 0.785 ±0.10 | 0.105 |
| 6.834 | 0.394 ± 0.08 | 0.405 |
| 6.930 | 0.446 ±0.03 | 0.351 |
| 7.154 | 0.210 ± 0.01 | 0.678 |
| 7.361 | 0.082 ± 0.01 | 1.086 |
| 7.525 | 0.050 ± 0.003 | 1.301 |
| 7.814 | 0.038 ± 0.02 | 1.417 |
| 7.948 | 0.047 ±0.31 | 1.328 |
| 8.267 | 0.037 ±0.003 | 1.432 |
| 8.360 | 0.028 ±0.003 | 1.553 |
| 8.598 | 0.026 ±0.001 | 1.585 |
| 8.755 | 0.078 ±0.001 | 1.106* |
| 9.181 | 0.085 ±0.002 | 1.073* |

***Table 19:*** *Dependence of $-\log_{10}K_{equ}$ on pH for the reaction of CysF9[93]β of* ***stripped donkey aquomethaemoglobin*** *with 5,5'-dithiobis(2-nitrobenzoate) at 25°C.*

*Conditions: phosphate buffers pH 5.6 – 7.8 and borate buffers pH 8.0 – 9.0 (ionic strength, 50 mmol $dm^{-3}$; added salt, NaCl); [stock DTNB] = 29 mmol $dm^{-3}$; [$HbO_2$] = 50 µmol (haem) $dm^{-3}$ (25 µmol $dm^{-3}$ in reactive sulphydryl groups); volume of haemoglobin used = 10 $cm^3$; wavelength, λ = 412 nm. A molar extinction of 14,000 $mol^{-1}$ $dm^3$ $cm^{-1}$ was assumed for 5-thio-2-nitrobenzoate ($TNB^-$) formed; $pK_{SH}$ = 8.3; $pK_{TNB}$ = 5.267*

| pH | $K_{equ}$ | $-\log_{10}K_{equ}$ |
|---|---|---|
| 5.592 | 0.654 ± 0.05 | 0.184 |
| 5.770 | 0.703 ± 0.06 | 0.153 |
| 5.989 | 0.429 ± 0.04 | 0.367 |
| 6.209 | 0.425 ± 0.03 | 0.371 |
| 6.470 | 0.327 ± 0.02 | 0.485 |
| 6.639 | 0.272 ± 0.004 | 0.565 |
| 6.798 | 0.397 ± 0.05 | 0.402 |
| 7.059 | 0.195 ± 0.08 | 0.711 |
| 7.193 | 0.112 ± 0.02 | 0.949 |
| 7.370 | 0.086 ± 0.01 | 1.066 |
| 7.530 | 0.071 ± 0.01 | 1.146 |
| 7.774 | 0.035 ± 0.07 | 1.457 |
| 7.957 | 0.014 ± 0.03* | 1.843 |
| 8.215 | 0.020 ± 0.02 | 1.692 |
| 8.416 | 0.026 ± 0.003 | 1.578 |
| 8.561 | 0.021 ± 0.002 | 1.670 |
| 8.802 | 0.027 ± 0.003 | 1.566 |
| 9.058 | 0.041 ± 0.002* | 1.382 |

***Table 20:*** *Dependence of $-\log_{10}K_{equ}$ on pH for the reaction of CysF9[93]β of* ***donkey oxyhaemoglobinin the presence of inositol-$P_6$*** *with 5,5'-dithiobis(2-nitrobenzoate) at 25°C.*

*Conditions: phosphate buffers pH 5.6 - 7.8 and borate buffers pH 8.0 – 9.0 (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [stock DTNB] = 29 mmol $dm^{-3}$; [$HbO_2$] = 50 µmol (haem) $dm^{-3}$ (25 µmol $dm^{-3}$ in reactive sulphydryl groups); volume of haemoglobin used = 3 $cm^3$; [inositol-$P_6$] = 50 µmol $dm^{-3}$; wavelength, λ = 412 nm. A molar extinction of 14,000 $mol^{-1}$ $dm^3$ $cm^{-1}$ was assumed for 5-thio-2-nitrobenzoate ($TNB^-$) formed; $pK_{SH}$ = 8.3; $pK_{TNB}$ = 5.267.*

| pH | $K_{equ}$ | $-\log_{10}K_{equ}$ |
|---|---|---|
| 5.600 | 86.084 ±10.87 | -1.935 |
| 5.887 | 51.417 ±6.88 | -1.711 |
| 6.036 | 11.714 ± 1.25 | -1.069 |
| 6.239 | 26.982 ± 2.12 | -1.431 |
| 6.315 | 9.832 ± 0.84 | -0.993 |
| 6.600 | 3.335 ± 0.58 | -0.523 |
| 6.779 | 2.191 ± 0.41 | -0.341 |
| 6.972 | 2.129 ±0.40 | -0.328 |
| 7.192 | 1.746 ± 0.22 | -0.242 |
| 7.389 | 0.800 ± 0.11 | 0.097 |
| 7.557 | 0.529 ± 0.07 | 0.276 |
| 7.776 | 0.425 ± 0.03 | 0.371 |
| 7.954 | 0.493 ± 0.06 | 0.307 |
| 8.182 | 0.675 ± 0.01 | 0.171 |
| 8.411 | 0.494 ± 0.09 | 0.306 |
| 8.555 | 0.586 ± 0.01 | 0.232 |
| 8.774 | 0.354 ±0.04 | 0.451 |
| 8.950 | 0.364 ± 0.02 | 0.439 |

***Table 21:*** *Dependence of $-\log_{10}K_{equ}$ on pH for the reaction of CysF9[93]β of* ***donkey carbonmonoxyhaemoglobinin the presence of inositol-$P_6$*** *with 5,5'-dithiobis(2-nitrobenzoate) at 25°C.*

*Conditions: phosphate buffers pH 5.6 - 7.8 and borate buffers pH 8.0 – 9.0 (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [stock DTNB] = 29 mmol $dm^{-3}$; [$HbO_2$] = 50 µmol (haem) $dm^{-3}$ (25 µmol $dm^{-3}$ in reactive sulphydryl groups); volume of haemoglobin used = 3 $cm^3$; [inositol-$P_6$] = 50 µmol $dm^{-3}$; wavelength, λ = 412 nm. A molar extinction of 14,000 $mol^{-1}$ $dm^3$ $cm^{-1}$ was assumed for 5-thio-2-nitrobenzoate ($TNB^-$) formed; $pK_{SH}$ = 8.3; $pK_{TNB}$ = 5.267.*

| pH | $K_{equ}$ | $-\log_{10}K_{equ}$ |
|---|---|---|
| 5.667 | 77.240 ±8.95 | -1.888 |
| 5.846 | 38.354 ± 7.38 | -1.584 |
| 5.967 | 24.679 ± 3.30 | -1.392 |
| 6.136 | 11.140 ± 0.96 | -1.047 |
| 6.313 | 7.327 ± 0.5 | -0.865 |
| 6.566 | 7.115 ± 1.04 | -0.852 |
| 6.764 | 2.128 ± 0.30 | -0.328 |
| 6.952 | 0.907 ± 0.11 | 0.042 |
| 7.199 | 0.484 ± 0.04 | 0.315 |
| 7.362 | 0.395 ± 0.03 | 0.403 |
| 7.555 | 0.353 ± 0.05 | 0.452 |
| 7.725 | 0.206 ± 0.02 | 0.687 |
| 7.998 | 0.193 ± 0.04 | 0.716 |
| 8.179 | 0.310 ± 0.02 | 0.509 |
| 8.357 | 0.257 ± 0.01 | 0.591 |
| 8.563 | 0.166 ± 0.02 | 0.779 |
| 8.800 | 0.175 ± 0.5 | 0.757 |
| 8.931 | 0.185 ± 0.02 | 0.734 |

***Table 22:*** *Dependence of $-\log_{10}K_{equ}$ on pH for the reaction of CysF9[93]β of* ***donkey aquomethaemoglobinin the presence of inositol-$P_6$*** *with 5,5'-dithiobis(2-nitrobenzoate) at 25°C.*

*Conditions: phosphate buffers pH 5.6 - 7.8 and borate buffers pH 8.0 – 9.0 (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [stock DTNB] = 29 mmol $dm^{-3}$; [$HbO_2$] = 50 µmol (haem) $dm^{-3}$ (25 µmol $dm^{-3}$ in reactive sulphydryl groups); volume of haemoglobin used = 3 $cm^3$; [inositol-$P_6$] = 50 µmol $dm^{-3}$; wavelength, λ = 412 nm. A molar extinction of 14,000 $mol^{-1}$ $dm^3$ $cm^{-1}$ was assumed for 5-thio-2-nitrobenzoate ($TNB^-$) formed; $pK_{SH}$ = 8.3; $pK_{TNB}$ = 5.267.*

| pH | $K_{equ}$ | $-\log_{10}K_{equ}$ |
|---|---|---|
| 5.647 | 270.969 ± 14.54 | -2.433 |
| 5.889 | 84.559 ±13.86 | -1.927 |
| 6.054 | 31.847 ±7.43 | -1.503 |
| 6.165 | 44.299 ±5.74 | -1.646 |
| 6.355 | 26.223 ±5.10 | -1.419 |
| 6.553 | 7.528 ±1.53 | -0.877 |
| 6.841 | 3.619 ±0.01 | -0.559 |
| 6.953 | 3.632 ±0.52 | -0.560 |
| 7.162 | 0.817 ±0.22 | 0.088 |
| 7.354 | 0.651 ±0.03 | 0.186 |
| 7.552 | 0.334 ±0.02 | 0.476 |
| 7.786 | 0.282 ±0.02 | 0.549 |
| 7.951 | 0.236 ± 0.02 | 0.627 |
| 8.193 | 0.285 ± 0.04 | 0.545 |
| 8.364 | 0.192 ± 0.08 | 0.716 |
| 8.550 | 0.204 ± 0.08 | 0.691 |
| 8.758 | 0.200 ± 0.01 | 0.700 |
| 8.958 | 0.290 ± 0.01 | 0.538 |

***Table 23**: Typical kinetic run for the reaction of 5,5'-dithiobis(2-nitrobenzoate) with the CysF9[93]β sulphydryl groups **stripped dog carbonmonoxyhaemoglobin** at 25°C. Conditions: phosphate buffer pH **5.57** (ionic strength, 50mmol $dm^{-3}$; added salt, NaCl); [$HbO_2$] = 10 µmol (haem) $dm^{-3}$ (5 µmol $dm^{-3}$ in reactive sulphydryl groups); [DTNB] = 300 µmol $dm^{-3}$; wavelength, λ = 412 nm.*

| Time (s) | Transmittance (%) | Absorbance | -ln($A_{equ}$ - $A_t$) |
|---|---|---|---|
| 2.00 | 94.00 | 0.027 | 2.484 |
| 4.00 | 92.20 | 0.035 | 2.590 |
| 6.00 | 94.10 | 0.026 | 2.478 |
| 8.00 | 90.50 | 0.043 | 2.704 |
| 10.00 | 90.60 | 0.043 | 2.697 |
| 12.00 | 92.10 | 0.036 | 2.596 |
| 14.00 | 90.20 | 0.045 | 2.726 |
| 16.00 | 89.50 | 0.048 | 2.779 |
| 18.00 | 88.10 | 0.055 | 2.895 |
| 20.00 | 91.20 | 0.040 | 2.655 |
| 22.00 | 90.50 | 0.043 | 2.704 |
| 24.00 | 90.60 | 0.043 | 2.697 |
| 26.00 | 91.10 | 0.040 | 2.662 |
| 28.00 | 89.40 | 0.049 | 2.787 |
| 30.00 | 84.80 | 0.072 | 3.252 |
| 32.00 | 87.60 | 0.057 | 2.941 |
| 34.00 | 87.40 | 0.058 | 2.960 |
| 36.00 | 87.50 | 0.058 | 2.951 |
| 38.00 | 88.00 | 0.056 | 2.904 |
| 40.00 | 86.50 | 0.063 | 3.051 |
| 50.00 | 86.00 | 0.066 | 3.106 |
| 60.00 | 85.90 | 0.066 | 3.117 |
| 70.00 | 84.90 | 0.071 | 3.239 |
| 100.00 | 81.70 | 0.088 | 3.793 |
| 110.00 | 81.80 | 0.087 | 3.770 |
| 130.00 | 80.40 | 0.095 | 4.163 |
| 140.00 | 80.80 | 0.093 | 4.034 |
| 160.00 | 80.00 | 0.097 | 4.313 |
| 220.00 | 80.60 | 0.094 | 4.096 |
| 240.00 | 80.40 | 0.095 | 4.163 |
| 260.00 | 79.70 | 0.099 | 4.443 |
| 280.00 | 78.80 | 0.103 | 4.987 |
| | | 0.110 ($A_{equ}$) | |

***Table 24**: Typical kinetic run for the reaction of 5,5'-dithiobis(2-nitrobenzoate) with the CysF9[93]β sulphydryl groups of **stripped dog carbonmonoxyhaemoglobin** at 25°C.*

*Conditions: borate buffer pH **9.11** (ionic strength, 50mmol $dm^{-3}$; added salt, NaCl); [$HbO_2$] = 10 µmol (haem) $dm^{-3}$ (5 µmol $dm^{-3}$ in reactive sulphydryl groups); [DTNB] = 300 µmol $dm^{-3}$; wavelength, λ = 412 nm.*

| Time (s) | Transmittance (%) | Absorbance | -ln($A_{equ}$ - $A_t$) |
|---|---|---|---|
| 6.00 | 90.00 | 0.046 | 2.670 |
| 8.00 | 89.00 | 0.051 | 2.743 |
| 10.00 | 88.60 | 0.053 | 2.774 |
| 12.00 | 88.30 | 0.054 | 2.798 |
| 14.00 | 88.10 | 0.055 | 2.814 |
| 16.00 | 91.10 | 0.040 | 2.597 |
| 18.00 | 91.30 | 0.040 | 2.584 |
| 20.00 | 89.90 | 0.046 | 2.677 |
| 22.00 | 88.80 | 0.052 | 2.758 |
| 24.00 | 90.80 | 0.042 | 2.616 |
| 26.00 | 89.60 | 0.048 | 2.699 |
| 28.00 | 90.20 | 0.045 | 2.656 |
| 30.00 | 88.80 | 0.052 | 2.758 |
| 32.00 | 87.70 | 0.057 | 2.847 |
| 34.00 | 90.60 | 0.043 | 2.629 |
| 36.00 | 89.30 | 0.049 | 2.720 |
| 38.00 | 90.80 | 0.042 | 2.616 |
| 60.00 | 84.10 | 0.075 | 3.224 |
| 70.00 | 86.70 | 0.062 | 2.937 |
| 80.00 | 82.20 | 0.085 | 3.511 |
| 90.00 | 83.30 | 0.079 | 3.334 |
| 100.00 | 81.00 | 0.092 | 3.751 |
| 110.00 | 83.10 | 0.080 | 3.364 |
| 120.00 | 80.20 | 0.096 | 3.954 |
| 130.00 | 81.60 | 0.088 | 3.624 |
| 140.00 | 80.80 | 0.093 | 3.798 |
| 160.00 | 78.50 | 0.105 | 4.618 |
| 180.00 | 79.00 | 0.102 | 4.372 |
| 240.00 | 77.90 | 0.108 | 5.030 |
| 260.00 | 80.00 | 0.097 | 4.012 |
| 300.00 | 77.50 | 0.111 | 5.449 |
| | | 0.115 ($A_{equ}$) | |

***Table 25**: Typical kinetic run for the reaction of 5,5'-dithiobis(2-nitrobenzoate) with the CysF9[93]β sulphydryl groups of **stripped donkey carbonmonoxyhaemoglobin** at 25°C.*

*Conditions: phosphate buffer pH **5.645** (ionic strength, 50mmol $dm^{-3}$; added salt, NaCl); [$HbO_2$] = 10 µmol (haem) $dm^{-3}$ (5 µmol $dm^{-3}$ in reactive sulphydryl groups); [DTNB] = 300 µmol $dm^{-3}$; wavelength, λ = 412 nm.*

| Time (s) | Transmittance (%) | Absorbance | -ln($A_{equ}$ - $A_t$) |
|---|---|---|---|
| 0.00 | 86.30 | 0.064 | 2.577 |
| 2.00 | 81.60 | 0.088 | 2.963 |
| 4.00 | 82.10 | 0.086 | 2.912 |
| 8.00 | 82.90 | 0.081 | 2.838 |
| 10.00 | 82.40 | 0.084 | 2.884 |
| 12.00 | 82.80 | 0.082 | 2.849 |
| 14.00 | 82.90 | 0.081 | 2.838 |
| 16.00 | 83.20 | 0.080 | 2.811 |
| 18.00 | 82.90 | 0.081 | 2.838 |
| 20.00 | 82.80 | 0.082 | 2.847 |
| 22.00 | 82.30 | 0.085 | 2.893 |
| 24.00 | 81.90 | 0.087 | 2.932 |
| 26.00 | 82.30 | 0.085 | 2.893 |
| 28.00 | 82.00 | 0.086 | 2.922 |
| 30.00 | 82.10 | 0.086 | 2.912 |
| 32.00 | 81.80 | 0.087 | 2.942 |
| 34.00 | 81.30 | 0.090 | 2.994 |
| 36.00 | 81.20 | 0.090 | 3.005 |
| 38.00 | 81.30 | 0.090 | 2.994 |
| 40.00 | 81.20 | 0.090 | 3.005 |
| 50.00 | 80.60 | 0.094 | 3.072 |
| 60.00 | 80.70 | 0.093 | 3.060 |
| 70.00 | 79.80 | 0.098 | 3.170 |
| 80.00 | 78.60 | 0.105 | 3.340 |
| 90.00 | 77.90 | 0.108 | 3.457 |
| 100.00 | 77.70 | 0.110 | 3.493 |
| 110.00 | 77.80 | 0.109 | 3.474 |
| 120.00 | 76.40 | 0.117 | 3.768 |
| 130.00 | 77.00 | 0.114 | 3.631 |
| 140.00 | 76.80 | 0.115 | 3.675 |
| 160.00 | 76.70 | 0.115 | 3.697 |
| 180.00 | 75.60 | 0.121 | 3.989 |
| 200.00 | 74.50 | 0.128 | 4.410 |
| 220.00 | 74.10 | 0.130 | 4.624 |
| 260.00 | 73.80 | 0.132 | 4.821 |
| 280.00 | 73.50 | 0.134 | 5.069 |
| | | 0.140 ($A_{equ}$) | |

***Table 26**: Typical kinetic run for the reaction of 5,5'-dithiobis(2-nitrobenzoate) with the CysF9[93]β sulphydryl groups of **stripped donkey carbonmonoxyhaemoglobin** at 25°C.*

*Conditions: borate buffer pH **8.956** (ionic strength, 50mmol $dm^{-3}$; added salt, NaCl); [$HbO_2$] = 10 µmol (haem) $dm^{-3}$ (5 µmol $dm^{-3}$ in reactive sulphydryl groups); [DTNB] = 300 µmol $dm^{-3}$; wavelength, λ = 412 nm.*

| Time (s) | Transmittance (%) | Absorbance | -ln($A_{equ}$ - $A_t$) |
|---|---|---|---|
| 2.00 | 83.20 | 0.080 | 2.657 |
| 4.00 | 83.50 | 0.078 | 2.635 |
| 8.00 | 84.60 | 0.073 | 2.559 |
| 10.00 | 84.30 | 0.074 | 2.579 |
| 12.00 | 84.90 | 0.071 | 2.539 |
| 14.00 | 82.60 | 0.083 | 2.703 |
| 16.00 | 82.40 | 0.084 | 2.719 |
| 18.00 | 82.10 | 0.086 | 2.744 |
| 20.00 | 81.60 | 0.088 | 2.786 |
| 22.00 | 82.70 | 0.082 | 2.696 |
| 24.00 | 81.10 | 0.091 | 2.830 |
| 26.00 | 81.20 | 0.090 | 2.821 |
| 28.00 | 80.70 | 0.093 | 2.867 |
| 30.00 | 81.00 | 0.092 | 2.839 |
| 32.00 | 81.50 | 0.089 | 2.794 |
| 34.00 | 80.40 | 0.095 | 2.896 |
| 36.00 | 80.00 | 0.097 | 2.936 |
| 38.00 | 80.70 | 0.093 | 2.867 |
| 40.00 | 80.10 | 0.096 | 2.926 |
| 50.00 | 80.70 | 0.093 | 2.867 |
| 60.00 | 78.30 | 0.106 | 3.129 |
| 70.00 | 76.40 | 0.117 | 3.408 |
| 80.00 | 76.50 | 0.116 | 3.391 |
| 90.00 | 77.40 | 0.111 | 3.251 |
| 100.00 | 75.70 | 0.121 | 3.537 |
| 110.00 | 77.50 | 0.111 | 3.236 |
| 120.00 | 74.80 | 0.126 | 3.734 |
| 130.00 | 75.60 | 0.121 | 3.557 |
| 140.00 | 74.30 | 0.129 | 3.864 |
| 160.00 | 73.50 | 0.134 | 4.117 |
| 180.00 | 74.10 | 0.130 | 3.921 |
| 200.00 | 73.10 | 0.136 | 4.275 |
| 220.00 | 73.70 | 0.133 | 4.047 |
| 240.00 | 73.80 | 0.132 | 4.014 |
| 260.00 | 73.00 | 0.137 | 4.318 |
| | | 0.150 ($A_{equ}$) | |

***Table 27**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog oxyhaemoglobin** at 25°C in phosphate buffer pH **5.612** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 3.63E-03* | 3.28E-02* | 1.16E-02 | 1.77E-02 | 1.47E-02 ± 1.5E-03 |
| 4.00E-04 | 1.68E-02 | 1.27E-02 | 1.58E-02 | - | 1.51E-02 ± 1.4E-03 |
| 5.00E-04 | 1.88E-02 | 1.60E-02 | 1.68E-02 | 1.55E-02 | 1.68E-02 ± 8.3E-04 |
| 6.00E-04 | 2.14E-02 | 1.50E-02 | 2.28E-02 | 1.13E-02 | 1.76E-02 ± 2.9E-03 |
| Slope = 1.05E+01 ± 1.6E+00; Intercept = 1.13E-02 ± 7.2E-04 | | | | | |

***Table 28**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog oxyhaemoglobin** at 25°C in phosphate buffer pH **5.831** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.74E-02 | 1.77E-02 | 1.78E-02 | 1.79E-02 | 1.77E-02 ± 1.38E-04* |
| 4.00E-04 | 1.23E-02 | 1.24E-02 | 1.24E-02 | 1.26E-02 | 1.24E-02 ± 7.50E-05 |
| 5.00E-04 | 1.35E-02 | 1.44E-02 | 1.55E-02 | 1.55E-02 | 1.47E-02 ± 5.13E-04 |
| 6.00E-04 | 1.76E-02 | 1.78E-02 | 1.80E-02 | 2.34E-02 | 1.92E-02 ± 1.46E-03 |
| Slope = 3.40E+01 ± 6.4E+00; Intercept = 1.55E-03 ± 3.2E-03 | | | | | |

***Table 29**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog oxyhaemoglobin** at 25°C in phosphate buffer pH **6.050** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.76E-02 | 1.36E-02 | 1.52E-02 | 1.56E-02* | 1.55E-02 ± 1.0E-03 |
| 4.00E-04 | 1.43E-02 | 1.49E-02 | 1.54E-02 | 1.52E-02 | 1.50E-02 ± 2.8E-04 |
| 5.00E-04 | 1.52E-02 | 1.49E-02 | 1.56E-02 | 1.56E-02 | 1.53E-02 ± 1.7E-04 |
| 6.00E-04 | 1.87E-02 | 1.89E-02 | 1.52E-02 | 1.53E-02 | 1.70E-02 ± 9.3E-04 |
| Slope = 1.04E+01 ± 3.9E+00; Intercept = 1.06E-02 ± 2.0E-03 | | | | | |

***Table 30**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog oxyhaemoglobin** at 25°C in phosphate buffer pH **6.208** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.23E-02 | 1.32E-02 | 1.35E-02 | 1.34E-02 | 1.31E-02 ± 2.8E-04 |
| 4.00E-04 | 1.38E-02 | 1.46E-02 | 1.48E-02 | 1.54E-02 | 1.47E-02 ± 3.9E-04 |
| 5.00E-04 | 1.52E-02 | 1.54E-02 | 1.55E-02 | 1.67E-02 | 1.57E-02 ± 3.7E-04 |
| 6.00E-04 | 1.59E-02 | 1.58E-02 | 1.60E-02 | 1.76E-02 | 1.63E-02 ± 4.5E-04 |
| Slope = 1.06E+01 ± 1.5E+00; Intercept = 1.02E-02 ± 7.0E-04 | | | | | |

***Table 31**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog oxyhaemoglobin** at 25°C in phosphate buffer pH **6.405** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.34E-02 | 1.46E-02 | 1.55E-02 | 1.63E-02 | 1.50E-02 ± 7.2E-04 |
| 4.00E-04 | 2.05E-02 | 1.33E-02 | 1.34E-02 | 1.36E-02 | 1.52E-02 ± 1.8E-03 |
| 5.00E-04 | 1.58E-02 | 1.51E-02 | 1.58E-02 | 1.60E-02 | 1.57E-02 ± 2.1E-04 |
| 6.00E-04 | 1.85E-02 | 1.75E-02 | 1.76E-02 | 1.78E-02 | 1.78E-02 ± 2.4E-04 |
| Slope = 9.11E+00 ± 3.2E+00; Intercept = 1.18E-02 ± 1.5E-03 | | | | | |

***Table 32**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog oxyhaemoglobin** at 25°C in phosphate buffer pH **6.605** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.13E-02 | 1.23E-02 | 1.33E-02 | 1.36E-02 | 1.26E-02 ± 5.6E-04 |
| 4.00E-04 | 1.44E-02 | 1.58E-02 | 1.59E-02 | 1.66E-02 | 1.57E-02 ± 5.5E-04 |
| 5.00E-04 | 1.48E-02 | 1.51E-02 | 1.68E-02 | 1.70E-02 | 1.59E-02 ± 5.3E-04 |
| 6.00E-04 | 1.54E-02 | 1.55E-02 | 1.67E-02 | 1.67E-02 | 1.61E-02 ± 3.3E-04 |
| Slope = 1.06E+01 ± 5.0E+00; Intercept = 1.03E-02 ± 2.3E-03 | | | | | |

***Table 33**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog oxyhaemoglobin** at 25°C in phosphate buffer pH **6.807** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.47E-02 | 1.41E-02 | 1.46E-02 | 1.50E-02 | 1.46E-02 ± 2.3E-04 |
| 4.00E-04 | 1.57E-02 | 1.60E-02 | 1.54E-02 | 1.70E-02 | 1.60E-02 ± 4.0E-04 |
| 5.00E-04 | 1.61E-02 | 1.67E-02 | 1.73E-02 | 1.75E-02 | 1.69E-02 ± 3.4E-04 |
| 6.00E-04 | 1.67E-02 | 1.76E-02 | 1.89E-02 | 1.99E-02 | 1.83E-02 ± 7.9E-04 |
| Slope = 1.20E+01 ± 7.3E-01; Intercept = 1.11E-02 ± 3.4E-04 | | | | | |

***Table 34**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog oxyhaemoglobin** at 25°C in phosphate buffer pH **7.005** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.55E-02 | 1.57E-02 | 1.58E-02 | 1.64E-02 | 1.58E-02 ± 2.4E-04 |
| 4.00E-04 | 1.54E-02 | 1.54E-02 | 1.64E-02 | 1.69E-02 | 1.60E-02 ± 3.7E-04 |
| 5.00E-04 | 1.56E-02 | 1.64E-02 | 1.67E-02 | 1.85E-02 | 1.68E-02 ± 7.2E-04 |
| 6.00E-04 | 1.99E-02 | 1.63E-02 | 1.96E-02 | 1.97E-02 | 1.89E-02 ± 9.1E-04 |
| Slope = 9.94E+00 ± 3.1E+00; Intercept = 1.24E-02 ± 1.4E-03 | | | | | |

***Table 35**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog oxyhaemoglobin** at 25°C in phosphate buffer pH **7.223** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.42E-02 | 1.63E-02 | 1.42E-02 | 1.43E-02 | 1.47E-02 ±5.2E-04 |
| 4.00E-04 | 1.54E-02 | 1.63E-02 | 1.64E-02 | 1.65E-02 | 1.61E-02 ± 2.7E-04 |
| 5.00E-04 | 1.43E-02 | 1.48E-02 | 1.74E-02 | 1.85E-02 | 1.62E-02 ± 1.1E-03 |
| 6.00E-04 | 1.99E-02 | 1.71E-02 | 1.73E-02 | 1.98E-02 | 1.85E-02 ± 6.8E-04 |
| Slope = 1.14E+01 ± 2.9E+00; Intercept = 1.13E-02 ± 1.3E-03 | | | | | |

***Table 36**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog oxyhaemoglobin** at 25°C in phosphate buffer pH **7.433** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.23E-02 | 1.24E-02 | 1.81E-02 | 1.24E-02 | 1.38E-02 ± 1.5E-03 |
| 4.00E-04 | 1.53E-02 | 1.55E-02 | 1.56E-02 | 1.56E-02 | 1.55E-02 ± 9.0E-05 |
| 5.00E-04 | 1.69E-02 | 1.69E-02 | 1.75E-02 | 1.78E-02 | 1.73E-02 ± 2.4E-04 |
| 6.00E-04 | 1.74E-02 | 1.81E-02 | 1.75E-02 | 1.79E-02 | 1.77E-02 ± 1.8E-04 |
| Slope = 1.35E+01 ± 2.2E+00; Intercept = 9.98E-03 ± 1.0E-03 | | | | | |

***Table 37**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog oxyhaemoglobin** at 25°C in phosphate buffer pH **7.614** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.30E-02 | 1.27E-02 | 1.40E-02 | 1.48E-02 | 1.36E-02 ± 5.3E-04 |
| 4.00E-04 | 1.50E-02 | 1.53E-02 | 1.54E-02 | 1.54E-02 | 1.53E-02 ± 8.3E-05 |
| 5.00E-04 | 1.65E-02 | 1.65E-02 | 1.66E-02 | 1.69E-02 | 1.66E-02 ± 1.1E-04 |
| 6.00E-04 | 2.01E-02 | 1.61E-02 | 1.61E-02 | 1.61E-02 | 1.71E-02 ± 1.0E-03 |
| Slope = 1.18E+01 ± 1.87E+00; Intercept = 1.04E-02 ± 8.7E-04 | | | | | |

***Table 38**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog oxyhaemoglobin** at 25°C in phosphate buffer pH **7.776** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.64E-02 | 1.65E-02 | 1.65E-02 | 1.66E-02 | 1.65E-02 ± 5.3E-05 |
| 4.00E-04 | 1.53E-02 | 1.73E-02 | 1.77E-02 | 1.77E-02 | 1.70E-02 ± 6.1E-04 |
| 5.00E-04 | 1.76E-02 | 1.79E-02 | 2.19E-02 | 2.48E-02 | 2.05E-02 ± 1.8E-03 |
| 6.00E-04 | 2.45E-02 | 2.46E-02 | 2.68E-02 | 2.75E-02 | 2.58E-02 ± 7.6E-04 |
| Slope = 3.16E+01 ± 7.7E+00 ; Intercept = 5.77E-03 ± 3.6E-03 | | | | | |

***Table 39**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog oxyhaemoglobin** at 25°C in borate buffer pH **8.003** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 9.25E-03 | 1.25E-02 | 1.37E-02 | 1.42E-02 | 1.24E-02 ± 1.2E-03 |
| 4.00E-04 | 1.33E-02 | 1.33E-02 | 1.36E-02 | 1.45E-02 | 1.37E-02 ± 3.2E-04 |
| 5.00E-04 | 1.82E-02 | 1.82E-02 | 1.48E-02 | 1.83E-02 | 1.74E-02 ± 8.6E-04 |
| 6.00E-04 | 1.51E-02 | 1.95E-02 | 1.97E-02 | 2.31E-02 | 1.94E-02 ± 2.0E-03 |
| Slope = 2.46E+01 ± 3.2E+00; Intercept = 4.62E-03 ± 1.5E-03 | | | | | |

***Table 40**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog oxyhaemoglobin** at 25°C in borate buffer pH **8.156** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.36E-02 | 1.55E-02 | 1.40E-02 | 1.47E-02 | 1.44E-02 ± 4.9E-04 |
| 4.00E-04 | 1.77E-02 | 1.46E-02 | 1.48E-02 | 1.57E-02 | 1.57E-02 ± 7.9E-04 |
| 5.00E-04 | 1.78E-02 | 1.51E-02 | 2.06E-02 | 1.63E-02 | 1.74E-02 ± 1.4E-03 |
| 6.00E-04 | 1.75E-02 | 1.75E-02 | 1.85E-02 | 2.52E-02 | 1.97E-02 ± 1.9E-03 |
| Slope = 1.74E+01 ± 1.6E+00; Intercept = 8.99E-03 ± 7.2E-04 | | | | | |

***Table 41**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog oxyhaemoglobin** at 25°C in borate buffer pH **8.408** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.08E-02 | 1.15E-02 | 1.30E-02 | 1.75E-02 | 1.32E-02 ± 1.7E-03 |
| 4.00E-04 | 1.53E-02 | 1.59E-02 | 1.63E-02 | 1.72E-02 | 1.62E-02 ± 4.8E-04 |
| 5.00E-04 | 1.47E-02 | 1.57E-02 | 1.83E-02 | 1.92E-02 | 1.69E-02 ± 1.1E-03 |
| 6.00E-04 | 1.57E-02 | 1.78E-02 | 1.82E-02 | 1.90E-02 | 1.77E-02 ± 8.2E-04 |
| Slope = 1.42E+01 ± 3.8E+00; Intercept = 9.61E-03 ± 1.8E-03 | | | | | |

***Table 42**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog oxyhaemoglobin** at 25°C in borate buffer pH **8.608** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.45E-02 | 1.53E-02 | 1.57E-02 | 1.57E-02 | 1.53E-02 ± 2.9E-04 |
| 4.00E-04 | 1.44E-02 | 1.57E-02 | 1.76E-02 | 1.85E-02 | 1.66E-02 ± 1.0E-03 |
| 5.00E-04 | 1.64E-02 | 1.67E-02 | 1.83E-02 | 2.05E-02 | 1.80E-02 ± 1.0E-03 |
| 6.00E-04 | 1.50E-02 | 1.54E-02 | 1.57E-02 | 1.61E-02 | 1.56E-02 ± 2.7E-04* |
| Slope = 1.34E+01 ± 2.8E-01; Intercept = 1.12E-02 ± 1.2E-04 | | | | | |

***Table 43**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog oxyhaemoglobin** at 25°C in borate buffer pH **8.838** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.43E-02 | 1.45E-02 | 1.52E-02 | 1.63E-02 | 1.51E-02 ± 5.1E-04 |
| 4.00E-04 | 1.55E-02 | 1.40E-02 | 1.59E-02 | 1.65E-02 | 1.55E-02 ± 6.2E-04 |
| 5.00E-04 | 1.53E-02 | 1.66E-02 | 1.68E-02 | 1.55E-02 | 1.60E-02 ± 3.8E-04 |
| 6.00E-04 | 2.05E-02 | 1.51E-02 | 1.88E-02 | 1.92E-02 | 1.84E-02 ± 1.4E-03 |
| Slope = 1.05E+01 ± 3.3E+00; Intercept = 1.15E-02 ± 1.5E-03 | | | | | |

***Table 44**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog oxyhaemoglobin** at 25°C in borate buffer pH **9.058** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.56E-02 | 1.65E-02 | 1.34E-02 | 1.52E-02 | 1.52E-02 ± 8.0E-04 |
| 4.00E-04 | 1.60E-02 | 1.62E-02 | 1.64E-02 | 1.65E-02 | 1.63E-02 ± 1.3E-04 |
| 5.00E-04 | 1.28E-02 | 1.53E-02 | 1.54E-02 | 1.57E-02 | 1.48E-02 ± 7.5E-04* |
| 6.00E-04 | 1.69E-02 | 1.76E-02 | 1.69E-02 | 1.70E-02 | 1.71E-02 ± 1.8E-04 |
| Slope = 6.02E+00 ± 1.8E+00; Intercept = 1.36E-02 ± 8.2E-04 | | | | | |

***Table 45**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog carbonmonoxyhaemoglobin** at 25°C in phosphate buffer pH **5.596** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.36E-02 | 1.44E-02 | 1.51E-02 | 1.54E-02 | 1.46E-02 ± 4.3E-04 |
| 4.00E-04 | 1.52E-02 | 1.52E-02 | 1.68E-02 | 1.76E-02 | 1.62E-02 ± 6.0E-04 |
| 5.00E-04 | 1.56E-02 | 1.58E-02 | 1.67E-02 | 1.68E-02 | 1.62E-02 ± 3.0E-0 |
| 6.00E-04 | 1.57E-02 | 1.70E-02 | 1.75E-02 | 1.89E-02 | 1.72E-02 ± 8.1E-0 |
| Slope = 7.87E+00 ± 2.0E+00; Intercept = 1.25E-02 ± 9.1E-04 | | | | | |

***Table 46**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog carbonmonoxyhaemoglobin** at 25°C in phosphate buffer pH **5.760** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.05E-02 | 1.09E-02 | 1.16E-02 | 1.17E-02 | 1.12E-02 ± 3.2E-04 |
| 4.00E-04 | 1.52E-02 | 1.53E-02 | 1.57E-02 | 1.59E-02 | 1.55E-02 ± 1.7E-04 |
| 5.00E-04 | 1.44E-02 | 1.52E-02 | 1.56E-02 | 1.57E-02 | 1.52E-02 ± 3.2E-04 |
| 6.00E-04 | 1.53E-02 | 1.58E-02 | 1.64E-02 | 1.66E-02 | 1.60E-02 ± 3.3E-0 |
| Slope = 1.43E+01 ± 6.9E+00; Intercept = 8.07E-03 ± 3.2E-03 | | | | | |

***Table 47**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog carbonmonoxyhaemoglobin** at 25°C in phosphate buffer pH **6.076** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.40E-02 | 1.40E-02 | 1.52E-02 | 1.61E-02 | 1.48E-02 ± 5.4E-04 |
| 4.00E-04 | 1.25E-02 | 1.33E-02 | 1.44E-02 | 1.46E-02 | 1.37E-02 ± 5.2E-04 |
| 5.00E-04 | 1.22E-02 | 1.76E-02 | 1.82E-02 | 1.89E-02 | 1.67E-02 ± 1.7E-03 |
| 6.00E-04 | 1.63E-02 | 1.89E-02 | 1.51E-02 | 1.98E-02 | 1.75E-02 ± 1.2E-03 |
| Slope = 1.34E+01 ± 1.4E+00; Intercept = 9.68E-03 ± 6.6E-04 | | | | | |

***Table 48**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog carbonmonoxyhaemoglobin** at 25°C in phosphate buffer pH **6.223** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.53E-02 | 1.54E-02 | 1.55E-02 | 1.57E-02 | 1.55E-02 ± 9.3E-05 |
| 4.00E-04 | 1.71E-02 | 1.56E-02 | 1.65E-02 | 1.66E-02 | 1.64E-02 ± 3.8E-04 |
| 5.00E-04 | 1.57E-02 | 1.57E-02 | 1.61E-02 | 1.63E-02 | 1.59E-02 ± 1.7E-04* |
| 6.00E-04 | 1.99E-02 | 1.76E-02 | 1.87E-02 | 1.87E-02 | 1.87E-02 ± 5.9E-04 |
| Slope = 1.08E+01 ± 4.5E-01; Intercept = 1.22E-02 ± 2.0E-04 | | | | | |

***Table 49**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog carbonmonoxyhaemoglobin** at 25°C in phosphate buffer pH **6.447** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.57E-02 | 1.58E-02 | 1.59E-02 | 1.59E-02 | 1.58E-02 ± 5.8E-05 |
| 4.00E-04 | 1.46E-02 | 1.55E-02 | 1.68E-02 | 1.86E-02 | 1.64E-02 ± 1.0E-03 |
| 5.00E-04 | 1.98E-02 | 1.65E-02 | 1.66E-02 | 1.66E-02 | 1.74E-02 ± 8.4E-04 |
| 6.00E-04 | 1.99E-02 | 1.86E-02 | 1.89E-02 | 1.68E-02 | 1.86E-02 ± 7.8E-0 |
| Slope = 9.26E+00 ± 1.0E+00; Intercept = 1.3E-02 ± 4.7E-04 | | | | | |

***Table 50**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog carbonmonoxyhaemoglobin** at 25°C in phosphate buffer pH **6.622** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.12E-02 | 1.21E-02 | 1.31E-02 | 1.32E-02 | 1.24E-02 ± 5.1E-04 |
| 4.00E-04 | 1.28E-02 | 1.31E-02 | 1.35E-02 | 1.40E-02 | 1.34E-02 ± 3.10-04 |
| 5.00E-04 | 1.30E-02 | 1.36E-02 | 1.38E-02 | 1.41E-02 | 1.36E-02 ± 2.6E-04 |
| 6.00E-04 | 1.38E-02 | 1.48E-02 | 1.57E-02 | 1.50E-02 | 1.49E-02 ± 4.7E-04 |
| Slope = 7.62E+00 ± 1.3E+00; Intercept = 1.01E-02 ± 5.8E-04 | | | | | |

***Table 51**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog carbonmonoxyhaemoglobin** at 25°C in phosphate buffer pH **6.799** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.65E-02 | 1.73E-02 | 1.75E-02 | 1.85E-02 | 1.74E-02 ± 5.0E-04 |
| 4.00E-04 | 1.83E-02 | 1.85E-02 | 1.87E-02 | 1.86E-02 | 1.85E-02 ± 8.5E-05* |
| 5.00E-04 | 1.80E-02 | 1.81E-02 | 1.83E-02 | 1.87E-02 | 1.83E-02 ± 1.7E-04 |
| 6.00E-04 | 1.86E-02 | 1.87E-02 | 1.89E-02 | 0.0.1846 | 1.87E-02 ± 7.0E-05 |
| Slope = 4.36E+00 ± 5.4E-02; Intercept = 1.61E-02 ± 2.6E-05 | | | | | |

***Table 52**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog carbonmonoxyhaemoglobin** at 25°C in phosphate buffer pH **6.974** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.26E-02 | 1.26E-02 | 1.28E-02 | 1.29E-02 | 1.27E-02 ± 9.0E-05 |
| 4.00E-04 | 1.24E-02 | 1.28E-02 | 1.32E-02 | 1.34E-02 | 1.29E-02 ± 2.5E-04 |
| 5.00E-04 | 1.22E-02 | 1.31E-02 | 1.35E-02 | 1.36E-02 | 1.31E-02 ± 3.5E-04 |
| 6.00E-04 | 1.33E-02 | 1.33E-02 | 1.33E-02 | 1.34E-02 | 1.33E-02 ± 2.0E-05 |
| Slope = 1.93E+00 ± 9.5E-02; Intercept = 1.22E-02 ± 4.4E-05 | | | | | |

***Table 53**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog carbonmonoxyhaemoglobin** at 25°C in phosphate buffer pH **7.196** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.27E-02 | 1.27E-02 | 1.26E-02 | 1.26E-02 | 1.26E-02 ± 2.5E-05 |
| 4.00E-04 | 1.22E-02 | 1.26E-02 | 1.33E-02 | 1.33E-02 | 1.28E-02 ± 2.7E-04 |
| 5.00E-04 | 1.24E-02 | 1.27E-02 | 1.36E-02 | 1.39E-02 | 1.31E-02 ± 3.7E-04 |
| 6.00E-04 | 1.28E-02 | 1.69E-02 | 1.33E-02 | 1.35E-02 | 1.41E-02 ± 1.0E-03 |
| Slope = 4.76E+00 ± 1.3E+00; Intercept = 1.10E-02 ± 6.1E-04 | | | | | |

***Table 54**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog carbonmonoxyhaemoglobin** at 25°C in phosphate buffer pH **7.375** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.59E-02 | 1.61E-02 | 1.61E-02 | 1.64E-02 | 1.61E-02 ± 1.4E-04 |
| 4.00E-04 | 1.62E-02 | 1.62E-02 | 1.65E-02 | 1.62E-02 | 1.63E-02 ± 7.8E-05 |
| 5.00E-04 | 1.72E-02 | 1.62E-02 | 1.72E-02 | 1.70E-02 | 1.69E-02 ± 2.5E-04 |
| 6.00E-04 | 1.86E-02 | 1.63E-02 | 1.72E-02 | 1.73E-02 | 1.73E-02 ± 5.8E-04 |
| Slope = 4.32E+00 ± 6.7E-01; Intercept = 1.47E-02 ± 3.1E-04 | | | | | |

***Table 55**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog carbonmonoxyhaemoglobin** at 25°C in phosphate buffer pH **7.591** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 2.05E-02 | 2.08E-02 | 2.08E-02 | 2.09E-02 | 2.08E-02 ± 1.15E-04 |
| 4.00E-04 | 2.04E-02 | 2.14E-02 | 2.14E-02 | 2.24E-02 | 2.14E-02 ± 5.00E-04 |
| 5.00E-04 | 2.06E-02 | 2.16E-02 | 2.26E-02 | 2.41E-02 | 2.22E-02 ± 8.78E-04 |
| 6.00E-04 | 2.13E-02 | 2.32E-02 | 2.39E-02 | 2.33E-02 | 2.29E-02 ± 6.45E-04 |
| Slope = 7.29E+00 ± 2.56E-01; Intercept = 1.85E-02 ± 1.19E-04 | | | | | |

***Table 56**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog carbonmonoxyhaemoglobin** at 25°C in phosphate buffer pH **7.792** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.55E-02 | 1.55E-02 | 1.54E-02 | 1.77E-02 | 1.60E-02 ± 5.8E-04 |
| 4.00E-04 | 1.62E-02 | 1.64E-02 | 1.64E-02 | 1.65E-02 | 1.64E-02 ± 7.0E-05 |
| 5.00E-04 | 1.68E-02 | 1.64E-02 | 1.67E-02 | 1.66E-02 | 1.66E-02 ± 1.0E-04 |
| 6.00E-04 | 2.02E-02 | 1.64E-02 | 1.65E-02 | 1.65E-02 | 1.74E-02 ± 9.4E-04 |
| Slope = 4.39E+00 ± 8.9E-01; Intercept = 1.46E-02 ± 4.1E-04 | | | | | |

***Table 57**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog carbonmonoxyhaemoglobin** at 25°C in borate buffer pH **7.971** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.36E-02 | 1.39E-02 | 1.52E-02 | 1.62E-02 | 1.48E-02 ± 6.5E-04 |
| 4.00E-04 | 1.51E-02 | 1.53E-02 | 1.91E-02 | 1.82E-02 | 1.69E-02 ± 9.9E-04 |
| 5.00E-04 | 1.51E-02 | 1.55E-02 | 1.92E-02 | 1.82E-02 | 1.70E-02 ± 1.0E-03 |
| 6.00E-04 | 1.78E-02 | 1.79E-02 | 1.87E-02 | 1.75E-02 | 1.80E-02 ± 3.0E-04 |
| Slope = 9.74E+00 ± 2.8E+00; Intercept = 1.23E-02 ± 1.3E-03 | | | | | |

***Table 58**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog carbonmonoxyhaemoglobin** at 25°C in borate buffer pH **8.218** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| 5 | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.21E-02 | 1.35E-02 | 1.46E-02 | 1.47E-02 | 1.37E-02 ± 6.6E-04 |
| 4.00E-04 | 1.48E-02 | 1.60E-02 | 1.70E-02 | 1.43E-02 | 1.55E-02 ± 6.8E-04 |
| 5.00E-04 | 1.42E-02 | 1.56E-02 | 1.81E-02 | 1.57E-02 | 1.59E-02 ± 9.7E-04 |
| 6.00E-04 | 1.77E-02 | 2.20E-02 | 2.25E-02 | 2.29E-02 | 2.12E-02 ± 1.3E-03* |
| Slope = 1.08E+01 ± 3.9E+00; Intercept = 1.07E-02 ± 1.6E-03 | | | | | |

***Table 59**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog carbonmonoxyhaemoglobin** at 25°C in borate buffer pH **8.381** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.32E-02 | 1.39E-02 | 1.41E-02 | 1.52E-02 | 1.41E-02 ± 5.0E-04 |
| 4.00E-04 | 1.51E-02 | 2.00E-02 | 1.55E-02 | 1.58E-02 | 1.66E-02 ± 1.2E-03 |
| 5.00E-04 | 1.63E-02 | 1.66E-02 | 1.75E-02 | 1.77E-02 | 1.70E-02 ± 3.5E-04 |
| 6.00E-04 | 1.49E-02 | 2.08E-02 | 1.60E-02 | 1.68E-02 | 1.71E-02 ± 1.5E-03 |
| Slope = 9.48E+00 ± 4.0E+00; Intercept = 1.20E-02 ± 1.9E-03 | | | | | |

***Table 60**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog carbonmonoxyhaemoglobin** at 25°C in borate buffer pH **8.604** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.59E-02 | 1.65E-02 | 1.66E-02 | 1.47E-02 | 1.59E-02 ± 4.9E-04 |
| 4.00E-04 | 1.67E-02 | 1.61E-02 | 1.65E-02 | 1.74E-02 | 1.67E-02 ± 3.3E-04 |
| 5.00E-04 | 1.72E-02 | 1.66E-02 | 1.76E-02 | 1.79E-02 | 1.73E-02 ± 3.0E-04 |
| 6.00E-04 | 1.99E-02 | 1.98E-02 | 1.98E-02 | 1.89E-02 | 1.96E-02 ± 2.6E-04 |
| Slope = 1.17E+01 ± 2.7E+00; Intercept = 1.21E-02 ± 1.2E-03 | | | | | |

***Table 61**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog carbonmonoxyhaemoglobin** at 25°C in borate buffer pH **8.815** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.23E-02 | 1.51E-02 | 1.72E-02 | 1.57E-02 | 1.51E-02 ± 1.2E-03 |
| 4.00E-04 | 1.31E-02 | 1.58E-02 | 1.59E-02 | 1.57E-02 | 1.51E-02 ± 7.20-04 |
| 5.00E-04 | 1.39E-02 | 1.58E-02 | 1.59E-02 | 2.24E-02 | 1.70E-02 ± 2.1E-03 |
| 6.00E-04 | 1.59E-02 | 2.28E-02 | 2.28E-02 | 2.29E-02 | 2.11E-02 ± 1.7E-03* |
| Slope = 9.59E+00 ± 5.4E+00; Intercept = 1.19E-02 ± 2.2E-03 | | | | | |

***Table 62**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog carbonmonoxyhaemoglobin** at 25°C in borate buffer pH **9.068** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.59E-02 | 1.61E-02 | 1.05E-02 | 1.70E-02 | 1.49E-02 ± 1.6E-03 |
| 4.00E-04 | 1.46E-01* | 1.33E-02 | 1.60E-02 | 1.66E-02 | 1.53E-02 ± 1.1E-03 |
| 5.00E-04 | 1.72E-02 | 1.48E-02 | 1.66E-02 | 1.72E-02 | 1.65E-02 ± 5.9E-04 |
| 6.00E-04 | 1.95E-02 | 1.65E-02 | 1.80E-02 | 1.92E-02 | 1.83E-02 ± 7.5E-04 |
| Slope = 1.44E+01 ± 3.9E+00; Intercept = 1.00E-02 ± 1.8E-03 | | | | | |

***Table 63**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog aquomethaemoglobin** at 25°C in phosphate buffer pH **5.618** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 8.76E-03 | 1.59E-02 | 1.51E-02 | 1.46E-02 | 1.36E-02 ± 1.8E-03 |
| 4.00E-04 | 1.54E-02 | 1.56E-02 | 1.39E-02 | 1.54E-02 | 1.51E-02 ± 4.1E-04 |
| 5.00E-04 | 1.80E-02 | 1.83E-02 | 1.25E-02 | 1.50E-02 | 1.59E-02 ± 1.4E-03 |
| 6.00E-04 | 2.20E-02 | 2.50E-02 | 1.88E-02 | 2.52E-02 | 2.28E-02 ± 1.6E-03 |
| Slope = 2.84E+01 ± 9.6E+00; Intercept = 4.03E-03 ± 4.5E-03 | | | | | |

***Table 64**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog aquomethaemoglobin** at 25°C in phosphate buffer pH **5.784** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.63E-02 | 1.44E-02 | 1.49E-02 | 1.38E-02 | 1.49E-02 ± 6.4E-04 |
| 4.00E-04 | 1.63E-02 | 1.34E-02 | 1.68E-02 | 1.58E-02 | 1.56E-02 ± 8.6E-04 |
| 5.00E-04 | 1.66E-02 | 1.60E-02 | 1.99E-02 | 1.74E-02 | 1.75E-02 ± 9.9E-04 |
| 6.00E-04 | 1.94E-02 | 1.72E-02 | 2.67E-02 | 1.83E-02 | 2.04E-02 ± 2.4E-03 |
| Slope = 1.85E+01 ± 3.5E+00; Intercept = 8.74E-03 ± 1.6E-03 | | | | | |

***Table 65**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog aquomethaemoglobin** at 25°C in phosphate buffer pH **6.074** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.24E-02 | 1.32E-02 | 1.35E-02 | 1.51E-02 | 1.36E-02 ± 6.8E-04 |
| 4.00E-04 | 1.35E-02 | 1.40E-02 | 1.44E-02 | 1.70E-02 | 1.47E-02 ± 8.8E-04 |
| 5.00E-04 | 1.29E-02 | 1.37E-02 | 1.56E-02 | 1.74E-02 | 1.49E-02 ± 1.1E-03 |
| 6.00E-04 | 1.63E-02 | 1.67E-02 | 1.90E-02 | 6.08E-02* | 1.73E-02 ± 8.8E-04 |
| Slope = 1.15E+01 ± 3.1E+00; Intercept = 9.95E-03 ± 1.4E-03 | | | | | |

***Table 66**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog aquomethaemoglobin** at 25°C in phosphate buffer pH **6.228** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.11E-02 | 1.14E-02 | 1.29E-02 | 1.51E-02 | 1.27E-02 ± 1.0E-03 |
| 4.00E-04 | 1.18E-02 | 1.34E-02 | 1.60E-02 | 1.62E-02 | 1.44E-02 ± 1.1E-03 |
| 5.00E-04 | 1.14E-02 | 1.44E-02 | 1.60E-02 | 1.84E-02 | 1.50E-02 ± 1.7E-03 |
| 6.00E-04 | 1.23E-02 | 1.57E-02 | 1.58E-02 | 1.75E-02 | 1.53E-02 ± 1.3E-03 |
| Slope = 8.68E+00 ± 2.3E+00; Intercept = 1.04E-02 ± 1.1E-03 | | | | | |

***Table 67**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog aquomethaemoglobin** at 25°C in phosphate buffer pH **6.416** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.37E-02 | 1.37E-02 | 1.51E-02 | 1.64E-02 | 1.47E-02 ± 6.8E-04 |
| 4.00E-04 | 1.38E-02 | 1.50E-02 | 1.69E-02 | 1.78E-02 | 1.59E-02 ± 1.0E-03 |
| 5.00E-04 | 1.38E-02 | 1.62E-02 | 1.65E-02 | 1.83E-02 | 1.62E-02 ± 1.1E-03 |
| 6.00E-04 | 1.59E-02 | 1.59E-02 | 1.70E-02 | 1.70E-02 | 1.65E-02 ± 2.9E-04 |
| Slope = 5.50E+00 ± 1.4E+00; Intercept = 1.33E-02 ± 6.6E-04 | | | | | |

***Table 68**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog aquomethaemoglobin** at 25°C in phosphate buffer pH **6.511** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.15E-02 | 1.45E-02 | 1.37E-02 | 1.47E-02 | 1.36E-02 ± 8.1E-04 |
| 4.00E-04 | 1.42E-02 | 2.01E-02 | 1.44E-02 | 1.48E-02 | 1.59E-02 ± 1.5E-03 |
| 5.00E-04 | 1.33E-02 | 1.92E-02 | 1.83E-02 | 1.38E-02 | 1.61E-02 ± 1.5E-03 |
| 6.00E-04 | 1.54E-02 | 1.82E-02 | 1.93E-02 | 1.85E-02 | 1.79E-02 ± 9.8E-04 |
| Slope = 1.31E+01 ± 2.6E+00; Intercept = 9.98E-03 ± 1.2E-03 | | | | | |

***Table 69**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog aquomethaemoglobin** at 25°C in phosphate buffer pH **6.765** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.80E-02 | 1.40E-02 | 1.30E-02 | 1.16E-02 | 1.41E-02 ± 1.6E-03 |
| 4.00E-04 | 1.67E-02 | 1.51E-02 | 1.41E-02 | 1.19E-02 | 1.45E-02 ± 1.2E-03 |
| 5.00E-04 | 1.69E-02 | 1.43E-02 | 1.70E-02 | 1.38E-02 | 1.55E-02 ± 7.8E-04 |
| 6.00E-04 | 1.22E-02 | 1.39E-02 | 1.55E-02 | 2.09E-02 | 1.56E-02 ± 2.2E-03 |
| Slope = 5.42E+00 ± 1.2E+00; Intercept = 1.25E-02 ± 5.5E-04 | | | | | |

***Table 70**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog aquomethaemoglobin** at 25°C in phosphate buffer pH **6.968** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.29E-02 | 1.59E-02 | 1.30E-02 | 1.31E-02 | 1.37E-02 ± 7.7E-04 |
| 4.00E-04 | 1.47E-02 | 1.67E-02 | 1.54E-02 | 1.43E-02 | 1.53E-02 ± 5.9E-04 |
| 5.00E-04 | 1.39E-02 | 1.44E-02 | 1.44E-02 | 2.01E-02 | 1.57E-02 ± 1.6E-03 |
| 6.00E-04 | 1.69E-02 | 1.53E-02 | 1.60E-02 | 1.60E-02 | 1.60E-02 ± 4.1E-04 |
| Slope = 7.30E+00 ± 2.0E+00; Intercept = 1.19E-02 ± 9.4E-04 | | | | | |

***Table 71**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog aquomethaemoglobin** at 25°C in phosphate buffer pH **7.174** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.29E-02 | 1.29E-02 | 1.67E-02 | 1.36E-02 | 1.41E-02 ± 9.5E-04 |
| 4.00E-04 | 1.61E-02 | 1.31E-02 | 1.34E-02 | 1.48E-02 | 1.44E-02 ± 7.4E-04 |
| 5.00E-04 | 1.92E-02 | 1.50E-02 | 1.51E-02 | 1.53E-02 | 1.61E-02 ± 1.0E-03 |
| 6.00E-04 | 1.88E-02 | 1.84E-02 | 1.46E-02 | 1.65E-02 | 1.71E-02 ± 1.0E-03 |
| Slope = 1.08E+01 ± 1.9E+00; Intercept = 1.06E-02 ± 9.0E-04 | | | | | |

***Table 72**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog aquomethaemoglobin** at 25°C in phosphate buffer pH **7.576** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.37E-02 | 1.48E-02 | 1.49E-02 | 1.62E-02 | 1.49E-02 ± 6.2E-04 |
| 4.00E-04 | 1.52E-02 | 1.78E-02 | 1.61E-02 | 1.34E-02 | 1.56E-02 ± 1.1E-03 |
| 5.00E-04 | 1.57E-02 | 1.66E-02 | 1.50E-02 | 1.64E-02 | 1.59E-02 ± 4.2E-04 |
| 6.00E-04 | 1.58E-02 | 1.61E-02 | 1.89E-02 | 1.55E-02 | 1.66E-02 ± 8.6E-04 |
| Slope = 5.32E+00 ± 4.9E-01; Intercept = 1.34E-02 ± 2.3E-04 | | | | | |

***Table 73**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog aquomethaemoglobin** at 25°C in phosphate buffer pH **7.777** ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.44E-02 | 1.42E-02 | 1.41E-02 | 1.22E-02 | 1.37E-02 ± 5.5E-04 |
| 4.00E-04 | 1.52E-02 | 1.29E-02 | 1.39E-02 | 1.55E-02 | 1.44E-02 ± 6.3E-04 |
| 5.00E-04 | 1.24E-02 | 1.63E-02 | 1.56E-02 | 1.39E-02 | 1.45E-02 ± 9.8E-04 |
| 6.00E-04 | 1.47E-02 | 1.57E-02 | 1.69E-02 | 1.55E-02 | 1.57E-02 ± 5.5E-04 |
| Slope = 5.99E+00 ± 1.3E+00; Intercept = 1.19E-02 ± 6.1E-04 | | | | | |

***Table 74**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog aquomethaemoglobin** at 25°C in borate buffer pH **7.958** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.44E-02 | 1.47E-02 | 1.49E-02 | 1.55E-02 | 1.49E-02 ± 2.8E-04 |
| 4.00E-04 | 1.58E-02 | 1.51E-02 | 1.54E-02 | 1.60E-02 | 1.56E-02 ± 2.2E-04 |
| 5.00E-04 | 1.67E-02 | 1.55E-02 | 1.58E-02 | 1.57E-02 | 1.59E-02 ± 3.0E-04 |
| 6.00E-04 | 1.62E-02 | 1.62E-02 | 1.73E-02 | 1.53E-02 | 1.62E-02 ± 5.2E-04 |
| Slope = 4.38E+00 ± 6.9E-01; Intercept = 1.37E-02 ± 3.2E-04 | | | | | |

***Table 75**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog aquomethaemoglobin** at 25°C in borate buffer pH **8.188** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.40E-02 | 1.25E-02 | 1.54E-02 | 1.21E-02 | 1.35E-02 ± 8.2E-04 |
| 4.00E-04 | 8.16E-02* | 1.24E-02 | 1.44E-02 | 1.67E-02 | 1.45E-02 ± 1.4E-03 |
| 5.00E-04 | 8.11E-03 | 1.34E-02 | 1.60E-02 | 1.66E-02 | 1.35E-02 ± 2.1E-03 |
| 6.00E-04 | 1.45E-02 | 1.73E-02 | 1.52E-02 | 1.63E-02 | 1.58E-02 ± 7.0E-04 |
| Slope = 7.77E+00 ± 8.4E-01; Intercept = 1.13E-02 ± 3.9E-04 | | | | | |

***Table 76**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog aquomethaemoglobin** at 25°C in borate buffer pH **8.394** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.40E-02 | 1.21E-02 | 1.59E-02 | 1.55E-02 | 1.44E-02 ± 9.4E-04 |
| 4.00E-04 | 1.53E-02 | 1.34E-02 | 1.45E-02 | 1.44E-02 | 1.44E-02 ± 4.6E-04 |
| 5.00E-04 | 1.60E-02 | 1.45E-02 | 1.39E-02 | 1.55E-02 | 1.50E-02 ± 5.2E-04 |
| 6.00E-04 | 1.45E-02 | 1.44E-02 | 1.64E-02 | 1.54E-02 | 1.52E-02 ± 5.0E-04 |
| Slope = 2.97E+00 ± 6.9E-01; Intercept = 1.34E-02 ± 3.2E-04 | | | | | |

***Table 77**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog aquomethaemoglobin** at 25°C in borate buffer pH **8.606** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.45E-02 | 1.50E-02 | 1.28E-02 | 1.32E-02 | 1.39E-02 ± 5.6E-04 |
| 4.00E-04 | 1.47E-02 | 1.50E-02 | 1.38E-02 | 1.30E-02 | 1.41E-02 ± 5.0E-04 |
| 5.00E-04 | 1.60E-02 | 1.21E-02 | 1.58E-02 | 1.57E-02 | 1.49E-02 ± 9.7E-04 |
| 6.00E-04 | 1.33E-02 | 1.45E-02 | 2.15E-02 | 1.34E-02 | 1.57E-02 ± 2.1E-03 |
| Slope = 6.29E+00 ± 9.3E-01; Intercept = 1.18E-02 ± 4.3E-04 | | | | | |

***Table 78**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog aquomethaemoglobin** at 25°C in borate buffer pH **8.786** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.38E-02 | 1.51E-02 | 1.72E-02 | 1.31E-02 | 1.48E-02 ± 1.0E-03 |
| 4.00E-04 | 1.74E-02 | 1.54E-02 | 1.47E-02 | 1.43E-02 | 1.55E-02 ± 7.9E-04 |
| 5.00E-04 | 1.30E-02 | 1.51E-02 | 1.56E-02 | 1.89E-02 | 1.56E-02 ± 1.5E-03 |
| 6.00E-04 | 1.66E-02 | 1.80E-02 | 1.66E-02 | 1.31E-02 | 1.61E-02 ± 1.2E-03 |
| Slope = 3.94E+00 ± 6.5E-01; Intercept = 1.37E-02 ± 3.0E-04 | | | | | |

***Table 79**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped dog aquomethaemoglobin** at 25°C in borate buffer pH **8.989** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.47E-02 | 1.27E-02 | 1.29E-02 | 1.42E-02 | 1.36E-02 ± 5.0E-04 |
| 4.00E-04 | 1.48E-02 | 1.51E-02 | 1.27E-02 | 1.45E-02 | 1.43E-02 ± 6.0E-04 |
| 5.00E-04 | 1.77E-02 | 1.33E-02 | 1.38E-02 | 1.49E-02 | 1.49E-02 ± 1.1E-03 |
| 6.00E-04 | 1.32E-02 | 1.53E-02 | 1.74E-02 | 1.55E-02 | 1.53E-02 ± 1.0E-03 |
| Slope = 4.14E+00 ± 4.7E-01; Intercept = 1.29E-02 ± 2.2E-04 | | | | | |

***Table 80**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog oxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **5.614** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.16E-02 | 1.22E-02 | 1.38E-02 | 1.48E-02 | 1.31E-02 ± 8.1E-04 |
| 4.00E-04 | 1.30E-02 | 1.32E-02 | 1.45E-02 | 1.54E-02 | 1.40E-02 ± 6.0E-04 |
| 5.00E-04 | 1.67E-02 | 1.34E-02 | 1.35E-02 | 1.63E-02 | 1.50E-02 ± 8.1E-04 |
| 6.00E-04 | 1.81E-02 | 1.42E-02 | 1.51E-02 | 1.59E-02 | 1.58E-02 ± 9.6E-04 |
| Slope = 9.12E+00 ± 1.8E-01; Intercept = 1.04E-02 ± 8.4E-05 | | | | | |

***Table 81**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog oxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **5.811** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.13E-02 | 1.29E-02 | 1.34E-02 | 1.43E-02 | 1.29E-02 ± 7.4E-04 |
| 4.00E-04 | 1.40E-02 | 1.41E-02 | 1.46E-02 | 1.48E-02 | 1.44E-02 ± 2.0E-04 |
| 5.00E-04 | 1.30E-02 | 1.30E-02 | 1.40E-02 | 1.47E-02 | 1.37E-02 ± 4.3E-04 |
| 6.00E-04 | 1.54E-02 | 1.62E-02 | 1.64E-02 | 1.69E-02 | 1.62E-02 ± 3.8E-04 |
| Slope = 9.09E+00 ± 4.1E+00; Intercept = 1.02E-02 ± 1.9E-03 | | | | | |

***Table 82**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog oxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **6.029** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.52E-02 | 1.51E-02 | 1.52E-02 | 1.54E-02 | 1.52E-02 ± 8.0E-05 |
| 4.00E-04 | 1.45E-02 | 1.60E-02 | 1.76E-02 | 1.53E-02 | 1.58E-02 ± 7.8E-04 |
| 5.00E-04 | 1.47E-02 | 1.57E-02 | 1.63E-02 | 1.74E-02 | 1.61E-02 ± 6.7E-04 |
| 6.00E-04 | 1.32E-02 | 1.77E-02 | 1.71E-02 | 1.93E-02 | 1.68E-02 ± 1.5E-03 |
| Slope = 5.01E+00 ± 7.2E-01; Intercept = 1.37E-02 ± 3.3E-04 | | | | | |

***Table 83**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog oxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **6.244** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.56E-02 | 1.60E-02 | 1.72E-02 | 1.85E-02 | 1.68E-02 ± 7.3E-04 |
| 4.00E-04 | 1.57E-02 | 1.67E-02 | 1.74E-02 | 1.87E-02 | 1.71E-02 ± 7.5E-04 |
| 5.00E-04 | 1.58E-02 | 1.77E-02 | 1.88E-02 | 1.98E-02 | 1.80E-02 ± 1.0E-03 |
| 6.00E-04 | 1.80E-02 | 1.89E-02 | 1.87E-02 | 1.93E-02 | 1.87E-02 ± 3.3E-04 |
| Slope = 6.68E+00 ± 8.3E-01; Intercept = 1.47E-02 ± 3.8E-04 | | | | | |

***Table 84**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog oxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **6.403** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.33E-02 | 1.34E-02 | 1.40E-02 | 1.67E-02 | 1.43E-02 ± 8.5E-04 |
| 4.00E-04 | 1.46E-02 | 1.61E-02 | 1.64E-02 | 1.49E-02 | 1.55E-02 ± 4.6E-04 |
| 5.00E-04 | 1.51E-02 | 1.57E-02 | 1.69E-02 | 1.63E-02 | 1.60E-02 ± 4.6E-04 |
| 6.00E-04 | 1.63E-02 | 1.92E-02 | 2.11E-02 | 2.49E-02 | 2.04E-02 ± 2.2E-03* |
| Slope = 8.25E+00 ± 2.0E+00; Intercept = 1.20E-02 ± 8.0E-04 | | | | | |

***Table 85**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog oxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **6.589** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.31E-02 | 1.33E-02 | 1.37E-02 | 1.64E-02 | 1.41E-02 ± 8.4E-04 |
| 4.00E-04 | 1.51E-02 | 1.60E-02 | 1.62E-02 | 1.63E-02 | 1.59E-02 ± 3.1E-04 |
| 5.00E-04 | 1.53E-02 | 1.63E-02 | 1.65E-02 | 1.65E-02 | 1.61E-02 ± 3.0E-04 |
| 6.00E-04 | 1.39E-02 | 1.43E-02 | 1.51E-02 | 1.53E-02 | 1.46E-02 ± 3.4E-04* |
| Slope = 1.02E+01 ± 4.4E+00; Intercept = 1.13E-02 ± 1.8E-03 | | | | | |

***Table 86**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog oxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **6.801** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.41E-02 | 1.52E-02 | 1.53E-02 | 1.65E-02 | 1.53E-02 ± 6.2E-04 |
| 4.00E-04 | 1.43E-02 | 1.70E-02 | 1.70E-02 | 1.72E-02 | 1.64E-02 ± 7.2E-04 |
| 5.00E-04 | 1.31E-02 | 1.56E-02 | 1.56E-02 | 2.24E-02 | 1.67E-02 ± 2.3E-03 |
| 6.00E-04 | 1.68E-02 | 1.74E-02 | 1.77E-02 | 1.78E-02 | 1.74E-02 ± 2.4E-04 |
| Slope = 6.78E+00 ± 1.1E+00; Intercept = 1.34E-02 ± 5.0E-04 | | | | | |

***Table 87**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog oxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **6.998** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.23E-02 | 1.26E-02 | 1.37E-02 | 1.48E-02 | 1.33E-02 ± 6.4E-04 |
| 4.00E-04 | 1.34E-02 | 1.35E-02 | 1.53E-02 | 1.51E-02 | 1.43E-02 ± 4.7E-04 |
| 5.00E-04 | 1.28E-02 | 1.69E-02 | 1.60E-02 | 1.43E-02 | 1.50E-02 ± 1.0E-03 |
| 6.00E-04 | 1.42E-02 | 1.62E-02 | 1.63E-02 | 1.46E-02 | 1.53E-02 ± 5.3E-04 |
| Slope = 6.60E+00 ± 1.0E+00; Intercept = 1.15E-02 ± 4.7E-04 | | | | | |

***Table 88**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog oxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **7.171** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.53E-02 | 1.59E-02 | 1.60E-02 | 1.62E-02 | 1.59E-02 ± 2.4E-04 |
| 4.00E-04 | 1.54E-02 | 1.63E-02 | 1.63E-02 | 1.63E-02 | 1.61E-02 ± 2.4E-04 |
| 5.00E-04 | 1.61E-02 | 1.60E-02 | 1.61E-02 | 1.61E-02 | 1.61E-02 ± 2.3E-05 |
| 6.00E-04 | 1.60E-02 | 1.60E-02 | 1.62E-02 | 1.62E-02 | 1.61E-02 ± 4.7E-05 |
| Slope = 7.00E-01 ± 3.0E-01; Intercept = 1.57E-02 ± 1.4E-04 | | | | | |

***Table 89**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog oxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **7.396** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.50E-02 | 1.51E-02 | 1.52E-02 | 1.54E-02 | 1.52E-02 ± 8.3E-05 |
| 4.00E-04 | 1.54E-02 | 1.55E-02 | 1.55E-02 | 1.56E-02 | 1.55E-02 ± 4.7E-05 |
| 5.00E-04 | 1.62E-02 | 1.64E-02 | 1.64E-02 | 1.65E-02 | 1.64E-02 ± 5.5E-05 |
| 6.00E-04 | 1.62E-02 | 1.73E-02 | 2.66E-02 | 1.98E-02 | 2.00E-02 ± 2.6E-03* |
| Slope = 6.04E+00 ± 1.7E+00; Intercept = 1.33E-02 ± 7.0E-04 | | | | | |

***Table 90**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog oxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **7.606** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.38E-02 | 1.53E-02 | 1.54E-02 | 1.54E-02 | 1.50E-02 ± 4.1E-04 |
| 4.00E-04 | 1.49E-02 | 1.51E-02 | 1.62E-02 | 1.68E-02 | 1.58E-02 ± 4.7E-04 |
| 5.00E-04 | 1.63E-02 | 1.63E-02 | 1.63E-02 | 1.64E-02 | 1.63E-02 ± 2.7E-05 |
| 6.00E-04 | 1.32E-02 | 1.61E-02 | 1.82E-02 | 1.91E-02 | 1.67E-02 ± 1.5E-03 |
| Slope = 5.59E+00 ± 7.0E-01; Intercept = 1.34E-02 ± 3.2E-04 | | | | | |

***Table 91**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog oxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **7.807** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.50E-02 | 1.52E-02 | 1.65E-02 | 1.65E-02 | 1.58E-02 ± 3.7E-04 |
| 4.00E-04 | 1.66E-02 | 1.57E-02 | 1.58E-02 | 1.65E-02 | 1.62E-02 ± 2.3E-04 |
| 5.00E-04 | 1.62E-02 | 1.81E-02 | 1.82E-02 | 1.64E-02 | 1.72E-02 ± 4.9E-04 |
| 6.00E-04 | 2.12E-02 | 1.72E-02 | 2.26E-02 | 2.53E-02 | 2.16E-02 ± 2.0E-03* |
| Slope = 7.11E+00 ± 2.1E+00; Intercept = 1.36E-02 ± 8.5E-04 | | | | | |

***Table 92**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog oxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in borate buffer pH **7.994** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 5.03E-02 | 1.62E-02 | 1.97E-02 | 1.72E-02 | 1.71E-02 ± 1.1E-03 |
| 4.00E-04 | 1.70E-02 | 1.73E-02 | 1.58E-02 | 7.70E-03 | 1.44E-02 ± 2.4E-03 |
| 5.00E-04 | 1.96E-02 | 1.65E-02 | 1.75E-02 | 1.54E-02 | 1.73E-02 ± 1.0E-03 |
| 6.00E-04 | 1.94E-02 | 2.16E-02 | 1.91E-02 | 1.54E-02 | 1.89E-02 ± 1.5E-03 |
| Slope = 6.34E+00 ± 1.1E+03; Intercept = 1.40E-02 ± 1.8E-03 | | | | | |

***Table 93**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog oxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in borate buffer pH **8.197** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.66E-02 | 1.80E-02 | 1.60E-02 | 1.50E-02 | 1.64E-02 ± 7.5E-04 |
| 4.00E-04 | 1.66E-02 | 1.77E-02 | 1.85E-02 | 1.60E-02 | 1.72E-02 ± 6.2E-04 |
| 5.00E-04 | 1.94E-02 | 1.49E-02 | 1.78E-02 | 1.98E-02 | 1.80E-02 ± 1.2E-03 |
| 6.00E-04 | 2.47E-02 | 1.42E-02 | 1.70E-02 | 1.67E-02 | 1.81E-02± 2.6E-03* |
| Slope = 7.56E+00 ± 0.5E-03; Intercept = 1.40E-02 ± 4.6E-04 | | | | | |

***Table 94**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog oxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in borate buffer pH **8.398** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.63E-02 | 1.67E-02 | 1.75E-02 | 1.09E-02 | 1.53E-02 ± 1.7E-03 |
| 4.00E-04 | 1.47E-02 | 1.62E-02 | 1.36E-02 | 1.70E-02 | 1.54E-02 ± 8.5E-04 |
| 5.00E-04 | 1.86E-02 | 1.62E-02 | 1.58E-02 | 1.61E-02 | 1.67E-02 ± 7.2E-04 |
| 6.00E-04 | 1.61E-02 | 1.70E-02 | 1.86E-02 | 1.93E-02 | 1.77E-02 ± 8.1E-04 |
| Slope = 8.47E+00 ± 1.9E+00; Intercept = 1.25E-02 ± 9.0E-04 | | | | | |

***Table 95**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog oxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in borate buffer pH **8.610** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.05E-02 | 1.57E-02 | 1.81E-02 | 1.75E-02 | 1.54E-02 ± 1.9E-03 |
| 4.00E-04 | 1.72E-02 | 1.43E-02 | 1.64E-02 | 1.76E-02 | 1.64E-02 ± 8.3E-04 |
| 5.00E-04 | 1.72E-02 | 2.01E-02 | 1.54E-02 | 1.70E-02 | 1.75E-02 ± 1.2E-03 |
| 6.00E-04 | 1.99E-02 | 1.72E-02 | 1.81E-02 | 1.82E-02 | 1.83E-02 ± 6.8E-04 |
| Slope = 9.75E+00 ± 2.9E-01; Intercept = 1.25E-02 ± 1.3E-04 | | | | | |

***Table 96**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog oxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in borate buffer pH **8.827** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.57E-02 | 1.35E-02 | 1.69E-02 | 1.40E-02 | 1.50E-02 ± 8.7E-04 |
| 4.00E-04 | 1.68E-02 | 1.45E-02 | 1.78E-02 | 1.65E-02 | 1.64E-02 ± 8.3E-04 |
| 5.00E-04 | 1.43E-02 | 1.91E-02 | 1.82E-02 | 1.61E-02 | 1.69E-02 ± 1.2E-03 |
| 6.00E-04 | 1.71E-02 | 1.83E-02 | 1.83E-02 | 1.71E-02 | 1.77E-02 ± 3.2E-04 |
| Slope = 8.61E+00 ± 1.3E+00; Intercept = 1.26E-02 ± 5.8E-04 | | | | | |

***Table 97**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog oxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in borate buffer pH **8.968** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.59E-02 | 1.45E-02 | 1.62E-02 | 1.58E-02 | 1.56E-02 ± 4.3E-04* |
| 4.00E-04 | 1.58E-02 | 1.67E-02 | 1.67E-02 | 1.43E-02 | 1.58E-02 ± 6.0E-04 |
| 5.00E-04 | 1.64E-02 | 1.68E-02 | 1.69E-02 | 1.55E-02 | 1.64E-02 ± 3.7E-04 |
| 6.00E-04 | 1.87E-02 | 1.88E-02 | 1.84E-02 | 1.63E-02 | 1.81E-02 ± 6.1E-04 |
| Slope = 1.11E+01 ± 3.3E+00; Intercept = 1.12E-02 ± 1.7E-03 | | | | | |

***Table 98**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog carbonmonoxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **5.605** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.16E-02 | 1.27E-02 | 1.37E-02 | 1.39E-02 | 1.30E-02 ± 5.8E-04 |
| 4.00E-04 | 9.01E-03 | 1.15E-02 | 1.51E-02 | 1.65E-02 | 1.30E-02 ± 1.9E-03 |
| 5.00E-04 | 1.31E-02 | 1.45E-02 | 1.53E-02 | 1.63E-02 | 1.48E-02 ± 8.0E-04 |
| 6.00E-04 | 1.50E-02 | 1.74E-02 | 1.79E-02 | 1.79E-02 | 1.71E-02 ± 7.3E-04 |
| Slope = 1.39E+01 ± 3.6E+00; Intercept = 8.20E-03 ± 1.7E-03 | | | | | |

***Table 99**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog carbonmonoxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **5.831** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 9.14E-03 | 9.23E-03 | 9.28E-03 | 1.14E-02 | 9.77E-03 ± 5.8E-04 |
| 4.00E-04 | 1.30E-02 | 1.34E-02 | 1.42E-02 | 1.49E-02 | 1.39E-02 ± 4.6E-04 |
| 5.00E-04 | 1.22E-02 | 1.32E-02 | 1.49E-02 | 1.53E-02 | 1.39E-02 ± 7.9E-04 |
| 6.00E-04 | 1.27E-02 | 1.33E-02 | 1.47E-02 | 1.57E-02 | 1.41E-02 ± 7.4E-04 |
| Slope = 1.29E+01 ± 6.9E+00; Intercept = 7.08E-03 ± 3.2E-03 | | | | | |

***Table 100**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog carbonmonoxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **6.014** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.28E-02 | 1.38E-02 | 1.47E-02 | 1.55E-02 | 1.42E-02 ± 6.8E-04 |
| 4.00E-04 | 1.37E-02 | 1.39E-02 | 1.50E-02 | 1.50E-02 | 1.44E-02 ± 3.3E-04 |
| 5.00E-04 | 1.40E-02 | 1.49E-02 | 1.51E-02 | 1.53E-02 | 1.48E-02 ± 3.4E-04 |
| 6.00E-04 | 1.35E-02 | 1.37E-02 | 1.58E-02 | 1.64E-02 | 1.49E-02 ± 7.3E-04* |
| Slope = 3.08E+00 ± 6.5E-01; Intercept = 1.32E-02 ± 2.7E-04 | | | | | |

***Table 101**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog carbonmonoxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **6.191** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.22E-02 | 1.23E-02 | 1.30E-02 | 1.42E-02 | 1.29E-02 ± 5.0E-04 |
| 4.00E-04 | 1.63E-02 | 1.25E-02 | 1.48E-02 | 1.59E-02 | 1.49E-02 ± 9.6E-04 |
| 5.00E-04 | 1.77E-02 | 1.42E-02 | 1.84E-02 | 1.85E-02 | 1.72E-02 ± 1.1E-03* |
| 6.00E-04 | 1.49E-02 | 1.59E-02 | 1.66E-02 | 1.66E-02 | 1.60E-02 ± 4.2E-04 |
| Slope = 9.42E+00 ± 3.4E+00; Intercept = 1.05E-02 ± 1.5E-03 | | | | | |

***Table 102**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog carbonmonoxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **6.383** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.34E-02 | 1.23E-02 | 1.42E-02 | 1.60E-02 | 1.40E-02 ± 9.1E-04 |
| 4.00E-04 | 1.34E-02 | 1.49E-02 | 1.53E-02 | 1.63E-02 | 1.50E-02 ± 7.2E-04 |
| 5.00E-04 | 1.42E-02 | 1.72E-02 | 1.80E-02 | 1.50E-02 | 1.61E-02 ± 9.6E-04 |
| 6.00E-04 | 1.36E-02 | 1.77E-02 | 1.54E-02 | 1.85E-02 | 1.63E-02 ± 1.2E-03 |
| Slope = 8.06E+00 ± 1.4E+00; Intercept = 1.17E-02 ± 6.7E-04 | | | | | |

***Table 103**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog carbonmonoxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **6.578** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.42E-02 | 1.45E-02 | 1.52E-02 | 1.66E-02 | 1.51E-02 ± 5.9E-04 |
| 4.00E-04 | 1.38E-02 | 1.45E-02 | 1.58E-02 | 1.67E-02 | 1.52E-02 ± 7.2E-04 |
| 5.00E-04 | 1.62E-02 | 1.64E-02 | 1.69E-02 | 1.69E-02 | 1.66E-02 ± 1.7E-04 |
| 6.00E-04 | 1.20E-02 | 1.50E-02 | 1.60E-02 | 1.69E-02 | 1.50E-02 ± 1.2E-03* |
| Slope = 7.30E+00 ± 3.7E+00; Intercept = 1.27E-02 ± 1.5E-03 | | | | | |

***Table 104**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog carbonmonoxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **6.841** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.26E-02 | 1.34E-02 | 1.40E-02 | 1.54E-02 | 1.38E-02 ± 7.2E-04 |
| 4.00E-04 | 1.13E-02 | 1.42E-02 | 1.46E-02 | 1.57E-02 | 1.39E-02 ± 1.1E-03 |
| 5.00E-04 | 1.30E-02 | 1.46E-02 | 1.48E-02 | 1.57E-02 | 1.45E-02 ± 6.7E-04 |
| 6.00E-04 | 1.52E-02 | 1.93E-02 | 1.23E-02 | 1.25E-02 | 1.48E-02 ± 1.8E-03 |
| Slope = 3.51E+00 ± 6.3E-01; Intercept = 1.27E-02 ± 2.9E-04 | | | | | |

***Table 105**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog carbonmonoxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **6.975** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.37E-02 | 1.41E-02 | 1.43E-02 | 1.54E-02 | 1.43E-02 ± 4.2E-04 |
| 4.00E-04 | 1.42E-02 | 1.42E-02 | 1.51E-02 | 1.61E-02 | 1.49E-02 ± 4.8E-04 |
| 5.00E-04 | 1.35E-02 | 1.49E-02 | 1.59E-02 | 1.59E-02 | 1.50E-02 ± 5.9E-04 |
| 6.00E-04 | 1.44E-02 | 1.47E-02 | 1.54E-02 | 1.59E-02 | 1.51E-02 ± 3.9E-04 |
| Slope = 2.34E+00 ± 8.4E-01; Intercept = 1.38E-02 ± 3.9E-04 | | | | | |

***Table 106**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog carbonmonoxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **7.187** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.36E-02 | 1.43E-02 | 1.49E-02 | 1.59E-02 | 1.47E-02 ± 5.7E-04 |
| 4.00E-04 | 1.37E-02 | 1.49E-02 | 1.54E-02 | 1.49E-02 | 1.47E-02 ± 4.4E-04 |
| 5.00E-04 | 1.57E-02 | 1.47E-02 | 1.57E-02 | 1.46E-02 | 1.52E-02 ± 2.9E-04 |
| 6.00E-04 | 1.46E-02 | 1.67E-02 | 1.67E-02 | 1.68E-02 | 1.62E-02 ± 5.7E-04 |
| Slope = 5.00E+00 ± 1.6E+00; Intercept = 1.29E-02 ± 7.5E-04 | | | | | |

***Table 107**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog carbonmonoxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **7.406** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.29E-02 | 1.34E-02 | 1.41E-02 | 1.45E-02 | 1.37E-02 ± 4.0E-04 |
| 4.00E-04 | 1.47E-02 | 1.51E-02 | 1.59E-02 | 1.61E-02 | 1.54E-02 ± 3.4E-04 |
| 5.00E-04 | 1.53E-02 | 1.59E-02 | 1.54E-02 | 1.55E-02 | 1.55E-02 ± 1.6E-04 |
| 6.00E-04 | 1.63E-02 | 1.71E-02 | 1.75E-02 | 1.69E-02 | 1.70E-02 ± 3.1E-04 |
| Slope = 9.85E+00 ± 2.2E+00; Intercept = 1.10E-02 ± 1.0E-03 | | | | | |

***Table 108**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog carbonmonoxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **7.614** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.39E-02 | 1.48E-02 | 1.56E-02 | 1.58E-02 | 1.50E-02 ± 4.7E-04* |
| 4.00E-04 | 1.38E-02 | 1.40E-02 | 1.48E-02 | 1.55E-02 | 1.45E-02 ± 4.4E-04 |
| 5.00E-04 | 1.73E-02 | 1.53E-02 | 1.54E-02 | 1.63E-02 | 1.60E-02 ± 5.0E-04 |
| 6.00E-04 | 1.51E-02 | 1.60E-02 | 1.74E-02 | 1.76E-02 | 1.65E-02 ± 6.3E-04 |
| Slope = 5.03E+00 ± 1.7E-01; Intercept = 1.35E-02 ± 8.4E-05 | | | | | |

***Table 109**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog carbonmonoxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **7.802** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.42E-02 | 1.44E-02 | 1.45E-02 | 1.48E-02 | 1.45E-02 ± 1.4E-04 |
| 4.00E-04 | 1.43E-02 | 1.43E-02 | 1.48E-02 | 1.48E-02 | 1.46E-02 ± 1.3E-04 |
| 5.00E-04 | 1.48E-02 | 1.58E-02 | 1.59E-02 | 1.39E-02 | 1.51E-02 ± 5.1E-04 |
| 6.00E-04 | 1.56E-02 | 1.58E-02 | 1.59E-02 | 1.59E-02 | 1.58E-02 ± 9.2E-05* |
| Slope = 3.12E+00 ± 1.2E+00; Intercept = 1.35E-02 ± 5.0E-04 | | | | | |

***Table 110**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog carbonmonoxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in borate buffer pH **8.016** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.30E-02 | 1.58E-02 | 1.42E-02 | 1.46E-02 | 1.44E-02 ± 6.9E-04 |
| 4.00E-04 | 1.40E-02 | 1.44E-02 | 1.50E-02 | 1.52E-02 | 1.47E-02 ± 3.0E-04 |
| 5.00E-04 | 1.62E-02 | 1.31E-02 | 1.44E-02 | 1.30E-02 | 1.42E-02 ± 8.1E-04* |
| 6.00E-04 | 1.44E-02 | 1.56E-02 | 1.32E-02 | 1.60E-02 | 1.48E-02 ± 7.1E-04 |
| Slope = 1.17E+00 ± 4.8E-01; Intercept = 1.41E-02 ± 2.2E-04 | | | | | |

***Table 111**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog carbonmonoxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in borate buffer pH **8.214** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.57E-02 | 1.60E-02 | 1.64E-02 | 1.68E-02 | 1.62E-02 ±2.7E-04 |
| 4.00E-04 | 1.20E-02 | 1.79E-02 | 1.79E-02 | 1.80E-02 | 1.64E-02 ±1.5E-03 |
| 5.00E-04 | 1.57E-02 | 1.58E-02 | 1.90E-02 | 1.58E-02 | 1.66E-02 ±8.3E-04 |
| 6.00E-04 | 1.72E-02 | 1.81E-02 | 1.72E-02 | 1.73E-02 | 1.75E-02 ± 2.2E-04 |
| Slope = 3.84E+00 ± 1.2E+00; Intercept = 1.49E-02 ± 5.5E-04 | | | | | |

***Table 112**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog carbonmonoxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in borate buffer pH **8.417** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.49E-02 | 1.66E-02 | 1.65E-02 | 1.68E-02 | 1.62E-02 ± 4.7E-04 |
| 4.00E-04 | 1.54E-02 | 1.67E-02 | 1.69E-02 | 1.69E-02 | 1.65E-02 ± 3.8E-04 |
| 5.00E-04 | 1.64E-02 | 1.68E-02 | 1.70E-02 | 1.67E-02 | 1.67E-02 ± 1.3E-04 |
| 6.00E-04 | 1.78E-02 | 1.82E-02 | 1.85E-02 | 1.85E-02 | 1.83E-02 ± 2.0E-04 |
| Slope = 6.44E+00 ± 2.2E+00; Intercept = 1.40E-02 ± 1.0E-03 | | | | | |

***Table 113**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog carbonmonoxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in borate buffer pH **8.603** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.49E-02 | 1.56E-02 | 1.57E-02 | 1.80E-02 | 1.60E-02 ± 7.8E-04 |
| 4.00E-04 | 1.42E-02 | 1.49E-02 | 1.53E-02 | 1.60E-02 | 1.51E-02 ± 4.3E-04* |
| 5.00E-04 | 1.54E-02 | 1.92E-02 | 1.56E-02 | 1.58E-02 | 1.65E-02 ± 9.5E-04 |
| 6.00E-04 | 1.52E-02 | 1.65E-02 | 1.83E-02 | 1.83E-02 | 1.71E-02 ± 7.9E-04 |
| Slope = 3.26E+00 ± 8.4E-01; Intercept = 1.50E-02 ± 4.1E-04 | | | | | |

***Table 114**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog carbonmonoxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in borate buffer pH **8.796** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.29E-02 | 1.33E-02 | 1.40E-02 | 1.42E-02 | 1.36E-02 ± 3.4E-04 |
| 4.00E-04 | 1.53E-02 | 1.54E-02 | 1.56E-02 | 1.58E-02 | 1.55E-02 ± 1.2E-04 |
| 5.00E-04 | 1.54E-02 | 1.55E-02 | 1.56E-02 | 1.58E-02 | 1.56E-02 ± 9.3E-05 |
| 6.00E-04 | 1.46E-02 | 1.56E-02 | 1.66E-02 | 1.76E-02 | 1.61E-02 ± 7.4E-04 |
| Slope = 7.57E+00 ± 2.8E+00; Intercept = 1.18E-02 ± 1.3E-03 | | | | | |

***Table 115**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog carbonmonoxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in borate buffer pH **8.988** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.43E-02 | 1.44E-02 | 1.44E-02 | 1.53E-02 | 1.46E-02 ± 2.5E-04 |
| 4.00E-04 | 1.44E-02 | 1.47E-02 | 1.47E-02 | 1.58E-02 | 1.49E-02 ± 3.7E-04 |
| 5.00E-04 | 1.63E-02 | 1.54E-02 | 1.54E-02 | 1.54E-02 | 1.56E-02 ± 2.4E-04 |
| 6.00E-04 | 1.49E-02 | 1.57E-02 | 1.58E-02 | 1.78E-02 | 1.60E-02 ± 7.3E-04 |
| Slope = 5.03E+00 ± 5.1E-01; Intercept = 1.30E-02 ± 2.4E-04 | | | | | |

***Table 116**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog aquomethaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **5.586** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.51E-02 | 1.36E-02 | 1.10E-02 | 1.09E-02 | 1.26E-02 ± 1.0E-03* |
| 4.00E-04 | 1.39E-02 | 1.42E-02 | 1.31E-02 | 1.32E-02 | 1.36E-02 ± 2.8E-04 |
| 5.00E-04 | 1.60E-02 | 1.18E-02 | 1.50E-02 | 1.34E-02 | 1.41E-02 ± 1.1E-03 |
| 6.00E-04 | 1.43E-02 | 1.56E-02 | 1.54E-02 | 1.12E-02 | 1.41E-02 ± 1.1E-03 |
| Slope = 2.64E+00 ± 1.2E+00; Intercept = 1.26E-02 ± 6.3E-04 | | | | | |

***Table 117**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog aquomethaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **5.825** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.21E-02 | 1.21E-02 | 1.39E-02 | 1.37E-02 | 1.30E-02 ± 4.3E-04 |
| 4.00E-04 | 1.21E-02 | 1.59E-02 | 1.52E-02 | 1.43E-02 | 1.44E-02 ± 9.5E-04 |
| 5.00E-04 | 1.39E-02 | 1.54E-02 | 1.45E-02 | 1.43E-02 | 1.45E-02 ± 3.7E-04 |
| 6.00E-04 | 1.54E-02 | 1.63E-02 | 1.44E-02 | 1.53E-02 | 1.54E-02 ± 4.8E-04 |
| Slope = 4.89E+00 ± 2.0E+00; Intercept = 1.23E-02 ± 1.0E-03 | | | | | |

***Table 118**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog aquomethaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **6.114** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.22E-02 | 1.49E-02 | 1.34E-02 | 1.41E-02 | 1.37E-02 ± 6.8E-04 |
| 4.00E-04 | 1.46E-02 | 1.30E-02 | 1.50E-02 | 1.29E-02 | 1.39E-02 ± 5.1E-04 |
| 5.00E-04 | 1.57E-02 | 1.55E-02 | 1.13E-02 | 1.38E-02 | 1.41E-02 ± 1.1E-03 |
| 6.00E-04 | 1.84E-02 | 1.46E-02 | 1.32E-02 | 1.33E-02 | 1.49E-02 ± 1.3E-03 |
| Slope = 3.88E+00 ± 1.1E+00; Intercept = 1.24E-02 ± 5.1E-04 | | | | | |

***Table 119**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog aquomethaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **6.212** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.14E-02 | 1.40E-02 | 1.33E-02 | 1.65E-02 | 1.38E-02 ± 1.3E-03 |
| 4.00E-04 | 1.82E-02 | 1.30E-02 | 1.49E-02 | 1.55E-02 | 1.54E-02 ± 1.3E-03 |
| 5.00E-04 | 1.69E-02 | 1.17E-02 | 1.23E-02 | 1.02E-02 | 1.28E-02 ± 1.7E-03* |
| 6.00E-04 | 1.41E-02 | 1.65E-02 | 1.67E-02 | 1.75E-02 | 1.62E-02 ± 8.6E-04 |
| Slope = 7.45E+00 ± 3.1E+00; Intercept = 1.19E-02 ± 1.4E-03 | | | | | |

***Table 120**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog aquomethaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **6.426** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.27E-02 | 1.36E-02 | 1.44E-02 | 1.58E-02 | 1.41E-02 ± 7.8E-04 |
| 4.00E-04 | 1.26E-02 | 1.41E-02 | 1.73E-02 | 1.63E-02 | 1.50E-02 ± 1.2E-03 |
| 5.00E-04 | 1.60E-02 | 1.71E-02 | 1.66E-02 | 1.70E-02 | 1.67E-02 ± 2.9E-04 |
| 6.00E-04 | 1.68E-02 | 1.77E-02 | 1.83E-02 | 1.69E-02 | 1.74E-02 ± 3.7E-04 |
| Slope = 1.16E+01 ± 1.1E+00; Intercept = 1.06E-02 ± 5.3E-04 | | | | | |

***Table 121**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog aquomethaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **6.571** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.24E-02 | 1.55E-02 | 1.32E-02 | 1.30E-02 | 1.35E-02 ± 7.7E-04 |
| 4.00E-04 | 1.49E-02 | 1.32E-02 | 1.53E-02 | 1.31E-02 | 1.41E-02 ± 5.5E-04 |
| 5.00E-04 | 1.77E-02 | 1.45E-02 | 1.59E-02 | 1.59E-02 | 1.60E-02 ± 7.9E-04 |
| 6.00E-04 | 1.61E-02 | 1.87E-02 | 1.58E-02 | 1.67E-02 | 1.68E-02 ± 7.2E-04 |
| Slope = 1.18E+01 ± 1.7E+00; Intercept = 9.80E-03 ± 7.9E-04 | | | | | |

***Table 122**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog aquomethaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **6.773** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.38E-02 | 1.23E-02 | 1.22E-02 | 1.39E-02 | 1.31E-02 ± 4.2E-04 |
| 4.00E-04 | 1.16E-02 | 1.21E-02 | 1.43E-02 | 1.52E-02 | 1.33E-02 ± 9.1E-04 |
| 5.00E-04 | 1.85E-02 | 1.15E-02 | 1.49E-02 | 1.54E-02 | 1.51E-02 ± 1.7E-03 |
| 6.00E-04 | 1.80E-02 | 1.22E-02 | 1.73E-02 | 1.82E-02 | 1.64E-02 ± 1.5E-03 |
| Slope = 1.19E+01 ± 2.3E+00; Intercept = 9.11E-03 ± 1.1E-03 | | | | | |

***Table 123**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog aquomethaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **6.986** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.33E-02 | 1.23E-02 | 1.39E-02 | 1.16E-02 | 1.28E-02 ± 5.8E-04 |
| 4.00E-04 | 2.08E-02 | 1.12E-02 | 1.29E-02 | 1.54E-02 | 1.51E-02 ± 2.4E-03 |
| 5.00E-04 | 2.15E-02 | 1.38E-02 | 1.41E-02 | 1.27E-02 | 1.55E-02 ± 2.2E-03 |
| 6.00E-04 | 1.70E-02 | 1.59E-02 | 1.40E-02 | 1.25E-02 | 1.48E-02 ± 1.1E-03* |
| Slope = 1.37E+01 ± 5.4E+00; Intercept = 8.98E-03 ± 2.2E-03 | | | | | |

***Table 124**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog aquomethaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **7.160** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 2.82E-02* | 1.16E-02 | 1.26E-02 | 1.36E-02 | 1.26E-02 ± 6.8E-04 |
| 4.00E-04 | 1.23E-02 | 1.10E-02 | 1.77E-02 | 1.24E-02 | 1.33E-02 ± 1.7E-03 |
| 5.00E-04 | 1.59E-02 | 1.47E-02 | 1.27E-02 | 1.35E-02 | 1.42E-02 ± 8.0E-04 |
| 6.00E-04 | 1.38E-02 | 2.27E-02 | 1.34E-02 | 1.52E-02 | 1.63E-02 ± 2.3E-03 |
| Slope = 1.19E+01 ± 2.3E+00; Intercept = 8.76E-03 ± 1.1E-03 | | | | | |

***Table 125**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog aquomethaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **7.354** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.31E-02 | 1.43E-02 | 1.44E-02 | 1.28E-02 | 1.36E-02 ± 4.2E-04 |
| 4.00E-04 | 1.42E-02 | 1.41E-02 | 1.67E-02 | 1.29E-02 | 1.45E-02 ± 9.3E-04 |
| 5.00E-04 | 1.38E-02 | 1.75E-02 | 1.39E-02 | 1.44E-02 | 1.49E-02 ± 9.3E-04 |
| 6.00E-04 | 1.38E-02 | 1.36E-02 | 2.99E-02 | 1.66E-02 | 1.85E-02 ± 4.1E-03 |
| Slope = 1.49E+01 ± 5.0E+00; Intercept = 8.68E-03 ± 2.3E-03 | | | | | |

***Table 126**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog aquomethaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **7.576** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.58E-02 | 1.60E-02 | 6.63E-03 | 1.52E-02 | 1.34E-02 ± 2.3E-03 |
| 4.00E-04 | 1.18E-02 | 1.49E-02 | 1.48E-02 | 1.39E-02 | 1.39E-02 ± 7.7E-04 |
| 5.00E-04 | 1.49E-02 | 2.55E-02 | 1.48E-02 | 9.25E-03 | 1.61E-02 ± 4.1E-03 |
| 6.00E-04 | 1.79E-02 | 1.57E-02 | 1.61E-02 | 1.65E-02 | 1.65E-02 ± 5.6E-04 |
| Slope =1.18E+01 ± 2.5E+00; Intercept = 9.68E-03 ± 1.2E-03 | | | | | |

***Table 127**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog aquomethaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **7.764** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.54E-02 | 1.28E-02 | 1.27E-02 | 1.02E-02 | 1.28E-02 ± 1.3E-03 |
| 4.00E-04 | 1.39E-02 | 1.65E-02 | 1.61E-02 | 1.37E-02 | 1.51E-02 ± 7.0E-04 |
| 5.00E-04 | 1.74E-02 | 1.24E-02 | 1.28E-02 | 1.83E-02 | 1.52E-02 ± 1.5E-03 |
| 6.00E-04 | 1.41E-02 | 2.00E-02 | 1.57E-02 | 1.68E-02 | 1.67E-02 ± 1.5E-03 |
| Slope = 1.18E+01 ± 2.8E+00; Intercept = 9.63E-03 ± 1.3E-03 | | | | | |

***Table 128**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog aquomethaemoglobin** in the presence of **inositol-$P_6$** at 25°C in borate buffer pH **7.968** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 9.97E-03 | 1.42E-02 | 1.20E-02 | 1.28E-02 | 1.22E-02 ± 1.1E-03 |
| 4.00E-04 | 1.50E-02 | 1.36E-02 | 1.41E-02 | 1.59E-02 | 1.46E-02 ± 5.7E-04 |
| 5.00E-04 | 1.85E-02 | 1.58E-02 | 1.80E-02 | 1.91E-02 | 1.79E-02 ± 8.3E-04 |
| 6.00E-04 | 3.79E-02 | 1.47E-02 | 1.46E-02 | 1.36E-02 | 2.02E-02 ± 6.1E-03 |
| Slope = 2.71E+01 ± 1.3E+00; Intercept = 4.02E-03 ± 6.0E-04 | | | | | |

***Table 129**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog aquomethaemoglobin** in the presence of **inositol-$P_6$** at 25°C in borate buffer pH **8.175** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.22E-02 | 1.31E-02 | 1.31E-02 | 1.38E-02 | 1.30E-02 ± 4.1E-04 |
| 4.00E-04 | 1.29E-02 | 1.34E-02 | 1.43E-02 | 1.16E-02 | 1.30E-02 ± 6.6E-04 |
| 5.00E-04 | 1.43E-02 | 1.16E-02 | 1.22E-02 | 1.43E-02 | 1.31E-02 ± 6.9E-04 |
| 6.00E-04 | 1.53E-02 | 1.50E-02 | 1.19E-02 | 1.22E-02 | 1.36E-02 ± 8.5E-04* |
| Slope = 3.35E-01 ± 1.5E-01; Intercept = 1.29E-02 ± 6.2E-05 | | | | | |

***Table 130**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog aquomethaemoglobin** in the presence of **inositol-$P_6$** at 25°C in borate buffer pH **8.407** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.80E-02 | 1.31E-02 | 1.20E-02 | 1.33E-02 | 1.41E-02 ± 1.5E-03 |
| 4.00E-04 | 1.49E-02 | 1.35E-02 | 1.43E-02 | 1.40E-02 | 1.42E-02 ± 3.6E-04 |
| 5.00E-04 | 1.35E-02 | 1.46E-02 | 1.76E-02 | 1.55E-02 | 1.53E-02 ± 1.0E-03 |
| 6.00E-04 | 1.85E-02 | 1.67E-02 | 1.75E-02 | 1.86E-02 | 1.78E-02 ± 5.0E-04 |
| Slope = 1.24E+01 ± 3.9E+00; Intercept = 9.76E-03 ± 1.8E-03 | | | | | |

***Table 131**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog aquomethaemoglobin** in the presence of **inositol-$P_6$** at 25°C in borate buffer pH **8.582** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.28E-02 | 1.48E-02 | 1.38E-02 | 1.38E-02 | 1.38E-02 ± 5.0E-04 |
| 4.00E-04 | 1.25E-02 | 1.41E-02 | 1.24E-02 | 1.68E-02 | 1.39E-02 ± 1.1E-03 |
| 5.00E-04 | 5.84E-02 | 1.32E-02 | 1.52E-02 | 1.55E-02 | 2.56E-02 ± 1.1E-02 |
| 6.00E-04 | 1.70E-02 | 1.92E-02 | 1.71E-02 | 1.91E-02 | 1.81E-02 ± 5.4E-04 |
| Slope = 1.36E+01 ± 5.5E+00; Intercept = 8.98E-03 ± 2.6E-03 | | | | | |

***Table 132**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog aquomethaemoglobin** in the presence of **inositol-$P_6$** at 25°C in borate buffer pH **8787** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.38E-02 | 1.68E-02 | 1.38E-02 | 1.45E-02 | 1.47E-02 ± 7.6E-04 |
| 4.00E-04 | 1.65E-02 | 1.91E-02 | 1.24E-02 | 1.28E-02 | 1.52E-02 ± 1.7E-03 |
| 5.00E-04 | 1.59E-02 | 1.55E-02 | 1.58E-02 | 1.65E-02 | 1.59E-02 ± 2.4E-04 |
| 6.00E-04 | 1.63E-02 | 1.98E-02 | 1.84E-02 | 1.88E-02 | 1.84E-02 ± 8.9E-04 |
| Slope = 1.16E+01 ± 3.2E+00; Intercept = 1.08E-02 ± 1.5E-03 | | | | | |

***Table 133**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **dog aquomethaemoglobin** in the presence of **inositol-$P_6$** at 25°C in borate buffer pH **9.001** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.46E-02 | 1.52E-02 | 1.32E-02 | 1.41E-02 | 1.43E-02 ± 4.8E-04 |
| 4.00E-04 | 1.66E-02 | 1.54E-02 | 1.40E-02 | 1.52E-02 | 1.53E-02 ± 6.6E-04 |
| 5.00E-04 | 1.25E-02 | 1.53E-02 | 1.53E-02 | 1.76E-02 | 1.52E-02 ± 1.3E-03 |
| 6.00E-04 | 1.88E-02 | 1.87E-02 | 1.72E-02 | 1.83E-02 | 1.82E-02 ± 4.0E-04 |
| Slope = 1.18E+01 ± 4.4E+00; Intercept = 1.04E-02 ± 2.1E-03 | | | | | |

***Table 134**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey oxyhaemoglobin** at 25°C in phosphate buffer pH **5.616** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.47E-02 | 1.57E-02 | 1.63E-02 | 1.75E-02 | 1.61E-02 ± 7.0E-04 |
| 4.00E-04 | 1.55E-02 | 1.55E-02 | 1.65E-02 | 1.70E-02 | 1.61E-02 ± 5.3E-04 |
| 5.00E-04 | 1.53E-02 | 1.59E-02 | 1.66E-02 | 1.70E-02 | 1.62E-02 ± 4.4E-04 |
| 6.00E-04 | 1.65E-02 | 1.76E-02 | 2.01E-02 | 2.22E-02 | 1.91E-02 ± 1.4E-03* |
| Slope = 7.50E-01 ± 9.8E-02; Intercept = 1.58E-02 ± 4.0E-05 | | | | | |

***Table 135**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey oxyhaemoglobin** at 25°C in phosphate buffer pH **5.831** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.35E-02 | 1.43E-02 | 1.67E-02 | 1.75E-02 | 1.55E-02 ± 1.0E-03 |
| 4.00E-04 | 1.42E-02 | 1.55E-02 | 1.56E-02 | 1.70E-02 | 1.56E-02 ± 9.2E-04 |
| 5.00E-04 | 1.39E-02 | 1.66E-02 | 1.70E-02 | 2.05E-02 | 1.70E-02 ± 1.7E-03 |
| 6.00E-04 | 1.82E-02 | 1.59E-02 | 1.65E-02 | 2.35E-02 | 1.85E-02 ± 1.9E-03 |
| Slope = 1.04E+01 ± 2.4E+00; Intercept = 1.20E-02 ± 1.1E-03 | | | | | |

***Table 136**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey oxyhaemoglobin** at 25°C in phosphate buffer pH **6.0740** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.33E-02 | 1.55E-02 | 1.67E-02 | 1.73E-02 | 1.57E-02 ± 9.9E-04 |
| 4.00E-04 | 1.46E-02 | 1.59E-02 | 1.64E-02 | 1.68E-02 | 1.59E-02 ± 7.2E-04 |
| 5.00E-04 | 1.71E-02 | 1.49E-02 | 1.65E-02 | 1.75E-02 | 1.65E-02 ± 6.6E-04 |
| 6.00E-04 | 1.47E-02 | 1.67E-02 | 1.80E-02 | 1.83E-02 | 1.69E-02 ± 9.0E-04 |
| Slope = 4.18E+00 ± 5.0E-01; Intercept = 1.44E-02 ± 2.3E-04 | | | | | |

***Table 137**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey oxyhaemoglobin** at 25°C in phosphate buffer pH **6.212** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.42E-02 | 1.45E-02 | 1.55E-02 | 1.65E-02 | 1.52E-02 ± 5.9E-04 |
| 4.00E-04 | 1.47E-02 | 1.47E-02 | 1.57E-02 | 1.68E-02 | 1.55E-02 ± 7.1E-04 |
| 5.00E-04 | 1.38E-02 | 1.57E-02 | 1.65E-02 | 1.71E-02 | 1.58E-02 ± 8.3E-04 |
| 6.00E-04 | 1.54E-02 | 1.60E-02 | 1.62E-02 | 1.73E-02 | 1.62E-02 ± 4.8E-04 |
| Slope = 3.50E+00 ± 2.7E-01; Intercept = 1.41E-02 ± 1.3E-04 | | | | | |

***Table 138**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey oxyhaemoglobin** at*

*25°C in phosphate buffer pH **6.400** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.41E-02 | 1.46E-02 | 1.51E-02 | 1.72E-02 | 1.52E-02 ± 7.9E-04 |
| 4.00E-04 | 1.49E-02 | 1.50E-02 | 1.61E-02 | 1.72E-02 | 1.58E-02 ± 7.6E-04 |
| 5.00E-04 | 1.44E-02 | 1.65E-02 | 1.79E-02 | 1.83E-02 | 1.68E-02 ± 9.7E-04 |
| 6.00E-04 | 5.69E-02* | 1.64E-02 | 1.75E-02 | 1.86E-02 | 1.75E-02 ± 7.3E-04 |
| Slope = 7.64E+00 ± 5.2E-01; Intercept = 1.29E-02 ± 2.4E-04 | | | | | |

***Table 139**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey oxyhaemoglobin** at 25°C in phosphate buffer pH **6.594** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.22E-02 | 1.44E-02 | 1.58E-02 | 1.72E-02 | 1.49E-02 ± 1.2E-03 |
| 4.00E-04 | 1.32E-02 | 1.43E-02 | 1.58E-02 | 1.84E-02 | 1.54E-02 ± 1.7E-03 |
| 5.00E-04 | 1.44E-02 | 1.52E-02 | 1.59E-02 | 1.77E-02 | 1.58E-02 ± 8.3E-04 |
| 6.00E-04 | 1.59E-02 | 1.69E-02 | 1.83E-02 | 2.07E-02 | 1.80E-02 ± 1.2E-03 |
| Slope = 9.65E+00 ± 3.0E+00; Intercept = 1.17E-02 ± 1.4E-03 | | | | | |

***Table 140**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey oxyhaemoglobin** at 25°C in phosphate buffer pH **6.807** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.33E-02 | 1.48E-02 | 1.52E-02 | 1.61E-02 | 1.49E-02 ± 7.1E-04 |
| 4.00E-04 | 1.48E-02 | 1.54E-02 | 1.65E-02 | 1.57E-02 | 1.56E-02 ± 5.8E-04 |
| 5.00E-04 | 1.31E-02 | 1.59E-02 | 1.63E-02 | 1.90E-02 | 1.60E-02 ± 1.5E-03 |
| 6.00E-04 | 1.55E-02 | 1.61E-02 | 1.73E-02 | 1.85E-02 | 1.68E-02 ± 7.5E-04 |
| Slope = 6.45E+00 ± 4.6E-01; Intercept = 1.29E-02 ± 2.1E-04 | | | | | |

***Table 141**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey oxyhaemoglobin** at 25°C in phosphate buffer pH **6.997** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.37E-02 | 1.46E-02 | 1.48E-02 | 1.70E-02 | 1.50E-02 ± 8.2E-04 |
| 4.00E-04 | 1.49E-02 | 1.56E-02 | 1.62E-02 | 1.63E-02 | 1.57E-02 ± 4.5E-04 |
| 5.00E-04 | 1.52E-02 | 1.62E-02 | 1.77E-02 | 1.88E-02 | 1.70E-02 ± 9.1E-04 |
| 6.00E-04 | 1.99E-02 | 1.55E-02 | 1.74E-02 | 1.77E-02 | 1.76E-02 ± 1.1E-03 |
| Slope = 8.16E+00 ± 1.6E+00; Intercept = 1.25E-02 ± 7.5E-04 | | | | | |

***Table 142:** The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey oxyhaemoglobin** at 25°C in phosphate buffer pH **7223** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.48E-02 | 1.52E-02 | 1.55E-02 | 1.64E-02 | 1.55E-02 ± 4.0E-04 |
| 4.00E-04 | 1.66E-02 | 1.57E-02 | 1.54E-02 | 1.58E-02 | 1.59E-02 ± 3.9E-04 |
| 5.00E-04 | 1.71E-02 | 1.92E-02 | 1.62E-02 | 1.49E-02 | 1.69E-02 ± 1.1E-03 |
| 6.00E-04 | 1.54E-02 | 2.44E-02 | 1.66E-02 | 1.53E-02 | 1.79E-02 ± 2.3E-03 |
| Slope = 8.36E+00 ± 1.1E+00; Intercept = 1.28E-02 ± 5.0E-04 | | | | | |

***Table 143**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey oxyhaemoglobin** at 25°C in phosphate buffer pH **7.410** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 8.15E-03* | 1.39E-02 | 1.56E-02 | 1.44E-02 | 1.46E-02 ± 5.8E-04 |
| 4.00E-04 | 1.34E-02 | 1.58E-02 | 1.31E-02 | 1.60E-02 | 1.46E-02 ± 9.7E-04 |
| 5.00E-04 | 1.64E-02 | 1.90E-02 | 1.48E-02 | 1.54E-02 | 1.64E-02 ± 1.1E-03 |
| 6.00E-04 | 1.60E-02 | 1.92E-02 | 1.48E-02 | 1.63E-02 | 1.66E-02 ± 1.1E-03 |
| Slope = 7.64E+00 ± 2.6E+00; Intercept = 1.21E-02 ± 1.2E-03 | | | | | |

***Table 144**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey oxyhaemoglobin** at 25°C in phosphate buffer pH **7.612** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.37E-02 | 1.79E-02 | 1.64E-02 | 1.54E-02 | 1.58E-02 ± 1.1E-03 |
| 4.00E-04 | 1.63E-02 | 1.65E-02 | 1.58E-02 | 1.69E-02 | 1.64E-02 ± 3.7E-04 |
| 5.00E-04 | 7.98E-02* | 1.82E-02 | 1.59E-02 | 1.86E-02 | 1.76E-02 ± 8.9E-04 |
| 6.00E-04 | 2.19E-02 | 1.50E-02 | 2.15E-02 | 2.17E-02 | 2.00E-02 ± 1.7E-03 |
| Slope = 8.61E+00 ± 2.0E+00; Intercept = 1.31E-02 ± 8.0E-04 | | | | | |

***Table 145**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey oxyhaemoglobin** at 25°C in phosphate buffer pH **7.768** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.54E-02 | 1.55E-02 | 1.57E-02 | 1.58E-02 | 1.56E-02 ± 9.7E-05 |
| 4.00E-04 | 1.73E-02 | 1.45E-02 | 1.77E-02 | 1.54E-02 | 1.62E-02 ± 1.1E-03 |
| 5.00E-04 | 1.58E-02 | 1.57E-02 | 1.75E-02 | 1.79E-02 | 1.67E-02 ± 5.4E-04 |
| 6.00E-04 | 1.38E-02 | 1.87E-02 | 1.88E-02 | 1.89E-02 | 1.75E-02 ± 1.3E-03 |
| Slope = 6.27E+00 ± 4.1E-01; Intercept = 1.37E-02 ± 1.9E-04 | | | | | |

***Table 146**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey oxyhaemoglobin** at 25°C in borate buffer pH **8.007** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.60E-02 | 1.34E-02 | 1.42E-02 | 1.39E-02 | 1.44E-02 ± 6.4E-04 |
| 4.00E-04 | 1.55E-02 | 1.89E-02 | 1.63E-02 | 1.43E-02 | 1.63E-02 ± 1.5E-03 |
| 5.00E-04 | 1.58E-02 | 1.91E-02 | 1.96E-02 | 1.23E-02 | 1.67E-02 ± 1.8E-03 |
| 6.00E-04 | 1.55E-02 | 1.70E-02 | 1.73E-02 | 1.74E-02 | 1.68E-02 ± 4.8E-04 |
| Slope = 7.77E+00 ± 2.9E+00; Intercept = 1.25E-02 ± 1.4E-03 | | | | | |

***Table 147**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey oxyhaemoglobin** at 25°C in borate buffer pH **8.169** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.69E-02 | 1.75E-02 | 1.47E-02 | 1.22E-02 | 1.53E-02 ± 1.3E-03 |
| 4.00E-04 | 1.54E-02 | 1.43E-02 | 1.42E-02 | 1.75E-02 | 1.53E-02 ± 1.1E-03 |
| 5.00E-04 | 1.60E-02 | 1.55E-02 | 1.89E-02 | 1.50E-02 | 1.63E-02 ± 9.8E-04 |
| 6.00E-04 | 1.62E-02 | 1.96E-02 | 1.61E-02 | 1.77E-02 | 1.74E-02 ± 8.9E-04 |
| Slope = 7.22E+00 ± 1.8E+00; Intercept = 1.28E-02 ± 8.3E-04 | | | | | |

***Table 148**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey oxyhaemoglobin** at 25°C in borate buffer pH **8.408** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.40E-02 | 1.32E-02 | 1.24E-02 | 1.55E-02 | 1.38E-02 ± 7.9E-04 |
| 4.00E-04 | 1.49E-02 | 1.72E-02 | 1.65E-02 | 1.32E-02 | 1.55E-02 ± 1.3E-03 |
| 5.00E-04 | 1.32E-02 | 1.59E-02 | 1.54E-02 | 1.81E-02 | 1.56E-02 ± 1.2E-03 |
| 6.00E-04 | 1.62E-02 | 1.73E-02 | 1.74E-02 | 1.61E-02 | 1.67E-02 ± 3.3E-04 |
| Slope = 9.03E+00 ± 2.0E+00; Intercept = 1.13E-02 ± 9.3E-04 | | | | | |

***Table 149**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey oxyhaemoglobin** at 25°C in borate buffer pH **8.606** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.53E-02 | 1.46E-02 | 1.42E-02 | 1.54E-02 | 1.48E-02 ± 2.9E-04 |
| 4.00E-04 | 1.41E-02 | 1.58E-02 | 1.68E-02 | 1.72E-02 | 1.60E-02 ± 1.0E-03 |
| 5.00E-04 | 1.60E-02 | 1.67E-02 | 1.61E-02 | 1.62E-02 | 1.63E-02 ± 1.8E-04 |
| 6.00E-04 | 1.61E-02 | 1.79E-02 | 1.71E-02 | 1.71E-02 | 1.71E-02 ± 4.6E-04 |
| Slope = 6.93E+00 ± 1.1E+00; Intercept = 1.29E-02 ± 5.1E-04 | | | | | |

***Table 150**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey oxyhaemoglobin** at 25°C in borate buffer pH **8.858** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.52E-02 | 1.48E-02 | | | 1.50E-02 ± 2.0E-04 |
| 4.00E-04 | 1.63E-02 | 1.64E-02 | 1.52E-02 | 1.51E-02 | 1.57E-02 ± 4.2E-04 |
| 5.00E-04 | 1.80E-02 | 1.61E-02 | 1.47E-02 | 1.55E-02 | 1.60E-02 ± 8.3E-04 |
| 6.00E-04 | 1.64E-02 | 1.85E-02 | 1.78E-02 | 1.79E-02 | 1.76E-02 ± 5.2E-04 |
| Slope = 8.16E+00 ± 1.8E+00; Intercept = 1.24E-02 ± 8.3E-04 | | | | | |

***Table 151**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey oxyhaemoglobin** at 25°C in borate buffer pH **8.996** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.15E-02 | 1.34E-02 | 1.47E-02 | 1.51E-02 | 1.37E-02 ± 9.2E-04 |
| 4.00E-04 | 1.21E-02 | 1.32E-02 | 1.62E-02 | 1.90E-02 | 1.51E-02 ± 2.3E-03 |
| 5.00E-04 | 1.38E-02 | 1.55E-02 | 1.72E-02 | 1.84E-02 | 1.62E-02 ± 1.2E-03 |
| 6.00E-04 | 1.34E-02 | 1.61E-02 | 1.74E-02 | 1.83E-02 | 1.63E-02 ± 1.2E-03 |
| Slope = 9.02E+00 ± 2.1E+00; Intercept = 1.13E-02 ± 9.9E-04 | | | | | |

***Table 152**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey carbonmonoxyhaemoglobin** at 25°C in phosphate buffer pH **5.704** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 9.13E-03 | 1.29E-02 | 1.40E-02 | 1.67E-02 | 1.32E-02 ± 1.9E-03 |
| 4.00E-04 | 1.34E-02 | 1.42E-02 | 1.49E-02 | 1.53E-02 | 1.45E-02 ± 6.2E-04 |
| 5.00E-04 | 1.36E-02 | 1.47E-02 | 1.53E-02 | 1.73E-02 | 1.52E-02 ± 9.0E-04 |
| 6.00E-04 | 1.12E-02 | 1.38E-02 | 1.53E-02 | 1.69E-02 | 1.43E-02 ± 1.42E-03* |
| Slope = 1.02E+01 ± 1.5E+00; Intercept = 1.02E-02 ± 6.2E-04 | | | | | |

***Table 153**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey carbonmonoxyhaemoglobin** at 25°C in phosphate buffer pH **5.872** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.04E-02 | 1.32E-02 | 1.62E-02 | 1.96E-02 | 1.49E-02 ± 2.3E-03 |
| 4.00E-04 | 1.20E-02 | 1.46E-02 | 1.59E-02 | 1.76E-02 | 1.50E-02 ± 1.9E-03 |
| 5.00E-04 | 1.37E-02 | 1.44E-02 | 1.59E-02 | 1.63E-02 | 1.51E-02 ± 6.6E-04* |
| 6.00E-04 | 1.44E-02 | 1.51E-02 | 1.61E-02 | 1.87E-02 | 1.61E-02 ± 1.1E-03 |
| Slope = 4.24E+00 ± 9.7E-01; Intercept = 1.35E-02 ± 4.4E-04 | | | | | |

***Table 154**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey carbonmonoxyhaemoglobin** at 25°C in phosphate buffer pH **6.049** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.49E-02 | 1.60E-02 | 1.64E-02 | 1.81E-02 | 1.63E-02 ± 8.0E-04 |
| 4.00E-04 | 1.76E-02 | 1.57E-02 | 1.67E-02 | 1.77E-02 | 1.69E-02 ± 6.8E-04 |
| 5.00E-04 | 1.77E-02 | 1.55E-02 | 1.66E-02 | 1.98E-02 | 1.74E-02 ± 1.1E-03 |
| 6.00E-04 | 1.84E-02 | 1.63E-02 | 1.86E-02 | 1.96E-02 | 1.82E-02 ± 8.2E-04 |
| Slope = 6.12E+00 ± 4.7E-01; Intercept = 1.45E-02 ± 2.2E-04 | | | | | |

***Table 155**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey carbonmonoxyhaemoglobin** at 25°C in phosphate buffer pH **6.206** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.28E-02 | 1.46E-02 | 1.63E-02 | 1.72E-02 | 1.52E-02 ± 1.1E-03 |
| 4.00E-04 | 1.50E-02 | 1.63E-02 | 1.63E-02 | 1.64E-02 | 1.60E-02 ± 4.5E-04 |
| 5.00E-04 | 1.51E-02 | 1.53E-02 | 1.73E-02 | 2.00E-02 | 1.69E-02 ± 1.2E-03 |
| 6.00E-04 | 1.27E-02 | 1.38E-02 | 1.65E-02 | 2.73E-02 | 1.76E-02 ± 3.6E-03 |
| Slope = 8.08E+00 ± 3.5E-01; Intercept = 1.28E-02 ± 1.6E-04 | | | | | |

***Table 156**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey carbonmonoxyhaemoglobin** at 25°C in phosphate buffer pH **6.404** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.18E-02 | 1.16E-02 | 1.56E-02 | 1.69E-02 | 1.40E-02 ± 1.3E-03 |
| 4.00E-04 | 1.06E-02 | 1.24E-02 | 1.60E-02 | 1.77E-02 | 1.42E-02 ± 2.4E-03 |
| 5.00E-04 | 1.30E-02 | 1.50E-02 | 1.61E-02 | 1.87E-02 | 1.57E-02 ± 1.4E-03 |
| 6.00E-04 | 1.34E-02 | 1.64E-02 | 1.76E-02 | 2.54E-02 | 1.82E-02 ± 3.0E-03 |
| Slope = 1.43E+01 ± 3.7E+00; Intercept = 9.09E-03 ± 1.7E-03 | | | | | |

***Table 157**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey carbonmonoxyhaemoglobin** at 25°C in phosphate buffer pH **6.554** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.16E-02 | 1.36E-02 | 1.66E-02 | 1.96E-02 | 1.53E-02 ± 2.0E-03 |
| 4.00E-04 | 1.35E-02 | 1.45E-02 | 1.45E-02 | 1.75E-02 | 1.50E-02 ± 1.0E-03* |
| 5.00E-04 | 1.33E-02 | 1.58E-02 | 1.80E-02 | 1.86E-02 | 1.64E-02 ± 1.3E-03 |
| 6.00E-04 | 1.68E-02 | 1.90E-02 | 1.90E-02 | 2.60E-02 | 2.02E-02 ± 2.3E-03 |
| Slope = 1.47E+01 ± 8.1E+00; Intercept = 1.04E-02 ± 3.9E-03 | | | | | |

***Table 158**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey carbonmonoxyhaemoglobin** at 25°C in phosphate buffer pH **6.827** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.56E-02 | 1.76E-02 | 1.80E-02 | 1.90E-02 | 1.75E-02 ± 8.5E-04 |
| 4.00E-04 | 1.66E-02 | 1.76E-02 | 1.91E-02 | 1.99E-02 | 1.83E-02 ± 1.1E-03 |
| 5.00E-04 | 1.34E-02 | 1.55E-02 | 1.85E-02 | 2.02E-02 | 1.69E-02 ± 1.7E-03* |
| 6.00E-04 | 1.65E-02 | 1.90E-02 | 2.54E-02 | 2.67E-02 | 2.19E-02 ± 2.6E-03 |
| Slope = 1.50E+01 ± 2.7E+00; Intercept = 1.27E-02 ± 1.2E-03 | | | | | |

***Table 159**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey carbonmonoxyhaemoglobin** at 25°C in phosphate buffer pH **7.087** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 6.74E-03 | 1.12E-02 | 1.27E-02 | 1.38E-02 | 1.11E-02 ± 1.8E-03 |
| 4.00E-04 | 1.12E-02 | 1.35E-02 | 1.63E-02 | 1.84E-02 | 1.48E-02 ± 2.4E-03 |
| 5.00E-04 | 9.85E-03 | 1.57E-02 | 1.62E-02 | 1.84E-02 | 1.50E-02 ± 2.1E-03 |
| 6.00E-04 | 1.22E-02 | 1.32E-02 | 1.34E-02 | 2.36E-02 | 1.56E-02 ± 2.9E-03 |
| Slope = 1.36E+01 ± 5.7E+00; Intercept = 8.04E-03 ± 2.7E-03 | | | | | |

***Table 160**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey carbonmonoxyhaemoglobin** at 25°C in phosphate buffer pH **7.228** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.04E-02 | 1.05E-02 | 1.25E-02 | 1.33E-02 | 1.16E-02 ± 7.2E-04 |
| 4.00E-04 | 1.30E-02 | 1.97E-02 | 1.20E-02 | 1.21E-02 | 1.42E-02 ± 2.6E-03 |
| 5.00E-04 | 1.30E-02 | 1.41E-02 | 1.58E-02 | 1.62E-02 | 1.48E-02 ± 8.1E-04 |
| 6.00E-04 | 1.37E-02 | 1.39E-02 | 1.54E-02 | 2.01E-02 | 1.58E-02 ± 1.6E-03 |
| Slope = 1.30E+01 ± 3.0E+00; Intercept = 8.23E-03 ± 1.4E-03 | | | | | |

***Table 161**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey carbonmonoxyhaemoglobin** at 25°C in phosphate buffer pH **7.380** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 2.45E-02 | 3.57E-02 | 3.91E-02 | 5.25E-02 | 3.80E-02 ± 7.0E-03 |
| 4.00E-04 | 1.55E-02 | 3.55E-02 | 4.26E-02 | 6.42E-02 | 3.94E-02 ± 1.6E-02 |
| 5.00E-04 | 2.45E-02 | 3.57E-02 | 3.85E-02 | 5.21E-02 | 3.77E-02 ± 6.9E-03* |
| 6.00E-04 | 1.48E-02 | 1.57E-02 | 5.83E-02 | 9.27E-02 | 4.54E-02 ± 1.9E-02 |
| Slope = 2.54E+01 ± 3.7E+00; Intercept = 2.99E-02 ± 1.7E-03 | | | | | |

***Table 162**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey carbonmonoxyhaemoglobin** at 25°C in phosphate buffer pH **7.570** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.29E-02 | 1.51E-02 | 1.57E-02 | 1.77E-02 | 1.54E-02 ± 1.2E-03 |
| 4.00E-04 | 1.32E-02 | 1.50E-02 | 1.57E-02 | 2.09E-02 | 1.62E-02 ± 2.6E-03 |
| 5.00E-04 | 1.50E-02 | 1.55E-02 | 1.75E-02 | 1.88E-02 | 1.67E-02 ± 9.5E-04 |
| 6.00E-04 | 1.91E-02 | 2.00E-02 | 2.16E-02 | 1.89E-02 | 1.99E-02 ± 6.8E-04 |
| Slope = 1.40E+01 ± 4.3E+00; Intercept = 1.07E-02 ± 2.0E-03 | | | | | |

***Table 163**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey carbonmonoxyhaemoglobin** at 25°C in phosphate buffer pH **7.824** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.21E-02 | 1.29E-02 | 1.40E-02 | 2.15E-02 | 1.51E-02 ± 2.3E-03 |
| 4.00E-04 | 1.25E-02 | 1.49E-02 | 1.67E-02 | 1.71E-02 | 1.53E-02 ± 1.5E-03 |
| 5.00E-04 | 2.32E-02 | 5.42E-02 | 6.52E-02 | 9.56E-02 | 5.95E-02 ± 1.8E-02* |
| 6.00E-04 | 1.97E-02 | 2.65E-02 | 3.59E-02 | 3.74E-02 | 2.99E-02 ± 4.4E-03 |
| Slope = 5.25E+01 ± 1.8E+01; Intercept = 2.68E-03 ± 8.0E-03 | | | | | |

***Table 164**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey carbonmonoxyhaemoglobin** at 25°C in borate buffer pH **8.059** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.15E-02 | 1.33E-02 | 1.63E-02 | 1.73E-02 | 1.46E-02 ± 1.5E-03 |
| 4.00E-04 | 1.33E-02 | 1.53E-02 | 1.73E-02 | 1.86E-02 | 1.61E-02 ± 1.8E-03 |
| 5.00E-04 | 1.59E-02 | 1.73E-02 | 1.80E-02 | 2.19E-02* | 1.71E-02 ± 6.8E-04 |
| 6.00E-04 | 1.63E-02 | 1.82E-02 | 2.15E-02 | 1.91E-02 | 1.88E-02 ± 1.3E-03 |
| Slope = 1.36E+01 ± 1.0E+00; Intercept = 1.05E-02 ± 4.6E-04 | | | | | |

***Table 165**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey carbonmonoxyhaemoglobin** at 25°C in borate buffer pH **8.144** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 9.23E-03 | 1.25E-02 | 1.44E-02 | 1.64E-02 | 1.31E -02 ± 1.8E-03 |
| 4.00E-04 | 1.16E-02 | 1.45E-02 | 1.59E-02 | 1.81E-02 | 1.50E-02 ± 2.1E-03 |
| 5.00E-04 | 1.29E-02 | 1.54E-02 | 1.72E-02 | 1.92E-02 | 1.62E-02 ± 1.6E-03 |
| 6.00E-04 | 1.30E-02 | 1.51E-02 | 1.81E-02 | 2.06E-02 | 1.67E-02 ± 1.9E-03 |
| Slope = 1.18E+01 ± 2.2E+00; Intercept = 9.93E-03 ± 1.0E-03 | | | | | |

***Table 166**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey carbonmonoxyhaemoglobin** at 25°C in borate buffer pH **8.364** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.22E-02 | 1.36E-02 | 1.44E-02 | 1.51E-02 | 1.38E-02 ± 7.3E-04 |
| 4.00E-04 | 1.29E-02 | 1.51E-02 | 1.76E-02 | 1.88E-02 | 1.61E-02 ± 2.0E-03 |
| 5.00E-04 | 1.03E-02 | 1.25E-02 | 1.60E-02 | 1.74E-02 | 1.41E-02 ± 1.8E-03* |
| 6.00E-04 | 1.21E-02 | 1.35E-02 | 1.83E-02 | 2.28E-02 | 1.67E-02 ± 2.7E-03 |
| Slope = 8.56E+00 ± 4.9E+00; Intercept = 1.18E-02 ± 2.2E-03 | | | | | |

***Table 167**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey carbonmonoxyhaemoglobin** at 25°C in borate buffer pH **8.552** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.24E-02 | 1.31E-02 | 1.51E-02 | 1.61E-02 | 1.42E-02 ± 9.3E-04 |
| 4.00E-04 | 1.45E-02 | 1.63E-02 | 1.79E-02 | 1.97E-02 | 1.71E-02 ± 1.7E-03 |
| 5.00E-04 | 1.61E-02 | 1.92E-02 | 1.98E-02 | 2.05E-02 | 1.89E-02 ± 1.1E-03 |
| 6.00E-04 | 1.24E-02 | 1.37E-02 | 1.43E-02 | 1.64E-02 | 1.42E-02 ± 9.9E-04* |
| Slope = 2.36E+01 3.3E+00± ; Intercept = 7.29E-03 ± 1.3E-03 | | | | | |

***Table 168**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey carbonmonoxyhaemoglobin** at 25°C in borate buffer pH **8.780** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.14E-02 | 1.35E-02 | 1.60E-02 | 1.40E-02 | 1.37E-02 ± 1.1E-03 |
| 4.00E-04 | 1.24E-02 | 1.58E-02 | 1.63E-02 | 1.65E-02 | 1.53E-02 ± 1.4E-03 |
| 5.00E-04 | 1.25E-02 | 1.85E-02 | 1.52E-02 | 1.65E-02 | 1.56E-02 ± 1.5E-03 |
| 6.00E-04 | 1.64E-02 | 1.78E-02 | 1.89E-02 | 1.97E-02 | 1.82E-02 ± 8.3E-04* |
| Slope = 9.62E+00 ± 3.3E+00; Intercept = 1.10E-02 ± 1.3E-03 | | | | | |

***Table 169**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey carbonmonoxyhaemoglobin** at 25°C in borate buffer pH **8.958** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.20E-02 | 1.48E-02 | 1.53E-02 | 1.69E-02 | 1.48E-02 ±1.2E-03 |
| 4.00E-04 | 1.31E-02 | 1.49E-02 | 1.65E-02 | 1.86E-02 | 1.58E-02 ±1.8E-03 |
| 5.00E-04 | 1.37E-02 | 1.49E-02 | 1.68E-02 | 1.79E-02 | 1.58E-02 ±1.1E-03 |
| 6.00E-04 | 1.27E-02 | 1.41E-02 | 1.77E-02 | 1.89E-02 | 1.59E-02 ±1.6E-03* |
| Slope = 5.38E+00± 2.9E+00; Intercept = 1.33E-02± 1.2E-03 | | | | | |

***Table 170**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey aquomethaemoglobin** at 25°C in phosphate buffer pH **5.614** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 9.81E-03 | 1.25E-02 | 1.49E-02 | 1.28E-02 | 1.25E-02 ± 1.3E-03 |
| 4.00E-04 | 1.22E-02 | 1.35E-02 | 1.58E-02 | 1.65E-02 | 1.45E-02 ± 1.4E-03 |
| 5.00E-04 | 1.34E-02 | 1.46E-02 | 1.58E-02 | 1.47E-02 | 1.46E-02 ± 8.6E-04 |
| 6.00E-04 | 1.46E-02 | 1.65E-02 | 1.56E-02 | 1.30E-02 | 1.49E-02 ± 6.0E-04 |
| Slope = 2.16E+00 ± 6.7E-01; Intercept = 1.36E-02 ± 3.4E-04 | | | | | |

***Table 171**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey aquomethaemoglobin** at 25°C in phosphate buffer pH **5.852** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.27E-02 | 1.44E-02 | 1.55E-02 | 1.70E-02 | 1.49E-02 ± 1.1E-03 |
| 4.00E-04 | 1.27E-02 | 1.40E-02 | 1.73E-02 | 1.89E-02 | 1.57E-02 ± 2.1E-03 |
| 5.00E-04 | 1.55E-02 | 1.71E-02 | 1.76E-02 | 1.90E-02 | 1.73E-02 ± 8.9E-04 |
| 6.00E-04 | 1.75E-02 | 2.11E-02 | 2.35E-02 | 2.43E-02 | 2.16E-02 ± 1.7E-03 |
| Slope = 2.17E+01 ± 5.8E+00; Intercept = 7.60E-03 ± 2.7E-03 | | | | | |

***Table 172**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey aquomethaemoglobin** at 25°C in phosphate buffer pH **6.061** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.34E-02 | 1.45E-02 | 1.64E-02 | 1.71E-02 | 1.53E-02 ± 9.3E-04 |
| 4.00E-04 | 1.30E-02 | 1.52E-02 | 1.58E-02 | 1.79E-02 | 1.55E-02 ± 1.6E-03 |
| 5.00E-04 | 1.40E-02 | 1.48E-02 | 1.63E-02 | 1.77E-02 | 1.57E-02 ± 9.2E-04 |
| 6.00E-04 | 1.42E-02 | 1.66E-02 | 1.75E-02 | 1.95E-02 | 1.69E-02 ± 1.3E-03 |
| Slope = 5.05E+00 ± 1.9E+00; Intercept = 1.36E-02 ± 8.8E-04 | | | | | |

***Table 173**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey aquomethaemoglobin** at 25°C in phosphate buffer pH **6.221** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.27E-02 | 1.42E-02 | 1.46E-02 | 1.77E-02 | 1.48E-02 ± 1.2E-03 |
| 4.00E-04 | 1.28E-02 | 1.56E-02 | 1.66E-02 | 1.85E-02 | 1.59E-02 ± 1.9E-03 |
| 5.00E-04 | 1.32E-02 | 1.57E-02 | 1.71E-02 | 1.85E-02 | 1.61E-02 ± 1.3E-03 |
| 6.00E-04 | 1.53E-02 | 1.76E-02 | 1.83E-02 | 1.87E-02 | 1.75E-02 ± 8.5E-04 |
| Slope = 8.38E+00 ± 1.5E+00; Intercept = 1.23E-02 ± 6.8E-04 | | | | | |

***Table 174**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey aquomethaemoglobin** at 25°C in phosphate buffer pH **6.425** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.25E-02 | 1.37E-02 | 1.66E-02 | 1.72E-02 | 1.50E-02 ± 1.2E-03 |
| 4.00E-04 | 1.36E-02 | 1.59E-02 | 1.76E-02 | 1.88E-02 | 1.64E-02 ± 1.7E-03 |
| 5.00E-04 | 1.59E-02 | 1.65E-02 | 1.85E-02 | 1.90E-02 | 1.75E-02 ± 7.6E-04 |
| 6.00E-04 | 1.76E-02 | 1.80E-02 | 1.90E-02 | 1.96E-02 | 1.85E-02 ± 5.0E-04 |
| Slope = 1.16E+01 ± 7.2E-01; Intercept = 1.16E-02 ± 3.4E-04 | | | | | |

***Table 175**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey aquomethaemoglobin** at 25°C in phosphate buffer pH **6.627** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.11E-02 | 1.36E-02 | 1.59E-02 | 1.76E-02 | 1.45E-02 ± 1.6E-03 |
| 4.00E-04 | 1.30E-02 | 1.40E-02 | 1.51E-02 | 1.61E-02 | 1.45E-02 ± 1.1E-03 |
| 5.00E-04 | 1.33E-02 | 1.60E-02 | 1.61E-02 | 1.70E-02 | 1.56E-02 ± 9.3E-04 |
| 6.00E-04 | 1.75E-02 | 1.86E-02 | 1.90E-02 | 1.96E-02 | 1.87E-02 ± 5.1E-04 |
| Slope = 1.35E+01 ± 5.0E+00; Intercept = 9.78E-03 ± 2.3E-03 | | | | | |

***Table 176**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey aquomethaemoglobin** at 25°C in phosphate buffer pH **6.816** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.15E-02 | 1.35E-02 | 1.45E-02 | 1.54E-02 | 1.37E-02 ± 9.8E-04 |
| 4.00E-04 | 1.16E-02 | 1.23E-02 | 1.46E-02 | 1.65E-02 | 1.37E-02 ± 1.6E-03 |
| 5.00E-04 | 1.33E-02 | 1.53E-02 | 1.80E-02 | 1.70E-02 | 1.59E-02 ± 1.2E-03 |
| 6.00E-04 | 1.71E-02 | 1.86E-02 | 1.88E-02 | 1.33E-02 | 1.69E-02 ± 1.4E-03 |
| Slope = 1.17E+01 ± 2.8E+00; Intercept = 9.79E-03 ± 1.3E-03 | | | | | |

***Table 177**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey aquomethaemoglobin** at 25°C in phosphate buffer pH **6.974** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.13E-02 | 1.49E-02 | 1.57E-02 | 1.77E-02 | 1.49E-02 ± 1.6E-03 |
| 4.00E-04 | 1.34E-02 | 1.42E-02 | 1.55E-02 | 1.90E-02 | 1.55E-02 ± 1.9E-03 |
| 5.00E-04 | 1.42E-02 | 1.53E-02 | 1.77E-02 | 1.88E-02 | 1.65E-02 ± 1.2E-03 |
| 6.00E-04 | 1.74E-02 | 1.77E-02 | 1.86E-02 | 1.91E-02 | 1.82E-02 ± 4.4E-04 |
| Slope = 1.09E+01 ± 1.7E+00; Intercept = 1.14E-02 ± 7.8E-04 | | | | | |

***Table 178**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey aquomethaemoglobin** at 25°C in phosphate buffer pH **7.150** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.31E-02 | 1.35E-02 | 1.46E-02 | 1.66E-02 | 1.44E-02 ± 8.8E-04 |
| 4.00E-04 | 1.19E-02 | 1.51E-02 | 1.69E-02 | 1.77E-02 | 1.54E-02 ± 1.9E-03 |
| 5.00E-04 | 1.28E-02 | 1.59E-02 | 1.68E-02 | 1.85E-02 | 1.60E-02 ± 1.4E-03 |
| 6.00E-04 | 1.91E-02 | 1.66E-02 | 1.86E-02 | 1.90E-02 | 1.83E-02 ± 6.2E-04 |
| Slope = 1.24E+01 ± 2.6E+00; Intercept = 1.05E-02 ± 1.2E-03 | | | | | |

***Table 179**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey aquomethaemoglobin** at 25°C in phosphate buffer pH **7.373** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 9.99E-03 | 1.16E-02 | 1.20E-02 | 1.56E-02 | 1.23E-02 ± 1.4E-03 |
| 4.00E-04 | 1.12E-02 | 1.22E-02 | 1.58E-02 | 2.18E-02 | 1.53E-02 ± 3.5E-03 |
| 5.00E-04 | 1.25E-02 | 1.42E-02 | 1.67E-02 | 1.87E-02 | 1.55E-02 ± 1.5E-03 |
| 6.00E-04 | 1.44E-02 | 1.64E-02 | 1.86E-02 | 1.88E-02 | 1.70E-02 ± 1.1E-03 |
| Slope = 1.45E+01 ± 3.6E+00; Intercept = 8.52E-03 ± 1.7E-03 | | | | | |

***Table 180**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey aquomethaemoglobin** at 25°C in phosphate buffer pH **7.552** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.13E-02 | 1.17E-02 | 1.35E-02 | 1.46E-02 | 1.27E-02 ± 8.0E-04 |
| 4.00E-04 | 1.19E-02 | 1.21E-02 | 1.33E-02 | 1.53E-02 | 1.32E-02 ± 1.2E-03 |
| 5.00E-04 | 1.13E-02 | 1.38E-02 | 1.55E-02 | 1.69E-02 | 1.44E-02 ± 1.4E-03 |
| 6.00E-04 | 1.23E-02 | 1.47E-02 | 1.78E-02 | 1.86E-02 | 1.58E-02 ± 1.6E-03 |
| Slope = 1.05E+01 ± 1.7E+00; Intercept = 9.31E-03 ± 7.9E-04 | | | | | |

***Table 181**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey aquomethaemoglobin** at 25°C in phosphate buffer pH **7.733** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.34E-02 | 1.41E-02 | 1.46E-02 | 1.66E-02 | 1.47E-02 ± 8.1E-04 |
| 4.00E-04 | 1.29E-02 | 1.35E-02 | 1.66E-02 | 1.94E-02 | 1.56E-02 ± 2.2E-03 |
| 5.00E-04 | 1.53E-02 | 1.70E-02 | 1.77E-02 | 1.93E-02 | 1.73E-02 ± 1.0E-03 |
| 6.00E-04 | 1.53E-02 | 1.75E-02 | 1.85E-02 | 1.89E-02 | 1.76E-02 ± 8.9E-04 |
| Slope = 1.04E+01 ± 1.9E+00; Intercept = 1.16E-02 ± 8.6E-04 | | | | | |

***Table 182**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey aquomethaemoglobin** at 25°C in borate buffer pH **8.008** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.12E-02 | 1.38E-02 | 1.60E-02 | 1.84E-02 | 1.48E-02 ± 1.8E-03 |
| 4.00E-04 | 1.24E-02 | 1.46E-02 | 1.71E-02 | 1.94E-02 | 1.59E-02 ± 2.3E-03 |
| 5.00E-04 | 1.31E-02 | 1.54E-02 | 1.78E-02 | 1.95E-02 | 1.65E-02 ± 1.6E-03 |
| 6.00E-04 | 1.44E-02 | 1.63E-02 | 1.79E-02 | 1.92E-02 | 1.70E-02 ± 1.2E-03 |
| Slope = 6.94E+00 ± 9.2E-01; Intercept = 1.29E-02 ± 4.3E-04 | | | | | |

***Table 183**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey aquomethaemoglobin** at 25°C in borate buffer pH **8.223** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.25E-02 | 1.44E-02 | 1.56E-02 | 1.76E-02 | 1.50E-02 ± 1.3E-03 |
| 4.00E-04 | 1.29E-02 | 1.48E-02 | 1.58E-02 | 1.76E-02 | 1.53E-02 ± 1.6E-03 |
| 5.00E-04 | 1.43E-02 | 1.73E-02 | 1.61E-02 | 1.63E-02 | 1.60E-02 ± 7.5E-04 |
| 6.00E-04 | 1.38E-02 | 1.57E-02 | 1.72E-02 | 1.83E-02 | 1.62E-02 ± 1.1E-03 |
| Slope = 4.45E+00 ± 7.8E-01; Intercept = 1.36E-02 ± 3.6E-04 | | | | | |

***Table 184**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey aquomethaemoglobin** at 25°C in borate buffer pH **8.421** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.16E-02 | 1.32E-02 | 1.54E-02 | 1.75E-02 | 1.44E-02 ± 1.5E-03 |
| 4.00E-04 | 1.15E-02 | 1.32E-02 | 1.56E-02 | 1.76E-02 | 1.45E-02 ± 2.0E-03 |
| 5.00E-04 | 1.11E-02 | 1.34E-02 | 1.68E-02 | 1.85E-02 | 1.50E-02 ± 1.8E-03 |
| 6.00E-04 | 1.36E-02 | 1.54E-02 | 1.62E-02 | 1.80E-02 | 1.58E-02 ± 1.1E-03 |
| Slope = 4.57E+00 ± 1.2E+00; Intercept = 1.29E-02 ± 5.7E-04 | | | | | |

***Table 185**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey aquomethaemoglobin** at 25°C in borate buffer pH **8.615** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.22E-02 | 1.45E-02 | 1.55E-02 | 1.76E-02 | 1.50E-02 ± 1.3E-03 |
| 4.00E-04 | 1.45E-02 | 1.54E-02 | 1.65E-02 | 1.75E-02 | 1.60E-02 ± 1.0E-03 |
| 5.00E-04 | 1.34E-02 | 1.57E-02 | 1.77E-02 | 1.77E-02 | 1.61E-02 ± 1.1E-03 |
| 6.00E-04 | 1.37E-02 | 1.57E-02 | 1.95E-02 | 1.86E-02 | 1.69E-02 ± 1.4E-03 |
| Slope = 5.86E+00 ± 1.2E+00; Intercept = 1.33E-02 ± 5.5E-04 | | | | | |

***Table 186**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey aquomethaemoglobin** at 25°C in borate buffer pH **8.713** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.12E-02 | 1.43E-02 | 1.63E-02 | 1.89E-02 | 1.52E-02 ± 1.9E-03 |
| 4.00E-04 | 1.35E-02 | 1.51E-02 | 1.60E-02 | 1.77E-02 | 1.55E-02 ± 1.4E-03 |
| 5.00E-04 | 1.44E-02 | 1.64E-02 | 1.72E-02 | 1.86E-02 | 1.67E-02 ± 1.0E-03 |
| 6.00E-04 | 1.54E-02 | 1.61E-02 | 1.78E-02 | 1.84E-02 | 1.69E-02 ± 7.3E-04 |
| Slope = 6.33E+00 ± 1.1E+00; Intercept = 1.32E-02 ± 5.2E-04 | | | | | |

***Table 187**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **stripped donkey aquomethaemoglobin** at 25°C in borate buffer pH **8.930** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.20E-02 | 1.46E-02 | 1.66E-02 | 1.06E-02 | 1.35E-02 ± 1.5E-03 |
| 4.00E-04 | 1.18E-02 | 1.36E-02 | 1.58E-02 | 1.88E-02 | 1.50E-02 ± 2.3E-03 |
| 5.00E-04 | 1.24E-02 | 1.43E-02 | 1.63E-02 | 1.96E-02 | 1.56E-02 ± 1.8E-03 |
| 6.00E-04 | 1.31E-02 | 1.53E-02 | 1.78E-02 | 1.96E-02 | 1.65E-02 ± 1.6E-03 |
| Slope = 9.68E+00 ± 1.3E+00; Intercept = 1.08E-02 ± 6.0E-04 | | | | | |

***Table 188**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey oxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **5.611** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.28E-02 | 1.41E-02 | 1.48E-02 | 1.49E-02 | 1.41E-02 ± 5.4E-04 |
| 4.00E-04 | 1.22E-02 | 1.46E-02 | 1.66E-02 | 1.92E-02 | 1.56E-02 ± 2.3E-03 |
| 5.00E-04 | 1.32E-02 | 1.82E-02 | 1.84E-02 | 1.93E-02 | 1.73E-02 ± 1.5E-03 |
| 6.00E-04 | 2.13E-02 | 1.59E-02 | 1.60E-02 | 1.67E-02 | 1.75E-02 ± 1.4E-03 |
| Slope = 1.16E+01 ± 2.4E+00; Intercept = 1.09E-02 ± 1.1E-03 | | | | | |

***Table 189**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey oxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **5.809** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 5.82E+00 | 5.81E+00 | 5.82E+00 | 5.81E+00 | 5.82E+00 ± 2.5E-03 |
| 4.00E-04 | 5.82E+00 | 5.81E+00 | 5.81E+00 | 5.82E+00 | 5.82E+00 ± 3.3E-03 |
| 5.00E-04 | 1.36E-02 | 1.66E-02 | 1.75E-02 | 1.87E-02 | 1.66E-02 ± 1.3E-03 |
| 6.00E-04 | 1.47E-02 | 1.69E-02 | 1.71E-02 | 1.86E-02 | 1.68E-02 ± 9.6E-04 |
| Slope = 5.80E+00 ± 1.9E+00; Intercept = 1.36E-02 ± 9.0E-04 | | | | | |

***Table 190**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey oxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **6.029** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.18E-02 | 1.63E-02 | 1.78E-02 | 1.47E-02 | 1.51E-02 ± 1.5E-03 |
| 4.00E-04 | 1.59E-02 | 1.75E-02 | 1.77E-02 | 1.41E-02 | 1.63E-02 ± 1.2E-03 |
| 5.00E-04 | 1.62E-02 | 1.73E-02 | 1.76E-02 | 1.98E-02 | 1.77E-02 ± 8.9E-04 |
| 6.00E-04 | 1.46E-02 | 1.49E-02 | 1.51E-02 | 1.55E-02 | 1.50E-02 ± 2.2E-04* |
| Slope = 1.29E+01 ± 8.8E-01; Intercept = 1.12E-02 ± 3.6E-04 | | | | | |

***Table 191**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey oxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **6.225** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.42E-02 | 1.57E-02 | 1.72E-02 | 1.75E-02 | 1.61E-02 ± 8.4E-04 |
| 4.00E-04 | 1.48E-02 | 1.49E-02 | 1.56E-02 | 2.19E-02 | 1.68E-02 ± 2.4E-03 |
| 5.00E-04 | 1.53E-02 | 1.67E-02 | 1.70E-02 | 1.88E-02 | 1.70E-02 ± 8.6E-04 |
| 6.00E-04 | 1.64E-02 | 1.76E-02 | 1.84E-02 | 2.74E-02 | 2.00E-02 ± 2.7E-03 |
| Slope = 1.16E+01 ± 4.4E+00; Intercept = 1.22E-02 ± 2.1E-03 | | | | | |

***Table 192**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey oxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **6.401** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.40E-02 | 1.56E-02 | 1.57E-02 | 1.71E-02 | 1.56E-02 ±7.8E-04 |
| 4.00E-04 | 1.42E-02 | 1.58E-02 | 1.63E-02 | 1.81E-02 | 1.61E-02 ±1.3E-03 |
| 5.00E-04 | 1.59E-02 | 1.62E-02 | 1.67E-02 | 1.67E-02 | 1.64E-02 ±2.1E-04 |
| 6.00E-04 | 1.39E-02 | 1.56E-02 | 1.64E-02 | 1.73E-02 | 1.58E-02 ±8.6E-04* |
| Slope = 3.88E+00 ± 5.2E-01; Intercept = 1.45E-02 ± 2.1E-04 | | | | | |

***Table 193**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey oxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **6.588** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.42E-02 | 1.51E-02 | 1.53E-02 | 1.63E-02 | 1.52E-02 ± 5.1E-04 |
| 4.00E-04 | 1.51E-02 | 1.57E-02 | 1.63E-02 | 1.74E-02 | 1.61E-02 ± 7.4E-04 |
| 5.00E-04 | 1.50E-02 | 1.57E-02 | 1.59E-02 | 2.10E-02 | 1.69E-02 ± 1.5E-03 |
| 6.00E-04 | 1.70E-02 | 1.79E-02 | 1.86E-02 | 1.88E-02 | 1.81E-02 ± 4.7E-04 |
| Slope = 9.24E+00 ± 6.1E-01; Intercept = 1.24E-02 ± 2.8E-04 | | | | | |

***Table 194**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey oxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **6.801** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.37E-02 | 1.49E-02 | 1.68E-02 | 2.02E-02 | 1.64E-02 ± 1.6E-03 |
| 4.00E-04 | 1.47E-02 | 1.55E-02 | 1.74E-02 | 2.01E-02 | 1.69E-02 ± 1.8E-03 |
| 5.00E-04 | 1.52E-02 | 1.55E-02 | 1.64E-02 | 2.80E-02 | 1.88E-02 ± 3.2E-03 |
| 6.00E-04 | 1.49E-02 | 1.61E-02 | 1.69E-02 | 1.83E-02 | 1.65E-02 ± 8.5E-04* |
| Slope = 1.21E+01 ± 4.0E+00; Intercept = 1.25E-02 ± 1.6E-03 | | | | | |

***Table 195**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey oxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **6.996** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.32E-02 | 1.34E-02 | 1.38E-02 | 1.60E-02 | 1.41E-02 ± 7.0E-04 |
| 4.00E-04 | 1.42E-02 | 1.43E-02 | 1.55E-02 | 1.67E-02 | 1.52E-02 ± 8.1E-04 |
| 5.00E-04 | 1.51E-02 | 1.54E-02 | 1.63E-02 | 1.79E-02 | 1.61E-02 ± 7.1E-04 |
| 6.00E-04 | 1.49E-02 | 1.51E-02 | 1.53E-02 | 1.55E-02 | 1.52E-02 ± 1.6E-04* |
| Slope = 1.00E+01 ± 2.4E-01; Intercept = 1.11E-02 ± 9.8E-05 | | | | | |

***Table 196**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey oxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **7.171** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.34E-02 | 1.65E-02 | 1.71E-02 | 1.84E-02 | 1.64E-02 ± 1.3E-03* |
| 4.00E-04 | 1.54E-02 | 1.60E-02 | 1.67E-02 | 1.71E-02 | 1.63E-02 ± 5.8E-04 |
| 5.00E-04 | 1.43E-02 | 1.63E-02 | 1.75E-02 | 1.81E-02 | 1.66E-02 ± 9.5E-04 |
| 6.00E-04 | 1.53E-02 | 1.70E-02 | 1.77E-02 | 2.20E-02 | 1.80E-02 ± 1.7E-03 |
| Slope = 8.52E+00 ± 3.4E+00; Intercept = 1.27E-02 ± 1.7E-03 | | | | | |

***Table 197**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey oxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **7.394** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.54E-02 | 1.58E-02 | 1.60E-02 | 1.77E-02 | 1.62E-02 ± 5.7E-04 |
| 4.00E-04 | 1.55E-02 | 1.68E-02 | 1.88E-02 | 1.97E-02 | 1.77E-02 ± 1.4E-03 |
| 5.00E-04 | 1.49E-02 | 1.59E-02 | 1.69E-02 | 1.81E-02 | 1.64E-02 ± 7.9E-04* |
| 6.00E-04 | 1.51E-02 | 1.79E-02 | 1.89E-02 | 2.03E-02 | 1.80E-02 ± 1.3E-03 |
| Slope = 5.43E+00 ± 3.1E+00; Intercept = 1.50E-02 ± 1.4E-03 | | | | | |

***Table 198**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey oxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **7.606** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.22E-02 | 1.46E-02 | 1.76E-02 | 1.80E-02 | 1.56E-02 ± 1.5E-03 |
| 4.00E-04 | 1.41E-02 | 1.58E-02 | 1.63E-02 | 1.78E-02 | 1.60E-02 ± 1.2E-03 |
| 5.00E-04 | 1.48E-02 | 1.50E-02 | 1.67E-02 | 1.81E-02 | 1.61E-02 ± 8.3E-04 |
| 6.00E-04 | 1.36E-02 | 1.59E-02 | 1.65E-02 | 1.97E-02 | 1.64E-02 ± 1.5E-03 |
| Slope = 2.53E+00 ± 2.9E-01; Intercept = 1.49E-02 ± 1.4E-04 | | | | | |

***Table 199**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey oxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **7.808** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.41E-02 | 1.55E-02 | 1.56E-02 | 1.64E-02 | 1.54E-02 ± 5.7E-04 |
| 4.00E-04 | 1.42E-02 | 1.72E-02 | 1.72E-02 | 1.75E-02 | 1.65E-02 ± 1.1E-03 |
| 5.00E-04 | 1.41E-02 | 1.46E-02 | 1.48E-02 | 1.54E-02 | 1.47E-02 ± 3.3E-04* |
| 6.00E-04 | 1.56E-02 | 1.60E-02 | 1.79E-02 | 1.85E-02 | 1.70E-02 ± 7.3E-04 |
| Slope = 4.87E+00 ± 2.1E+00; Intercept = 1.42E-02 ± 9.7E-04 | | | | | |

***Table 200**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey oxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in borate buffer pH **7.994** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.50E-02 | 1.52E-02 | 1.55E-02 | 1.63E-02 | 1.55E-02 ± 3.3E-04 |
| 4.00E-04 | 1.49E-02 | 1.55E-02 | 1.57E-02 | 1.63E-02 | 1.56E-02 ± 4.7E-04 |
| 5.00E-04 | 1.49E-02 | 1.61E-02 | 1.63E-02 | 1.64E-02 | 1.59E-02 ± 3.6E-04 |
| 6.00E-04 | 1.85E-02 | 1.54E-02 | 1.62E-02 | 1.86E-02 | 1.71E-02 ± 8.1E-04 |
| Slope = 5.32E+00 ± 1.8E+00; Intercept = 1.37E-02 ± 8.4E-04 | | | | | |

***Table 201**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey oxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in borate buffer pH **8.196** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.24E-02 | 1.34E-02 | 1.58E-02 | 1.58E-02 | 1.44E-02 ± 8.6E-04 |
| 4.00E-04 | 1.37E-02 | 1.65E-02 | 1.72E-02 | 1.75E-02 | 1.62E-02 ± 1.3E-03 |
| 5.00E-04 | 1.64E-02 | 1.67E-02 | 1.67E-02 | 1.71E-02 | 1.67E-02 ± 1.7E-04 |
| 6.00E-04 | 1.01E-02 | 1.53E-02 | 1.78E-02 | 2.42E-02 | 1.68E-02 ± 3.5E-03 |
| Slope = 7.95E+00 ± 2.9E+00; Intercept = 1.25E-02 ± 1.3E-03 | | | | | |

***Table 202**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey oxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in borate buffer pH **8.399** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 9.05E-03 | 1.46E-02 | 1.76E-02 | 1.82E-02 | 1.49E-02 ± 2.3E-03 |
| 4.00E-04 | 1.62E-02 | 1.65E-02 | 1.68E-02 | 1.59E-02 | 1.64E-02 ± 2.9E-04 |
| 5.00E-04 | 1.47E-02 | 1.84E-02 | 1.73E-02 | 1.83E-02 | 1.72E-02 ± 9.1E-04 |
| 6.00E-04 | 1.65E-02 | 1.68E-02 | 1.74E-02 | 1.97E-02 | 1.76E-02 ± 8.0E-04 |
| Slope = 8.87E+00 ± 1.8E+00; Intercept = 1.25E-02 ± 8.2E-04 | | | | | |

***Table 203**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey oxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in borate buffer pH **8.607** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.42E-02 | 1.48E-02 | 1.58E-02 | 1.68E-02 | 1.54E-02 ± 6.7E-04 |
| 4.00E-04 | 1.45E-02 | 1.50E-02 | 1.53E-02 | 1.71E-02 | 1.55E-02 ± 8.4E-04 |
| 5.00E-04 | 1.63E-02 | 1.71E-02 | 1.79E-02 | 1.82E-02 | 1.74E-02 ± 4.8E-04 |
| 6.00E-04 | 1.68E-02 | 1.70E-02 | 1.89E-02 | 1.99E-02 | 1.81E-02 ± 7.7E-04 |
| Slope = 1.01E+01 ± 2.4E+00; Intercept = 1.20E-02 ± 1.1E-03 | | | | | |

***Table 204**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey oxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in borate buffer pH **8.832** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.41E-02 | 1.46E-02 | 1.65E-02 | 1.66E-02 | 1.54E-02 ± 6.3E-04 |
| 4.00E-04 | 1.30E-02 | 1.42E-02 | 1.56E-02 | 1.91E-02 | 1.55E-02 ± 2.0E-03 |
| 5.00E-04 | 1.15E-02 | 1.67E-02 | 1.71E-02 | 1.78E-02 | 1.58E-02 ± 1.6E-03 |
| 6.00E-04 | 1.54E-02 | 1.56E-02 | 1.57E-02 | 1.64E-02 | 1.58E-02 ± 2.7E-04 |
| Slope = 1.34E+00 ± 4.0E-01; Intercept = 1.50E-02 ± 1.9E-04 | | | | | |

***Table 205**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey oxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in borate buffer pH **8.968** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.43E-02 | 1.52E-02 | 1.55E-02 | 1.66E-02 | 1.54E-02 ± 5.7E-04 |
| 4.00E-04 | 1.44E-02 | 1.54E-02 | 1.58E-02 | 1.64E-02 | 1.55E-02 ± 6.7E-04 |
| 5.00E-04 | 1.49E-02 | 1.56E-02 | 1.57E-02 | 1.67E-02 | 1.57E-02 ± 4.4E-04 |
| 6.00E-04 | 1.45E-02 | 1.55E-02 | 1.72E-02 | 1.86E-02 | 1.64E-02 ± 1.0E-03 |
| Slope = 3.37E+00 ± 1.0E+00; Intercept = 1.42E-02 ± 4.7E-04 | | | | | |

***Table 206**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey carbonmonoxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **5.616** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.55E-02 | 1.55E-02 | 1.65E-02 | 1.47E-02 | 1.56E-02 ± 4.5E-04 |
| 4.00E-04 | 1.66E-02 | 1.57E-02 | 1.63E-02 | 1.59E-02 | 1.61E-02 ± 3.0E-04 |
| 5.00E-04 | 1.53E-02 | 1.75E-02 | 1.70E-02 | 1.70E-02 | 1.67E-02 ± 5.5E-04 |
| 6.00E-04 | 1.65E-02 | 1.76E-02 | 2.01E-02 | 2.22E-02 | 1.91E-02 ± 1.4E-03 |
| Slope = 5.22E+00 ± 3.7E-01; Intercept = 1.40E-02 ± 1.7E-04 | | | | | |

***Table 207**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey carbonmonoxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **5.831** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.35E-02 | 1.43E-02 | 1.67E-02 | 1.75E-02 | 1.55E-02 ± 1.0E-03 |
| 4.00E-04 | 1.42E-02 | 1.55E-02 | 1.56E-02 | 1.70E-02 | 1.56E-02 ± 9.2E-04 |
| 5.00E-04 | 1.39E-02 | 1.66E-02 | 1.70E-02 | 2.05E-02 | 1.70E-02 ± 1.7E-03 |
| 6.00E-04 | 1.42E-02 | 1.59E-02 | 1.65E-02 | 2.35E-02 | 1.75E-02 ± 2.3E-03 |
| Slope = 7.38E+00 ± 1.7E+00; Intercept = 1.31E-02 ± 8.1E-04 | | | | | |

***Table 208**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey carbonmonoxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **6.040** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.46E-02 | 1.59E-02 | 1.64E-02 | 1.68E-02 | 1.59E-02 ± 5.4E-04* |
| 4.00E-04 | 1.33E-02 | 1.55E-02 | 1.67E-02 | 1.73E-02 | 1.57E-02 ± 1.3E-03 |
| 5.00E-04 | 1.71E-02 | 1.49E-02 | 1.65E-02 | 1.75E-02 | 1.65E-02 ± 6.6E-04 |
| 6.00E-04 | 1.47E-02 | 1.67E-02 | 1.80E-02 | 1.83E-02 | 1.69E-02 ± 9.0E-04 |
| Slope = 6.00E+00 ± 1.0E+00; Intercept = 1.34E-02 ± 5.3E-04 | | | | | |

***Table 209**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey carbonmonoxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **6.212** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.42E-02 | 1.45E-02 | 1.55E-02 | 1.65E-02 | 1.52E-02 ± 5.9E-04 |
| 4.00E-04 | 1.38E-02 | 1.57E-02 | 1.65E-02 | 1.71E-02 | 1.58E-02 ± 1.1E-03 |
| 5.00E-04 | 1.54E-02 | 1.60E-02 | 1.62E-02 | 1.73E-02 | 1.62E-02 ± 4.8E-04 |
| 6.00E-04 | 1.47E-02 | 1.47E-02 | 1.57E-02 | 1.68E-02 | 1.55E-02 ± 5.4E-04 |
| Slope = 5.38E+00 ± 4.2E-01; Intercept = 1.36E-02 ± 1.7E-04 | | | | | |

***Table 210**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey carbonmonoxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **6.400** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.41E-02 | 1.46E-02 | 1.51E-02 | 1.72E-02 | 1.52E-02 ± 7.9E-04 |
| 4.00E-04 | 1.49E-02 | 1.50E-02 | 1.61E-02 | 1.72E-02 | 1.58E-02 ± 7.6E-04 |
| 5.00E-04 | 1.44E-02 | 1.65E-02 | 1.79E-02 | 1.83E-02 | 1.68E-02 ± 9.7E-04 |
| 6.00E-04 | 5.69E-02* | 1.64E-02 | 1.75E-02 | 1.86E-02 | 1.75E-02 ± 7.3E-04 |
| Slope = 7.64E+00 ± 5.2E-01; Intercept = 1.29E-02 ± 2.4E-04 | | | | | |

***Table 211**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey carbonmonoxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **6.594** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.22E-02 | 1.44E-02 | 1.58E-02 | 1.72E-02 | 1.49E-02 ± 1.2E-03 |
| 4.00E-04 | 1.32E-02 | 1.43E-02 | 1.58E-02 | 1.84E-02 | 1.54E-02 ± 1.7E-03 |
| 5.00E-04 | 1.44E-02 | 1.52E-02 | 1.59E-02 | 1.77E-02 | 1.58E-02 ± 8.3E-04 |
| 6.00E-04 | 1.59E-02 | 1.69E-02 | 1.33E-02 | 2.07E-02 | 1.67E-02 ± 1.9E-03 |
| Slope = 5.90E+00 ± 8.0E-01; Intercept = 1.31E-02 ± 3.7E-04 | | | | | |

***Table 212**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey carbonmonoxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **6.807** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.33E-02 | 1.48E-02 | 1.52E-02 | 1.61E-02 | 1.49E-02 ± 7.1E-04 |
| 4.00E-04 | 1.48E-02 | 1.54E-02 | 1.65E-02 | | 1.56E-02 ± 5.8E-04 |
| 5.00E-04 | 1.31E-02 | 1.59E-02 | 1.63E-02 | 1.90E-02 | 1.60E-02 ± 1.5E-03 |
| 6.00E-04 | 1.55E-02 | 1.61E-02 | 1.73E-02 | 1.85E-02 | 1.68E-02 ± 7.5E-04 |
| Slope = 6.45E+00 ± 4.6E-01; Intercept = 1.29E-02 ± 2.1E-04 | | | | | |

***Table 213**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey carbonmonoxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **6.997** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.37E-02 | 1.46E-02 | 1.48E-02 | 1.70E-02 | 1.50E-02 ± 8.2E-04 |
| 4.00E-04 | 1.49E-02 | 1.56E-02 | 1.62E-02 | - | 1.56E-02 ± 4.2E-04 |
| 5.00E-04 | 1.39E-02 | 1.55E-02 | 1.74E-02 | 1.77E-02 | 1.61E-02 ± 9.4E-04 |
| 6.00E-04 | 1.52E-02 | 1.62E-02 | 1.77E-02 | 1.88E-02 | 1.70E-02 ± 9.1E-04 |
| Slope = 6.20E+00 ± 6.1E-01; Intercept = 1.32E-02 ± 2.8E-04 | | | | | |

***Table 214**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey carbonmonoxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **7.223** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.48E-02 | 1.52E-02 | 1.55E-02 | 1.64E-02 | 1.55E-02 ± 4.0E-04 |
| 4.00E-04 | 1.66E-02 | 1.57E-02 | 1.54E-02 | 1.58E-02 | 1.59E-02 ± 3.9E-04 |
| 5.00E-04 | 1.71E-02 | 1.92E-02 | 1.62E-02 | 1.49E-02 | 1.69E-02 ± 1.1E-03 |
| 6.00E-04 | 1.54E-02 | 2.44E-02 | 1.66E-02 | 1.53E-02 | 1.79E-02 ± 2.3E-03 |
| Slope = 8.36E+00 ± 1.1E+00; Intercept = 1.28E-02 ± 5.0E-04 | | | | | |

***Table 215**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey carbonmonoxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **7.411** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.39E-02 | 8.15E-03 | 1.56E-02 | 1.44E-02 | 1.30E-02 ± 1.9E-03 |
| 4.00E-04 | 1.34E-02 | 1.58E-02 | 1.31E-02 | 1.60E-02 | 1.46E-02 ± 9.7E-04 |
| 5.00E-04 | 1.64E-02 | 1.60E-02 | 1.48E-02 | 1.54E-02 | 1.57E-02±4.1E-04* |
| 6.00E-04 | 1.90E-02 | 1.32E-02 | 1.48E-02 | 1.63E-02 | 1.58E-02 ± 1.4E-03 |
| Slope = 8.90E+00 ± 9.3E+04; Intercept = 1.0E-02 ± 1.0E-03 | | | | | |

***Table 216**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey carbonmonoxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **7.612** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.37E-02 | 1.79E-02 | 1.64E-02 | 1.54E-02 | 1.58E-02 ± 1.1E-03 |
| 4.00E-04 | 1.63E-02 | 1.65E-02 | 1.58E-02 | 1.69E-02 | 1.64E-02 ± 3.7E-04 |
| 5.00E-04 | 7.98E-02* | 1.82E-02 | 1.59E-02 | 1.86E-02 | 1.76E-02 ± 8.9E-04 |
| 6.00E-04 | 2.19E-02 | 1.50E-02 | 2.15E-02 | 2.17E-02 | 2.00E-02 ± 1.7E-03 |
| Slope = 1.38E+01 ± 3.1E+00;Intercept = 1.12E-02 ± 1.5E-03 | | | | | |

***Table 217**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey carbonmonoxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **7.768** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.54E-02 | 1.55E-02 | 1.57E-02 | 1.58E-02 | 1.56E-02 ± 9.7E-05 |
| 4.00E-04 | 1.73E-02 | 1.45E-02 | 1.77E-02 | 1.54E-02 | 1.62E-02 ± 1.1E-03 |
| 5.00E-04 | 1.38E-02 | 1.87E-02 | 1.88E-02 | 1.89E-02 | 1.75E-02 ± 1.3E-03 |
| 6.00E-04 | 1.58E-02 | 1.57E-02 | 1.75E-02 | 1.79E-02 | 1.67E-02 ± 5.4E-04* |
| Slope = 9.62E+00 ± 1.9E+00; Intercept = 1.26E-02 ± 7.9E-04 | | | | | |

***Table 218**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey carbonmonoxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in borate buffer pH **8.007** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.60E-02 | 1.34E-02 | 1.42E-02 | 1.39E-02 | 1.44E-02 ± 6.4E-04 |
| 4.00E-04 | 1.55E-02 | 1.89E-02 | 1.63E-02 | 1.43E-02 | 1.63E-02 ± 1.5E-03 |
| 5.00E-04 | 1.58E-02 | 1.91E-02 | 1.96E-02 | 1.23E-02 | 1.67E-02 ± 1.8E-03 |
| 6.00E-04 | 1.55E-02 | 1.70E-02 | 1.73E-02 | 1.74E-02 | 1.68E-02 ± 4.8E-04 |
| Slope = 7.77E+00 ± 2.9E+00; Intercept = 1.25E-02 ± 1.4E-03 | | | | | |

***Table 219**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey carbonmonoxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in borate buffer pH **8.169** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 1.69E-02 | 1.75E-02 | 1.47E-02 | 1.22E-02 | 1.53E-02* | 1.69E-02 ± 1.3E-03 |
| 1.54E-02 | 1.43E-02 | 1.42E-02 | 1.75E-02 | 1.53E-02 | 1.54E-02 ± 1.1E-03 |
| 1.60E-02 | 1.55E-02 | 1.89E-02 | 1.50E-02 | 1.63E-02 | 1.60E-02 ± 9.8E-04 |
| 1.52E-02 | 1.96E-02 | 1.61E-02 | 1.56E-02 | 1.66E-02 | 1.52E-02 ± 1.1E-03 |
| Slope = 6.40E+00 ± 2.0E+00; Intercept = 1.29E-02 ± 1.0E-03 | | | | | |

***Table 220**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey carbonmonoxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in borate buffer pH **8.408** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.40E-02 | 1.32E-02 | 1.24E-02 | 1.55E-02 | 1.38E-02 ± 7.9E-04 |
| 4.00E-04 | 1.32E-02 | 1.59E-02 | 1.54E-02 | 1.32E-02 | 1.44E-02 ± 9.0E-04 |
| 5.00E-04 | 1.49E-02 | 1.72E-02 | 1.65E-02 | 1.81E-02 | 1.67E-02 ± 7.9E-04 |
| 6.00E-04 | 1.62E-02 | 1.73E-02 | 1.74E-02 | 1.81E-02 | 1.72E-02 ± 4.9E-04 |
| Slope = 1.27E+01 ± 2.4E+00; Intercept = 9.83E-03 ± 1.1E-03 | | | | | |

***Table 221**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey carbonmonoxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in borate buffer pH **8.606** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.53E-02 | 1.46E-02 | 1.42E-02 | 1.54E-02 | 1.48E-02 ± 2.9E-04 |
| 4.00E-04 | 1.41E-02 | 1.58E-02 | 1.68E-02 | 1.72E-02 | 1.60E-02 ± 1.0E-03 |
| 5.00E-04 | 1.60E-02 | 1.67E-02 | 1.61E-02 | 1.62E-02 | 1.63E-02 ± 1.8E-04 |
| 6.00E-04 | 1.61E-02 | 1.79E-02 | 1.71E-02 | 1.71E-02 | 1.71E-02 ± 4.6E-04 |
| Slope = 6.93E+00 ± 1.1E+00; Intercept = 1.29E-02 ± 5.1E-04 | | | | | |

***Table 222**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey carbonmonoxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in borate buffer pH **8.858** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.35E-02 | 1.48E-02 | 1.52E-02 | 1.76E-02 | 1.53E-02 ± 1.0E-03 |
| 4.00E-04 | 1.63E-02 | 1.64E-02 | 1.52E-02 | 1.51E-02 | 1.57E-02 ± 4.2E-04 |
| 5.00E-04 | 1.80E-02 | 1.61E-02 | 1.47E-02 | 1.55E-02 | 1.60E-02 ± 8.3E-04 |
| 6.00E-04 | 1.64E-02 | 1.46E-02 | 1.78E-02 | 1.79E-02 | 1.67E-02 ± 8.2E-04 |
| Slope = 4.45E+00 ± 3.9E-01; Intercept = 1.39E-02 ± 1.8E-04 | | | | | |

***Table 223**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey carbonmonoxyhaemoglobin** in the presence of **inositol-$P_6$** at 25°C in borate buffer pH **9.010** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.32E-02 | 1.38E-02 | 1.44E-02 | 1.54E-02 | 1.42E-02 ± 5.5E-04 |
| 4.00E-04 | 1.35E-02 | 1.55E-02 | 1.58E-02 | 1.59E-02 | 1.51E-02 ± 8.1E-04 |
| 5.00E-04 | 1.45E-02 | 1.65E-02 | 1.79E-02 | 1.84E-02 | 1.68E-02 ± 9.9E-04* |
| 6.00E-04 | 1.33E-02 | 1.57E-02 | 1.63E-02 | 1.70E-02 | 1.56E-02 ± 9.1E-04 |
| Slope = 4.21E+00 ± 1.7E+00; Intercept = 1.32E-02 ± 7.7E-04 | | | | | |

***Table 224**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey aquomethaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **5.622** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.25E-02 | 1.28E-02 | 1.42E-02 | 1.56E-02 | 1.38E-02 ± 7.7E-04 |
| 4.00E-04 | 1.21E-02 | 1.33E-02 | 1.46E-02 | 1.68E-02 | 1.42E-02 ± 1.6E-03 |
| 5.00E-04 | 1.29E-02 | 1.32E-02 | 1.48E-02 | 1.63E-02 | 1.43E-02 ± 8.4E-04 |
| 6.00E-04 | 1.45E-02 | 1.75E-02 | 1.82E-02 | 1.86E-02 | 1.72E-02 ± 1.0E-03* |
| Slope = 2.59E+00 ± 8.3E-01; Intercept = 1.30E-02 ± 3.4E-04 | | | | | |

***Table 225**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey aquomethaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **5.813** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.32E-02 | 1.37E-02 | 1.45E-02 | 1.65E-02 | 1.45E-02 ± 8.2E-04 |
| 4.00E-04 | 1.14E-02 | 1.32E-02 | 1.65E-02 | 1.68E-02 | 1.45E-02 ± 1.8E-03 |
| 5.00E-04 | 1.29E-02 | 1.36E-02 | 1.55E-02 | 1.71E-02 | 1.48E-02 ± 1.0E-03 |
| 6.00E-04 | 1.35E-02 | 1.56E-02 | 1.60E-02 | 1.72E-02 | 1.56E-02 ± 9.2E-04 |
| Slope = 3.64E+00 ± 1.2E+00; Intercept = 1.32E-02 ± 5.8E-04 | | | | | |

***Table 226**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey aquomethaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **6.003** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.06E-02 | 1.23E-02 | 1.34E-02 | 1.60E-02 | 1.31E-02 ± 1.3E-03 |
| 4.00E-04 | 1.15E-02 | 1.27E-02 | 1.32E-02 | 1.52E-02 | 1.32E-02 ± 1.2E-03 |
| 5.00E-04 | 1.09E-02 | 1.43E-02 | 1.63E-02 | 1.71E-02 | 1.47E-02 ± 1.6E-03 |
| 6.00E-04 | 1.11E-02 | 1.38E-02 | 1.51E-02 | 1.91E-02 | 1.48E-02 ± 2.0E-03 |
| Slope = 6.57E+00 ± 2.0E+00; Intercept = 1.10E-02 ± 9.2E-04 | | | | | |

***Table 227**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey aquomethaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **6.226** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.09E-02 | 1.21E-02 | 1.37E-02 | 1.71E-02 | 1.35E-02 ± 1.6E-03 |
| 4.00E-04 | 1.20E-02 | 1.36E-02 | 1.42E-02 | 1.56E-02 | 1.38E-02 ± 1.2E-03 |
| 5.00E-04 | 1.29E-02 | 1.50E-02 | 1.55E-02 | 1.66E-02 | 1.50E-02 ± 9.3E-04 |
| 6.00E-04 | 1.26E-02 | 1.46E-02 | 1.76E-02 | 1.85E-02 | 1.58E-02 ± 1.5E-03 |
| Slope = 8.19E+00 ± 1.0E+00; Intercept = 1.08E-02 ± 4.7E-04 | | | | | |

***Table 228**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey aquomethaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **6.423** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.15E-02 | 1.26E-02 | 1.46E-02 | 1.60E-02 | 1.36E-02 ± 1.1E-03 |
| 4.00E-04 | 1.21E-02 | 1.47E-02 | 1.69E-02 | 1.78E-02 | 1.54E-02 ± 1.9E-03 |
| 5.00E-04 | 1.37E-02 | 1.44E-02 | 1.66E-02 | 1.86E-02 | 1.58E-02 ± 1.2E-03 |
| 6.00E-04 | 1.36E-02 | 1.57E-02 | 1.62E-02 | 1.85E-02 | 1.60E-02 ± 1.2E-03 |
| Slope = 7.47E+00 ± 2.6E+00; Intercept = 1.19E-02 ± 1.2E-03 | | | | | |

***Table 229**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey aquomethaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **6.571** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.06E-02 | 1.26E-02 | 1.43E-02 | 1.60E-02 | 1.34E-02 ± 1.4E-03 |
| 4.00E-04 | 1.15E-02 | 1.34E-02 | 1.53E-02 | 1.75E-02 | 1.44E-02 ± 2.0E-03 |
| 5.00E-04 | 1.24E-02 | 1.46E-02 | 1.57E-02 | 1.75E-02 | 1.50E-02 ± 1.3E-03 |
| 6.00E-04 | 1.47E-02 | 1.65E-02 | 1.75E-02 | 1.91E-02 | 1.70E-02 ± 1.1E-03 |
| Slope = 1.14E+01 ± 1.9E+00; Intercept = 9.84E-03 ± 8.7E-04 | | | | | |

***Table 230**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey aquomethaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **6.774** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.18E-02 | 1.26E-02 | 1.35E-02 | 1.40E-02 | 1.30E-02 ± 5.5E-04* |
| 4.00E-04 | 1.09E-02 | 1.35E-02 | 1.37E-02 | 1.45E-02 | 1.31E-02 ± 1.2E-03 |
| 5.00E-04 | 1.27E-02 | 1.38E-02 | 1.40E-02 | 1.42E-02 | 1.37E-02 ± 3.7E-04 |
| 6.00E-04 | 1.25E-02 | 1.46E-02 | 1.54E-02 | 1.79E-02 | 1.51E-02 ± 1.4E-03 |
| Slope = 9.87E+00 ± 2.6E+00; Intercept = 9.05E-03 ± 1.3E-03 | | | | | |

***Table 231**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey aquomethaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **6.958** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.03E-02 | 1.33E-02 | 1.41E-02 | 1.46E-02 | 1.31E-02 ± 1.1E-03 |
| 4.00E-04 | 1.17E-02 | 1.35E-02 | 1.40E-02 | 1.58E-02 | 1.38E-02 ± 1.4E-03 |
| 5.00E-04 | 1.22E-02 | 1.34E-02 | 1.41E-02 | 1.46E-02 | 1.36E-02 ± 5.9E-04* |
| 6.00E-04 | 1.54E-02 | 1.47E-02 | 1.68E-02 | 1.76E-02 | 1.61E-02 ± 7.3E-04 |
| Slope = 1.02E+01 ± 1.3E+00; Intercept = 9.89E-03 ± 5.7E-04 | | | | | |

***Table 232**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey aquomethaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **7.170** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.29E-02 | 1.48E-02 | 1.56E-02 | 1.78E-02 | 1.53E-02 ± 1.2E-03 |
| 4.00E-04 | 9.50E+01* | 1.36E-02 | 1.54E-02 | 1.68E-02* | 1.45E+01 ± 3.2E+01* |
| 5.00E-04 | 1.47E-02 | 1.55E-02 | 1.66E-02 | 1.80E-02 | 1.62E-02 ± 8.2E-04 |
| 6.00E-04 | 1.16E-02 | 1.86E-02 | 2.16E-02 | 2.09E-02 | 1.81E-02 ± 2.5E-03 |
| Slope = 9.62E+00 ± 3.1E+00; Intercept = 1.19E-02 ± 1.4E-03 | | | | | |

***Table 233**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey aquomethaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **7.355** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.02E-02 | 1.27E-02 | 1.44E-02 | 1.55E-02 | 1.32E-02 ± 1.3E-03 |
| 4.00E-04 | 1.23E-02 | 1.36E-02 | 1.53E-02 | 1.66E-02 | 1.44E-02 ± 1.4E-03 |
| 5.00E-04 | 1.24E-02 | 1.46E-02 | 1.67E-02 | 1.76E-02 | 1.53E-02 ± 1.3E-03 |
| 6.00E-04 | 1.22E-02 | 1.46E-02 | 1.75E-02 | 2.02E-02 | 1.61E-02 ± 2.0E-03 |
| Slope = 9.73E+00 ± 6.5E-01; Intercept = 1.04E-02 ± 3.0E-04 | | | | | |

***Table 234**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey aquomethaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **7.578** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.36E-02 | 1.44E-02 | 1.52E-02 | 1.76E-02 | 1.52E-02 ± 9.9E-04 |
| 4.00E-04 | 1.37E-02 | 1.45E-02 | 1.59E-02 | 1.85E-02 | 1.57E-02 ± 1.6E-03 |
| 5.00E-04 | 1.46E-02 | 1.51E-02 | 1.73E-02 | 1.94E-02 | 1.66E-02 ± 1.2E-03 |
| 6.00E-04 | 1.55E-02 | 1.75E-02 | 1.89E-02 | 2.21E-02 | 1.85E-02 ± 1.6E-03 |
| Slope = 1.09E+01 ± 2.3E+00; Intercept = 1.16E-02 ± 1.1E-03 | | | | | |

***Table 235**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey aquomethaemoglobin** in the presence of **inositol-$P_6$** at 25°C in phosphate buffer pH **7.761** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.26E-02 | 1.36E-02 | 1.57E-02 | 1.71E-02 | 1.47E-02 ± 1.1E-03 |
| 4.00E-04 | 1.54E-02 | 1.65E-02 | 1.78E-02 | 1.85E-02 | 1.70E-02 ± 1.0E-03 |
| 5.00E-04 | 1.16E-02 | 1.36E-02 | 1.52E-02 | 2.91E-02 | 1.73E-02 ± 4.4E-03 |
| 6.00E-04 | 1.56E-02 | 1.61E-02 | 1.85E-02 | 1.96E-02 | 1.74E-02 ± 9.8E-04 |
| Slope = 8.44E+00 ± 3.7E+00; Intercept = 1.28E-02 ± 1.7E-03 | | | | | |

***Table 236**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey aquomethaemoglobin** in the presence of **inositol-$P_6$** at 25°C in borate buffer pH **7.958** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.14E-02 | 1.49E-02 | 1.73E-02 | 1.94E-02 | 1.57E-02 ± 2.0E-03 |
| 4.00E-04 | 1.49E-02 | 1.53E-02 | 1.61E-02 | 1.76E-02 | 1.59E-02 ± 8.9E-04 |
| 5.00E-04 | 1.46E-02 | 1.66E-02 | 1.88E-02 | 1.96E-02 | 1.74E-02 ± 1.2E-03 |
| 6.00E-04 | 1.26E-02 | 1.49E-02 | 1.67E-02 | 1.86E-02 | 1.57E-02 ± 1.5E-03* |
| Slope = 8.24E+00 ± 3.6E+00; Intercept = 1.31E-02 ± 1.5E-03 | | | | | |

***Table 237**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey aquomethaemoglobin** in the presence of **inositol-$P_6$** at 25°C in borate buffer pH **8.208** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.12E-02 | 1.30E-02 | 1.34E-02 | 1.52E-02 | 1.32E-02 ± 1.0E-03 |
| 4.00E-04 | 1.16E-02 | 1.39E-02 | 1.60E-02 | 1.79E-02 | 1.49E-02 ± 2.1E-03 |
| 5.00E-04 | 1.30E-02 | 1.42E-02 | 1.49E-02 | 1.78E-02 | 1.50E-02 ± 1.2E-03 |
| 6.00E-04 | 1.46E-02 | 1.73E-02 | 1.86E-02 | 2.00E-02 | 1.76E-02 ± 1.4E-03 |
| Slope = 1.34E+01 ± 3.3E+00; Intercept = 9.12E-03 ± 1.5E-03 | | | | | |

***Table 238**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey aquomethaemoglobin** in the presence of **inositol-$P_6$** at 25°C in borate buffer pH **8.427** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.18E-02 | 1.37E-02 | 1.57E-02 | 1.85E-02 | 1.49E-02 ± 1.7E-03 |
| 4.00E-04 | 1.46E-02 | 1.63E-02 | 1.74E-02 | 1.89E-02 | 1.68E-02 ± 1.4E-03 |
| 5.00E-04 | 1.46E-02 | 1.70E-02 | 1.79E-02 | 1.90E-02 | 1.71E-02 ± 1.1E-03 |
| 6.00E-04 | 1.66E-02 | 1.72E-02 | 1.81E-02 | 1.87E-02 | 1.76E-02 ± 5.3E-04 |
| Slope = 8.35E+00 ± 2.5E+00; Intercept = 1.29E-02 ± 1.1E-03 | | | | | |

***Table 239**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey aquomethaemoglobin** in the presence of **inositol-$P_6$** at 25°C in borate buffer pH **8.661** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.45E-02 | 1.65E-02 | 1.86E-02 | 1.98E-02 | 1.73E-02 ± 1.3E-03 |
| 4.00E-04 | 1.49E-02 | 1.66E-02 | 1.92E-02 | 1.96E-02 | 1.75E-02 ± 1.6E-03 |
| 5.00E-04 | 1.37E-02 | 1.62E-02 | 1.96E-02 | 2.12E-02 | 1.77E-02 ± 1.9E-03 |
| 6.00E-04 | 1.55E-02 | 1.85E-02 | 1.69E-02 | 2.19E-02 | 1.82E-02 ± 1.6E-03 |
| Slope = 2.70E+00 ± 6.4E-01; Intercept = 1.65E-02 ± 3.0E-04 | | | | | |

***Table 240**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey aquomethaemoglobin** in the presence of **inositol-$P_6$** at 25°C in borate buffer pH **8.724** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.34E-02 | 1.59E-02 | 1.66E-02 | 1.75E-02 | 1.58E-02 ± 1.0E-03 |
| 4.00E-04 | 1.44E-02 | 1.59E-02 | 1.67E-02 | 1.96E-02 | 1.67E-02 ± 1.8E-03 |
| 5.00E-04 | 1.30E-02 | 1.56E-02 | 1.82E-02 | 2.00E-02 | 1.67E-02 ± 1.8E-03 |
| 6.00E-04 | 1.55E-02 | 1.60E-02 | 1.85E-02 | 1.95E-02 | 1.74E-02 ± 1.0E-03 |
| Slope = 4.72E+00 ± 1.0E+00; Intercept = 1.45E-02 ± 4.7E-04 | | | | | |

***Table 241**: The dependence of the pseudo-first order rate constant, $k_{obs}$, on the DTNB concentration for the reaction of DTNB with **donkey aquomethaemoglobin** in the presence of **inositol-$P_6$** at 25°C in borate buffer pH **8.928** (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [Hb] = 10 µmol (haem) $dm^{-3}$(5 µmol $dm^{-3}$ reactive sulphydryl groups).*

| [DTNB] (mol $dm^{-3}$) | $k_{1obs}$ $s^{-1}$ | $k_{2obs}$ $s^{-1}$ | $k_{3obs}$ $s^{-1}$ | $k_{4obs}$ $s^{-1}$ | Mean $k_{obs}$ $s^{-1}$ |
|---|---|---|---|---|---|
| 3.00E-04 | 1.34E-02 | 1.60E-02 | 1.76E-02 | 1.89E-02 | 1.65E-02 ± 1.4E-03 |
| 4.00E-04 | 1.45E-02 | 1.63E-02 | 1.79E-02 | 1.96E-02 | 1.71E-02 ± 1.7E-03 |
| 5.00E-04 | 1.44E-02 | 1.66E-02 | 1.87E-02 | 1.91E-02 | 1.72E-02 ± 1.2E-03 |
| 6.00E-04 | 1.55E-02 | 1.60E-02 | 1.85E-02 | 2.01E-02 | 1.75E-02 ± 1.2E-03 |
| Slope = 3.39E+00 ± 6.1E-01; Intercept = 1.55E-02 ± 2.8E-04 | | | | | |

***Table 242:*** *Dependence of $k_F$, the apparent forward second order rate constant, on pH for the reaction of 5,5'-dithiobis(2-nitrobenzoic acid) with* ***stripped dog oxyhaemoglobin*** *at 25°C.*

*Conditions: phosphate buffers pH 5.6 – 7.8 and borate buffer pH 8.0 – 9.0 (ionic strength 50mmol $dm^{-3}$; added salt, NaCl); [$HbO_2$] = 10 µmol (haem) $dm^{-3}$; [DTNB] = 300 µmol $dm^{-3}$ – 600 µmol $dm^{-3}$.*

| pH | $k_F$ ($mol^{-1}$ $dm^3$ $s^{-1}$) |
|---|---|
| 5.612 | 10.520 ± 1.6 |
| 5.831 | 33.950 ± 6.4* |
| 6.050 | 10.350 ± 3.9 |
| 6.208 | 10.616 ± 1.5 |
| 6.405 | 9.105 ± 3.2 |
| 6.605 | 10.607 ± 5.0 |
| 6.807 | 11.958 ± 0.7 |
| 7.005 | 9.943 ± 3.1 |
| 7.223 | 11.440 ± 2.9 |
| 7.433 | 13.515 ± 2.2 |
| 7.614 | 11.797 ± 1.9 |
| 7.776 | 31.557 ± 7.7* |
| 8.003 | 24.647 ± 3.2* |
| 8.156 | 17.384 ± 1.6 |
| 8.408 | 14.195 ± 3.8 |
| 8.608 | 13.390 ± 0.3 |
| 8.838 | 10.484 ± 3.3 |
| 9.058 | 6.016 ± 1.8 |

***Table 243:*** *Dependence of $k_F$, the apparent forward second order rate constant, on pH for the reaction of 5,5'-dithiobis(2-nitrobenzoic acid) with* ***stripped dog carbonmonoxyhaemoglobin*** *at 25°C.*

*Conditions: phosphate buffers pH 5.6 – 7.8 and borate buffer pH 8.0 – 9.0 (ionic strength 50mmol $dm^{-3}$; added salt, NaCl); [$HbO_2$] = 10 µmol (haem) $dm^{-3}$; [DTNB] = 300 µmol $dm^{-3}$ – 600 µmol $dm^{-3}$.*

| pH | $k_F$ ($mol^{-1}$ $dm^3$ $s^{-1}$) |
|---|---|
| 5.596 | 7.871 ± 2.0* |
| 5.760 | 14.258 ± 6.9 |
| 6.076 | 13.370 ± 1.4 |
| 6.223 | 10.830 ± 0.4 |
| 6.447 | 9.257 ± 1.0 |
| 6.622 | 7.617 ± 1.3 |
| 6.799 | 4.363 ± 0.1 |
| 6.974 | 1.934 ± 0.1* |
| 7.196 | 4.761 ± 1.3 |
| 7.375 | 4.319 ± 0.7 |
| 7.591 | 7.288 ± 0.3 |
| 7.792 | 4.385 ± 0.9 |
| 7.971 | 9.739 ± 2.8 |
| 8.218 | 10.840 ± 3.9 |
| 8.381 | 9.476 ± 4.0 |
| 8.604 | 11.660 ± 2.7 |
| 8.815 | 9.585 ± 5.4* |
| 9.068 | 14.390 ± 3.9 |

***Table 244:*** *Dependence of $k_F$, the apparent forward second order rate constant, on pH for the reaction of 5,5'-dithiobis(2-nitrobenzoic acid) with* ***stripped dog aquomethaemoglobin*** *at 25°C.*

*Conditions: phosphate buffers pH 5.6 – 7.8 and borate buffer pH 8.0 – 9.0 (ionic strength 50mmol $dm^{-3}$; added salt, NaCl); [$HbO_2$] = 10 µmol (haem) $dm^{-3}$; [DTNB] = 300 µmol $dm^{-3}$ – 600 µmol $dm^{-3}$.*

| pH | $k_F$ ($mol^{-1}$ $dm^3$ $s^{-1}$) |
|---|---|
| 5.618 | 28.447 ± 9.6 |
| 5.784 | 18.520 ± 3.5 |
| 6.074 | 11.512 ± 3.1 |
| 6.228 | 8.682 ± 2.3 |
| 6.416 | 5.496 ± 1.4 |
| 6.511 | 13.101 ± 2.6* |
| 6.765 | 5.423 ± 1.2 |
| 6.968 | 7.299 ± 2.0 |
| 7.174 | 10.758 ± 1.9* |
| 7.369 | 12.436 ± 3.3 |
| 7.576 | 5.317 ± 0.5 |
| 7.777 | 5.989 ± 1.3 |
| 7.958 | 4.384 ± 0.7 |
| 8.189 | 7.771 ± 0.8 |
| 8.394 | 2.973 ± 0.7 |
| 8.606 | 6.293 ± 0.9 |
| 8.786 | 3.939 ± 0.7 |
| 8.989 | 4.139 ± 0.5 |

***Table 245:*** *Dependence of $k_F$, the apparent forward second order rate constant, on pH for the reaction of 5,5'-dithiobis(2-nitrobenzoic acid) with* ***dog oxyhaemoglobin in the presence of inositol-$P_6$*** *at 25°C.*

*Conditions: phosphate buffers pH 5.6 – 7.8 and borate buffer pH 8.0 – 9.0 (ionic strength 50mmol $dm^{-3}$; added salt, NaCl); [HbCO] = 10 µmol (haem) $dm^{-3}$; [DTNB] = 300 µmol $dm^{-3}$ – 600 µmol $dm^{-3}$.*

| pH | $k_F$ ($mol^{-1}$ $dm^3$ $s^{-1}$) |
|---|---|
| 5.614 | 9.120 ± 0.2 |
| 5.811 | 9.089 ± 4.1 |
| 6.029 | 5.010 ± 0.7* |
| 6.224 | 6.677 ± 0.8 |
| 6.403 | 8.250 ± 2.0 |
| 6.589 | 10.160 ± 4.4* |
| 6.801 | 6.779 ± 1.1 |
| 6.998 | 6.598 ± 1.0 |
| 7.171 | 0.700 ± 0.3* |
| 7.396 | 6.035 ± 1.7 |
| 7.606 | 5.590 ± 0.7 |
| 7.807 | 7.110 ± 2.1 |
| 7.994 | 6.344 ± 3.5 |
| 8.197 | 7.560 ± 1.0 |
| 8.398 | 8.465 ± 1.9 |
| 8.61 | 9.747 ± 0.3 |
| 8.827 | 8.607 ± 1.3 |
| 8.968 | 11.075 ± 3.3* |

***Table 246:*** *Dependence of $k_F$, the apparent forward second order rate constant, on pH for the reaction of 5,5'-dithiobis(2-nitrobenzoic acid) with* ***dog carbonmonoxyhaemoglobin in the presence of inositol-$P_6$*** *at 25°C.*

*Conditions: phosphate buffers pH 5.6 – 7.8 and borate buffer pH 8.0 – 9.0 (ionic strength 50mmol $dm^{-3}$; added salt, NaCl); [HbCO] = 10 µmol (haem) $dm^{-3}$; [DTNB] = 300 µmol $dm^{-3}$ – 600 µmol $dm^{-3}$.*

| pH | $k_F$ ($mol^{-1}$ $dm^3$ $s^{-1}$) |
|---|---|
| 5.605 | 13.922 ± 3.6 |
| 5.831 | 12.940 ± 6.9 |
| 6.014 | 3.080 ± 0.7* |
| 6.191 | 9.416 ± 3.4 |
| 6.383 | 8.055 ± 1.4 |
| 6.578 | 7.300 ± 3.7 |
| 6.841 | 3.508 ± 0.6 |
| 6.975 | 2.338 ± 0.8 |
| 7.187 | 4.995 ± 1.6 |
| 7.406 | 9.850 ± 2.2* |
| 7.614 | 5.034 ± 0.2 |
| 7.802 | 3.115 ± 1.2 |
| 8.016 | 1.168 ± 0.5* |
| 8.214 | 3.841 ± 1.2 |
| 8.417 | 6.441 ± 2.2 |
| 8.603 | 3.264 ± 0.8 |
| 8.796 | 7.569 ± 2.8 |
| 8.988 | 5.026 ± 0.5 |

***Table 247:*** *Dependence of $k_F$, the apparent forward second order rate constant, on pH for the reaction of 5,5'-dithiobis(2-nitrobenzoic acid) with* ***dog aquomethaemoglobin in the presence of inositol-$P_6$*** *at 25°C.*

*Conditions: phosphate buffers pH 5.6 – 7.8 and borate buffer pH 8.0 – 9.0 (ionic strength 50mmol $dm^{-3}$; added salt, NaCl); [HbCO] = 10 µmol (haem) $dm^{-3}$; [DTNB] = 300 µmol $dm^{-3}$ – 600 µmol $dm^{-3}$.*

| pH | $k_F$ ($mol^{-1}$ $dm^3$ $s^{-1}$) |
|---|---|
| 5.586 | 2.640 ± 1.2 |
| 5.825 | 4.885 ± 2.0 |
| 6.114 | 3.878 ± 1.1 |
| 6.212 | 7.446 ± 3.1 |
| 6.426 | 11.592 ± 1.1 |
| 6.571 | 11.823 ± 1.7 |
| 6.773 | 11.884 ± 2.3 |
| 6.986 | 13.655 ± 5.4 |
| 7.16 | 11.867 ± 2.3 |
| 7.354 | 14.866 ± 5.0 |
| 7.576 | 11.752 ± 2.5 |
| 7.764 | 11.787 ± 2.8 |
| 7.968 | 27.100 ± 1.3* |
| 8.175 | 0.335 ± 0.2* |
| 8.407 | 12.403 ± 3.9 |
| 8.582 | 13.632 ± 5.5 |
| 8.787 | 11.601 ± 3.2 |
| 9.001 | 11.799 ± 4.4 |

***Table 248:*** *Dependence of $k_F$, the apparent forward second order rate constant, on pH for the reaction of 5,5'-dithiobis(2-nitrobenzoic acid) with* ***stripped donkey oxyhaemoglobin*** *at 25°C.*

*Conditions: phosphate buffers pH 5.6 – 7.8 and borate buffer pH 8.0 – 9.0 (ionic strength 50mmol $dm^{-3}$; added salt, NaCl); [$HbO_2$] = 10 µmol (haem) $dm^{-3}$; [DTNB] = 300 µmol $dm^{-3}$ – 600 µmol $dm^{-3}$.*

| pH | $k_F$ ($mol^{-1}$ $dm^3$ $s^{-1}$) |
|---|---|
| 5.616 | 0.750 ± 0.1 |
| 5.831 | 10.379 ± 2.4* |
| 6.040 | 4.175 ± 0.5 |
| 6.212 | 3.495 ± 0.3 |
| 6.400 | 7.643 ± 0.5 |
| 6.594 | 9.653 ± 3.0 |
| 6.807 | 6.451 ± 0.5 |
| 6.997 | 8.156 ± 1.6 |
| 7.223 | 8.360 ± 1.1 |
| 7.411 | 7.643 ± 2.6 |
| 7.612 | 8.610 ± 2.0 |
| 7.768 | 6.269 ± 0.4 |
| 8.007 | 7.769 ± 2.9 |
| 8.169 | 7.223 ± 1.8 |
| 8.408 | 9.034 ± 2.0 |
| 8.606 | 6.926 ± 1.1 |
| 8.858 | 8.163 ± 1.8 |
| 8.996 | 9.022 ± 2.1 |

***Table 249:*** *Dependence of $k_F$, the apparent forward second order rate constant, on pH for the reaction of 5,5'-dithiobis(2-nitrobenzoic acid) with* ***stripped donkey carbonmonoxyhaemoglobin*** *at 25°C.*

*Conditions: phosphate buffers pH 5.6 – 7.8 and borate buffer pH 8.0 – 9.0 (ionic strength 50mmol $dm^{-3}$; added salt, NaCl); [$HbO_2$] = 10 µmol (haem) $dm^{-3}$; [DTNB] = 300 µmol $dm^{-3}$ – 600 µmol $dm^{-3}$.*

| pH | $k_F$ ($mol^{-1}$ $dm^3$ $s^{-1}$) |
|---|---|
| 5.704 | 10.230 ± 1.5* |
| 5.872 | 4.237 ± 1.0 |
| 6.049 | 6.119 ± 0.5 |
| 6.206 | 8.082 ± 0.4 |
| 6.404 | 14.252 ± 3.7 |
| 6.554 | 14.725 ± 8.1 |
| 6.827 | 14.995 ± 2.7 |
| 7.087 | 13.575 ± 5.7 |
| 7.228 | 13.044 ± 3.0 |
| 7.380 | 25.364 ± 3.7* |
| 7.570 | 14.038 ± 4.3 |
| 7.824 | 52.543 ± 18.0* |
| 8.059 | 13.560± 1.0 |
| 8.144 | 11.822 ± 2.2 |
| 8.364 | 8.561 ± 4.9 |
| 8.552 | 23.600 ± 3.3* |
| 8.780 | 9.615± 3.3 |
| 8.958 | 5.375± 2.9 |

***Table 250:*** *Dependence of $k_F$, the apparent forward second order rate constant, on pH for the reaction of 5,5'-dithiobis(2-nitrobenzoic acid) with* ***stripped donkey aquomethaemoglobin*** *at 25°C.*

*Conditions: phosphate buffers pH 5.6 – 7.8 and borate buffer pH 8.0 – 9.0 (ionic strength 50mmol $dm^{-3}$; added salt, NaCl); [$HbO_2$] = 10 µmol (haem) $dm^{-3}$; [DTNB] = 300 µmol $dm^{-3}$ – 600 µmol $dm^{-3}$.*

| pH | $k_F$ ($mol^{-1}$ $dm^3$ $s^{-1}$) |
|---|---|
| 5.614 | 2.160 ± 0.7 |
| 5.852 | 21.736 ± 5.8* |
| 6.061 | 5.050 ± 1.9 |
| 6.221 | 8.384 ± 1.5 |
| 6.425 | 11.649 ± 0.7 |
| 6.627 | 13.464 ± 5.0 |
| 6.816 | 11.747 ± 2.8 |
| 6.974 | 10.924 ± 1.7 |
| 7.150 | 12.350 ± 2.6 |
| 7.373 | 14.454 ± 3.6 |
| 7.552 | 10.486 ± 1.7 |
| 7.733 | 10.404 ± 1.9 |
| 8.008 | 6.936 ± 0.9 |
| 8.223 | 4.450 ± 0.8 |
| 8.421 | 4.570 ± 1.2 |
| 8.615 | 5.862 ± 1.2 |
| 8.713 | 6.331 ± 1.1 |
| 8.930 | 9.680 ± 1.3 |

***Table 251:*** *Dependence of $k_F$, the apparent forward second order rate constant, on pH for the reaction of 5,5'-dithiobis(2-nitrobenzoic acid) with* ***donkey oxyhaemoglobin in the presence of inositol-$P_6$*** *at 25°C.*

*Conditions: phosphate buffers pH 5.6 – 7.8 and borate buffer pH 8.0 – 9.0 (ionic strength 50mmol $dm^{-3}$; added salt, NaCl); [HbCO] = 10 µmol (haem) $dm^{-3}$; [DTNB] = 300 µmol $dm^{-3}$ – 600 µmol $dm^{-3}$.*

| pH | $k_F$ ($mol^{-1}$ $dm^3$ $s^{-1}$) |
|---|---|
| 5.611 | 11.570 ± 2.4 |
| 5.809 | 5.795 ± 2.0* |
| 6.029 | 12.925 ± 0.9 |
| 6.225 | 11.620 ± 4.4 |
| 6.401 | 3.875 ± 0.5* |
| 6.588 | 9.241 ± 0.6 |
| 6.801 | 12.050 ± 4.0 |
| 6.996 | 10.035 ± 0.2 |
| 7.171 | 8.515 ± 3.4 |
| 7.394 | 5.429 ± 3.1 |
| 7.606 | 2.525 ± 0.3* |
| 7.808 | 4.871 ± 2.1 |
| 7.994 | 5.320 ± 1.8 |
| 8.196 | 7.951 ± 2.9 |
| 8.399 | 8.866 ± 1.8 |
| 8.607 | 10.132 ± 2.4 |
| 8.832 | 1.344 ± 0.48* |
| 8.968 | 3.373 ± 1.0* |

***Table 252:*** *Dependence of $k_F$, the apparent forward second order rate constant, on pH for the reaction of 5,5'-dithiobis(2-nitrobenzoic acid) with* ***donkey carbonmonoxyhaemoglobin in the presence of inositol-$P_6$*** *at 25°C.*

*Conditions: phosphate buffers pH 5.6 – 7.8 and borate buffer pH 8.0 – 9.0 (ionic strength 50mmol $dm^{-3}$; added salt, NaCl); [HbCO] = 10 µmol (haem) $dm^{-3}$; [DTNB] = 300 µmol $dm^{-3}$ – 600 µmol $dm^{-3}$.*

| pH | $k_F$ ($mol^{-1}$ $dm^3$ $s^{-1}$) |
|---|---|
| 5.616 | 5.222 ± 0.4 |
| 5.831 | 7.379 ± 1.7* |
| 6.040 | 6.000 ± 1.0 |
| 6.212 | 5.375 ± 0.4 |
| 6.400 | 7.643 ± 0.5* |
| 6.594 | 5.903 ± 0.8 |
| 6.807 | 6.451 ± 0.5 |
| 6.997 | 6.200 ± 0.6 |
| 7.223 | 8.360 ± 1.1 |
| 7.411 | 8.902 ± 2.2 |
| 7.612 | 13.796 ± 3.1* |
| 7.768 | 9.615 ± 1.9 |
| 8.007 | 7.769 ± 2.9 |
| 8.169 | 6.400 ± 2.0 |
| 8.408 | 12.664 ± 2.4* |
| 8.606 | 6.926 ± 1.1 |
| 8.858 | 4.449 ± 0.4 |
| 9.010 | 4.213 ± 1.7 |

***Table 253:*** *Dependence of $k_F$, the apparent forward second order rate constant, on pH for the reaction of 5,5'-dithiobis(2-nitrobenzoic acid) with* ***donkey aquomethaemoglobin in the presence of inositol-$P_6$*** *at 25°C.*

*Conditions: phosphate buffers pH 5.6 – 7.8 and borate buffer pH 8.0 – 9.0 (ionic strength 50mmol $dm^{-3}$; added salt, NaCl); [HbCO] = 10 µmol (haem) $dm^{-3}$; [DTNB] = 300 µmol $dm^{-3}$ – 600 µmol $dm^{-3}$.*

| pH | $k_F$ ($mol^{-1}$ $dm^3$ $s^{-1}$) |
|---|---|
| 5.622 | 2.590 ± 0.8 |
| 5.813 | 3.639 ± 1.2 |
| 6.003 | 6.572 ± 2.0 |
| 6.226 | 8.194 ± 1.0 |
| 6.423 | 7.472 ± 2.6 |
| 6.571 | 11.360 ± 1.9* |
| 6.774 | 9.865 ± 2.6 |
| 6.958 | 10.244 ± 1.3 |
| 7.170 | 9.620 ± 3.1 |
| 7.355 | 9.727 ± 0.6 |
| 7.578 | 10.926 ± 2.3* |
| 7.761 | 8.435 ± 3.7 |
| 7.958 | 8.235 ± 3.6 |
| 8.208 | 13.394 ± 3.3* |
| 8.427 | 8.353 ± 2.5 |
| 8.661 | 2.697 ± 0.6 |
| 8.724 | 4.716 ± 1.0 |
| 8.928 | 3.386 ± 0.6 |

***Table 254:*** *Dependence of $log_{10}k_R$ on pH for the reaction of 5,5'-dithiobis(2-nitrobenzoic acid) with* ***stripped dog oxyhaemoglobin*** *at 25°C.*

*Conditions: phosphate buffers pH 5.6 – 7.8 and borate buffer pH 8.0 – 9.0 (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl).*

| pH | $Log_{10}k_R$ |
|---|---|
| 5.646 | -0.482 |
| 5.876 | 0.221 |
| 6.075 | -0.428 |
| 6.224 | 1.817* |
| 6.408 | 0.363 |
| 6.578 | 0.314 |
| 6.834 | 0.477 |
| 7.003 | 0.759 |
| 7.222 | 0.861 |
| 7.377 | 0.699 |
| 7.592 | 1.084 |
| 7.773 | 1.969 |
| 8.052 | 1.764 |
| 8.233 | 1.921 |
| 8.414 | 1.951 |
| 8.584 | 1.972 |
| 8.834 | 1.844 |
| 9.099 | 1.841 |

***Table 255:*** *Dependence of $log_{10}k_R$ on pH for the reaction of 5,5'-dithiobis(2-nitrobenzoic acid) with* ***stripped dog carbonmonoxyhaemoglobin*** *at 25°C.*
*Conditions: phosphate buffers pH 5.6 – 7.8 and borate buffer pH 8.0 – 9.0 (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl).*

| pH | $Log_{10}k_R$ |
|---|---|
| 5.623 | -0.637 |
| 5.810 | -0.186 |
| 6.038 | -0.296 |
| 6.207 | -0.061 |
| 6.459 | 0.324 |
| 6.571 | 0.277 |
| 6.810 | 0.571 |
| 7.042 | 0.732 |
| 7.183 | 1.154 |
| 7.378 | 1.117 |
| 7.561 | 1.303 |
| 7.786 | 1.456 |
| 8.046 | 2.304 |
| 8.199 | 2.386 |
| 8.386 | 2.405 |
| 8.582 | 2.209 |
| 8.768 | 2.164 |
| 8.999 | 2.604 |

***Table 256:*** *Dependence of $log_{10}k_R$ on pH for the reaction of 5,5'-dithiobis(2-nitrobenzoic acid) with* ***stripped dog aquomethaemoglobin*** *at 25°C.*

*Conditions: phosphate buffers pH 5.6 – 7.8 and borate buffer pH 8.0 – 9.0 (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl).*

| pH | $Log_{10}k_R$ |
|---|---|
| 5.609 | 0.165 |
| 5.852 | 0.392 |
| 6.067 | 0.449 |
| 6.234 | 0.437 |
| 6.403 | 0.637 |
| 6.536 | 0.859* |
| 6.748 | 0.664 |
| 6.949 | 0.535* |
| 7.157 | 0.911 |
| 7.335 | 1.176 |
| 7.613 | 1.078 |
| 7.754 | 1.180 |
| 7.994 | 1.368 |
| 8.170 | 1.909* |
| 8.367 | 1.589 |
| 8.558 | 1.907 |
| 8.818 | 1.791 |
| 9.020 | 1.702 |

***Table 257:*** *Dependence of $log_{10}k_R$ on pH for the reaction of 5,5'-dithiobis(2-nitrobenzoic acid) with* ***dog oxyhaemoglobin in the presence of inositol-$P_6$*** *at 25°C. Conditions: phosphate buffers pH 5.6 – 7.8 and borate buffer pH 8.0 – 9.0 (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl).*

| pH | $Log_{10}k_R$ *[inositol-$P_6$],10µmol $dm^{-3}$* |
|---|---|
| 5.567 | -0.618 |
| 5.776 | -0.563 |
| 5.985 | 0.038 |
| 6.237 | 0.141 |
| 6.402 | 0.469 |
| 6.590 | 1.018 |
| 6.811 | 0.913 |
| 7.019 | 0.902 |
| 7.201 | -0.085* |
| 7.423 | 1.241 |
| 7.653 | 1.419 |
| 7.764 | 1.933 |
| 7.877 | 2.580 |
| 8.239 | 3.534* |
| 8.409 | 2.020 |
| 8.560 | 2.354 |
| 8.814 | 2.470 |
| 9.044 | 2.562 |

***Table 258:*** *Dependence of $log_{10}k_R$ on pH for the reaction of 5,5'-dithiobis(2-nitrobenzoic acid) with* ***dog carbonmonoxyhaemoglobin in the presence of inositol-P$_6$*** *at 25°C.*

*Conditions: phosphate buffers pH 5.6 – 7.8 and borate buffer pH 8.0 – 9.0 (ionic strength 50 mmol dm$^{-3}$; added salt, NaCl).*

| pH | $Log_{10}k_R$ *[inositol-$P_6$], 10µmol dm$^{-3}$* |
|---|---|
| 5.578 | -0.282 |
| 5.866 | -0.377 |
| 6.067 | -0.162 |
| 6.256 | 0.153 |
| 6.437 | 0.398 |
| 6.634 | 0.685 |
| 6.821 | 0.311 |
| 7.003 | 0.766 |
| 7.179 | 1.173 |
| 7.393 | 1.649* |
| 7.557 | 2.696* |
| 7.801 | 1.008 |
| 8.018 | 0.901* |
| 8.172 | 1.575 |
| 8.404 | 1.809 |
| 8.592 | 1.637 |
| 8.808 | 1.886 |
| 8.979 | 2.101 |

***Table 259:*** *Dependence of $log_{10}k_R$ on pH for the reaction of 5,5'-dithiobis(2-nitrobenzoic acid) with* ***dog aquomethaemoglobin in the presence of inositol-$P_6$*** *at 25°C.*

*Conditions: phosphate buffers pH 5.6 – 7.8 and borate buffer pH 8.0 – 9.0 (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl).*

| pH | $Log_{10}k_R$ *[inositol-$P_6$], 10µmol $dm^{-3}$* |
|---|---|
| 5.633 | -1.111 |
| 5.838 | -0.592 |
| 6.122 | -0.097 |
| 6.206 | -0.097 |
| 6.468 | 0.482 |
| 6.616 | 0.661 |
| 6.782 | 0.434 |
| 6.938 | 1.324 |
| 7.130 | 0.979 |
| 7.377 | 1.077 |
| 7.638 | 1.188 |
| 7.777 | 1.576 |
| 8.009 | 2.289 |
| 8.183 | 0.716* |
| 8.454 | 2.178 |
| 8.566 | 2.178 |
| 8.794 | 2.255 |
| 9.016 | 2.573 |

***Table 260:*** *Dependence of $log_{10}k_R$ on pH for the reaction of 5,5'-dithiobis(2-nitrobenzoic acid) with* ***stripped donkey oxyhaemoglobin*** *at 25°C.*

*Conditions: phosphate buffers pH 5.6 – 7.8 and borate buffer pH 8.0 – 9.0 (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl).*

| pH | $Log_{10}k_R$ |
|---|---|
| 5.781 | 0.841* |
| 6.024 | 0.569 |
| 6.209 | 0.339 |
| 6.367 | 0.834 |
| 6.585 | 1.009 |
| 6.825 | 1.226 |
| 7.041 | 1.220 |
| 7.226 | 1.761 |
| 7.415 | 2.315* |
| 7.603 | 1.811 |
| 7.603 | 2.143 |
| 7.753 | 1.998 |
| 8.001 | 2.257 |
| 8.184 | 2.246 |
| 8.410 | 2.509 |
| 8.643 | 2.539 |
| 8.841 | 2.249 |
| 8.970 | 2.524 |

***Table 261:*** *Dependence of $log_{10}k_R$ on pH for the reaction of 5,5'-dithiobis(2-nitrobenzoic acid) with* ***stripped donkey carbonmonoxyhaemoglobin*** *at 25°C.*
*Conditions: phosphate buffers pH 5.6 – 7.8 and borate buffer pH 8.0 – 9.0 (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl).*

| pH | $Log_{10}k_R$ |
|---|---|
| 5.652 | -0.095 |
| 5.845 | -0.103 |
| 5.992 | 0.314 |
| 6.185 | 0.729 |
| 6.404 | 1.165 |
| 6.545 | 1.273 |
| 6.831 | 1.580 |
| 7.009 | 1.483 |
| 7.191 | 1.793 |
| 7.371 | 2.490 |
| 7.548 | 2.448 |
| 7.819 | 3.137* |
| 8.004 | 2.497 |
| 8.206 | 2.504 |
| 8.362 | 2.485 |
| 8.575 | 2.958 |
| 8.768 | 2.162 |
| 9.070 | 1.531* |

***Table 262:*** *Dependence of $log_{10}k_R$ on pH for the reaction of 5,5'-dithiobis(2-nitrobenzoic acid) with* ***stripped donkey aquomethaemoglobin*** *at 25°C.*

*Conditions: phosphate buffers pH 5.6 – 7.8 and borate buffer pH 8.0 – 9.0 (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl).*

| pH | $Log_{10}k_R$ |
|---|---|
| 5.603 | 0.519 |
| 5.811 | 1.490* |
| 6.025 | 1.070 |
| 6.215 | 1.295 |
| 6.448 | 1.552 |
| 6.633 | 1.694 |
| 6.807 | 1.471 |
| 7.017 | 1.749 |
| 7.172 | 2.041 |
| 7.372 | 2.226 |
| 7.541 | 2.167 |
| 7.754 | 2.474 |
| 7.983 | 2.684 |
| 8.219 | 2.341 |
| 8.419 | 2.237 |
| 8.588 | 2.438 |
| 8.758 | 2.367 |
| 8.994 | 2.368 |

***Table 263:*** *Dependence of $log_{10}k_R$ on pH for the reaction of 5,5'-dithiobis(2-nitrobenzoic acid) with* ***donkey oxyhaemoglobin in the presence of inositol-$P_6$*** *at 25°C.*

*Conditions: phosphate buffers pH 5.6 – 7.8 and borate buffer pH 8.0 – 9.0 (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl), [inositol-$P_6$], 10µmol $dm^{-3}$*

| pH | $Log_{10}k_R$ *[inositol-$P_6$], 10µmol $dm^{-3}$* |
|---|---|
| 5.606 | -0.872 |
| 5.848 | -0.948 |
| 6.033 | 0.043 |
| 6.232 | -0.366 |
| 6.358 | -0.404 |
| 6.594 | 0.443 |
| 6.790 | 0.740 |
| 6.984 | 0.673 |
| 7.182 | 0.688 |
| 7.392 | 0.832 |
| 7.582 | 0.679 |
| 7.792 | 1.059 |
| 7.974 | 1.033 |
| 8.189 | 1.071 |
| 8.405 | 1.254 |
| 8.581 | 1.238 |
| 8.803 | 0.579* |
| 8.959 | 0.967 |

***Table 264:*** *Dependence of $log_{10}k_R$ on pH for the reaction of 5,5'-dithiobis(2-nitrobenzoic acid) with* ***donkey carbonmonoxyhaemoglobin in the presence of inositol-$P_6$*** *at 25°C.*

*Conditions: phosphate buffers pH 5.6 – 7.8 and borate buffer pH 8.0 – 9.0 (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [inositol-$P_6$], 10µmol $dm^{-3}$*

| pH | $Log_{10}k_R$ *[inositol-$P_6$],10µmol $dm^{-3}$* |
|---|---|
| 5.642 | -1.170 |
| 5.839 | -0.716 |
| 6.004 | -0.614 |
| 6.174 | -0.317 |
| 6.357 | 0.018 |
| 6.580 | -0.081 |
| 6.786 | 0.482 |
| 6.975 | 0.835 |
| 7.211 | 1.237 |
| 7.387 | 1.381 |
| 7.584 | 1.592 |
| 7.747 | 1.669 |
| 8.003 | 1.606 |
| 8.174 | 1.315 |
| 8.383 | 1.693 |
| 8.585 | 1.619 |
| 8.829 | 1.405 |
| 8.971 | 1.358 |

***Table 265:*** *Dependence of $log_{10}k_R$ on pH for the reaction of 5,5'-dithiobis(2-nitrobenzoic acid) with* ***donkey aquomethaemoglobin in the presence of inositol-$P_6$*** *at 25°C.*

*Conditions: phosphate buffers pH 5.6 – 7.8 and borate buffer pH 8.0 – 9.0 (ionic strength 50 mmol $dm^{-3}$; added salt, NaCl); [inositol-$P_6$], 10 µmol $dm^{-3}$*

| pH | $Log_{10}k_R$ *[inositol-$P_6$], 10µmol $dm^{-3}$* |
|---|---|
| 5.635 | -2.020 |
| 5.851 | -1.366 |
| 6.029 | -0.685 |
| 6.196 | -0.733 |
| 6.389 | -0.545 |
| 6.562 | 0.179 |
| 6.808 | 0.436 |
| 6.956 | 0.450 |
| 7.166 | 1.071 |
| 7.355 | 1.174 |
| 7.565 | 1.515 |
| 7.774 | 1.475 |
| 7.955 | 1.543 |
| 8.201 | 1.672 |
| 8.396 | 1.638 |
| 8.606 | 1.122* |
| 8.741 | 1.374 |
| 8.943 | 1.067* |

## APPENDIX II

### Programme for Calculating Equilibrium Constant, $K_{equ}$, of the Reaction of Haemoglobin with DTNB

IndVars: pH, EXT, DTNBVOL

DepVars: TNBCONC, KEQ

Params: pKTNB, pKSH

pKTNB = 5.267

pKSH = 8.3

K1TNB = 1 + (10^(-pH)/10^(-pKTNB))

K1SH = 1 + (10^(-pH)/10^(-pKSH))

HBTOT = 25E-6

TNBCONC = EXT/28000

DTNBTOT = 0.029*DTNBVOL/(3000 + DTNBVOL)

NUM = TNBCONC*TNBCONC*K1TNB*K1SH

DENOM = (HBTOT - TNBCONC*K1TNB)*(DTNBTOT - TNBCONC*K1TNB)

KEQ = NUM/DENOM

***

**Programme for fitting pH dependent Equilibrium Constant of the Reaction of Haemoglobin with DTNB (n = 2)**

IndVars: PH

DepVars: PKEQ

Params: PQ1R, PQ2R, PQ1T, PQ2T, QUOT1, QUOT2, PKE3, KRT

Q1R = 10^(-PQ1R)

Q2R = 10^(-PQ2R)

Q1T = 10^(-PQ1T)

Q2T = 10^(-PQ2T)

KE3 = 10^(-PKE3)

FIRSTTOP = 1+ 10^(-PH)/10^(-PQ2R)

SECTOP = ((10^(-PH))^2)/(10^(-PQ1R)*(10^(-PQ2R)))

THIRDTOP = (1 + 10^(-PH)/10^(-PQ2T))

KRT3TOP = KRT*THIRDTOP

FOTOP = (((10^(-PH))^2)/(10^(-PQ1T)*(10^(-PQ2T))))

KRT4TOP = KRT*FOTOP

TOP = FIRSTTOP + SECTOP + KRT3TOP + KRT4TOP

NUM = KE3*TOP

QUOT1 = KE3/KE2

QUOT2 = KE3/KE1

FIRSTBOT = 1+ (QUOT1*10^(-PH)/10^(-PQ2R))

SECBOT = QUOT2*((10*(-PH))^2)/10^(-PQ1R)*10^(-PQ2R)

DENOM = FIRSTBOT + SECBOT

KEQ = NUM/DENOM

PKEQ = -LOG10(KEQ)

***

**Program for fitting forward kinetics of the reaction of DTNB with haemoglobin sulphydryl groups at various pH values (profile n = 1)**

IndVars: PH

DepVars:KF

Params: K1, K2, PQ1R, PQ1T, PQ1H, PQS2, KRT3

Q1R =10^(-PQ1R)

Q1T = 10^(-PQ1T)

Q1H = 10^(-PQ1H)

QS2 = 10^(-PQS2)

A = (K1*(10^(-PH)))/(10^(-PQ1R))

B = (10^(-PH))/(10^(-PQ1R))

C = (10^(-PH))/(10^(-PQS2))

D = (10^(-PH))/(10^(-PQ1H))

E = 1 + D

FOTBOT = E*(10^(-PH))/(10^(-PQS2))

F = (10^(-PH))/(10^(-PQ1T))

THIRDBOT = 1 + F

TFBOT = THIRDBOT + FOTBOT

BOTPROD = KRT3*TFBOT

FIRSTBOT = 1 + B

NUM = K1*B + K2

DENOM = FIRSTBOT + BOTPROD

KF = NUM/DENOM

***

**Program for fitting forward kinetics of the reaction of DTNB with haemoglobin sulphydryl groups at various pH values (profile n = 2)**

IndVars: PH

DepVars:KF

Params: K1, K2, K3, PQ1R, PQ2R, PQ1T, PQ2T, PQ1H, PQ2H, PQS3, KRT3

Q1R =10^(-PQ1R)

Q2R = 10^(-PQ2R)

Q1T = 10^(-PQ1T)

Q2T =10^(-PQ2T)

Q1H = 10^(-PQ1H)

Q2H = 10^(-PQ2H)

QS3 = 10^(-PQS3)

A = (K1*((10^(-PH))^2))/((10^(-PQ1R))*(10^(-PQ2R)))

B = (K2*(10^(-PH)))/(10^(-PQ2R))

C = ((10^(-PH))^2)/((10^(-PQ1R))*(10^(-PQ2R)))

E = (10^(-PH))/(10^(-PQ2R))

F = (10^(-PH))/(10^(-PQS3))

G = ((10^(-PH))^2)/(10^(-PQ1H) *(10^(-PQ2H)))

H = (10^(-PH))/(10^(-PQ2H))

I = 1 + H + G

FOTBOT = I*(10^(-PH))/(10^(-PQS3))

J = (10^(-PH))/(10^(-PQ2T))

L = ((10^(-PH))^2)/((10^(-PQ1T))*(10^(-PQ2T)))

THIRDBOT = 1 + J + L

TFBOT = THIRDBOT + FOTBOT

BOTPROD = KRT3*TFBOT

FIRSTBOT = 1 + C + E

NUM = K1*C + K2*E + K3

DENOM = FIRSTBOT + BOTPROD

KF = NUM/DENOM

***

**Program for fitting reverse kinetics of the reaction of DTNB with haemoglobin sulphydryl groups at various pH values (complex profile n=2)**

IndVars: PH

DepVars: LOGKR

Params: PQ1R, PQ2R, PQ1T, PQ2T, KRT3, K1, K2, K3

Q1R = 10^(-PQ1R)

Q2R= 10^(-PQ2R)

Q1T = 10^(-PQ1T)

Q2T= 10^(-PQ2T)

PQTNB = 5.267

QTNB = 10^(-PQTNB)

QTNBH = 10^(-PH) + 10^(-PQTNB)

RATIOTNB = QTNB/QTNBH

TOP =K3 + K2*10^(-PH)/(10^(-PQ2R)) + K1*(10^(-PH)^2)/((10^(-PQ1R))*(10^(-PQ2R)))

NUM = RATIOTNB*TOP

BOT1 = 1 + 10^(-PH)/(10^(-PQ2R)) + (10^(-PH)^2)/((10^(-PQ1R))*(10^(-PQ2R)))

BOT2 = 1 + 10^(-PH)/(10^(-PQ2T)) + (10^(-PH)^2)/((10^(-PQ1T))*(10^(-PQ2T)))

DENOM=BOT1+ (BOT2*KRT3)

KR = NUM/DENOM

LOGKR=LOG10(KR)

***

## 4.5. REVERSE KINETICS

The reaction of haemoglobin with 5,5'–dithiobis(2-nitrobenzoate), DTNB, consists of two opposing second order processes:

The reverse reaction is at present difficult to carry out experimentally because both $TNB^-$ and PS.ST are produced *in situ* and cannot both be isolated. However, the equilibrium and apparent second order forward rate constants for the reaction of haemoglobin with DTNB have been reported in the earlier pages of this thesis as functions of pH. It is therefore possible to determine the apparent second order reverse rate constant, $k_R$, at each pH from the relation; $k_R = \frac{k_F}{K_{equ}}$.

### 4.5.1 pH dependence of the apparent second order reverse rate constant, $k_R$, for the reverse of the reaction of the CysF9[93]β sulphydryl groups of donkey haemoglobin with DTNB

Inositol-$P_6$ had very little effect on the DTNB affinity of all dog haemoglobin derivatives and therefore a study of the dependences of $k_R$ on pH for the reverse reaction of the CysF9[93]β sulphydryl groups with DTNB was ignored.

The dependences of $k_R$ on pH for the reverse reaction of the CysF9[93]β sulphydryl groups of *stripped* donkey haemoglobin with DTNB are shown in Figs. 4.53, 4.54 and 4.55 for the oxy, carbonmonoxy and aquomet derivatives, respectively. It is seen that $\log_{10}k_R$ exhibits a strong pH dependence for each derivative and varies between two and four orders of magnitude between pH 5.6 and 9.0.

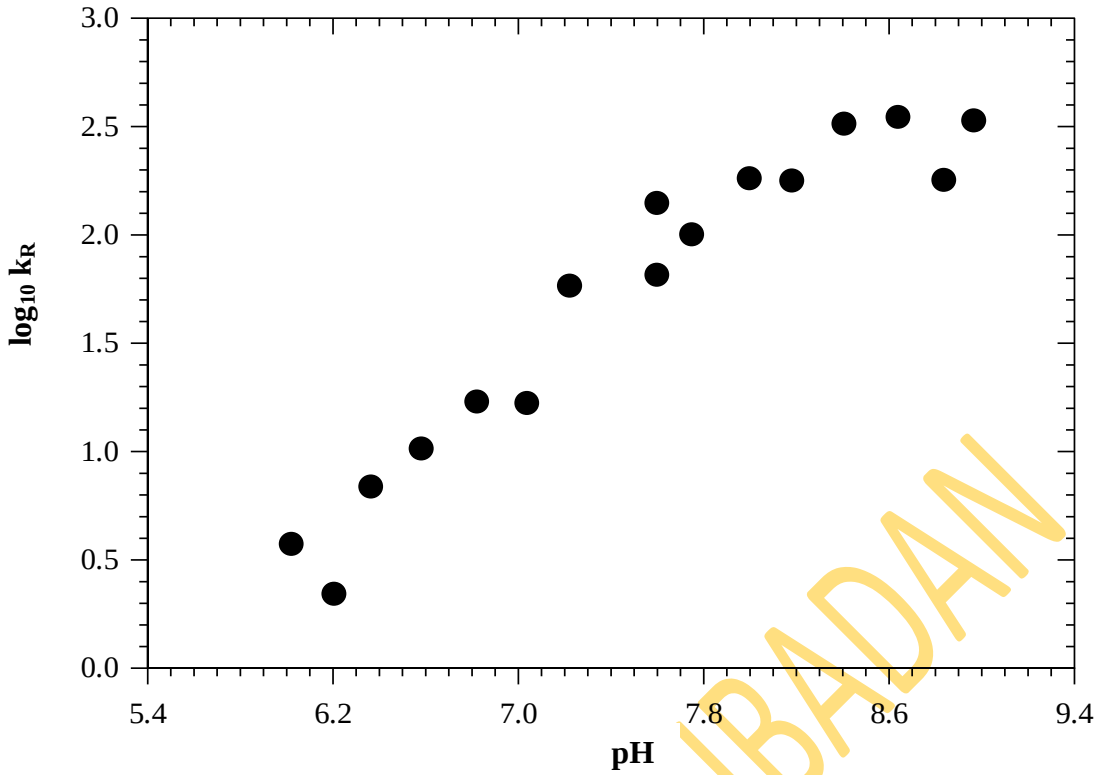



***Figure 4.53**: Dependence of $log_{10}k_R$ on pH for the reverse of the reaction of CysF9[93]β of **stripped donkey oxyhaemoglobin** with DTNB at 25°C. Conditions: phosphate buffers, pH 5.6 – 7.8; borate buffers, pH 8.0 – 9.0 (ionic strength, 50 mmol $dm^{-3}$; added salt, NaCl).*

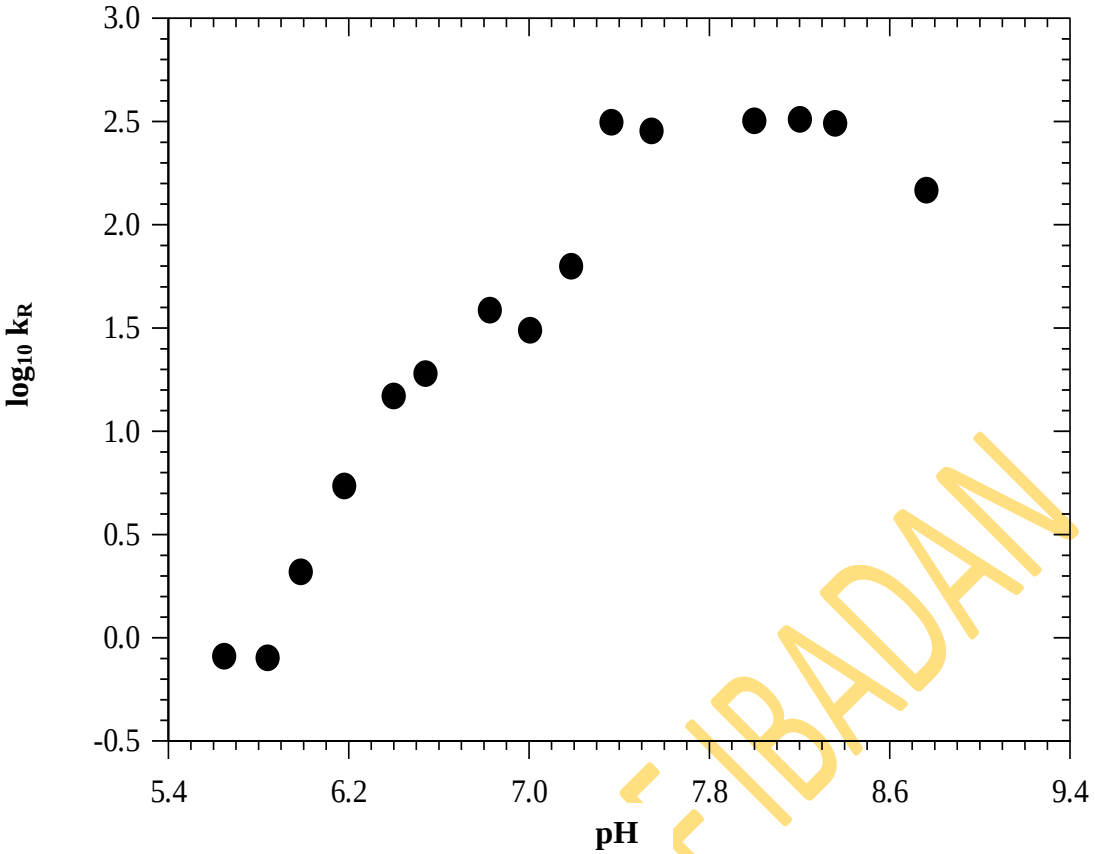



***Figure 4.54**: Dependence of $log_{10}k_R$ on pH for the reverse of the reaction of CysF9[93]β of **stripped donkey carbonmonoxyhaemoglobin** with DTNB at 25°C. Conditions: phosphate buffers, pH 5.6 – 7.8; borate buffers, pH 8.0 – 9.0 (ionic strength, 50 mmol $dm^{-3}$; added salt, NaCl).*

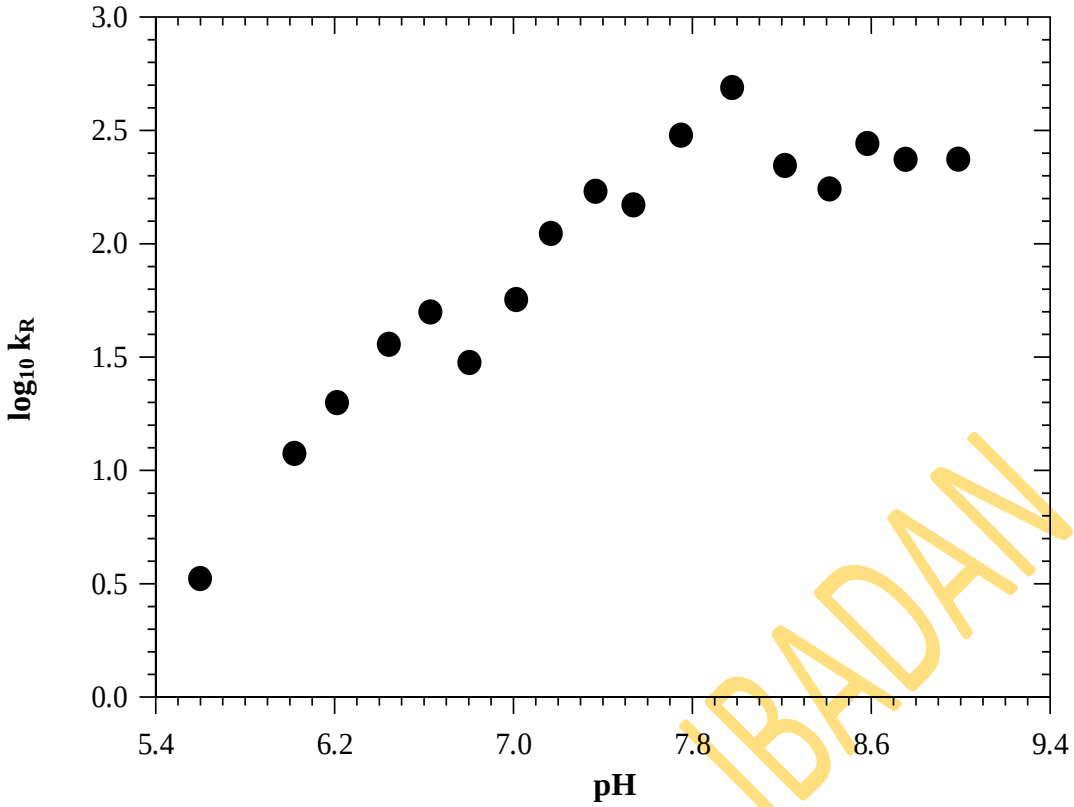



***Figure 4.55**: Dependence of $log_{10}k_R$ on pH for the reverse of the reaction of CysF9[93]β of **stripped donkey aquomethaemoglobin** with DTNB at 25°C. Conditions: phosphate buffers, pH 5.6 – 7.8; borate buffers, pH 8.0 – 9.0 (ionic strength, 50 mmol $dm^{-3}$; added salt, NaCl).*

### 4.5.2 Effect of inositol hexakisphosphate on the pH dependence of $k_R$: Donkey haemoglobin

Figs. 4.56, 4.57 and 4.58 show the $k_R$ data for donkey haemoglobin. $\log_{10}k_R$ exhibits a strong pH dependence for each derivative and varies between two and four orders of magnitude between pH 5.6 and 9.0.

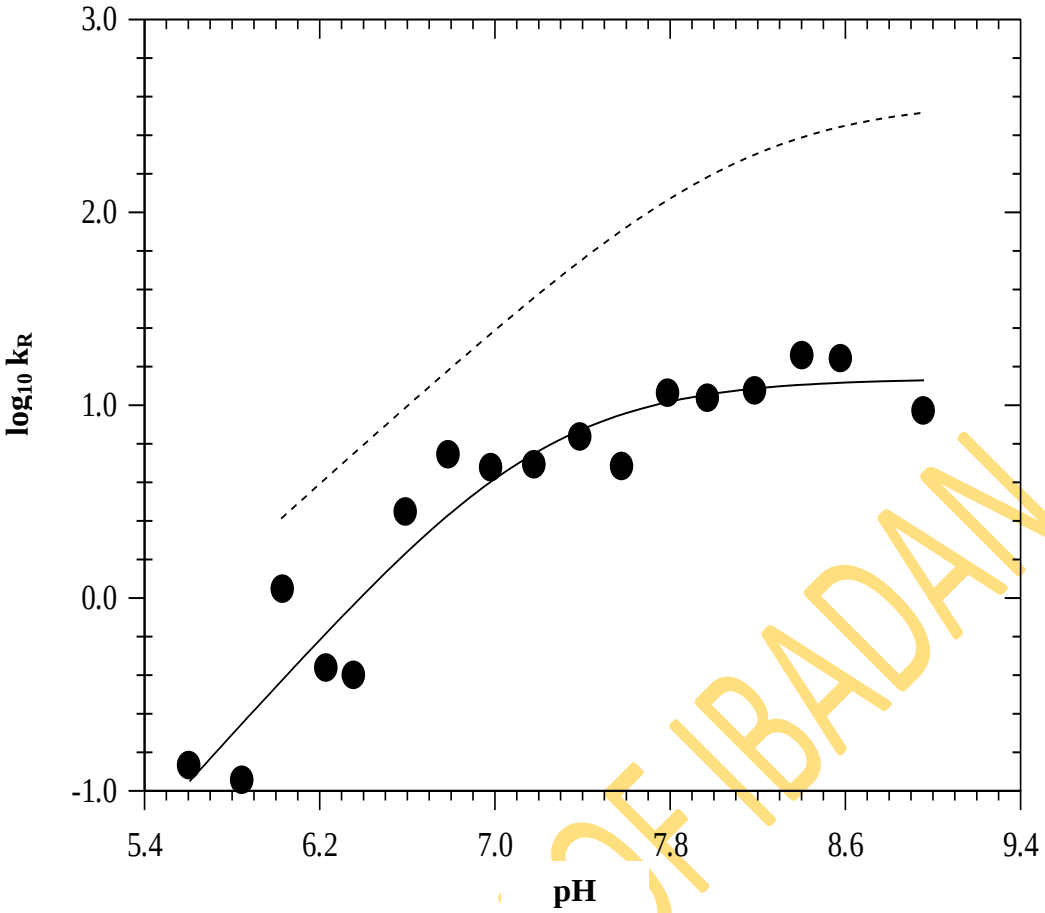



***Figure 4.56**: Dependence of $log_{10}k_R$ on pH for the reverse of the reaction of CysF9[93]β of **donkey oxyhaemoglobin** with DTNB in the presence of **inositol-$P_6$** at 25°C.Conditions: phosphate buffers, pH 5.6 – 7.8; borate buffers, pH 8.0 – 9.0 (ionic strength, 50 mmol $dm^{-3}$; added salt, NaCl), [inositol-$P_6$], 10 µmol $dm^{-3}$. The dashed line shows the corresponding profile for stripped donkey oxyhaemoglobin (cf Fig.4.53).*

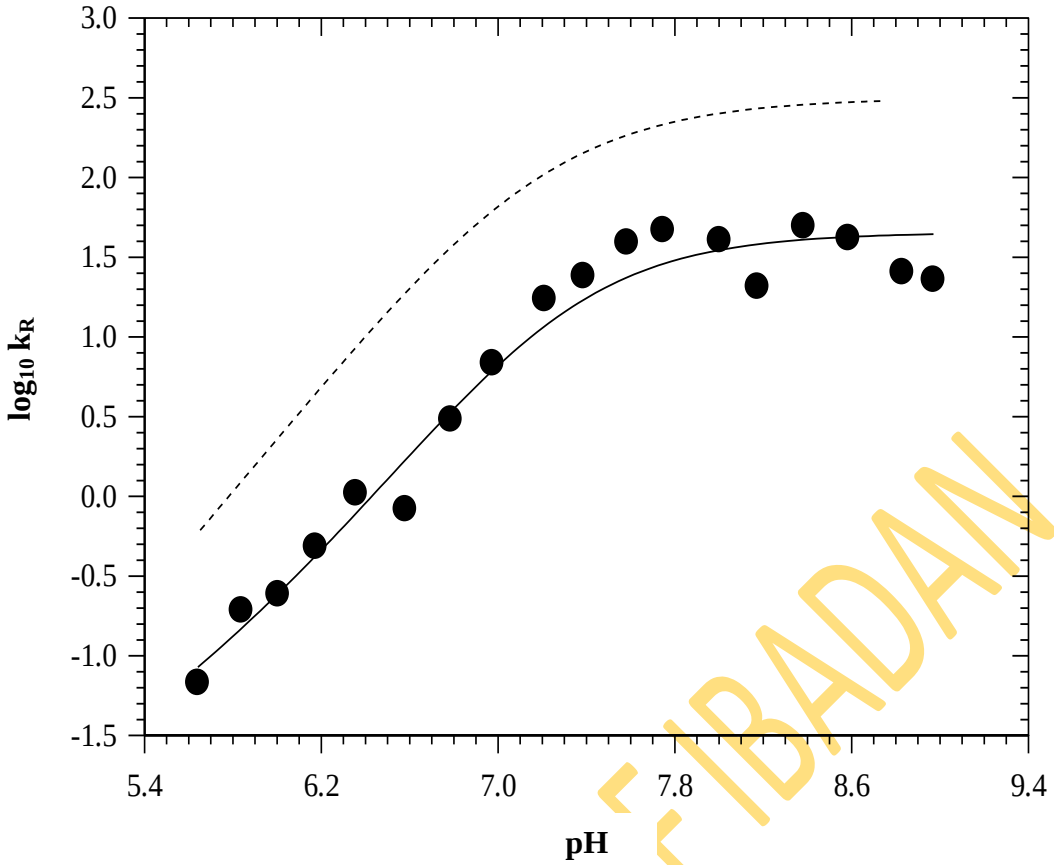



***Figure 4.57**: Dependence of $log_{10}k_R$ on pH for the reverse of the reaction of CysF9[93]β of **donkey carbonmonoxyhaemoglobin** with DTNB in the presence of **inositol-$P_6$** at 25°C.Conditions: phosphate buffers, pH 5.6 – 7.8; borate buffers, pH 8.0 – 9.0 (ionic strength, 50 mmol $dm^{-3}$; added salt, NaCl), [inositol-$P_6$], 10 µmol $dm^{-3}$. The dashed line shows the corresponding profile for stripped donkey carbonmonoxyhaemoglobin (cf Fig.4.54).*

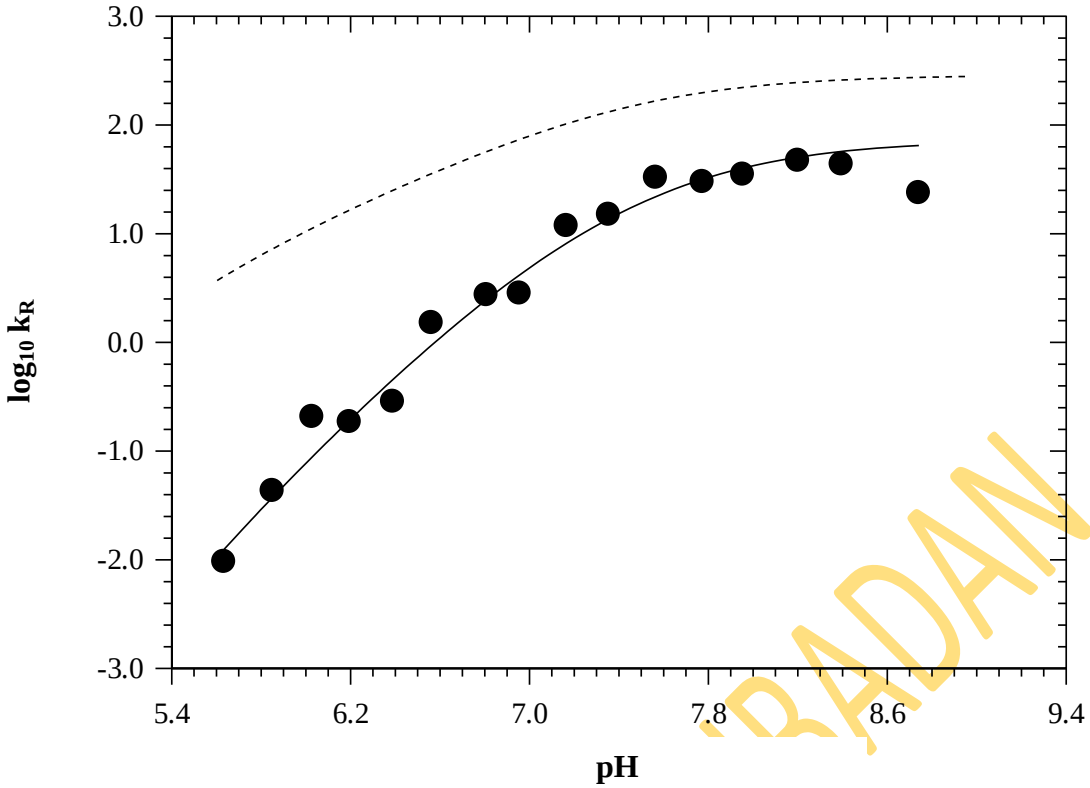
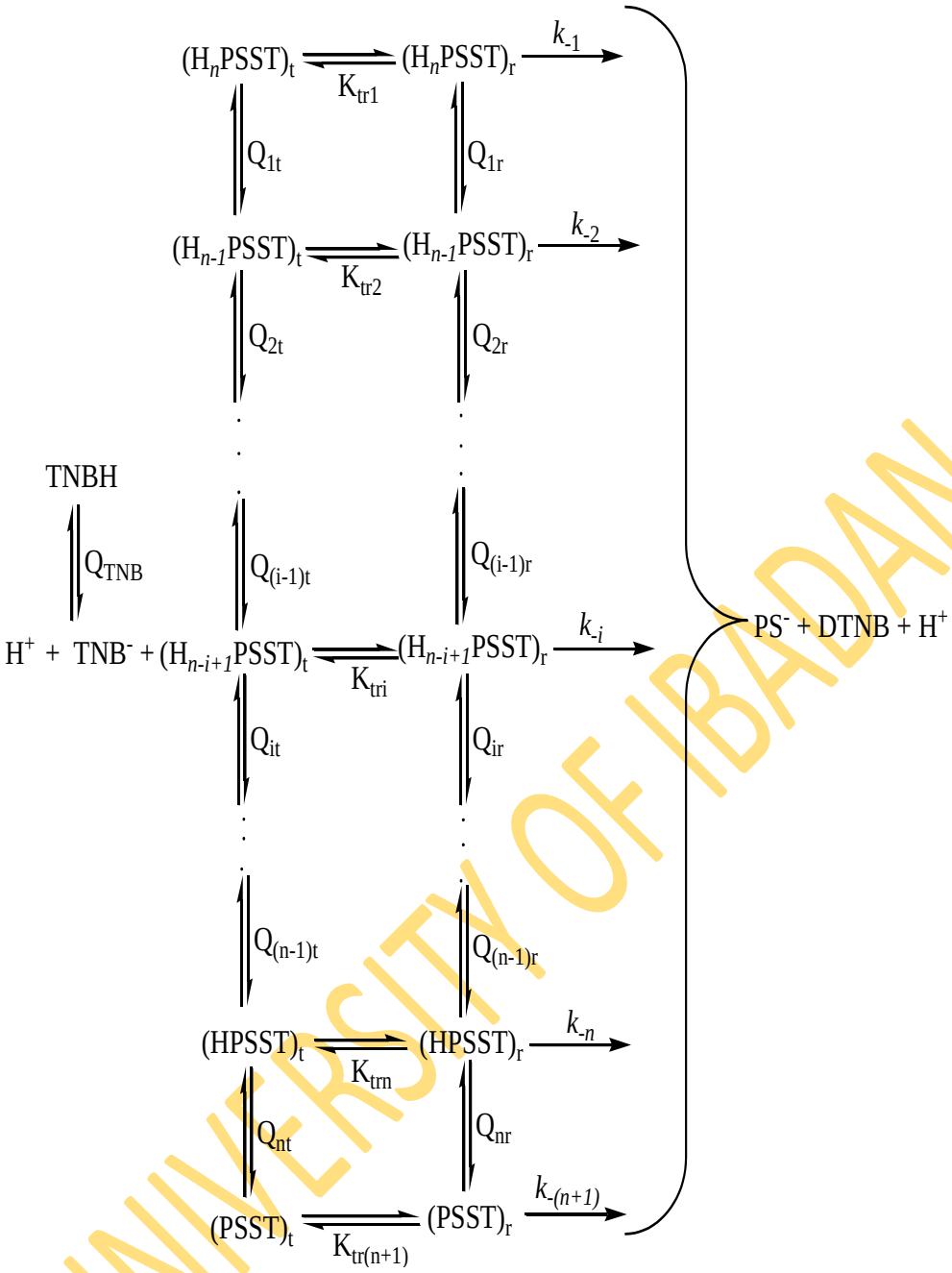



***Figure 4.58**: Dependence of $log_{10}k_R$ on pH for the reverse of the reaction of CysF9[93]β of **donkey aquomethaemoglobin** with DTNB in the presence of **inositol-$P_6$** at 25°C.Conditions: phosphate buffers, pH 5.6 – 7.8; borate buffers, pH 8.0 – 9.0 (ionic strength, 50 mmol $dm^{-3}$; added salt, NaCl), [Inositol-$P_6$], 10 µmol $dm^{-}$]. The dashed line shows the corresponding profile for stripped donkey aquomethaemoglobin. (cf Fig. 4.55).*

### 4.5.3 Theoretical analyses of the pH dependence of $k_R$:

### Donkey haemoglobin

The strong pH dependence of $\log_{10}k_R$ (Figs.4.53 – 4.58) indicates that the reverse of the DTNB reaction is coupled to the ionization of groups on the protein. To determine the nature and the number of these groups, Scheme 4.3 was proposed (Okonjo *et al.*, 2007). The dependence of $k_R$ on pH was analysed using Scheme 4.3.




***Scheme 4.3***

In Scheme 4.3, ($H_nPS.ST$) (n = 1, 2,…, n) are the mixed disulphide species in solution; $k_{-i}$ ($i$ = *1, 2,..., n+1*) are the second order reverse rate constants for the reactions of these species with $TNB^-$; $Q_{jr}$ (j = 1, 2,…, n) are the dissociation constants for the release of the jth proton from $H_{n-i+1}PS.ST$ in the ***r*** tertiary conformation; $Q_{jt}$ (j = 1, 2,…, n) are the corresponding dissociation constants for the release of the jth proton from $H_{n-i+1}PS.ST$ in the ***t*** tertiary conformation; $K_{rti}$ are the tertiary conformation transition constants. Species marked with ***r*** and ***t*** are in the ***r*** and ***t*** tertiary conformations, respectively. For the sake of clarity, the protons involved in the protolytic steps of Scheme 4.3 have been omitted. Assuming that the rates of the protolytic steps are much faster than the rates of the reverse of the DTNB reaction, the apparent second order reverse rate constant, $k_R$, is related to the various parameters of Scheme 4.3 by Eqn. 4.33.

$$k_R = \frac{\left[\frac{Q_{TNB}}{Q_{TNB}+\left[H^+\right]}\right]\left(k_{-(n+1)} + \sum_{i=1}^{n} k_{-1}\left[H^+\right]^{n-i+1}\prod_{j=i}^{n}\left(Q_{jr}\right)^{-1}\right)}{\left(1+\sum_{i=1}^{n} k_{-1}\left[H^+\right]^{n-i+1}\prod_{j=i}^{n}\left(Q_{jr}\right)^{-1}\right)+\frac{1}{K_{tr(n+1)}}\left(1+\sum_{i=1}^{n} k_{-1}\left[H^+\right]^{n-i+1}\prod_{j=i}^{n}\left(Q_{jt}\right)^{-1}\right)} \quad \text{.....4.33}$$

The dependence of $k_R$ on pH for each of the three haemoglobin derivatives (stripped and in the presence of inositol-$P_6$) was analyzed using Eqn. 4.33. The best-fit lines drawn through the points reported in Figs.4.41 – 4.46 were calculated with Eqn. 4.30 using n = 2. For n = 2, Eqn. 4.30 becomes:

$$k_R = \frac{\left[\frac{Q_{TNB}}{Q_{TNB}+\left[H^+\right]}\right]\left\{k_{-3}+\frac{k_{-1}\left[H^+\right]^2}{Q_{1r}Q_{2r}}+\frac{k_{-2}\left[H^+\right]}{Q_{2r}}\right\}}{\left\{1+\frac{\left[H^+\right]^2}{Q_{1r}Q_{2r}}+\frac{\left[H^+\right]}{Q_{2r}}\right\}+\frac{1}{K_{tr3}}\left\{1+\frac{\left[H^+\right]^2}{Q_{1t}Q_{2t}}+\frac{\left[H^+\right]}{Q_{2t}}\right\}} \quad \text{.....4.34}$$

For stripped donkey haemoglobin derivatives, the pH dependence of the reverse rate constant gave the theoretical best-fit lines shown in Figs. 4.53– 4.55. These were calculated using Eqn. 4.34. The best-fit parameters are reported in Table 4.11 and the mean values of $pQ_{1r}$, $pQ_{2r}$, $pQ_{1t}$, $pQ_{2t}$ and $K_{rt3}$ are 6.01 ± 0.33, 8.10 ± 0.04, 6.74 ± 0.28, 7.57 ± 0.31 and 21.50 ± 6.53, respectively.

***Table 4.12**: Reverse of the reaction of DTNB with CysF9[93]β of **stripped donkey** haemoglobin at 25°C. Best-fit parameters for the data reported in Figs. 4.53 – 4.55 (cf Eqn.4.34) for n = 2.*

| Parameters | Haemoglobin Derivatives | | | Mean |
|---|---|---|---|---|
| | Oxy | Carbonmonoxy | Aquomet | |
| $pQ_{1r}$ | 5.68 | 6.67 | 5.68 | 6.01 ± 0.3 |
| $pQ_{1t}$ | 8.04 | 8.15 | 8.11 | 8.10 ± 0.04 |
| $pQ_{2r}$ | 7.10 | 6.88 | 6.25 | 6.74 ± 0.4 |
| $pQ_{2t}$ | 8.18 | 7.28 | 7.24 | 7.57 ± 0.3 |
| $k_{-1}$(mol$^{-1}$ dm$^3$ s$^{-1}$) | 380.21 | 0.00 | 0.00 | |
| $k_{-2}$(mol$^{-1}$ dm$^3$ s$^{-1}$) | 1244.75 | 38.86 | 38.65 | |
| $k_{-3}$(mol$^{-1}$ dm$^3$ s$^{-1}$) | 11164.36 | 8824.70 | 2844.46 | |
| $K_{rt3}$ | 2.07 | 9.54 | 10.09 | 21.50 ± 6.5 |

For donkey haemoglobin derivatives in the presence of inositol-$P_6$, the pH dependence of the reverse rate constant gave the theoretical best-fit lines shown in Figs. 4.56 – 4.58. These were calculated using Eqn. 4.34. The best-fit parameters are reported in Table 4.13: the mean values of $pQ_{1r}$, $pQ_{2r}$, $pQ_{1t}$, $pQ_{2t}$ *and* $K_{rt3}$ are 6.16 ± 0.3, 7.56 ± 0.1, 6.99 ± 0.2, 7.50 ± 0.2 and 8.62 ± 2.5, respectively.

***Table 4.13**: Reverse of the reaction of DTNB with CysF9[93]β of **donkey** haemoglobin **in the presence of inositol-$P_6$** at 25°C. Best-fit parameters for the data reported in Figs. 4.56 – 4.58 (cf Eqn. 4.34) for n = 2.*

| Parameters | Haemoglobin Derivatives | | | Mean |
|---|---|---|---|---|
| | Oxy | Carbonmonoxy | Aquomet | |
| $pQ_{1r}$ | 5.63 | 6.46 | 6.39 | 6.16 ± 0.3 |
| $pQ_{1t}$ | 7.55 | 7.68 | 7.45 | 7.56 ± 0.1 |
| $pQ_{2r}$ | 6.69 | 7.21 | 7.07 | 6.99 ± 0.2 |
| $pQ_{2t}$ | 7.26 | 7.41 | 7.84 | 7.50 ± 0.2 |
| $k_{-1}$(mol$^{-1}$ dm$^3$ s$^{-1}$) | 0.65 | 1.30 | 0.00 | |
| $k_{-2}$(mol$^{-1}$ dm$^3$ s$^{-1}$) | 4.56 | 18.86 | 0.00 | |
| $k_{-3}$(mol$^{-1}$ dm$^3$ s$^{-1}$) | 87.94 | 631.24 | 618.86 | |
| $K_{rt3}$ | 5.40 | 12.93 | 7.54 | 8.62 ± 2.5 |