OF COLOURS, COLOURANTS AND COLOURED SUBSTANCES: A PHARMACIST'S TRIPODAL TRAWL

AN INAUGURAL LECTURE, 2015/2016

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OF COLOURS, COLOURANTS AND COLOURED SUBSTANCES: A PHARMACIST'S TRIPODAL TRAWL

An inaugural lecture delivered at the University of Ibadan

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By

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The Vice-Chancellor, Deputy Vice-Chancellor (Administration), Deputy Vice-Chancellor (Academic), Registrar, Librarian, Provost of the College of Medicine, Dean of the Faculty of Pharmacy, Dean of the Postgraduate School, Deans of other Faculties and of Students, Directors of Institutes, Distinguished Ladies and Gentlemen.

Preamble

I owe a debt of gratitude to God, The Almighty, for the privilege to stand in this auditorium to present this inaugural lecture on behalf of the Faculty of Pharmacy. This is the 12th inaugural lecture coming from the Faculty since the first one presented in 1984 by my mentor and indefatigable, indisputable and industrious postgraduate studies supervisor, who over the years has doubled as a father to me, Emeritus Professor Ajibola Akinyemi Olaniyi. This is the fourth from the Department of Pharmaceutical Chemistry. I consider myself as one who has merely and solely received the grace of God to stand in the same boat as others of noble birth and upbringing to contribute to the history of Pharmacy Education at Ibadan. Born with no hope, no future, no class, no inheritance, no nobility and nothing by worldly standards to be reckoned with. But that it might be by the purest crystalline grace, God has brought this base thing of the world and things that are despised to confound the wise, the rich and the endowed so that I may boldly declare that I am truly a trophy of God's grace. The story of my life is one that testifies of the mercy, love and grace of God. Left without a father from the age of one; a vegetable hawker till 10; a bar waiter till 15; a shop tender till 25 and becoming a Professor at 42 can only come through the endowment of God, Most High. Thus, this evening, I return all the glory, for all I have been and hope to be, to God, The Almighty. God granted me the wisdom to carry out all you will hear today and the strength and grace to have "*laboured more abundantly than they all: yet not I, but the grace of God which was with me*". To the apologetics of the existence of a mighty hand who

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rules in the affairs of men and the supposed atheist, I declare God can take any soul from obscurity to the limelight of His design for "*He raiseth up the poor out of the dust, and lifteth the needy out of the dunghill; That he may set him with princes, even with the princes of his people*".

The topic for this inaugural lecture was conceived as far back as 2008 when the picture of the various contributions I was making to Pharmaceutical Chemistry became apparent. This topic was not confirmed until about two years ago when my last daughter insisted "I have a gift for you" instead of joining us at the family altar at around 10 pm. Out came a paper beautifully painted with multiple colours resembling that of a rainbow. Hence, by the mouths of two witnesses the topic for this inaugural was confirmed.

Pharmacy as a Profession

The profession of Pharmacy is one saddled with the responsibility of providing appropriate medicines, drug information and pharmaceutical care for the patients. The Physician is left to no choice following diagnoses to seek for drugs that will be used for the treatment of the patient. The drug itself has been defined as any substance or mixture of substances that is manufactured, sold and offered for sale, for use in the treatment, mitigation, prevention or diagnosis of diseases, either in man or animal. It could also be used for restoration, correction or modification of organic functions in man or animal. On the other hand, a drug product is a finished dosage form, for instance, tablet, capsule, solution or suppository which contains the drug (commonly called active pharmaceutical or drug ingredient) usually, but not necessarily, in association with inactive ingredients (excipients).

Pharmacy has several sub-specialties, including Pharmacognosy, Pharmaceutical Chemistry, Pharmaceutics and Industrial Pharmacy, Pharmacology, Pharmaceutical Microbiology, Clinical Pharmacy and Pharmacy Administration. All these sub-specialties are designed towards optimising the benefits derivable from the administration of a given drug and in particular towards a holistic understanding

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of drugs. In addition, the Pharmacist is also trained in the rudiments of management, forensic science and accounting.

Pharmaceutical Chemistry is an important component of Applied Chemistry; it deals with the study of chemistry of drugs. Since all drugs are chemical compounds (whether organic or inorganic), Pharmaceutical Chemistry dwells on the physical, chemical, biopharmaceutical, medicinal properties of drugs as well as drug designs and discoveries. The two main areas of Pharmaceutical Chemistry are Medicinal Chemistry and Pharmaceutical Analysis. ***Medicinal Chemistry***, a multidisciplinary subject, has been defined as a basic science which brings together the knowledge and skills gained from the disciplines of chemistry, biology and biochemistry and apply them to the problem of drug chemistry and activity (Olaniyi 2005).

Pharmaceutical Analysis deals with the adoption of various physical and chemical test procedures to ensure that a given drug or dosage form contains what it purports to contain (Kar 2005). It emphasizes all the issues relating to quality of medicines. There are conventional agreements compiled in Pharmacopoeias and other official Compendia on the level of purity expected of each drug, excipient and drug product. The discipline of Pharmaceutical Analysis has been burdened with the choice of an appropriate method for the purity check and determination of quality of drugs and drug products. The choice of a particular method of analysis is based on such factors as physicochemical properties of the drugs, sample type (matrix) and size (lot or a little); data required (qualitative/quantitative); expected level(s) of analyte(s); precision and accuracy expected; likely interferences; number and frequency of samples for analysis and, of course, analyst preference.

Two broad categories of methods adopted are classical and instrumental methods of analysis. The classical methods utilise the age-long titrimetric and gravimetric methods, while instrumental techniques adopt the physical properties resulting from the chemistry of the compounds. Hence, the latter is often referred to as physicochemical methods of drug

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analysis. This category includes refractometry, electrochemical techniques, chromatographic techniques and spectroscopic techniques. Modern design of most techniques utilises amalgamation of the separation power of chromatography with the sensitivity of electrochemical and spectroscopic techniques.

Spectroscopy

Of the instrumental techniques available for the analyst, spectroscopy has been the subject of much improvement over the years. Spectroscopy is the study of interactions of electromagnetic radiation with matter. The various components of the electromagnetic spectrum are presented in figure 1.

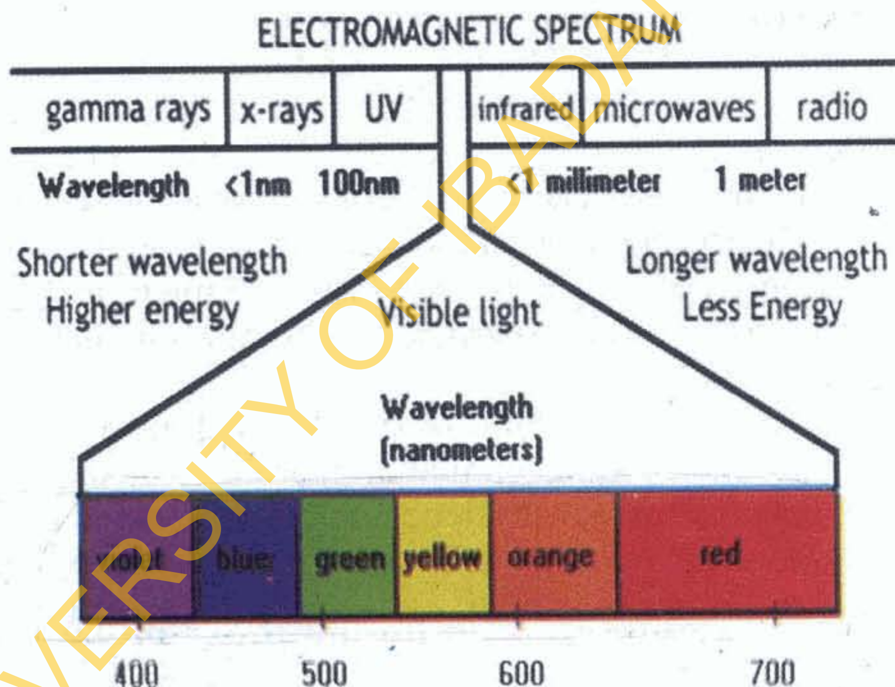

Fig. 1: Components of electromagnetic spectrum.

I have focused my research career on the utilisation of various spectroscopic techniques for the quality assessment of pharmaceuticals. Since spectroscopy is also an important technique available to the organic and medicinal chemists, I have had the privilege of contributing to the structure

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elucidations of both natural products and synthetic compounds.

The interactions of energy with organic (and inorganic alike) molecules lead to changes in the electronic, vibrational or rotational energies inherent in the molecules. Combinations of these energies constitute the internal energy of molecules. I will attempt as much as possible to reduce to the barest minimum a discussion on spectroscopy as many of my students in time past have wondered if such interactions are possible and often asked; "how are you sure of what you are discussing"? Mr. Vice-Chancellor Sir, I must state that to understand spectroscopy takes some guts and some imaginative thinking. I have had to demonstrate and indeed "gyrate" in class to describe what this supposed "abstract" science is all about.

Molecules and indeed matter can take on two orientations in space; it can be in the ground or excited state. We all know that humans can be in the excited mood, and this is often associated with some degree of elation and profound joy resulting from several causes that are positively incorporated and interjected into normal-day occurrences. An elated individual feels *on top of the world*, and he can utter statements or carry out some actions which he may later rejoice about or regret. In such moods, Kings in the Biblical times had promised and even offered "up to half their kingdom" if a riddle could be solved or when he was just excited by wine or display of beauty or loyalty. However, as everyone of us in this auditorium will realise and agree with me, such elated states do not last forever; we are soon back to our normal-day activities and if not guided by some higher devotion and powers, depression may set in (*the morning after* syndrome). Different events lead to different levels of elation in humans. And often, we may not be able to generalize what excites all humans equally alike. Are there some individuals who don't seem excited by anything? If there are, it may mean that what will excite them has not been provided or has not occurred. It is the same with molecules.

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Molecules will prefer to occupy the ground state which corresponds to a state of lowest energy. It is not at rest, as supposed originally, but it is involved in some *flip-flop* movement hoping to be excited. This state is referred to as the Highest Occupied Molecular Orbital (HOMO) state. Appropriate energy that separates the ground state from the excited state must be supplied to allow the excitation to occur. This energy is usually in quantized forms and ordered. No transition will occur except energies of appropriate values are supplied that counterbalance the energy differential between the states. On excitation, molecules occupy the Lowest Unoccupied Molecular Orbital (LUMO) state (fig. 2). Various spectroscopic techniques take advantage of the internal energies of atoms and molecules. Such spectroscopic techniques include atomic absorption and emission spectroscopy; ultraviolet-visible; infra-red; nuclear magnetic resonance spectroscopy and spectrofluorimetry.

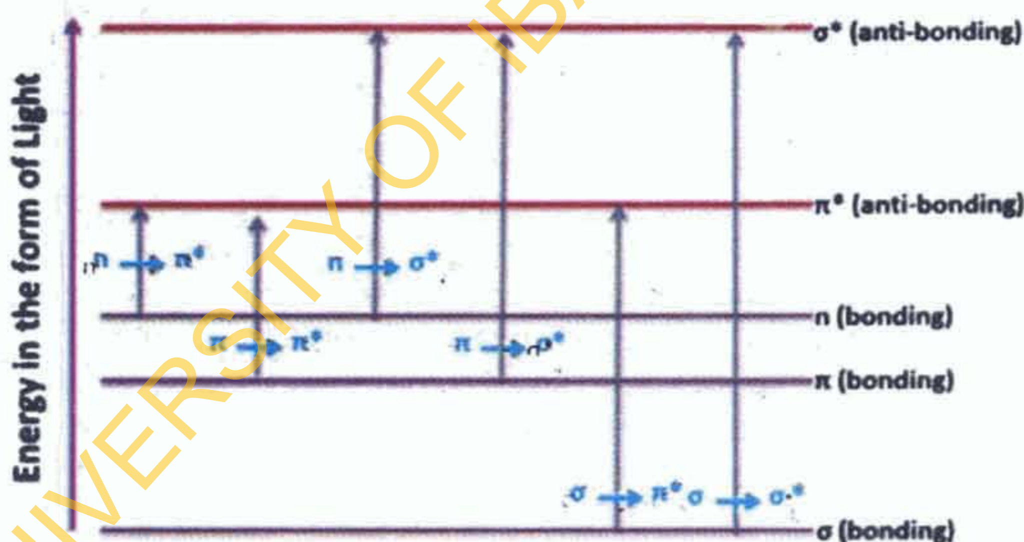

Fig. 2: Electronic transitions and energy states.

Signals and Measurements

The analytical process is a procedure of gaining information. At least, samples contain only latent information on the composition and structure, namely by their intrinsic

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properties. By interactions between the sample and the measuring system, this information is transformed step-by-step into signals, measured results and useful chemical information (Danzer 2004). Information is always connected with signals. In general, signals are definite states or processes of material systems. They can, therefore, be differentiated into static and dynamic signals. Examples of static signals are script, colours, images, figures and buildings. On the other hand, dynamic signals result from electrical, thermal, optical, acoustic, or chemical interactions. Nowadays, these signals are converted in each case into electric signals, in which form they may be treated and transmitted. Finally, the essential signal characteristics are recorded in a suitable form. The process of signal generation and evaluation is given by the chemical measurement process (Danzer 2007).

Analytical signals are generated by interactions between species of the analyte, to be precise, between certain forms of intrinsic energy of them and an external system of matter and energy, respectively. The systems and resulting interactions are summarized in table 1. The signals often generated are in some instances minute and are subjected to some degree of transduction and enhancement to make them visible and in essence make sense. But to the uninitiated, they appear nothing more than spikes or lines (fig. 3). The typical signals generated by some of the spectroscopic and spectrometric techniques carried out by Adegoke (2005) are presented in figure 4.

Herein lies the confusion and the allusion to the fact that spectroscopy is an abstract science. But I must confess I have been at home with the subject of signal generation and interpretation since my introduction to the subject matter during Medical Laboratory Sciences training.

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Table 1: Overview of Various Forms of Interactions taking place between Measuring Samples and different forms of Matter and Energy to produce Analytical Signals

Molecules Atoms	Chemical Reaction	
Ions (Solvated) Electrons	Electrochemical process	
Electron beams Ion beams	Particle-matter interactions	(a) Isoelastic interaction (energy transfer with sample species: (SPECTROSCOPY) (b) Elastic interactions (energy transfer): DIFFRACTION, MICROSCOPY)
Electromagnetic Radiation	Radiation-matter interactions	
Heat	Thermal interactions	
Directed movement Energy	Interactions between phases (partition, adsorption)	

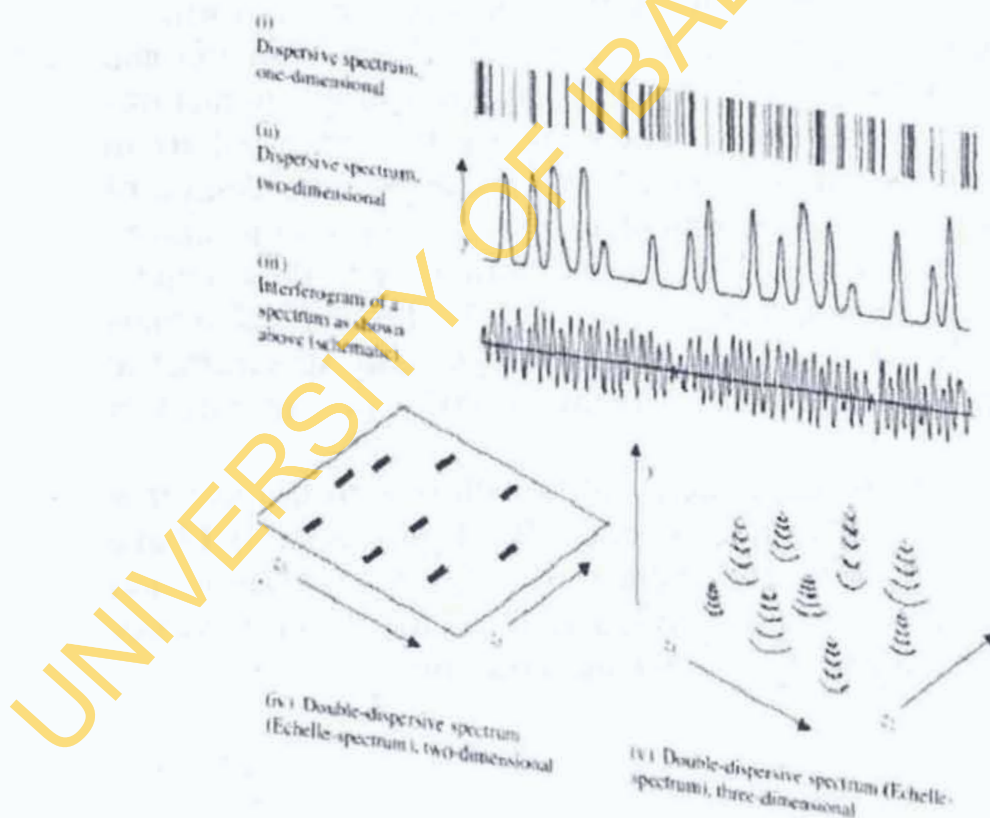

Fig. 3: Different types of signals recordings (schematic) obtained by various instruments and registering.

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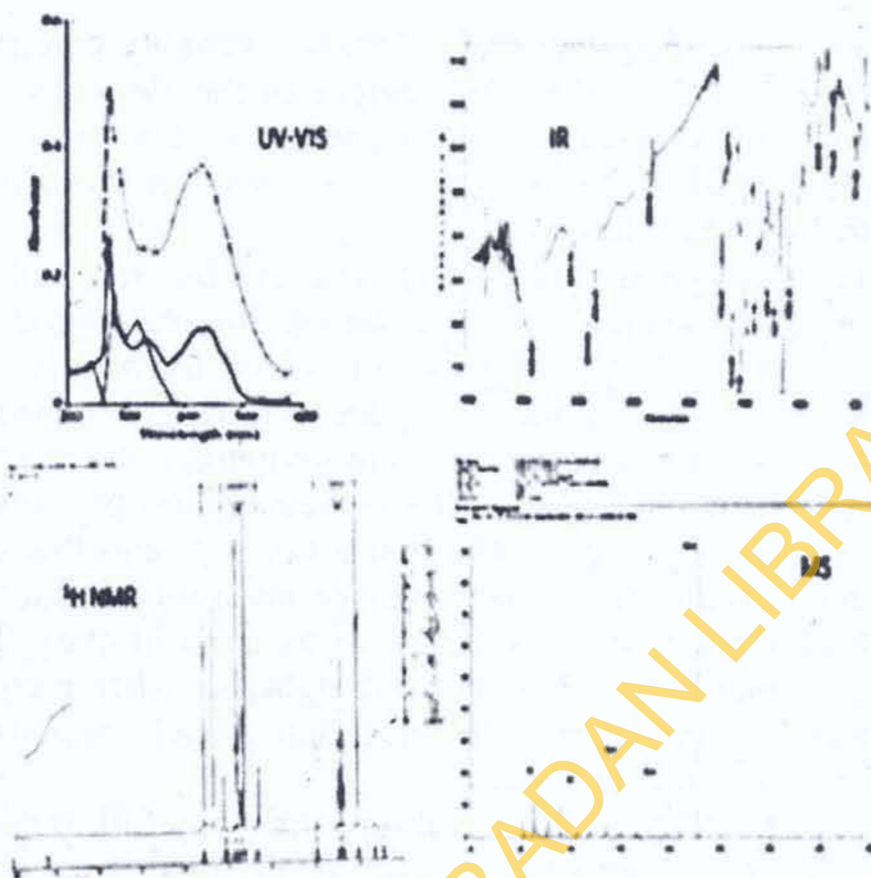

Fig. 4: Typical UV, IR, NMR and MS spectra.

Ultraviolet and Visible Spectroscopy

Mr. Vice-Chancellor, I started my research career mostly in the realm of the applications of Ultra-Violet and Visible spectroscopy (UV-Vis), and I will give a very brief description here. In UV-VIS absorption spectroscopy, many organic compounds absorb quite strongly with only a limited number of inorganic ions doing same (Willard et al. 1988). The practice is to use reagents to make majority of inorganic ions without inherent colours to absorb radiation. The entire process involved in majority of UV-Vis spectroscopy has to do with absorption of radiation to produce electronic transition. In reality, the molecules are as energetic as the modern teenagers. They invariably rock, roll, twist, jerk and bend, and if the music is of the right rhythm, choice and frequency, the electrons within the molecule shall move from the '*ground state*' to the '*excited state*'. Electrons generally

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found in the conjugated double bonds invariably give rise to spectra in the UV and visible regions of the electromagnetic spectrum. It is pertinent to mention here that an excited electron normally returns to the ground state in about 10^{-9} to 10^{-8} seconds (Kar 2005a).

The absorption of radiant energy by molecules is influenced by several factors, including absorbing groups (or chromophores), solvent effects, effect of temperature, and inorganic ions. Adegoke and Idowu (2010) demonstrated comprehensively the effects of chromophore, auxochromes, solvents and temperature on the UV absorption patterns of a group of four novel dyes. Other functional groups that either increase or reduce the wavelength or intensity of absorption of the chromophore are referred to as auxochromes. These species in themselves do not absorb light, but when present in preferred positions on molecules dramatically modify the absorption of such molecules.

The applications of UV-VIS spectroscopy fall under two main categories, qualitative and quantitative analyses. In structure determination, UV-VIS spectroscopy is used to detect the presence of chromophores like dienes, aromatics, polyenes, conjugated ketones and the likes. The technique used to be popular in determining identity and purity of drug molecules until the utilization of infra-red spectroscopy came to the fore.

Adegoke (2005) copiously adopted UV-VIS spectroscopy for the quantitative analyses of pharmaceutical phenol ethers using two main laws, Lambert's (equation 1) and Beer's (equation 2) laws. The quantum of the absorption is designated in terms of the absorption, A, that is represented by equations 1-3.

$$log_{10}\frac{I_0}{I} = \frac{K_\lambda}{2.303}l \qquad \text{-----} (1)$$

where, Io = Intensity of radiation passing into the absorbing layer, and
I = Intensity of radiation passing out of the absorbing layer
K_λ is a constant and *l* is the thickness (path length).

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$$log_{10}\frac{I_0}{I} = \frac{K_\lambda}{2.303}C \text{ -- (2)}$$

Combining equations 1 and 2 gives 3 which is Beer-Lambert's law or simply Beer's law when l = 1cm.

i.e. $log_{10}\frac{I_0}{I}$ = Absorbance, $A = -\frac{K_\lambda}{2.303}C\,l$ -------- (3)

or $A = a.c.l.$

a is called the molar absorptivity, ε (when c is in mol dm^{-3}) and $A^{1\%}_{1cm}$ (when c is in % w/v). Bearing in mind the ease in calculations and also the convenience of reference, the absorption of a 1-cm layer of a 1% w/v solution is usually recommended in most of the official compendia for many pharmaceutical substances and is evaluated by equation 4.

$$A^{1\%}_{1cm} = \frac{A}{C\,l} \text{ -- (4)}$$

where, c = Concentration of the absorbing substance represented as a percentage (w/v); and l = Thickness of the absorbing layer (cm).

Mr. Vice-Chancellor Sir, majority of the pharmaceuticals do not absorb intensely in the region of less interference (visible region). Absorptions of most pharmaceutical compounds fall under the UV region. Working in this region is often fraught with the difficulties of preventing non-specific absorption by most solvents, excipients, and since majority of substances, no matter how poorly endowed electronically will absorb here, scientists have over the years converted such molecules into newer ones through a variety of reactions. This conversion is however not limited to UV-VIS spectrophotometry.

When a compound is inherently coloured, colorimetric analysis is readily adoptable. This technique was developed

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from the age-long visual comparative tests. However, with the development of simple colorimeters, reduction or complete elimination of visual errors was made possible. The choice of a colorimetric procedure for the determination of a substance will depend upon such conditions as the concentration of the analyte in question, the condition under application and in particular the presence of interfering matrices (Basset et al. 1978).

For compounds that are not inherently coloured, an initial reaction may be necessary to make applications of colorimetric methods possible. The challenge in such reaction(s) is the need to keep the various steps as simple as possible and to ensure that a complete conversion of the analyte of interest is made possible. The criteria for a satisfactory colorimetric analysis include specificity of the reaction, proportionality between colour and concentration, stability of the colour, reproducibility, clarity of the solution and high sensitivity. Thus, in many instrumental techniques, it might be desirable to assay particular compounds in forms that are readily handled to improve sensitivity or selectivity. Usually, conversion of functional groups within the molecule to others more readily adaptable to the technique being adopted is preferred. This procedure called derivatization or derivative formation is applied in UV-VIS spectroscopy, gas chromatography (GC) and high performance liquid chromatography (HPLC) (Adegoke 2012a). This has been the mainstay of my primary research focus.

In such reactions involving applications of UV-VIS spectrophotometry, colours are produced and having selected the λ_{max} by a spectrophotometer, colorimeters are used for quantifying the amount of absorbing species. While this method has been popular for inorganic ions such as iron (II), Ni, Si, Zn and Co, colorimetric measurements of drugs and other organic compounds are now common after specific derivatization procedures. Although very few reactions are specific for a particular substance, many reactions are quite selective, or can be rendered selective through the

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introduction of masking agents, control of pH, solvent extraction, adjustment of the oxidation state, or prior removal of interferents (Willard et al. 1988).

Chemical derivatization fall under the category of indirect spectrophotometric analysis and the compounds of interest are often converted to those with different spectral properties. Chemical derivatization may be adopted in instances where the analyte absorbs weakly in the UV region as is common with most drugs, where interference by irrelevant absorption is present, where there is a need to improve selectivity of the procedure or when cost implications will favour adoption of a colorimetric method to that of a UV-VIS spectrophotometer (Davidson 1997).

General Classification of Chemical Derivatization Reactions

A wide range of reactions have been adopted for the chemical derivatization of inorganic and organic pharmaceuticals. Majority of these reactions are colour-producing reactions (Adegoke 2012a). Colorimetric methods can selectively transform a drug, its impurity or metabolite so that the spectrum is shifted to the visible region and away from interference caused by another drug, formulation components or biological substances, thereby conferring a further degree of specificity. Moreover, a drug with little or no useful absorption can be more sensitively determined by modifying it to a more highly absorptive chromophore (Fell 1986).

Some reactions have enjoyed wide applicability in chemical derivatization for the UV-VIS spectrophotometric determination of pharmaceuticals. The major reactions are: ion-pair formation, complexation reactions, acid-base procedures, enzymatic reactions, oxidation-reduction reactions and some miscellaneous methods as well as azo dye derivatization, hydrazine derivatization/Schiff base formation and organic charge transfer reactions/complexation (Adegoke 2012a).

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Ion Pair Analysis

Ion pair formation, initially investigated in physical chemistry, was found extremely interesting for chemical analysis, including pharmaceutical analysis. Modern analytical methods proved that the formation of ion pairs is a consequence of the electrostatic, hydrophobic and charge transfer interactions and allowed optimal experimental conditions setting for their formation (Willard et al. 1988).

Bromocresol blue (BCB), bromocresol purple (BCP) and bromocresol green (BCG) have found great relevance as ion pair donors in most reported methods on pharmaceuticals, such as guanethidine sulphate, guanfacine hydrochloride, guanoclor sulphate, guanoxan sulphate and debrisoquine sulphate (Wahbi et al. 1993); zolmitriptan in tablets (Aydogmus and Inanli 2007) and phenothiazine derivatives in bulk drug and their pharmaceutical preparations (Basavaiah and Krishnamurthy 1998).

Complexation Reactions

Complexation reactions have been adopted as an age-long approach for the analysis of metals and metalloids in water, pharmaceutical preparations and other matrices. The procedure usually involves the selection of appropriate complexing agents, controlling the pH and appropriately selecting the temperature and solvents required. Once the metal ions bind the ligand, specific colour changes are observed, which can be quantitated as a function of the amount of metal ions present. This procedure is also utilized for the gravimetric analysis of metal ions. In recent times, the ability of some organic pharmaceuticals to serve as ligands has also been utilized for the estimation of these drugs Complexation reactions have been used for the determination of such metals as cerium subgroups (Zhen et al. 1999), lansoprazole with Fe^{3+} (Basavaiah et al. 2007), some cephalosporins with Fe^{3+} (Okoye et al. 2007).

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Oxidation-reduction Reactions

Oxidation-reduction reactions have been used in some derivatization techniques. Amantadine HCl has been determined by oxidation of the drug by ammonium molybdate (Darwish et al. 2006). Adegoke and Balogun (2010) demonstrated the ability of cerium ammonium sulphate to accurately determine some quinolones. Cerium ammonium sulphate has also found relevance in oxidation-reduction spectrophotometry. It is commonly adopted for the oxidation of drugs that possess reducible moieties and, in turn, it gets oxidized to the cerate ion which has an intense yellow colour. In some determinations, the amount of residual cerium sulphate is determined by further reactions with such compounds as indigo carmine, methyl orange or *p*-dimethylaminobenzaldehyde (Basavaiah et al. 2007).

Other Methods

Some other reagents have been used for the determination of a wide range of pharmaceuticals and include 4-chloro-7-nitro-2,1,3-benzoxadiazole (NBD-Cl), sodium 1,2-naphthoquinone-4-sulphonic acid (NQS), sodium nitroprusside and 3-methyl-2-benzothiazolinone hydrazine (MBTH) HCl.

Mr. Vice-Chancellor Sir, I have deliberately placed azo dye formation, hydrazine derivatization and charge-transfer complexation last in this discussion on various techniques that have found usefulness in chemical derivatization procedures. This is because on this tripod have rested my long search for honours, and they have formed my tripodal trawl. A trawl when used as a verb refers to catching a fish or catching with a net or seine or more commonly to search thoroughly, and when used as a noun connotes an act of drawing out fish or a catch. To me, trawl therefore means to search through a large amount of information or many possibilities; it also implies an investigation, hunt, rummage, scan or thorough look. I have indeed put out my senses in all ramifications and set lines in preferred orders to catch possibilities of developing new methodologies from existing

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and new reagents. I have focused on azo dye derivatization, Schiff base formation and charge transfer complexation for some eighteen years as a device to catch degrees and papers that served as pivots for my Masters' and Ph.D degrees and on which the declaration of my outstanding Professorial contributions rested.

I must openly confess that in order to carry out any chemical derivatization procedure for an organic compound, a sound knowledge base of Organic Chemistry is important. I appreciate God for the wonderful teachers I had in Organic Chemistry in my undergraduate school days. I doff my hat for Professor T. Shambe who taught me Organic Chemistry (CHE 131) in 100 level and whose knowledge of the subject I admire so much that I used to feel I should have enrolled for B.Sc Organic Chemistry. Then, I passionately remember Professor V.C. Agwada in PCH 301 and 302 back then in University of Jos; he would come to class without notebook; he also ignited my interest in Organic Chemistry. Thus, while some run away from organic chemistry, these Chemists made me a lover of the complicated science involved in understanding how reactions proceed and how electrons transpose themselves to ensure that a reaction is completed. The foundation in Organic Chemistry, Mr. Vice-Chancellor Sir, became the tunic required to know what reaction will proceed or which will not, and has made me, with all sense of modesty, the only pharmacist around who is not tired of trying out organic reactions for not only colorimetric analysis but for synthesis of colours, colourants and coloured substances. Organic chemists may not see the electrons moving, but by careful understanding of laid-down principles, we feel the impact in reactions and of course in observable manifestations of colours in many instances. The big question, Mr. Vice-Chancellor Sir, is: Do we see all things before we attest to their significance and relevance? Obviously Not! Hence, we may not see electrons and their disposition, but we obviously see the end results of their transposition.

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Azo Dye Derivatization

Majority of the derivatization procedures I have carried out is based on formation of azo dyes. The azo dyes give rise to intensely coloured substances; they are characterized by the presence of the diazo linkage which brings two rings into conjugation and thereby extending the maximum wavelength (λ_{max}) to the visible region (fig. 5). As a result of such dye formation, lower detection limits are obtained, while at the same time, some measure of selectivity is afforded. Azo dyes are produced by the diazo coupling reaction of a diazonium salt with a neutral, activated or deactivated skeleton. The formation of an azo dye proceeds by preparation of diazonium salt and coupling of the latter with a suitable compound.

Diazonium Salts

Diazonium salts are produced from amines. Each class of amines yields a different kind of product in its reaction with nitrous acid, HONO. This unstable reagent is generated in the presence of the amine by the action of mineral acids on sodium nitrite. Peter Griess first discovered the original reaction in 1858 (Morrison and Boyd 1992). When primary aromatic amines are treated with nitrous acid, diazonium salts are formed. Incidentally, structure I contributes more to the hybrid II, as shown by bond-distance measurements (March 1992a).

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Fig. 5: Colorimetric determination of naproxen following azo dye derivatization with CDNBD (Idowu et al. 2009).

Preparation of Diazonium Salts

The preparation of diazonium salts is by a process known as diazotization, and a salt of the ion results depending on the mineral acid used (Saunders 1949; Herbst and Hunger 1997).

$$Ar\text{-}NH_2 + NaNO_2 + 2HX \xrightarrow{\text{cold}} Ar{-}N{\equiv}N^{+}X^{-} + NaX + 2H_2O$$

A diazonium salt

X^- could be HSO_4^- or Cl^- if H_2SO_4 or HCl is used.

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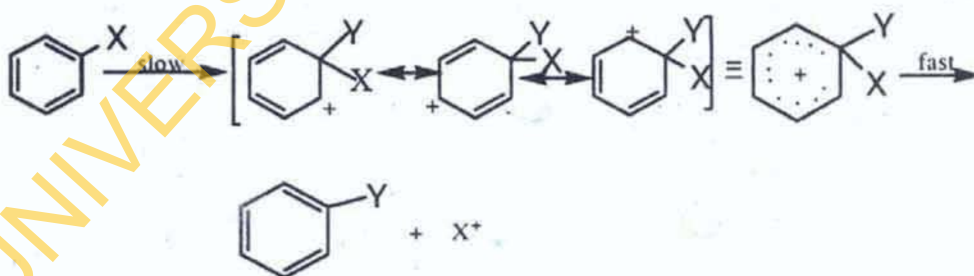
Reactions of Diazonium Salts

The large number of reactions undergone by diazonium salts may be divided into two classes:

(i) Replacement reaction in which nitrogen is lost as N_2 and some other atom or group becomes attached to the ring in its place. This is the best way to introduce F, Cl, Br, I, CN, OH and H into aromatic rings (March 1992b).

(ii) Diazo-coupling reaction in which the nitrogen is retained in the product (Morrison and Boyd 1992b). Under the proper conditions, diazonium ions react with certain aromatic compounds to yield products of the general formula Ar-N=N-Ar, called azo compounds or azo adducts. In this reaction, known as coupling reaction, the nitrogen of the diazonium group is retained in the product, in contrast to the replacement reactions, in which nitrogen is lost. The reaction on the aromatic substrate (coupler) proceeds by aromatic electrophilic substitution of the diazonium ion on sites on the ring predetermined by substituents present on the coupler as shown in Scheme 1 (March 1992b). The C-azo, O-azo and S-azo are equally possible.



Scheme 1: Electrophilic substitution on benzene ring

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Applications of Azo-dye Formation in Colorimetric Analysis

Two procedures are commonly adopted in the application of diazo coupling reaction for colorimetric analyses of pharmaceuticals:

(a) Analysis of drugs involving preliminary diazotization of the drug before coupling to a suitable reagent
(b) Analyses in which the diazonium salt is the reagent.

Diazotization of Drugs before Coupling

In this method, the drug contains either a free primary amino group or other derivatives such as amide and nitro groups. I have alongside some of my students developed novel reactions through diazotization of drugs before coupling (Adegoke and Umoh 2009; Adegoke and Quadri 2012; Thomas and Adegoke 2012; Thomas and Adegoke 2015). One of the reactions is presented in Scheme 2 for determination of cephalosporins. The earliest procedure is the development of a new coupling component for sulphanilamide determination by Bratton and Marshall (Bratton and Marshall 1939). The method involves colorimetric determination of sulphanilamide in blood or urine by first diazotizing the sulpha drug after extraction and coupling with N-(1-naphthyl) ethylenediamine dihydrochloride, a compound which later gained popularity as Bratton-Marshal reagent.

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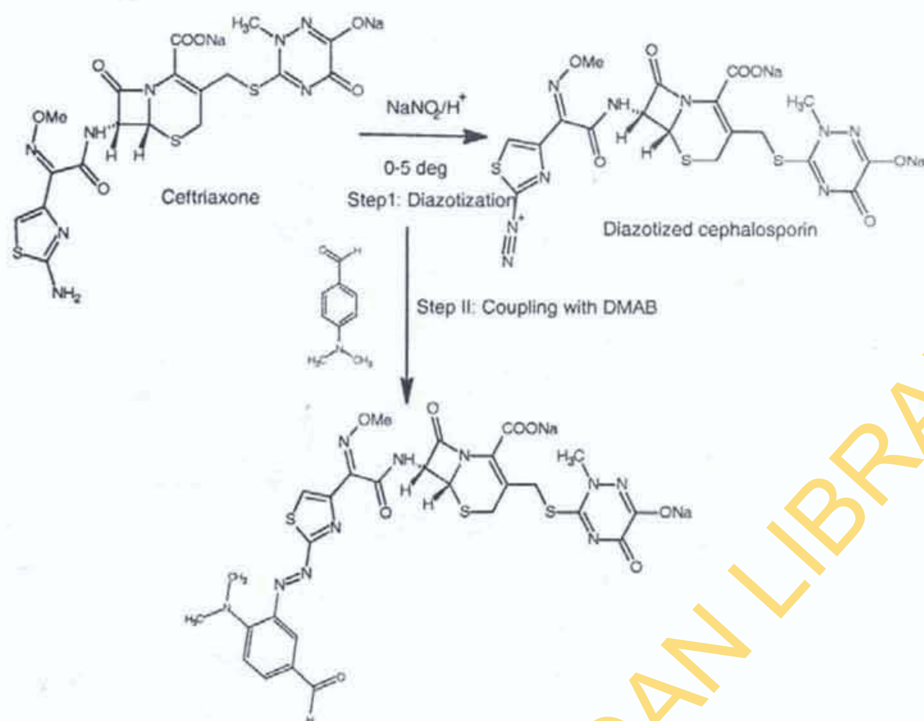

Scheme 2: Formation of Azo adduct between ceftriaxone and DMAB (Adegoke & Quadri 2012)

Diazotized Compound as the Reagent

This was the main procedure I carried out during my Ph.D research (Adegoke 2005). This procedure has a broader application. Here, the reagent possessing a free primary aromatic amino group is diazotized using appropriate method. The reagent is now coupled with drugs possessing appropriate activated aromatic skeleton. Earliest reagents in this category include diazotized *p*-nitroaniline (Smith and King 1964) and diazotized sulphanilic acid (Kozlov et al. 1969). Diazotized *p*-nitroaniline and diazotized sulphanilic acid have been used in an alkaline medium to determine ritodrine HCl and its pharmaceutical preparation (Revanasiddappa and Manju 2001). Scheme 3 shows the reaction between aceclofenac and 4-carboxyl-2,6-dinitrobenzene diazonium ion (CDNBD) as carried out by Aderibigbe et al. (2012a).

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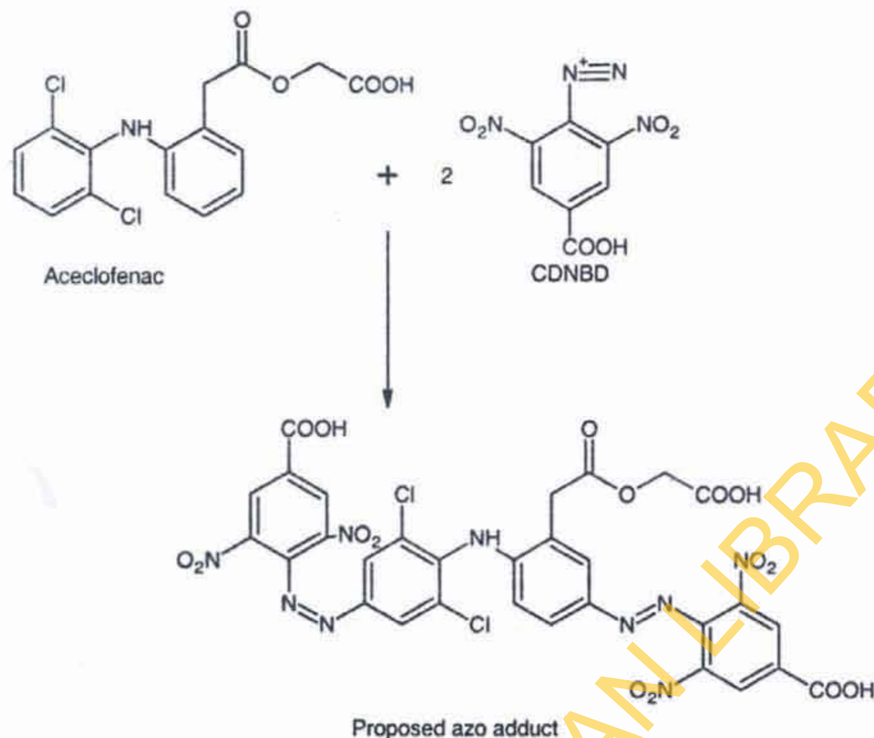

Scheme 3: Coupling reaction pattern between aceclofenac and CDNBD (Aderibigbe et al. 2012a)

Schiff Base Formation

Many colorimetric procedures are based on condensation reactions under suitable conditions between amines and carbonyls to generate Schiff's bases, hydrazones, semicarbazones or oximes (Scheme 4). Miwa, Yamamoto and Momose (1980) have extensively used 2-nitrophenylhydrazine to estimate carboxylic acids including salicylic acid produced in hydrolysis of aspirin.

When R" = Alkyl or aryl, the product is a Schiff's base
R" = NH_2 (hydrazine), the product is a hydrazone
R" = $NHCONH_2$ (semicarbazone), the product is a semicarbazide
R" = OH (hydroxylamine), the product is an oxime

Scheme 4: Schiff Base Formation

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The intensely coloured hydrazides produced are often determined colorimetrically. Prominent carbonyl group donors have been *p*-dimethylaminobenzaldehyde (Adegoke and Nwoke 2008) and *p*-dimethylaminocinnamaldehyde (Zawilla et al. 2002). Similarly, dihydralazine has been determined in pharmaceuticals after derivatization with 2-hydroxy-1-naphthaldehyde (Pous Miralles et al. 1993).

Charge Transfer Complexation

Certain substances combine in a 1:1 molar ratio to form addition products. The molecular addition compound is held together by weak forces, such as Van der Waals. The two new molecular orbitals formed are illustrated in the figure 6. Since the formation of these complexes involves transfer of electronic charge from an 'electron-rich' molecule (a Lewis-base donor) to an 'electron-deficient' molecule (a Lewis-acid acceptor), they are called charge-transfer complexes (Kemp 1984). The structure of most charge-transfer complexes can be visualized as a face-to-face association on a 1:1 donor: acceptor basis: only thus, for example, can maximum overlap of aromatic π-orbitals take place.

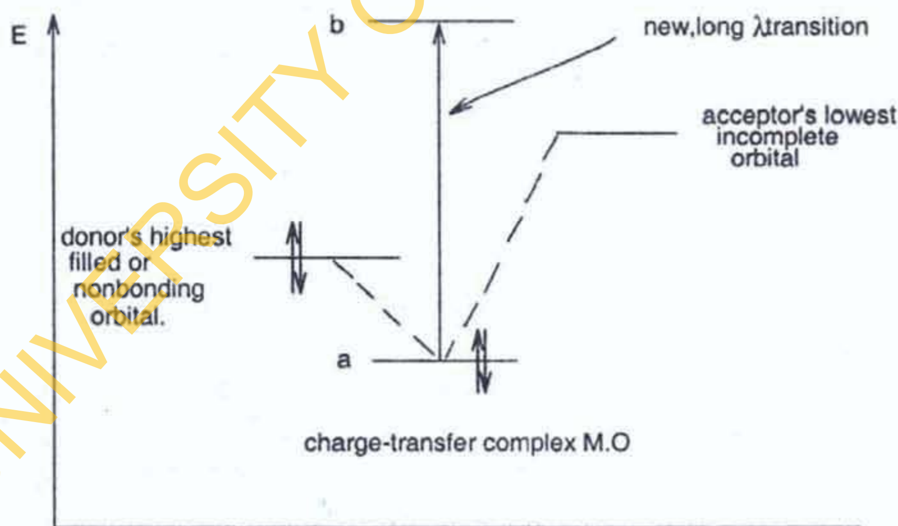

Fig. 6: Electronic transitions for charge-transfer complexes. Donor and acceptor orbitals combine to form two orbitals (a and b) for the complex. New electronic transitions for long λ are then possible between *a* and *b*.

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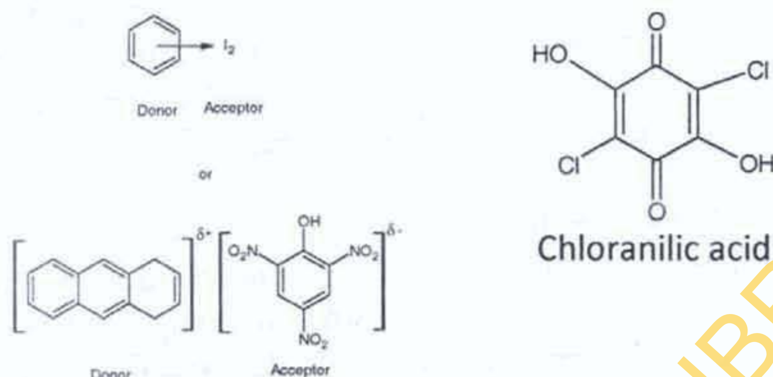
This kind of structure is difficult to draw, and most representations use one or other of the conventions shown below (Kemp 1984).



Chloranilic acid (2, 5-dichloro-3, 6-dihydroxy-*p*-benzoquinone) has enjoyed the widest application as a π-electron acceptor, while drugs possessing excess π-electrons or those with non-bonding *n*-electrons readily pair with it to form brilliantly coloured purple or pink adducts which are measured colorimetrically as a means of quantitation of the amount of analyte present. Several reactions of chloranilic acid with drugs have been reported.

Benesi-Hildebrand equation (Equation 5) has found particular usefulness in the estimation of the formation constant of molecular complexes and it is commonly utilized for this estimation.

$$\left(\frac{[A]_0}{A} = \frac{1}{K_{CT}\varepsilon_{CT}} \cdot \frac{1}{[D]0} + \frac{1}{\varepsilon_{CT}}\right) \quad \text{------------------------- (5)}$$

Where, $[A]_0$ is the initial concentration of the acceptor (charge-transfer reagent), A is the absorbance of the charge transfer band, $[D]_0$ is the initial concentration of the donor (drug or chemical), K_{CT} is the formation constant of the new charge transfer band and ε_{CT} is the molar absorptivity. A plot of $[A]_0$ /A against $1/[D]_0$ will yield intercept as $1/\varepsilon$ and the slope as $1/K\ \varepsilon$ from where the formation constant and the molar absorptivity are obtained. CT complexes are associated

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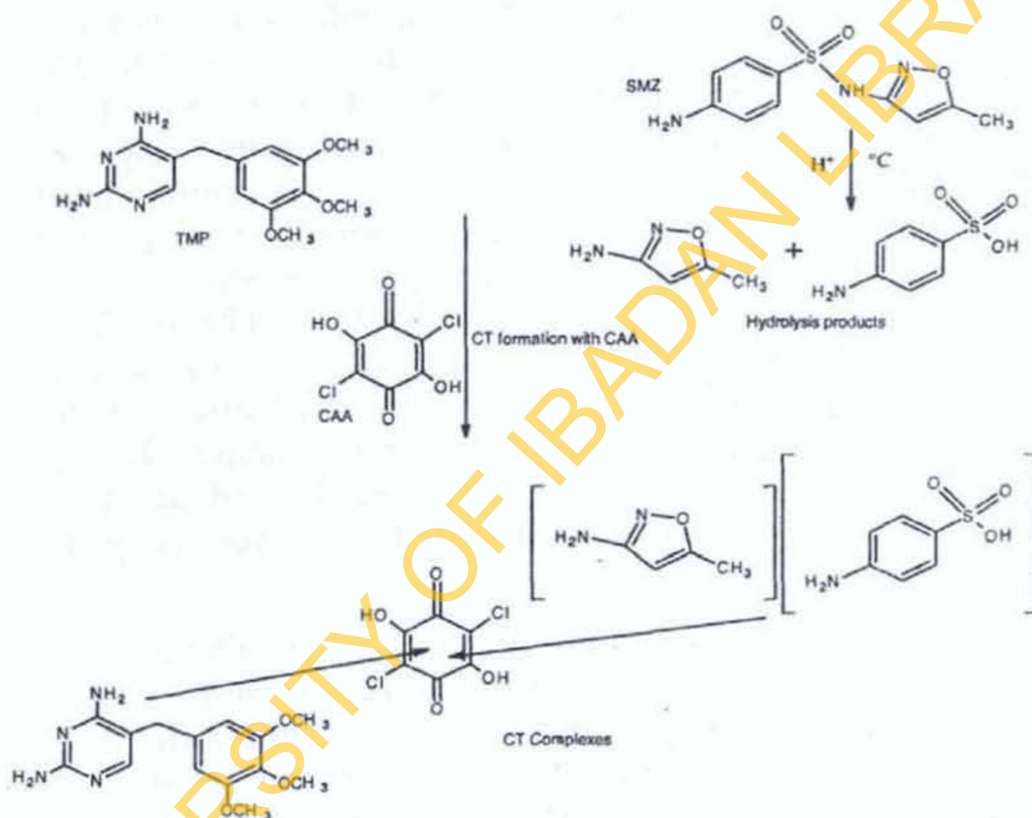
with the appearance of new UV-VIS absorption bands (Mulliken and Person 1969). CT complexes are sometimes produced as reaction intermediates (Ross and Kuntz 1954; Coloter 1963; Khan and Ahmad 2009), and most often they exist as stable donor-acceptor adducts (Andrews 1954; AL-Attas 2009; Yarwood 1973). One common mechanism for the CT complex formation carried out by Adegoke et al. (2014) for the determination of trimethoprim/ sulphamethoxazole is illustrated in Scheme 5.



Scheme 5: CT complexation formation between CAA and trimethoprim/ sulphamethoxazole (Adegoke et al. 2014)

Mr. Vice-Chancellor Sir, the application of the tripodal reactions of azo dye formation, Schiff base formation and charge transfer complexation, among other reactions, give rise to colours. Colours could serve as colourants, and their application in colorimetric analyses has led to the utilization of coloured substances for simple, sensitive, selective and accurate determination of compounds of pharmaceutical

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interest. In the next few paragraphs, I will give a brief overview of colours and colourants before considering my specific contributions to colour chemistry.

Colours

Colours have been with man from the ancient times. Due to its attractiveness, everyone is thrilled by the brilliance and alluring look of coloured substances. A life without colours appears bland and blank. Thus, the appropriate aesthetics to any object in nature is judged by the allure or the colour it possesses and gives out. Many people are obviously fascinated by colours. The word translated "*colour*" in *The Holy Bible* literally connotes "*an eye*". Colours arise from white light. "White" light contains the entire range of wavelengths within the visible region of the spectrum and if shone through a prism splits into a rainbow of respective colours. This rainbow of colours has been popularly given the acronym; ROYGBIV (Red, Orange, Yellow, Green, Blue, Indigo and Violet), and the colours constitute the visible spectrum of the electromagnetic radiation. The origin of the names of the colours has been an intriguing subject. Dating back to centuries, the names of our everyday colours have origins in the earliest known languages. According to linguists:

> There was a time when there were no colour-names as such . . . and that not very remote in many cases, when the present colour-words were terms that could be used in describing quite different qualities [including] gay, lively, smart, dashy, loud, gaudy . . . dull, dead, dreary . . . tarnished, stained, spotted, dirty, smeared . . . faint, faded [and feeble] (Melissa 2014).

Recent research in this area has demonstrated that this hierarchy matches human's reaction to different frequencies in the visible spectrum; that is, the stronger our reaction to that colour's frequency, the earlier it was named in the culture or as Loreto et al. (2012) put it:

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> The colour spectrum clearly exists at a physical level of wavelengths, humans tend to react most saliently to certain parts of this spectrum often selecting exemplars for them, and finally comes the process of linguistic colour naming, which adheres to universal patterns resulting in a neat hierarchy...

Similar to other cultures, English words for the colours generally followed that same pattern, with black and white coming first, and purple, orange and pink coming last (Melissa 2014). The names given to different colours have their origin from the parents of modern English language such as Proto-Indo-European (PIE), Proto-Germanic and Old English languages.

Black derives from .words invariably meaning the colour black, as well as dark, ink and "to burn." Originally meaning, burning, blazing, glowing and shining, in PIE was *bhleg*. This was changed to *blakkaz* in Proto-Germanic, to *blaken* in Dutch and *blaec*, in Old English. This last word, *blaec,* also meant ink, as did *blak* (Old Saxon) and *black* (Swedish).

White began its life in PIE as *kwintos* and meant simply as white or bright. This had changed to *khwitz* in Proto-Germanic, and later languages transformed it into *hvitr* (Old Norse), *hwit* (Old Saxon) and *wit* (Dutch). By the time Old English developed, the word was *kwit*. For red colour, in PIE, red was *reudh* and meant red and ruddy. In Proto-Germanic, red was *rauthaz*, and in its derivative languages *raudr* (Old Norse), *rod* (Old Saxon) and *rØd* (Danish). In Old English, it was written *read*. Green means grow, in PIE, it was *ghre*. Subsequent languages wrote it *grene* (Old Frisian), *graenn* (Old Norse) and *grown* (Dutch). In Old English, it was *grene* and meant the colour green as well as young and immature.

Thousands of years ago, yellow was considered to be closely related to green, and in PIE, it was *ghel* and meant both yellow and green. In Proto-Germanic, the word was *gelwaz*. Subsequent incarnations of German had the word as

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gulr (Old Norse), *gel* (Middle High German) and *gelo* (Old High German). As late as Old English, yellow was written *geolu* and *geolwe*. Blue was also often confused with yellow back in the day. The PIE word was *bhle-was* and meant "light-coloured, blue, blond yellow" and had its root as *bhel* which meant to shine. In Proto-Germanic, the word was *blaewaz*, and in Old English, it was *blaw*. English also gets some of its words from French, and blue is one of them. In Old French (one of the vulgar Latin dialects whose height was between the 9th and 13th centuries AD), blue was written *bleu* and *blew* and meant a variety of things including the colour blue.

Brown is derived from the Old Germanic for either or both a dark colour and a shining darkness *(brunoz* and *bruna)*, brown is a recent addition to English language. In Old English, it was *brun* or *brune*, and its earliest known writing was in about 1000 AD. Purple also skipped the PIE and seems to have sprung up in the 9th century AD, in Old English as *purpul*. Borrowed from the Latin word *purpura,* purple originally meant alternately, "purple colour, purple-dyed cloak, purple dye . . . a shellfish from which purple was made . . . [and] splendid attire generally."

Orange colour's name derives from the Sanskrit word for the fruit *naranga*. Thus, the colour orange was named after the fruit, not the other way around. This transformed into the Arabic and Persian *naranj,* and by the time of Old French to *pomme d'orenge*. It was originally recorded in English as the name of the colour in 1512. Before then, the English speaking world referred to the orange colour as *geoluhread*, which literally translates to "yellow-red."

Pink is one of the most recent colours to gain a name, pink was first recorded as describing the "pale rose colour" in 1733. In the 16th century, pink was the common name to describe a plant whose petals had a variety of colours (*Dianthus*), and it originally may have come from a Dutch word of the same spelling that meant small (Melissa 2014). The values of a colour are recognised by making its tints and shades. *Tints* are light values that are made by mixing a

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colour with white. For example, pink is a tint of red, and light blue is a tint of blue. *Shades* are dark values that are made by mixing a colour with black. Maroon is a shade of red, and navy is a shade of blue. In addition, colours are evaluated based on the hue (the percentage of primary colours); the chroma (richness of the colour) and the lightness (this refers to the amount of light reflected). The desire of humans to have any object they appreciate to be coloured led to the use of colourants.

Colourants

Colourants are pigments or dyes used in giving acceptable aesthetic value to an object. A dye by definition is a coloured compound that adheres to cloth and retains its colour against the attack of light, moisture and soap under normal conditions of wear (Nathan and Murthy 1968). Pigments are inorganic or organic, coloured, white or black materials which are practically insoluble in the medium in which they are incorporated. Dyes, unlike pigments, dissolve during their application, and in the process lose their crystal or particulate structure. It is thus by physical characteristics, rather than by chemical composition, that pigments are differentiated from dyes (Herbst and Hunger 1997).

The history of pigment application dates back to pre-historic cave paintings, which provides evidence of the use of ocher, hematite, brown iron ore and other mineral-based pigments more than 30,000 years ago. Cinnabar, azurite, malachite and lapis lazuli have been traced back to the third-millennium BC in China and Egypt (Herbst and Hunger 1997). The beginning of organic pigment application dates back to antiquity. It is certain that the art of using plant or animal "pigments" to extend the spectral range of available inorganic colourants by a selection of more brilliant shades had been practised thousands of years ago. Uses of dyes are diverse. They have been used as indicators of solvent polarity, chemical equilibria, and molecular environment as well as indicators of aggregation and molecular order

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(Olaniyi and Ogungbamila 1991; Herbst and Hunger 1997; Buss and Eggers 2000; Rageh 2004).

Classification of Organic Pigments and Dyes

Classification of organic pigments is commonly done either by chemical constitution or by colouristic properties. Strict separation of the two classification systems is however not very practical. Synthetic organic dyes span a wide range of varying chemical structures including among others, azo-, anthraquinones, carbonyls, nitros, triphenylmethane and phthalocyanines. About 50% of all the dyes manufactured come under the class of azo dyes prepared from diazonium salts.

Azoaromatic compounds have attracted much attention in recent times due to their emerging applications in dye stuff industry, for being excellent photo-aligning substrates for liquid crystals, highly efficient photorefractive media, acid–base, redox and metallochromic indicators, optical activators and optical storage media. Azo dyes have also been used in photo-catalytic reactions as catalyser exposing to ultraviolet light (Rau 1990; Kumar 1992; Nakamura et al. 2000; Yaroschchuk et al. 2001; Sakthivel et al. 2003; Masoud et al. 2004; Muruganandham and Swaminathan 2004; Muruganandham et al. 2006). They have also been widely employed as histological stains, such as: acid alizarin violet and Sudan series (Buss and Eggers 2000).

One other major application of dyes, in particular azo dyes, is their use as colour additives in food, cosmetics and pharmaceuticals (Sasaki et al. 2002). It is said that we "eat with our eyes as much as with our mouths," and that's certainly the case when we walk down the aisles of a supermarket (fig. 7) or shopping malls (Kobylewski and Jacobson 2010).

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Fig 7: Various consumer goods made aesthetically acceptable by colourants (FDA 2007).

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Despite extensive public awareness against artificial colouring, colourants have found usefulness in improving the aesthetic value of products. A variety of consumer goods are dyed with azo dyes. The consumer goods include foods, beverages, lipsticks, stick-on, tattoos, toothpastes, hair products, and the like. Some of these dyes present potential risk to man and his environment. The adverse effects of certain azo dyes on human health were first apparent when cases of bladder cancer were detected in workers involved in the manufacturing and use of benzidine-based dyes (Oh et al. 1997; Borros et al. 1999). Prior to that period, many benzidine-based dyes with disazo and triazo structures were used as direct dyes. Nowadays, however, dyes based on benzidine and its congeners can no longer be manufactured for textiles in the United States due to safety concerns (Bae and Freeman 2002).

Two prominent terms associated with the assessment of the toxicological properties of azo dyes are mutagenicity and genotoxicity. Mutagenicity refers to the induction of permanent transmissible changes in the amount or structure of the genetic material of cells and/or organisms. Genotoxicity,

on the other hand, is a broader term which refers to potentially harmful effects on genetic material which are not necessarily associated with mutagenicity. Thus, tests for genotoxicity include indication of induced damage to DNA, such as unscheduled DNA synthesis, sister chromatid exchange and DNA strand breaks (European Chemical Bureau 2003). Until recently, these tests were frequently used. However, a more useful approach for assessing DNA damage is the single-cell gel (SCG) or Comet assay (Tice et al. 2000). The terms "SCG" or "comet" are used interchangeably, and it refers to the assessment of individual cell DNA migration patterns produced after treatment with candidate genotoxins when compared with negative and positive controls.

The mutagenicity of azo dyes has been said to arise from the reduction of the azo group by the action of intestinal anaerobic bacteria or the hepatic azo reductases, which releases aromatic amines. Therefore, dye mutagenicity is often related to the mutagenic properties of the aromatic amines employed in the synthetic steps or present as degradation products (Oh et al. 1997; Borros et al. 1999). With regards to the genotoxicity of aromatic amines, it is known that the presence of ring substituents in the position *ortho* to the amino ($-NH_2$) group reduces mutagenicity (Gong et al. 2002). These studies have led to non-mutagenic benzidine analogs containing bulky alkoxyl groups *ortho* to the amino group and amino derivatives of the dihydrophenophosphazine ring systems (Bello et al. 2000).

The few azo dyes that have been certified as approved colourants for preparations intended for systemic and topical use are those that have been shown to be sufficiently safe in animal and clinical studies (Bello et al. 2000). The dyes include sunset yellow (IV) and allura red (V). The two dyes share a common parent structure; sulfonated phenylazo-hydroxynaphthalenes.

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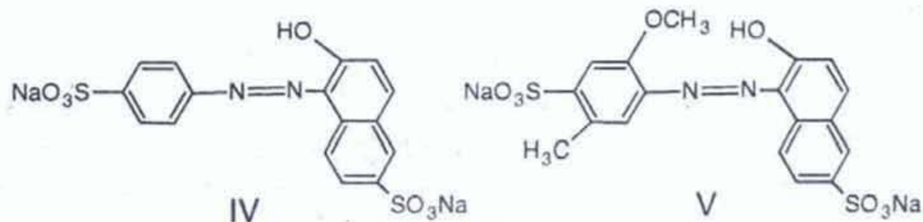

Due to the extensive awareness and publications of toxicological data yearly, attention is now shifting to naturally obtained colourants for incorporation into products likely to gain contact with man through food, inhalation, skin and mucous membrane (Francis and Markakis 2009; Wissgott and Bortlik 1996; Espín et al. 2000). The natural colourant area can be subdivided into anthocyanins, betalains, chlorophylls, carotenoids, flavonoids, polyphenols, Monascus, hemes, quinones, biliproteins, safflower, turmeric and miscellaneous.

The debate however is still on-going. One clear fact is that there is a need for international harmonization of various standards and acceptable admissible daily exposure to colour additives. It must be emphasized that approval of one colour additive for a product does not necessarily transfer such approvals to other colourants. Everyone must be educated with regards to what can be tolerated, as idiosyncrasies have been associated with the use of some products containing colourants.

Mr. Vice-Chancellor Sir, having examined the basis of colours, colourants and coloured substances, I hereby, present my contributions to Colour Chemistry. My contributions to Colour Chemistry run through the main tripodal trawl of azo dye derivatization, Schiff base formation and charge transfer complexation. I will along the line describe some other extensions to this story.

Old Reagent, New Applications

Para-dimethylaminobenzaldehyde, DMAB (Ehrlich's reagent), has found usefulness in a wide range of applications from analytical, biochemical to synthetic procedures and processes alongside other miscellaneous applications. The unique structural feature containing a *para*-dimethylamino

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substituent to an aldehyde moiety renders it highly reactive towards a wide range of compounds, and this has made it useful as a condensation reagent and oxidizable and reducible reactant in many reactions. Adegoke (2011a) presented a comprehensive review of the diverse applications of DMAB spanning over a century with a prospect that this compound will be more relevant in years to come in microbiology, chemical pathology and organic synthesis, as well as pharmaceutical and analytical chemistry. The diverse potentials of this compound led to my trawling into its applications, and, indeed, I have found new applications for the compound. The ability of DMAB to form condensation products with both primary and secondary amino groups have been explored for the development of simple and rapid analytical techniques for clinically useful drugs. DMAB was used for the assay of hydralazine hydrochloride in bulk and dosage forms (fig. 8) based on condensation reaction to generate an instant greenish-yellow coloured product (Adegoke and Nwoke 2008).

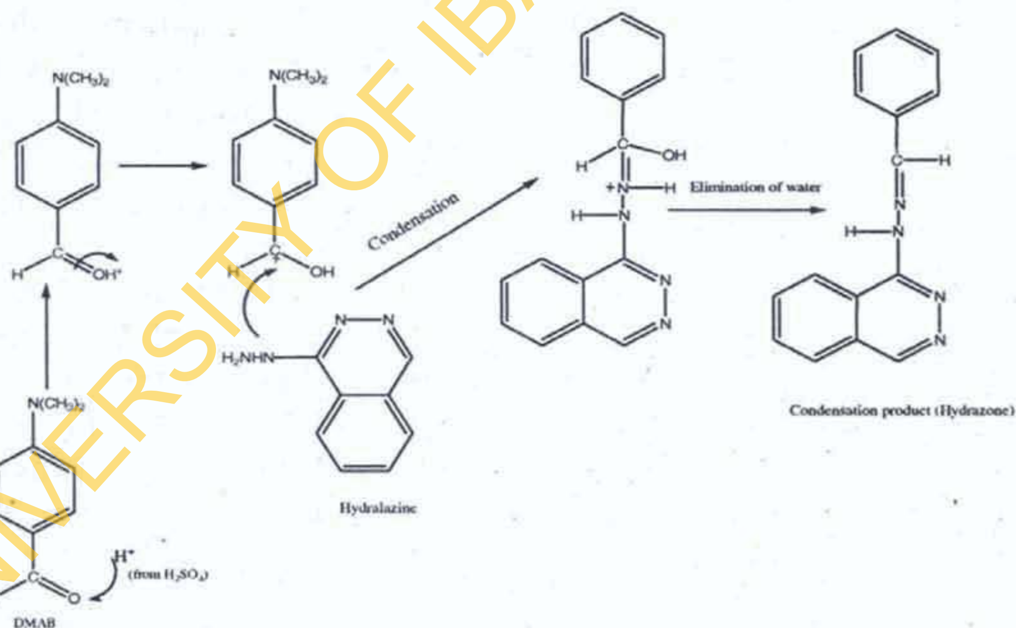

Fig. 8: Formation of hydrazone between hydralazine and DMAB.

In another report, a new, simple, cost-effective spectrophotometric method was developed for the determination of olanzapine in pharmaceuticals (Adegoke et al. 2014a). The

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ability of DMAB to be reduced and oxidized through the aldehyde group has also been utilized for accurate determination of some drugs. Adegoke and Osoye (2011) developed an alternative simple, accurate and precise method for the determination of artesunate and dihydroartemisinin in bulk samples and dosage forms. The method involves the reaction of the reactive methylene centres generated *in situ* from the acid decomposition of the artemisinin derivatives with *p*-dimethylaminobenzaldehyde (DMAB). DMAB was reduced to the purple-coloured alcohol, and this was quantitatively used to estimate the concentrations of the artemisinin derivatives (fig. 9). This reaction represents the first ever report of a full colorimetric determination of artemisinin derivatives in literature.

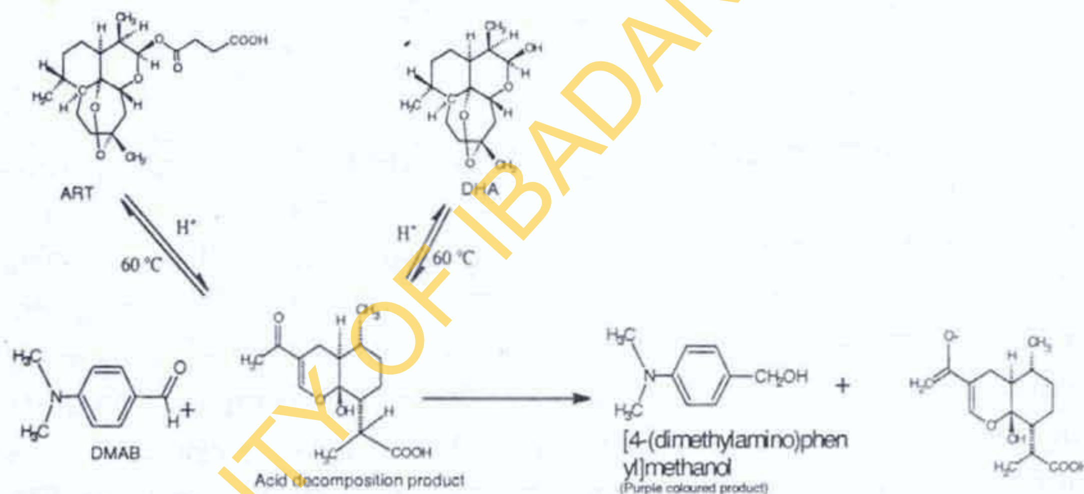

Fig. 9: Formation of a purple coloured product from DMAB following reaction with artemisinin derivatives.

A simple, accurate and sensitive spectrophotometric method was also developed for the determination of ciprofloxacin, pefloxacin and sparfloxacin. The method was based on the oxidation of these drugs with cerium (IV) in the presence of perchloric acid and subsequent measurement of the excess Ce (IV) by its reaction with *DMAB* to give a brownish coloured product. The decrease in the absorption intensity (ΔA) of the coloured product due to the presence of

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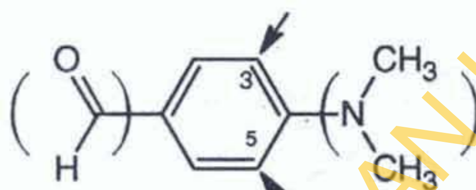
the drug was correlated with drug concentration in the sample solution (Adegoke and Balogun 2010).

Old Reagent, Novel Applications

Of the varied applications that have been reported for the utilization of DMAB in analytical and synthetic chemistry, its application as a coupling component has not been reported. The compound has a peculiar structure, and its ability to direct in-coming electrophile is determined by its 1,4-disubstituents; the aldehyde group and the dimethylamino substituent.



By theoretical considerations, positions 3 and 5 (arrowed) should be able to accept an electrophile to give an azo adduct. This is made possible by the deactivating effect of the aldehyde group (directs to *meta* positions; 3 and 5) and the positive inductive effect of the dimethylamino fragment (directs *ortho* positions; 3 and 5); therefore, both substituents augment each other. As sound as this theory may seem, in practice, azo adducts, if formed, are not stable and readily decompose. Thus, Adegoke and Umoh (2009) discovered a new approach to the application of DMAB in azo adduct formation. Since the aldehyde group withdraws electron and possesses an internal mesomeric effect, any factor that will diminish this inherent behaviour will dramatically enhance and pronounce the *ortho*-directing influence of the dimethylamino fragment. This mesomeric effect, we found out, gets pronounced in aqueous systems and is destroyed in polar aprotic solvents like methanol. Thus, the first ever report in literature of the ability of DMAB to function in this regard was reported by my group. The mechanism envisaged was that coupling of the diazotized skeletons with DMAB in

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methanol (as opposed to water) destroys the deactivating internal mesomeric effect of the aldehyde functional group thus permitting optimal activating influence of the dimethylamino group. The end result is usually the formation of coloured azo adducts that are determined using the visible wavelength range. This opened wide spheres for spectrophotometric analyses of drugs.

The first application of this mechanism was for the determination of reduced and diazotized metronidazole and tinidazole (Adegoke and Umoh 2009). The procedure involved coupling of diazotized nitroimidazoles with *p*-dimethylaminobenzaldehyde (DMAB) to form a greenish-yellow solution. The persistent yellowish-green colour produced was stable for seven days. This colour is completely different from the salmon-pink produced after condensation reaction between reduced nitroimidazoles and DMAB. Figure 10 presents the structures of the new compounds produced between DMAB and MZ and TZ. The possibility of steric hindrance on substitution by diazotized nitroimidazoles is eliminated as the dimethylamino group is staggered from the diazo linkage (bond angle between N8, C14 and C12 for the TZ adduct is 120.456° and that of the MZ adduct between N18, C10 and C4 is 122.665°). This shows that the angles around a main chain nitrogen atom (the diazo linkage) are all approximately equal to 120 degrees: consequently, the group is planar. Likewise, the torsion angles are 0.94 and 0.339°, respectively, for the TZ-DMAB and MZ-DMAB adducts. Since the torsion is between 0 and +90°, the stereochemical arrangement is of the *syn* type. Both molecules are therefore of the trigonal planar skeleton. Both the bond and torsion angles favour the formations of a stable adduct.

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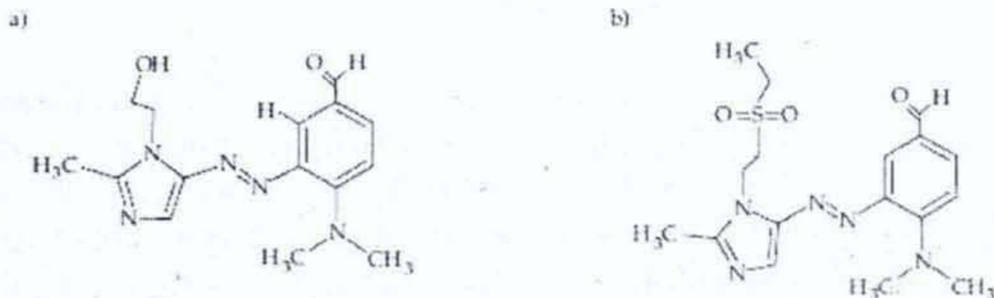

Fig. 10: Structure of the azo-adducts formed between DMAB and (a) Metronidazole and (b) Tinidazole.

In another of such applications, a new simple, accurate and cost-effective spectrophotometric method was also developed for the analysis of some cephalosporins (ceftriaxone, ceftazidime, cefixime, cefotaxime and cefuroxime) in bulk samples and pharmaceutical dosage forms (Adegoke and Quadri 2012). The spectra are presented in figure 11. Two antiviral agents have also been successfully determined using this reaction: acyclovir (Thomas and Adegoke 2012) and ganciclovir in figure 12 (Thomas and Adegoke 2015). Both methods are simple, inexpensive, reproducible and fast. The reaction has also been applied to the simultaneous determination of trimethoprim and sulphamethoxazole using solid phase extraction as a preliminary selective step (Olaifa 2014). Similarly, the reaction has been successfully adopted for the accurate determination of gabapentin in bulk and dosage forms (Aiyenale 2016).

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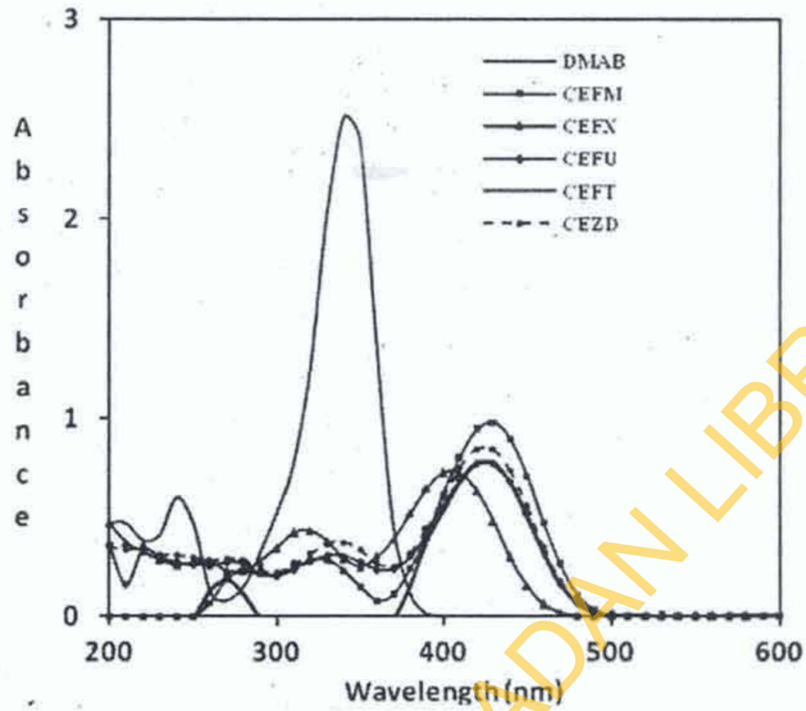

Fig. 11: Overlaid absorption spectra of azo adducts formed between the cephalosporins and DMAB.

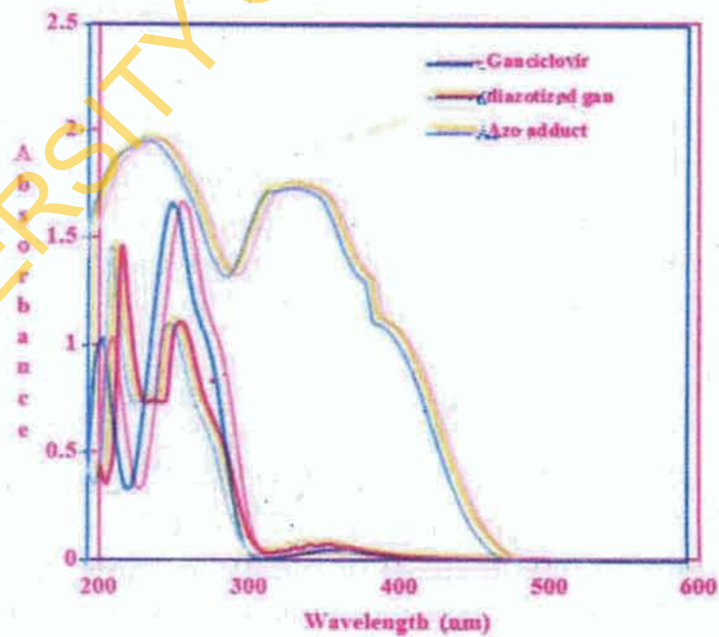

Fig. 12: Overlaid absorption spectra of ganciclovir, diazotized ganciclovir and azo adduct formed with DMAB.

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Old Reagent, Old Applications, New Devises

Chloranilic acid ranks the highest in the order of priority of reagents that have been used in the application of charge-transfer complexation (CTC) reaction of acceptor molecules. While this reagent is an old one and the applications well established, I have, along with my students, devised some new ways to evaluate the possibility of CTC formation or otherwise through the applications of chemometrics. I have found out that there is a relationship among several factors and parameters which will determine and support charge transfer complexation. Thus, CTC reactions have been applied for the accurate determination of metronidazole, tinidazole (Adegoke et al. 2010a; Adegoke 2011b), lumefantrine (Adegoke et al. 2011), trimethoprim and sulphamethoxazole (Adegoke et al. 2014), as well as olanzapine (Adegoke et al. 2015).

We have discovered that the estimation of the formation constant as a function of temperature gives an indication of the stability or otherwise of the new CTC. In addition, more practical approach has been the physicochemical parameterization of the factors that can account for the stability of the CTC and thus permitting ready and accurate determination of the drug molecules in complex matrices. In the determination of olanzapine, OLP (Adegoke et al. 2015), chemometrics were conducted and provided accurate explanation to the formed complex. The first physicochemical parameter calculated was the transition energy of the complex which is obtained from the expression $h\nu_{CT}$ where h is Planck's constant and ν_{CT} is the wavenumber of the absorption peak of the CT complex. The transition energy was found to be 2.303 eV.

Two other physicochemical parameters estimated were oscillator strength and transition dipole moment. The oscillator strength (f) is a dimensionless quantity used to express the transition probability of the CT band and the transition dipole moment (μ_{EN}) of the CT complex (Martin et al. 1983). Both parameters are obtained from equations 6 and 7 respectively.

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$$f = 4.32 \times 10^{-9}[\varepsilon \Delta v_{1/2}] \quad (6)$$

$$\mu_{EN} = 0.095 \left[\frac{\varepsilon CT \Delta v_{1/2}}{\Delta v}\right]^{1/2} \quad (7)$$

where, $\Delta v_{1/2}$ is the half-width i.e. the width of the band at the half the maximum absorption, and Δv ≈wavenumber at the absorption maximum.

The oscillator strength, f and the transition dipole moment obtained are 7.289 and 0.892 Debye respectively. The ionization potential, I_D, of the donor in the charge transfer complex was another physicochemical parameter calculated using the empirical equation derived by Aloisi and Pigantro, 1973 (presented in equation 8).

$$I_{D\,(ev)} = 5.76 + 1.53 x 10^{-4}\, v_{CT} \quad (8)$$

Where, v_{CT} is the wavenumber of the CT band in cm^{-1}. I_D was found to be 6.054 eV. The resonance energy of the complex (R_N) in the ground state is obtained from the theoretical equation derived by Brieglab (1961) given in equation 9.

$$\varepsilon_{CT} = 7.7 \times 10^4 / [hv_{CT}] / R_N - 3.5 \quad (9)$$

where, ε_{CT} is the molar absorptivity of the complex at the maximum of the CT absorption, hv_{CT} is the transition energy of the complex.

The resonance energy was calculated as 4.687 eV for the new molecular complex. The dissociation energy (W) of the formed CT complex between OLP and CAA was calculated from the transition energy (hv_{CT}), ionization potential of the donor (I_D) and the electron affinity of CAA (E_A = 1.1) using the relationship in equation 10 (McConnel et al. 1953).

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$$h\nu_{CT} = I_D - E_A - W \text{ --------------------------------------- } (10)$$

The dissociation energy was found to be 2.651 eV.

From the results presented in table 2, some observable trends are clearly evident from these physicochemical parameters. The values obtained point to the good stability of the complex formed between CAA as acceptor and olanzapine as donor. The ionization potential of the donor gave a high value of 6.054 eV denoting that OLP is a good *n*-electron donor and making the electrons readily available for donation to an acceptor such as CAA.

Table 2: Physicochemical Parameters for the Formation of CT-complex between Olanzapine and Chloranilic Acid

Drug	CT λ_{max} (nm)	$h\nu_{CT}$ (eV)	f	μ_{EN} (Debye)	R_N (eV)	I_D (eV)	W (eV)
Olanzapine	520	2.303	7.289	0.892	4.687	6.054	2.651

The secondary amino group found in olanzapine has proven to be a useful electron donor from the results of the I_D obtained. The transition energy is about two times less than this ionization energy of the CT band; hence, the energy barrier required for electronic transition is readily surmounted, and the complex is produced readily. This further confirms the avidity at which the complex was produced. The dissociation energy (W) was also found to be far lesser than I_D. Thus, the spontaneous decomposition of the CT complex will be minimal especially at room temperature where these values were obtained from. The high resonance energy of the formed complex will produce a stabilization effect on the complex, and this is expected considering the bulky nature of OLP and the tendency to be stable upon donation of a lone pair electron to CAA.

In addition to the foregoing, we have also estimated the thermodynamic parameters, and they have given us opportunity to adequately discover the propensity or

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otherwise of CTC formation. The thermodynamic functions, standard free energy change (ΔG^0), the enthalpy change (ΔH) and the entropy change (ΔS) were obtained from the well-established equations 11, 12 and 13 respectively.

$$-\Delta G^0 = 2.303 \text{ RT} \log K_{CT} \quad \text{------------------------------} (11)$$

$$Log\, K = -\frac{\Delta H}{2.303}\left(\frac{1}{T}\right) + Constant \quad \text{--------------------} (12)$$

$$-\Delta G^0 = \Delta H \; - \text{T}\, \Delta S \quad \text{--} (13)$$

The enthalpy of the CT formation was obtained by plotting the Log of formation constant against the reciprocal of absolute temperature. The plot is presented in figure 13. Table 3 contains the various thermodynamic parameters obtained alongside the molar absorptivities and formation constants at the four temperature levels. The standard free energy gave a negative value at all the four temperature conditions considered in this experimental design. This result points to the exothermic nature of the complex formation. This thus explains why higher temperature values led to decrease in the absorbance of the complex. The Gibbs free energy became increasingly higher with increase in temperature denoting that it becomes difficult to generate the complex at higher temperatures. Since it is established that the formation of the complex occurs through an exothermic process, higher temperature will prevent the avidity of reaction and the spontaneity of the charge transfer complexation. The high Gibbs free energy obtained even at room temperature however attests to the ease of formation of the complex. Though relatively small, the enthalpy change also points to the possibilities of ease of formation of the CT complexes. The enthalpy of formation for the molecular complex was found to be -0.8803 $KJMol^{-1}$. The entropy also gave relatively high values with the value reducing as temperature increased.

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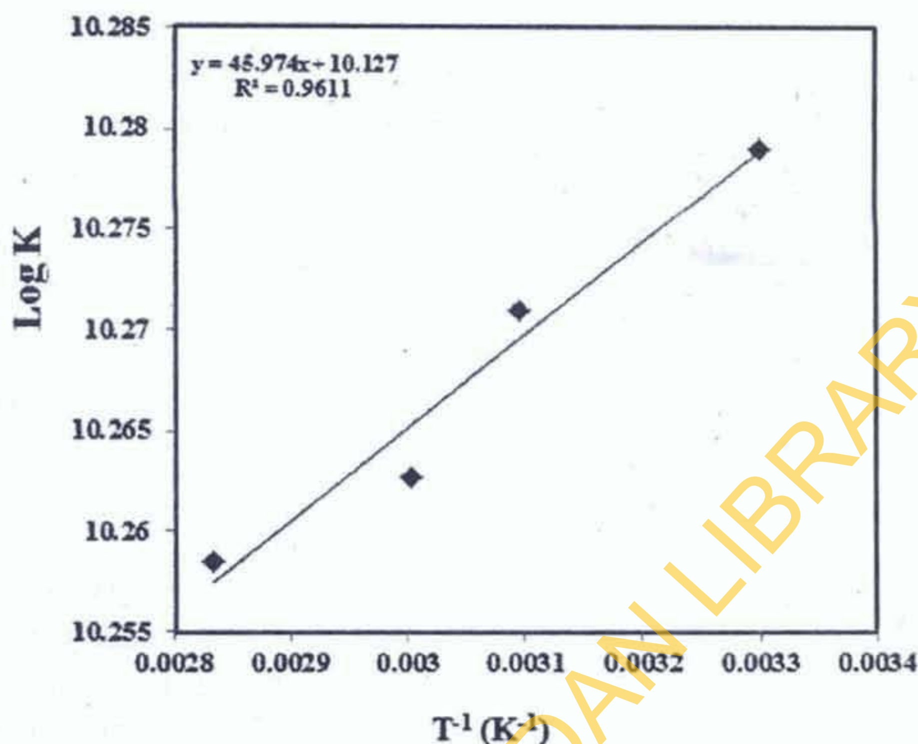

Fig. 13: Plot of Log K of OLP-CAA complexes as a function of 1/T (K^{-1}).

Table 3: Thermodynamic Changes following Complexation between Olanzapine and Chloranilic Acid

Temperature (Kelvin)	Molar Absorptivity (ε) L Mol^{-1} Cm^{-1}	Formation Constant (K) M^{-1}	ΔG^0 ($KJMol^{-1}$)	ΔH ($KJMol^{-1}$)	ΔS ($KJMol^{-1}$)
303	1.687×10^3	1.9014×10^{10}	-59.635		0.1997
323	1.656×10^3	1.8661×10^{10}	-63.521	-0.8803	0.1994
333	1.625×10^3	1.8310×10^{10}	-65.435		0.1991
353	1.609×10^3	1.8133×10^{10}	-69.369		0.1990

ΔG^0 is the free energy change: ΔH is the enthalpy change and ΔS is the entropy change

With the successes recorded adopting this old reagent, chloranilic acid, we are currently developing some new nitronaphthalenes as charge transfer acceptors. This is the thrust of the Ph.D. research of one of my students, Offiong Umoh, who has made tremendous progress on the adoption of novel hydroxyl nitronaphthalenes of varying structures as charge transfer acceptor for clinically useful drugs.

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New Reagent, Novel Applications

Mr. Vice-Chancellor Sir, following the successful completion of my M.Sc degree in 1999 with a project utilizing diazotized 4-amino-3,5-dinitrobenzoic acid (ADBA) developed by Idowu (1998). I received a terrible discouragement as journal outlets rejected the supposed new methodology reported for aspirin. The comment was that there was no distinction between the spectrum of aspirin and that of salicylic acid following reaction with diazotized ADBA. This same report was received for halofantrine reported by Idowu (1998), and no success was recorded for paracetamol.

With a sound knowledge base in organic chemistry, I threw my trawl line into literature and sought for drugs that possess activated skeletons. Also, from a thorough knowledge of drug metabolism, I opined that sites liable to deactivation through functional group addition in drug molecules during metabolism could be possible sites for attack by an electrophile. The thorough search of literature produced about 250 drug molecules that could be likely candidates. About 50 compounds were eventually sourced in the local market. The challenge I was left with was to produce the precursor amine (ADBA) in a high state of purity. This was accomplished and attested to by Idowu's collaborators in Japan with the ADBA produced having a 99.99% purity. Several refinements of the diazotization process led to the possibility of a reagent grade diazonium ion. This provided the platform to be involved in the training of several B. Pharm, PGD and M.Sc. students over the years, while it made a platform available for me to also complete my Doctoral research.

The first successful report for the application of diazotized ADBA was one of the candidate drugs I trawled into the chemical library of likely drugs for analysis by diazotized ADBA. Mefenamic acid was successfully determined by colorimetric means (Idowu et al. 2002). The diazonium ion was re-christened 4-carboxyl-2,6-dinitrobenzene diazonium ion (CDNBD) to emphasize the species involved in the reaction with activated skeletons. The

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applications of 4-carboxyl-2,6-dinitrobenzene diazonium (CDNBD) ion as a derivatizing reagent was extensively adopted as an off-shoot of my MSc research and for my Ph.D research endeavour. One chemical group that was analyzed with CDNBD is the heterogeneous skeletons referred to as pharmaceutical phenol ether homologues. The use of the reagent led to the ready determination of pharmaceutical phenol ethers whose determination hithèrto as a chemical group was not possible by azo dye formation since phenol ethers being weakly activating do not react with majority of diazonium ions. Also, CDNBD when compared with diazotized sulphanilic acid and *para*-nitroaniline gave clearly defined peaks for such pharmaceutical phenol ethers as indomethacin, propranolol, nimesulide, reserpine, pindolol, astemizole, naproxen, nabumetone, griseofulvin, nadolol and clomiphene (Adegoke et al. 2005). The exceptionally high reactivity of CDNBD was demonstrated by the accurate determination of propranolol (Idowu et al. 2004), indomethacin (Adegoke et al. 2006a), nadolol (Adegoke et al. 2006b), reserpine (Adegoke et al. 2007a) and nabumetone (Adegoke et al, 2007b). The spectra produced on coupling of these phenol ethers with CDNBD are presented in figure 14.

Varying stoichiometric ratios were obtained and in particular for indomethacin, a 2:1 mole ratio of CDNBD to indomethacin was observed. Spectroscopic characterizations using NMR and mass spectrometry of its azo adduct revealed that simultaneous substitution on the indomethacin molecule must have taken place. This may be due to the isolated nature of the rings present in indomethacin (fig. 15). This is however a remarkable discovery as substitution of an electrophile on a ring deactivates the ring and makes multiple substitutions difficult. The high reactivity of CDNBD also led to the formation of azo dyes with artemisinin derivatives. This was also the first report of azo dye formation by artemisinin derivatives (Adegoke et al. 2010b).

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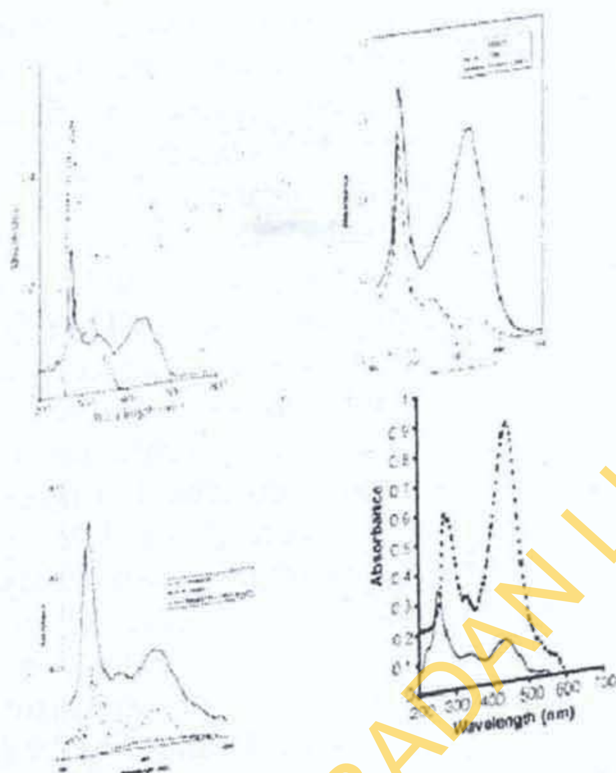

Fig. 14: UV-VIS Spectra of the azo adducts of pharmaceutical phenol ethers overlaid on CDNBD.

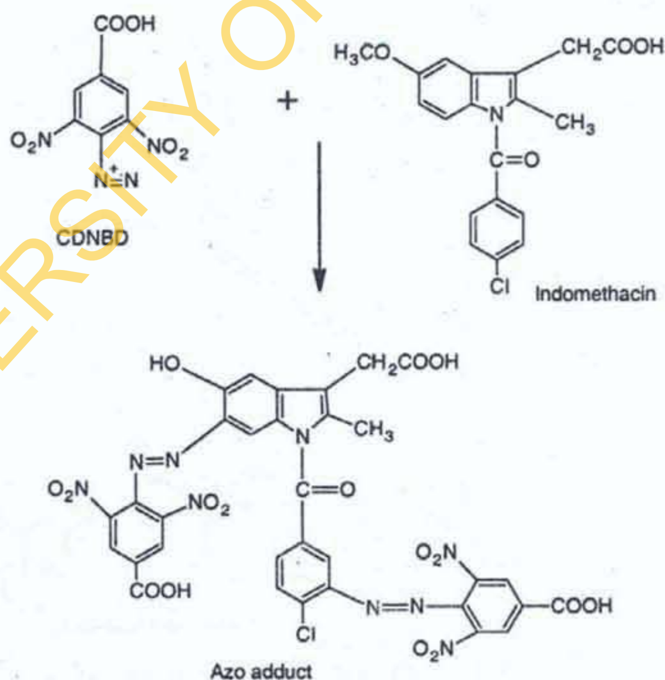

Fig. 15: Diazo coupling reaction between indomethacin and CDNBD.

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This once again represents a significant contribution as the artemisinin derivatives do not belong to the class of skeletons for which azo dye formation might be plausible. The mechanism envisaged was that conversion of artemisinin derivatives takes place in the presence of strong acids, and this produces reactive methylene centres. The reactive methylene centres are known to form azo adducts with highly reactive diazonium ions of which class CDNBD falls. The evidence for the formation of new azo adducts was provided by the formation of hydrophobic brown coloured azo adducts and proton NMR established the formation of the azo adducts. The reaction pathway is presented in figure 16. Once good detection limits are recorded in UV, pre-column derivatization is carried out to improve sensitivity in HPLC (Adegoke 2012b). The success recorded in the UV spectrophotometric work led to the extension to liquid chromatographic analyses (fig. 17) of artemisinin (ATS), artesunate (ART), artemether (ATM) and dihydroartemisinin (DHA) with good sensitivity recorded (Adegoke et al. 2012c). This is the first practical application of CDNBD as a pre-column derivatization reagent for the estimation of pharmaceuticals.

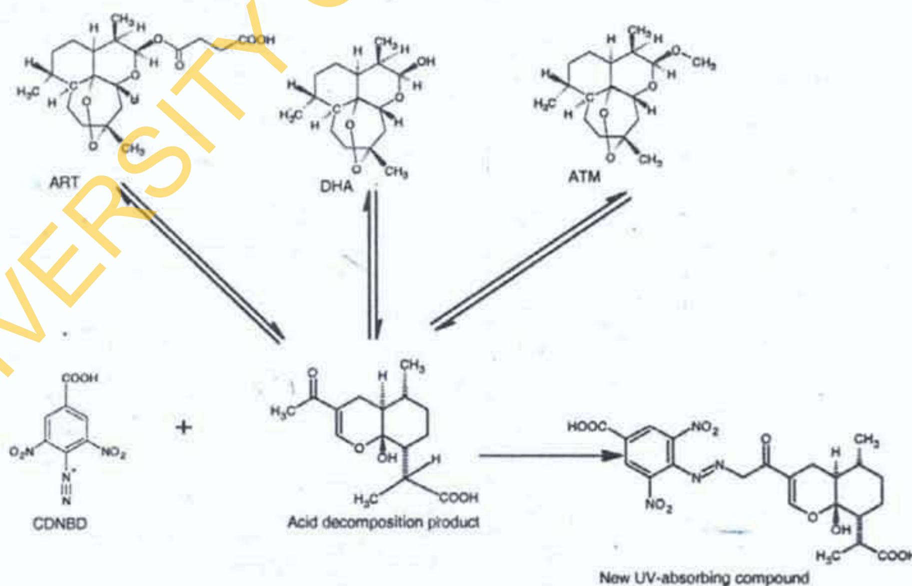

Fig. 16: Coupling reaction pattern for the formation of azo adducts between artemisinins and CDNBD.

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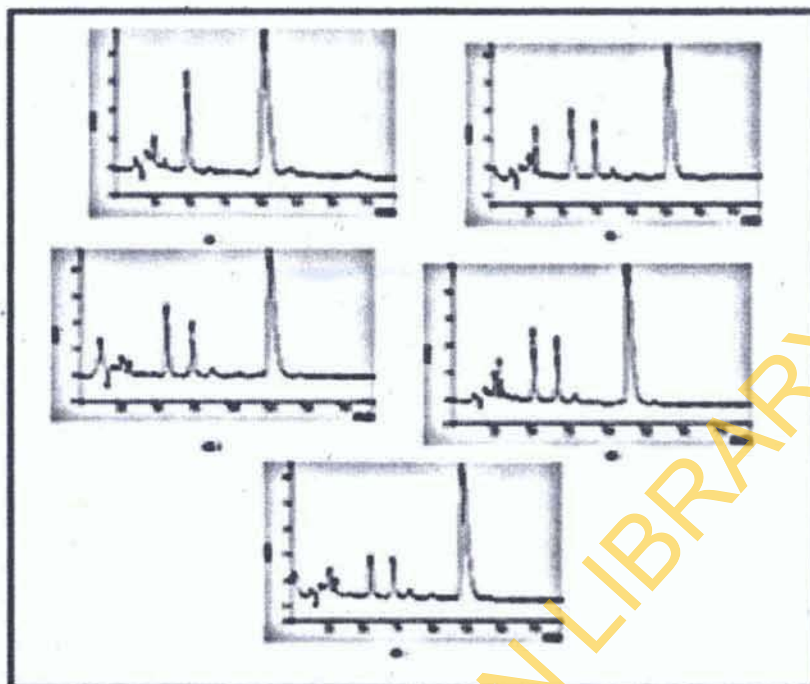

Fig. 17: Representative chromatograms using optimized chromatographic separation conditions; (a) CDNBD, (b) ATS (CDNBD-ATS peak 7.45 min), (c) ART (CDNBD-ART peak 7.45 min), (d) DHA (CDNBD-DHA peak 7.51 min) and (e) ATM (CDNBD-ATM peak 7.45 min).

The CDNBD reagent has also been demonstrated to possess some versatility as it was found to couple readily with aceclofenac (Aderibigbe et al. 2012a) and reduced nifedipine (Aderibigbe et al. 2012b).

New Chemical Entities

Mr. Vice-Chancellor Sir, a critical requirement for the completion of my Ph.D research was to demonstrate the mechanism underlying the diazo coupling reaction between CDNBD and the pharmaceutical phenol ethers. Majority of phenol ethers do not couple with diazonium ion because they belong to the class of moderately to weakly activating substituents. For over three years of working on the bench for my Ph.D degree, what I observed was the change of colours of the drugs upon contact with CDNBD (Adegoke 2005). As stated by Saunders (1949b), a notable characteristic of coupling of phenol ethers with highly reactive diazonium ions

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is that the diazo coupling reaction proceeds with partial or complete removal of the alkyl group of the ether, a remarkable occurrence as phenol ethers are by no means easily hydrolyzed.

They are hydrolyzed by the strong acid, hydroiodic acid (HI). While the proportion of the side-chain removed depends on the diazo-compound, it was hitherto not clear whether re-substitution takes place or that the diazonium displaces the OH remaining. In actual fact, a dramatic experience occurred with propranolol that had colour changes observed in its coupling with CDNBD from blue to violet to reddish-pink which could therefore be attributed to the cleavage of the side chain. Thus, the blue colour may represent the azo adduct of intact propranolol and other colours to various stages of cleavage. From the NMR of propranolol-CDNBD azo adduct, it is clear that the re-substitution of the alkyloxamine side chain does not take place and the presence of a strong $O\text{-}H_{str}$ in the IR shows a residual OH on the ring. Literatures that I could not source and access in three years of Ph.D work became handy in three days! For this I remain grateful to God for the MacArthur short duration support to visit the Department of Pharmaceutical Sciences, University of Strathclyde, Glasgow, Scotland in 2004.

Mr. Vice-Chancellor Sir, I pray daily for our Universities in Nigeria that we will get to that advanced state of getting both ancient and timely literatures for research endeavours. I sincerely believe that forum like this must uphold the truth and make proclamations about results of research that were generated to earn publications leading to Professorship. I can *play to the gallery* to earn applause from a distinguished audience as this through well-crafted oratory proclamations of what was done and not done to attain achievements. But then we as academics must recognize that the worth of a man's life is not based on the abundance of attainments, but rather on the uniqueness, peculiarity and value he has been created with. The truth is that I never set out to design dyes during my Ph.D programme, but rather just laden with the burden and the requirements to carry out spectroscopic

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investigations into the isolated dyes following reactions between CDNBD and some pharmaceutical phenol ethers. This however turned out to be a blessing. Thus, spectroscopic characterization of all the azo adducts for the first time established the scission of the ether linkage on diazo coupling to generate naphthols. This was a remarkable observation as confusion seems to exist in literature as to the exact mechanism of coupling and if the ethers are formed again after coupling reaction. Adegoke et al. (2008) clearly demonstrated that the scission of ether linkage occurs without reconversion back to the ether but leaving behind a residual hydroxyl group. However, this led to the *serendipitous discovery* of a new class of azo dye series named phenylazohydroxynaphthalenes. These dyes have similar structures to approved colourants such as sunset yellow and allura red (fig. 18). The dyes were obtained from the reaction of the diazonium ion, 4-carboxyl-2,6-dinitrobenzene diazonium ion (CDNBD) with naphthalene derivative obtained from β-naphthol, α-naphthol (and propranolol), naproxen and nabumetone respectively to produce AZ-01, AZ-02, AZ-03 and AZ-04 (Scheme 6).

Some properties of the Scheme 6 dyes were investigated and reported (Idowu et al. 2007). In particular, there are subtle differences in the hydrophobic properties of the dyes with the proximity effect of the hydroxyl group to the azo linkage in AZ-01, -03 and -04 conferring different migration patterns on TLC compared to AZ-02. The dyes were thereafter used as excellent solvent probes in the assessment of solvatochromic behaviours of solvents of varying polarities by Adegoke and Idowu (2010). Some excellent structure-spectra correlations were afforded by the dyes. The results of the curve fitting coefficients for the solvatochromic assessments enabled the classification of the various interactions of solvents with the dyes and relate the solvatochromic behaviours to the substituent effects on the dye molecules. Charge-transfer complexation occurring between one of the congeners (AZ-02) and N,N`-dimethylformamide was extensively studied and discovered

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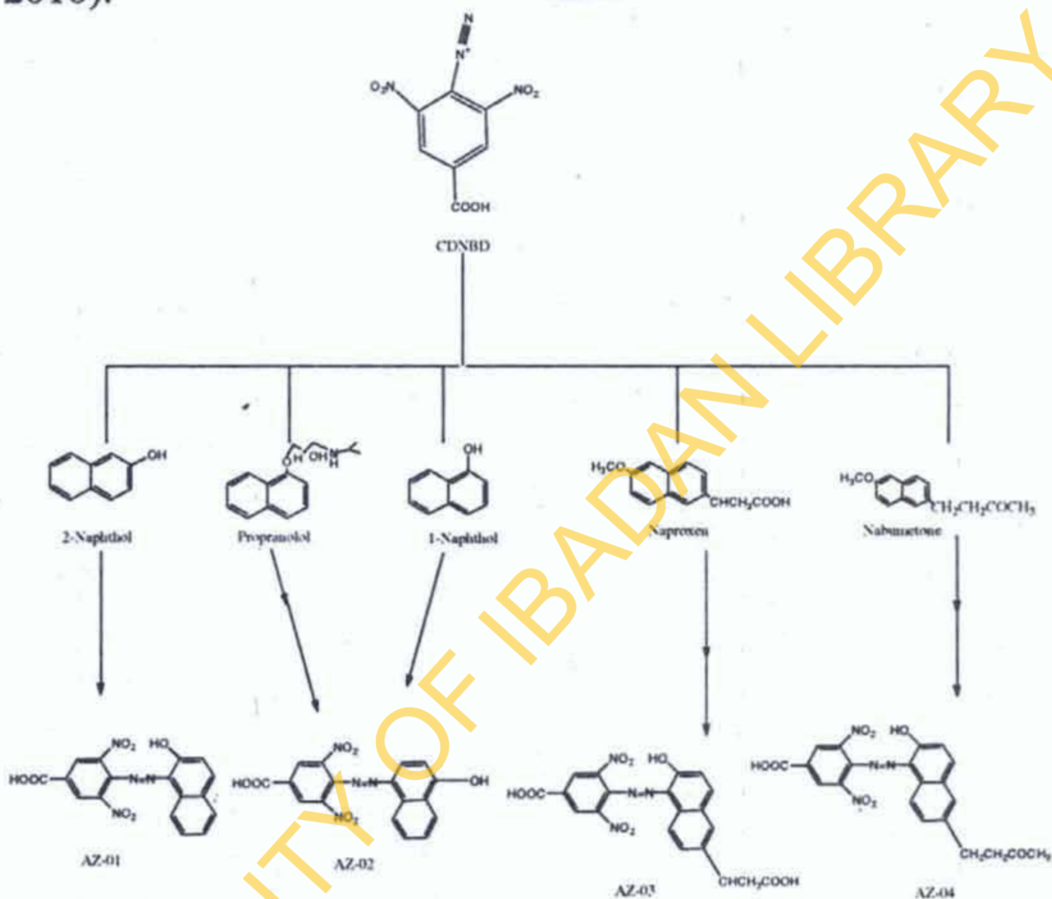
to be both concentration- and temperature-dependent. The electronic character and the chemical nature of the solvents as well as the chemical nature of the other substituents, apart from the common hydroxyl group, are important factors for the observed solvatochromic properties of the 4-carboxyl-2, 6-dinitrophenylazohydroxynaphthalenes (Adegoke and Idowu 2010).



Scheme 6: Synthesis of 4-carboxyl-2,6-dinitrophenylazohydroxynaphthalenes

Azo-hydrazone tautomerism (fig. 19) was found by NMR studies to exist in AZ-01, -03 and -04 (Adegoke et al. 2008). The phenomenon of azo–hydrazone tautomerism in azo dyes and other dye molecules has been studied by several authors. A hydrazone is a tautomer of an azo compound. Since the azo and hydrazone tautomers show different optical and physical properties; their tautomerization has been extensively studied regarding colour and other related industries. The three dyes

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gave two main bands in the 50:50 mixture of DMF with other solvents consisting of a high energy band at 250–382 nm, while the low energy bands for the dyes occurred at 415–485 nm. Spectral shifts in the binary solvent mixtures were related to the solvent dipolarity, basicity of the less polar component relative to DMF, substituent type, molar transition energy, formation constant for the hydrogen-bonding solvated complexes and the standard free energy change for hydrogen bonding with DMF. The relative predominance of the hydrazone tautomer bears a direct relationship to the basicity of the solvent, presence of hydrogen bond donor substituent and was associated with high molar transition energies and low formation constant. The microenvironment surrounding the dye molecules played a major role in the stability of one tautomer relative to the other (Adegoke 2011c).

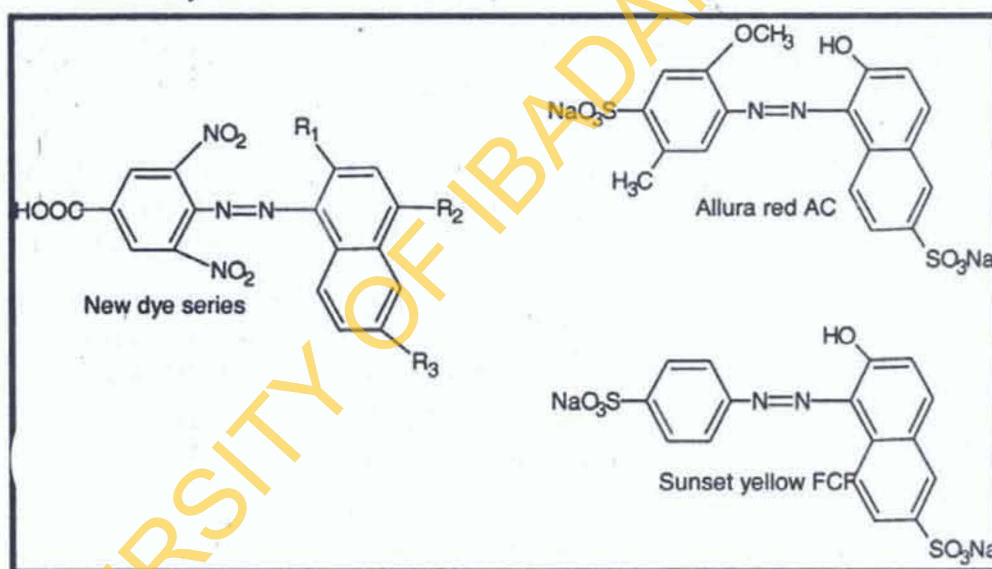

Fig. 18: New dye series {R_1=OH, R_2=R_3=H [AZ-01];
R_2=OH, R_1, R_3=H [AZ-02];
R_1=OH, R_2=H, R_3= $CH(CH_3)COOH$ [AZ-03];
R_1=OH, R_2=H, R_3=($CH_2CH_2COCH_3$) [AZ-04]} alongside allura red and sunset yellow

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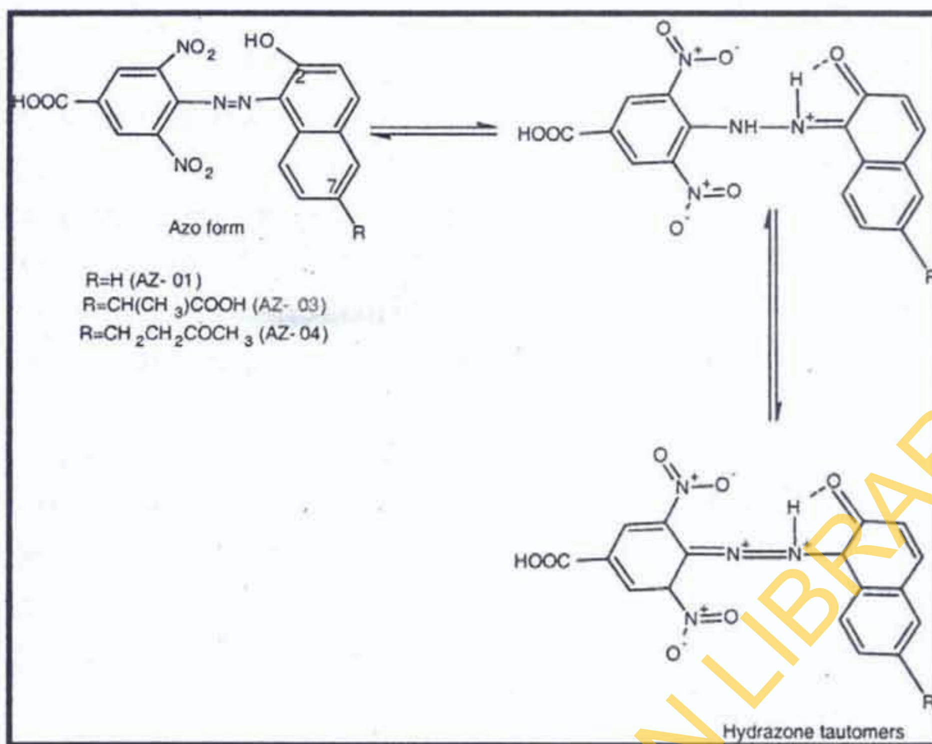

Fig. 19: Azo-hydrazone tautomerism in the three congeners AZ-01, AZ-03 and AZ-04.

The prominent effects of solvated H-bonding complexes were observed for the low energy bands of the dyes in the binary solvent mixture. Table 4 shows the variation of formation constants (K_f) and Gibbs free energy (ΔG) for the dyes in the binary liquid pair. K_f shows a negative correlation with ΔG for AZ-01 (Y = –07896X + 4.66907, R^2 = 0.9942) for non-polar solvents implying that as the formation constant of the solvated complexes increases, the spontaneity of its formation reduces. Similar effects were observed in polar solvents ($Y = -0.3341X + 4.564, R^2 = 0.9345$).

Mr. Vice-Chancellor Sir, antimicrobial properties of incorporated dyes in food, beverages, cosmetics and fabrics have become desirable as such potentials prolong the utility of such products by inhibiting bacterial and fungal growth. Thus, Adegoke et al. (2010c) investigated the antimicrobial properties of these new dyes with a view to providing dual roles as dyes and preservatives. A remarkable contribution to knowledge in this field was the demonstration of the ability of these new investigative dyes to serve as antimicrobial agent against multidrug resistant *Staphylococcus aureus*.

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Table 4: Variation of Low Energy band with K_f, $-\Delta G$ and $E_T(30)$

Binary mixture	AZ-01			AZ-03			AZ-04		
	K_f	$-\Delta G$	$E_T(30)$	K_f	$-\Delta G$	$E_T(30)$	K_f	$-\Delta G$	$E_T(30)$
DMF-CCl_4	1.07	4.62	65.58	7.36	4.14	59.94	2.72	4.39	59.94
DMF-Toluene	0.66	4.75	64.25	2.49	4.41	64.39	1.84	4.49	64.54
DMF-$CHCl_3$	172.64	3.33	61.35	80.4	3.54	68.40	2.19	3.28	58.35
DMF-Acetonitrile	26.16	3.82	-	0	0	68.73	26.01	3.82	59.07
DMF-Acetone	1.09	4.62	64.69	11.6	4.03	64.69	28.80	3.79	64.69
DMF-Ethanol	3.03	4.36	69.73	9.89	4.63	69.06	46.00	3.68	59.07
DMF-Methanol	20.93	3.87	69.91	10.1	4.06	59.44	658.8	3.05	59.07

The next sets of tests that were carried out on the four congeners were aimed at discovering the suitability of these dyes molecules as colourants for food, beverage or cosmetics' if they lack genotoxic effects. Combes and Haveland-Smith (1982) chronicled published data for azo, triphenylmethane and xanthene dyes from short-term assays for muta-carcinogenicity. The extent of agreement between data from different tests and correlations with animal cancer assays were considered. Synthetic dyes from the three major structural classes exhibit genotoxicity, whilst only two natural colours have proved active to induce genotoxicity. Activity may be due to the presence of certain functional groups, notably nitro- and amino-substituents which are metabolized to ultimate electrophiles that may be stabilized by electronic interaction with aryl rings. Metabolic processes, such as azo-reduction, may be activating or detoxifying as shown by reviewed literatures. The studies have emphasized the need to control the level of these dyes in food products (Nevado et al. 1998; Opinion of Scientific panel 2005). Some other recent studies have indicated that some of the approved food colours such as tartrazine and sunset yellow are not mutagenic or clastogenic (Ould Elhkim et al. 2007; Poul et al. 2009; Thomas and Adegoke 2015). In another recent study (Mpountoukas et al. 2010), with amaranth, tartrazine and

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erythrosine, toxicity potentials to human lymphocytes *in vitro* was suggested to be due to direct binding to DNA. Therefore, continuous evaluation of food additives is warranted to ensure safety of the users.

The few azo dyes that have been certified as approved colourants for preparations intended for systemic and topical use are those that have been shown to be sufficiently safe in animal and clinical studies. Based on this background, *in vitro* and *in vivo* genotoxicity assessments were carried out on our four dyes. Distinguished audience, these sets of tests led me deep into the realm of Biology and provided a platform to advance in Chemical Biology. My collaborators at the Laboratory of Genetic Toxicology, University of Calcutta, Kolkata, India, could not believe a Chemist will prepare slides, stain, carry out single-cell gel electrophoresis and use the microscope with utmost proficiency. But I was not surprised as my two-year' stay in Medical Laboratory Technology came on handy. While I have no certificate to show for those rigorous training in routine and special diagnostics procedures in Haematology, Chemical pathology, Blood banking, Parasitology, Microbiology and Histopathology, the skills acquired are still part of me till today. So I urge parents not to despise the few essential skills your wards can gain while searching for their dream course of study in a University.

The *in vitro* genotoxicity of the new monoazo dye series was evaluated using human lymphocytes by alkaline comet assay. Cytotoxicity tests using trypan blue dye exclusion method were also carried out (Adegoke et al. 2012b). The viability test showed greater than 80% in all cases ensuring validity of the assay procedure. Varied results were obtained following exposure of the human lymphocytes to the five concentration levels of AZ-01, -02, -03 and -04. The influence of the dye concentrations on and tail extent moment (TEM) and olive tail moment (OTM) are presented in table 5 relative to the negative control.

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Table 5: Variation of Tail Extent Moment (TEM) and Olive Tail Moment with Concentrations of the Monoazo Dyes

Dye conc. (µM)	Tail Extent Moment [TEM] (± S.D.)				Olive Tail Moment [OTM](± S.D.)			
	AZ-01	AZ-02	AZ-03	AZ-04	AZ-01	AZ-02	AZ-03	AZ-04
0	0.03 ±0.01	0.04± 0.01	0.02±0.01	0.04 ±0.01	0.06 ±0.01	0.06 ±0.01	0.06 ±0.01	0.06 ±0.01
31.25	1.44±1.18	0.15 ±0.02	0.19 ±0.28	0.01 ±0.01	0.73± 0.61	0.16 ±0.01	0.14 ±0.13	0.04 ±0.02
62.5	1.51 ±0.55	0.29 ±0.10	0.05± 0.03	0.01 ±0.01	0.67 ±0.15	0.24± 0.01	0.07 ±0.02	0.03 ±0.02
125	2.19 ±0.74*	0.41 ±0.29	0.09 ±0.05	0.05 ±0.07	1.14 ± 0.26*	0.31 ±0.16	0.09 ±0.05	0.11 ±0.10
250	5.62 ±0.54*	1.18 ±0.26*	0.01 ±0.0	0.07 ±0.05	2.83± 0.32*	0.52± 0.09*	0.05 ±0.01	0.12 ±0.04
500	5.90 ±1.36*	1.06 ±0.73*	0.06 ±0.023	0.07± 0.03	2.91± 0.32*	0.52 ±0.31*	0.08 ±0.02	0.13 ±0.03

**Significant at $p = 0.05$*, value for MMS for TEM (100 µM) = 2.77±0.34; value for MMS for OTM (100 µM) = 16.11±3.93

The effect of the dyes on % tail DNA following Comet assay is presented in figure 20. In general, two of the congeners (AZ-01 and AZ-02) which are positional isomers in which the common OH group is *ortho* and *para* to the azo linkage gave differing results in terms of the three parameters of DNA damage studied-% tail DNA, OTM and TEM.

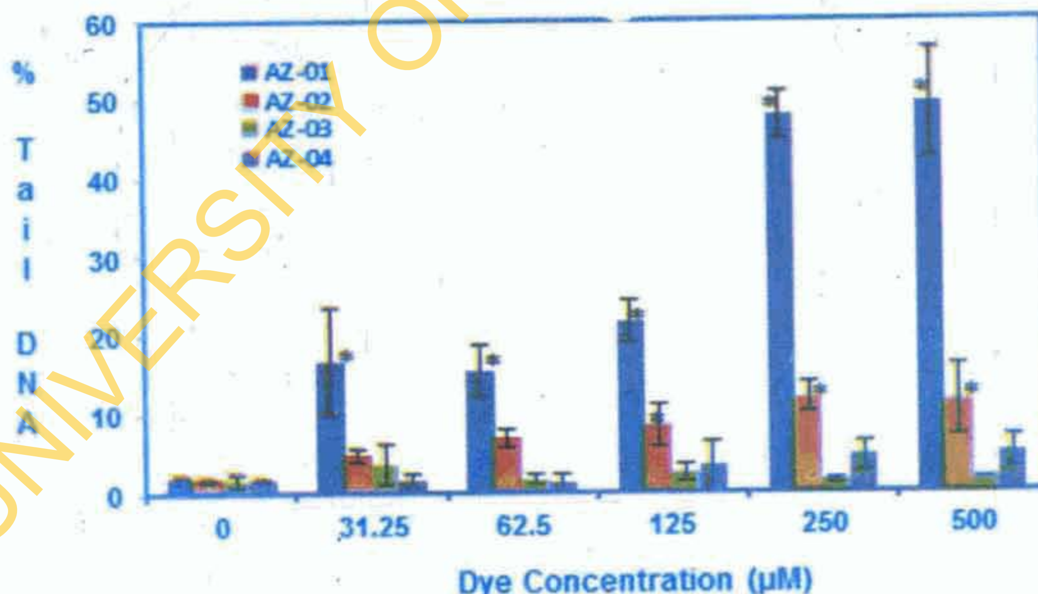

Fig. 20: Effects of AZ-01 – AZ-04 on % Tail DNA (* significant at p = 0.05), [a] Value for MMS (100µM = 45.63 ±8.529).

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The dye AZ-01 produced a concentration-dependent increase in DNA damage at all concentration levels with results obtained for the % tail DNA being statistically significant. The influence of structure on the biological properties of the test dyes became evident with the results obtained for AZ-02. This congener did not produce any statistically significant DNA damage at concentrations of 31.25 and 62.5 μM with respect to % tail DNA and at concentrations of 31.25, 62.5 and 125 μM for TEM ($p > 0.05$). The results obtained for AZ-03 and AZ-04 showed a marked departure from that of AZ-01 and AZ-02. At all the five concentration levels, the two dyes (AZ-03 and -04) did not produce any cellular damage. The biological properties of these four dyes bear a good relationship with their structure. AZ-01 and AZ-02 are obtained from the diazo coupling reaction of β- and α- naphthols with CDNBD respectively. The naphthols and phenols having terminal hydroxyl groups could readily ionize. The ionization may in some instances generate reactive oxygen species (·OH) which is known to be genotoxic (Bandyopadhyay et al. 1999).

However, both β- and α- naphthols have not been proven to be either mutagenic or genotoxic *in vitro* and *in vivo* though their presence in the environment, consumer goods and cosmetics are limited by the European Union (SCCNFP 2001; OECD SIDS 2006). Intracellular hydrolysis of azo dyes has been reported to increase the toxicity of azo dyes (Gottlieb et al. 2003). Thus, if AZ-01 gets hydrolyzed or reduced within the cells, any of the observed DNA damage may have occurred in addition to the presence of the hydroxyl group. AZ-02, from our previous study (Adegoke and Idowu 2010), was found to have a high polarity and it readily ionizes to donate protons to the environment. Thus, the lack of toxicity at lower concentrations may be due to the existence of the dyes in the ionic form which expectedly limits intracellular crossing except if it is coupled to an ion channel in an active transport process.

The other two congeners (AZ-03 and AZ-04) did not produce any statistically significant difference in DNA damage relative to the negative control at all the 5-

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concentration levels adopted in this study. These dyes, in addition to the common hydroxyl substituent, contain at the 7th positions the propionic (AZ-03) and butanone (AZ-04) substituents. Both dyes also exist in the hydrazone tautomer similar to AZ-01. However, presence of the additional C-7 substituents appears to have completely modified their toxicity. In earlier developments of sourcing for dyes which contain skeletons different from the mutagenic and genotoxic benzidine molecules, it was discovered that the presence of ring substituents in the position *ortho* to the amino ($-NH_2$) group of the precursor amine reduces mutagenicity (Gong et al. 2002). These studies have also led to non-mutagenic benzidine analogs containing bulky alkoxyl groups *ortho* to the amino group and amino derivatives of the dihydrophenophosphazine ring systems (Bello et al. 2000). The implication of these differences in structure is that processes that will be responsible for the breakdown of the dyes to toxic end-products will be slowed down or completely eliminated with the consequence that the dyes become non-toxic. The results of the comet assays are presented in figure 21 for the lowest and highest dose.

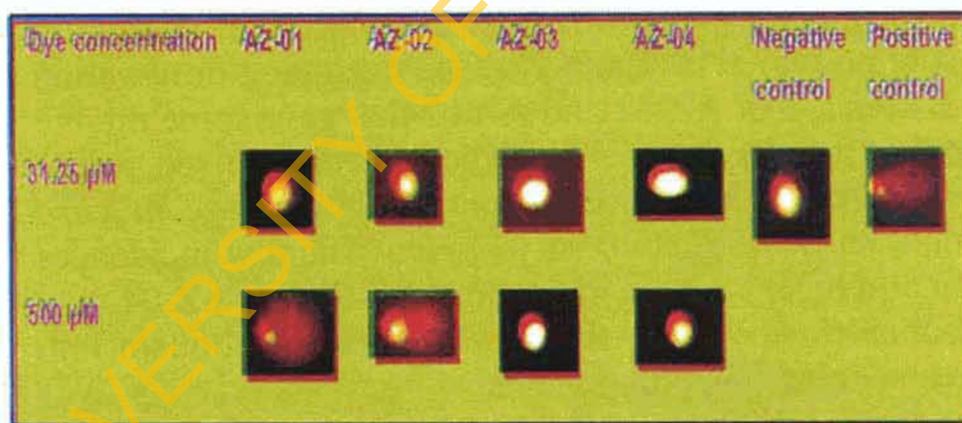

Fig. 21: Comet assay results with the four monoazo dyes using human lymphocytes at the two extreme doses

Mr. Vice-Chancellor Sir, following these results obtained in the *in vitro* assessment of the genotoxicity of these title dyes, a short-term *in vivo* genotoxicity evaluation was carried out in mice. Aqueous colloidal solutions of the dyes were administered to mice on each day for 5 successive days using

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gastric gavages. Two end-point assessments of the genotoxicity potentials of the dyes were assessed; comet assay and chromosomal aberration studies (Adegoke et al. 2014b). The dyes were well tolerated at the doses investigated, as there were no deaths or any adverse pharmacotoxic events. Dose-dependent DNA damage (in terms of percentage of tail DNA and Olive tail moment) occurred with AZ-01 and AZ-02, although the effects were significant only with the highest doses. AZ-03 gave similar patterns with those of AZ-01 and AZ-02, while replacement with butanone in AZ-04 altered the observed pattern. Minimal chromosomal damages were obtained for the four dyes, with AZ-01 and AZ-02 giving non-significant damages, while the highest dose of AZ-03 produced significant aberrations in terms of breaks. The results of the chromosomal aberration studies are presented in tables 6 and 7 for the four dyes. Some minor isochromatid breaks and gaps were also noticed in the dye-treated mice. Mitotic indices in all cases were not significantly different from concomitantly administered vehicle control showing lack of cytotoxicity of the monoazo dyes at these doses.

Representative plates for the chromosomal aberration studies are presented in figure 22. One intriguing observation with the dyes is that AZ-01 and AZ-02 that gave some minute genotoxicity results *in vitro* had the effects reversed, while AZ-03 and AZ-04 that produced no DNA damage *in vitro* gave some levels of genotoxicity *in vivo*. The few incidences of chromatid breaks observed for both AZ-03 and AZ-04 may be accounted for by the possibility of their metabolism into the precursor coupling components.

The dyes, AZ-03 and AZ-04 were obtained from potent non-steroidal anti-inflammatory drugs naproxen and nabumetone, respectively (Adegoke et al. 2008). If these precursors are produced when AZ-03 and AZ-04 are metabolized, then some level of DNA damage or CAs may be anticipated as most drugs are known to be genotoxic at some doses. In order to further understand the kind of damage that

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was taking place and possibly the mechanisms responsible, *in vitro* mechanistic-based binding studies of the dyes with calf-thymus (CT) double-strand DNA (ds-DNA) and bovine serum albumin (BSA) were carried out to provide a clue to the mode of interactions of the new dyes with macromolecules.

Table 6: Chromosome Aberrations Induced byAZ-01 and AZ-02 *in vivo* in Bone Marrow of Mice

Treatment (mg/kg body weight)	Total Chromosome aberrations[a]					CA/cell[b]	% DC[c]	MI[d]
	G′	G″	B′	B″	RR	Mean±S.D.	Mean±S.D.	Mean±S.D.
Negative Control[e]	6	4	2	-	-	0.006± 0.01	0.67± 0.01	3.32±0.64
AZ-01								
2.0	4	6	4	-	-	0.013±0.01	1.33±0.94	3.95±0.22
4.0	10	4	10	2	-	0.04±0.03	3.33±1.89	3.43±0.26
20.0	8	8	10	-	-	0.033±0.01	3.33±0.94	3.48±0.19
AZ-02								
2.0	8	4	8	-	-	0.027±0.01	2.33±1.25	3.90±0.14
4.0	7	6	11	1	-	0.036±0.01	3.34±0.94	3.19±0.26
20.0	6	4	12	1	-	0.04±0.02	4.34±2.49	3.68±0.47
Mitomycin C								
1.0	40	14	54	7	-	0.203±0.64*	12.67±6.53*	1.59±0.37*

Table 7: Chromosome Aberrations Induced byAZ-03 and AZ-04 *in vivo* in Bone Marrow of Mice

Treatment (mg/kg body weight)	Total Chromosome aberrations[a]					CA/cell[b]	% DC[c]	MI[d]
	G′	G″	B′	B″	RR	Mean±S.D.	Mean±S.D.	Mean±S.D.
Negative Control[e]	4	2	4	-	-	0.013± 0.01	1.33± 1.16	3.47±0.28
AZ-03								
2.25	6	4	6	2	-	0.027±0.012	2.67±1.16	4.11±0.46
4.50	4	4	12	4	-	0.053±0.012	5.34±1.16	3.42±0.32
22.5	20	8	22	8	1	0.103±0.01*	9.33±4.16*	3.54±0.26
AZ-04								
2.25	6	2	6	-	-	0.020±0.01	2.00±1.63	3.66±0.25
4.50	10	4	6	2	-	0.027±0.01	2.67±1.16	3.84±0.34
22.5	10	2	18	-	-	0.05±0.015	4.67±1.16	3.51±0.34
Mitomycin C								
2.0	40	16	46	16	2	0.213±0.08*	20.00±6.80*	2.76±0.57*

Legends for Tables 4 & 5: G′, G″: chromatid gap and isochromatid gaps; B′, B″: chromatid breaks and isochromatid breaks; RR: chromatid rearrangements. [a] 100 metaphase cells/animal (3 animals/dose). [b] Number of CAs/cell excluding gaps.

[c] % DC: percentage of damaged metaphase cells with at least one CA (excluding gaps). [d] Mitotic Index (%).

[e] Distilled water (negative control). * Significantly different from concurrent control data at $p \leq 0.05$

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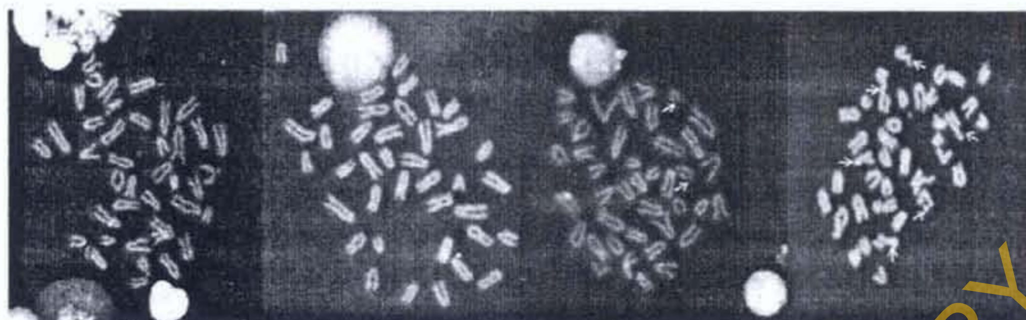

Fig. 22: Representative plates for chromosomal aberration studies (negative control; dye treatments and positive control; arrows show aberrations).

For the DNA binding studies, the modes of interaction of the four congeneric monoazo dyes, with CT ds-DNA in aqueous medium was investigated spectrophotometrically. Characteristic spectral changes observed at two temperatures (293 and 310 K) were assigned to either change in absorption intensity or change in wavelengths. The changes bear a direct relationship with the structures of the dyes. All the dyes gave good binding constants with DNA. Varying stoichiometric ratios were established between the dyes and DNA. Thermodynamic considerations enabled the delineation of the binding modes to be hydrogen bonding (AZ-01 and -03), electrostatic interactions (AZ-03) and hydrophobic bonding (AZ-04). Correlation of the formation constant with Massieu–Planck's constant established the hydrophobic interaction between DNA and AZ-04 (Adegoke et al. 2012c). It was observed that the binding of AZ-01 and AZ-02 with DNA involves a negative ΔG, $\Delta H<0$ and $\Delta S<0$, while for AZ-03 negative ΔG, $\Delta H<0$ and $\Delta S>0$ were observed. For AZ-04, negative ΔG, $\Delta H>0$ and $\Delta S>0$ were observed. The binding thermodynamics, as observed by Ahmadi et al (2009), reflect a subtle balance between the hydrogen, Van der Waals or multiple bonds and entropic effects. The change in entropy is governed by the release of counter ions and water from DNA and the dye molecules. For transfer of small molecules from polar to non-polar environments, hydrophobic interactions usually give $\Delta H>0$and $\Delta S>0$ with negative ΔG (Bekker and

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Norden 1997). For the interaction with bovine serum albumin, the focus was on the biophysical interactions of these monoazo dyes with bovine serum albumin (BSA) that play an important role in drug transport and storage in vertebrates. The molecular interactions of the monoazo dyes with BSA were conducted by spectroscopic means in order to gain in-sight into the intermolecular binding forces responsible for the observed biological profile of the dyes. Adegoke et al (2012d) observed trends in the spectra of unbound and bound monoazo dyes (tables 8 and 9) which suggest that the dye molecules are interacting with BSA through a wide range of mechanisms and for which the structures of the dyes played key roles in determining the observed patterns.

Table 8: High Energy Electronic Absorption Spectral Data of the Complexes Formed by the Monoazo Dyes with Varying Ratios of BSA

Dye-BSA Complex	λ_{max}, nm ($\log \varepsilon_{max}$)					
	BSA mole ratio					
	0	1.25	2.5	5.0	10.0	20.0
AZ 01-BSA	240 (3.99) 288 (3.80)*	274 (3.67)	274 (3.46)	238 (4.20) 276 (3.84)	278 (4.01)	274 (4.20)
AZ 02-BSA	242 (3.75) 270 (3.66)	240 (3.89) 276 (3.97)	242 (4.06) 272 (3.85)	240 (4.16) 280 (3.97)	238 (4.31) 280 (4.07)	238 (4.50) 280 (4.26)
AZ 03-BSA	242 (4.02) 296 (3.81)	240 (4.21) 270 (3.92)* 290 (3.88)*	240 (4.21) 296 (3.92)	240 (4.21) 278 (4.01)	240 (4.37) 280 (4.15)	240 (4.51) 278 (4.29)
AZ 04 BSA	242 (4.04) 292 (3.86)*	240 (4.07) 278 (2.57)	240 (4.04) 280 (3.50)	240 (3.99) 280 (3.77)	240 (4.18) 280 (3.94)	238 (4.41) 280 (4.15)

**shoulders at this wavelength*

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Table 9: Electronic Absorption Spectral Data for the Low Energy Transition of the Complexes Formed between the Monoazo Dyes and Varying Ratios of BSA

Dye-BSA Complex	λ_{max}, nm ($\log \varepsilon_{max}$)					
	BSA mole ratio					
	0	1.25	2.5	5.0	10.0	20.0
AZ 01-BSA	482 (3.89)	480 (3.61)	480 (3.68)	480 (3.63)	482 (3.44)	480 (3.21)
AZ 02-BSA	464 (3.34)	468 (3.38) 600 (2.96)	468 (3.45) 600 (2.84)	466 (3.53) 600 (2.96)	466 (3.57) 468 (3.74)	468 (3.74) 600 (3.29)
AZ 03-BSA	486 (4.90)	486 (3.91)	488 (3.93)	488 (3.93)	488 (4.01)	498 (4.03)
AZ 04 BSA	490 (3.91)	494 (3.20)	494 (3.17)	494 (3.35)	494 (3.35)	494 (3.29)

One congener (AZ-02) gave a minor peak characteristic of charge-transfer complexation. The binding constants of the four monoazo dyes were estimated and found to vary according to the dye structure and temperature of investigation. AZ-01 and -04 combined with BSA at approximately 1:1 mole ratio, while the other two congeners with additional proton donors gave greater than this mole ratio. Thermodynamic considerations established that the dyes utilized the various forms of binding modes; hydrogen bonding, hydrophobic bonding, van der Waals and AZ-03 was particularly involved in electrostatic interactions giving positive entropy change for a small enthalpy change.

Mr. Vice-Chancellor Sir, one structural defect observed with the new azo dye series is lack of water solubilizing agents. I proposed two approaches: use of sulphonated dye intermediates as coupling components for CDNBD and diazotization of amino-sulphonated intermediates prior to diazo coupling reaction with activated skeletons. The first approach yielded beautifully coloured azo dyes, but isolation and yield from the acidic media became a tough issue to handle. However, the second approach has produced about ten (10) novel azo dyes for which preliminary studies demonstrated their suitability as colourants and indicators of acid-base equilibria. Spectroscopic characterizations have yielded the structures of the novel azo dyes. One of my Ph.D students and a colleague in the Department, Pharm. Segun Thomas, is presently in Kolkata, India not just for the fun of

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enjoying Bengali cuisines, but primarily to establish the genotoxicity potentials or otherwise of these new dye entities.

New Reagents, Emerging Applications

Mr. Vice-Chancellor Sir, one new emerging application that has fallen within our trawl line is the development and applications of new molecules as sensitive and selective colorimetric chemosensors. Colorimetric sensors have become very popular in recent years due to their capability to detect and, in some instances, to semi-quantitate analyte by naked eye detection without resorting to expensive instrumentation (Zhang et al. 2006). The colorimetric chemosensors are therefore considered as one of the most effective analytical methods for environmental monitoring, particularly detection of major cationic and anionic species whose presence in the environment have deleterious consequences. Chemosensors include integrated chemosensors, conjugated chemosensors and colorimetric chemosensors.

Colorimetric chemosensors are designed in such a way that the receptor and signaling units are either fused together into one unit or connected by some unsaturated groups. Generally, electron donor-acceptor moieties connected by π-conjugation are chosen as suitable system for this purpose. Recognition of analyte by the chemosensor is expected to affect the conjugation between electron-donor and acceptor segment of the molecule substantially and thereby give rise to drastic colour changes which can be recognized.

Three of the four investigative monoazo dyes, possess the azo-hydrazone tautomerism. This property makes the molecules capable of serving as colorimetric chemosensors provided we can find environmentally important inorganic species that can carry out the intramolecular tautomeric switch and produce a stable colour. The first inorganic species candidate we were able to detect to carry out this conversion of the azo dyes was cyanide. The dyes were therefore evaluated as a highly selective colorimetric chemosensor for cyanide ion. Adegoke et al. (2014c) discovered that presence of cyanide ion gave an obvious colour change to produce purple or liliac colour in acetone.

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Under the optimum conditions, linear relationships between the CN^- concentrations and light absorption were established. Using these azo-hydrazone molecular switch entities, excellent selectivity towards the detection of CN^- in aqueous solution over miscellaneous competitive anions was observed. Such selectivity mainly results from the possibility of exclusive nucleophilic attack on the azo-hydrazone chemosensors by cyanide anions in aqueous system, not afforded by other competing anions (fig. 23). The nucleophilic addition product was supported by the extension of chromophore and the brilliant colour produced. If proton abstraction had taken place, the colour will not be intense as observed.

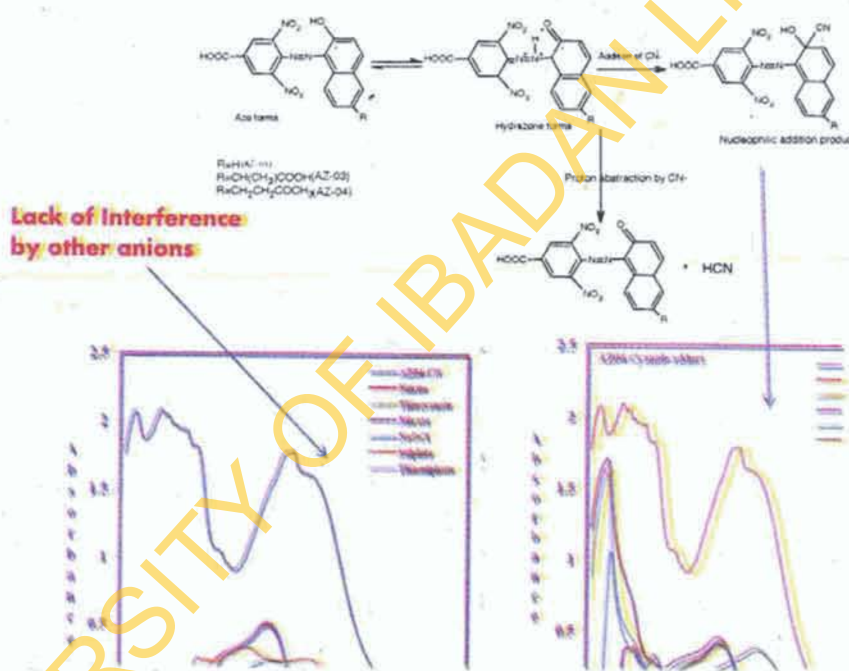

Fig. 23: Colorimetric chemosensor determination of cyanide with 4-carboxyl-2,6 –dinitrophenylazohydroxynaphthalenes and lack of interference by other anions.

Heavy metals are known to pose serious environmental and health hazards when present at levels exceeding the normal limits set by the World Health Organization. In

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addition, heavy metals are known to catalyze the decomposition of pharmaceuticals, cosmetics and beverages. The current effort we are making is to develop specific azo and azomethine moieties for the rapid colorimetric chemosensing of these heavy metals. The current design (fig. 24) is focused on highly functionalized tricyclic skeletons with the azo and azomethine linkages providing conjugated skeletons for colour formation following binding of the metal ions. It is hoped that novel molecules will be reported from these investigations soon.

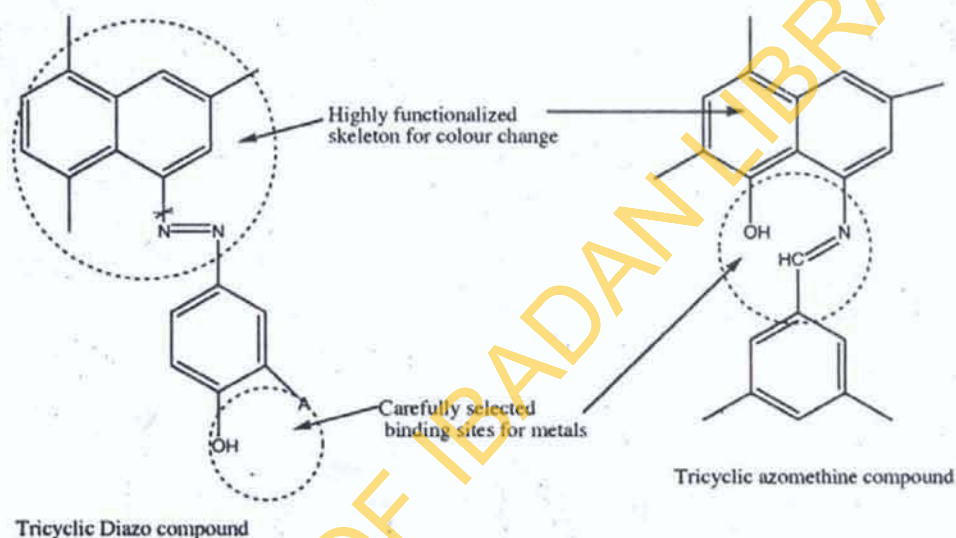

Fig. 24: Novel azo and azomethine tricyclic skeletons as colorimetric chemosensors for metals.

New Chemical Entities, Improved Properties

Mr. Vice-Chancellor Sir, distinguished audience, Schiff bases, known to be good chelating agents, are easily prepared and characterized. Little interest has been given to their uses for analytical purposes because of two serious drawbacks; they are insoluble in aqueous solutions and they decompose easily in acidic solutions, limiting their use to basic conditions. In synthetizing Schiff bases, two conditions are critical: the pH of the medium and steric and electronic effects of the reactants.

We sought to design novel Schiff bases that will be soluble in water and at the same time provide analytical platforms for the detection of some environmentally

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important inorganic cations and anions, while at the same time serving as excellent solvent probes. We made a great success designing, synthetising and characterising nine novel Schiff bases (fg. 25). This research was sponsored by the last ever Senate Research grant (2010) of the University of Ibadan. Well, I am still grateful at least for having my University sponsoring some nine-made-in-University-of-Ibadan Novel Schiff bases. We went further to thoroughly investigate one of the molecules, and we found it to be an excellent solvent probe that could distinguish between ethanol and methanol, and also serve as a highly selective colorimetric chemosensor for nitrite in dried meat and cat fishes prepared by smoking.

Mr. Vice-Chancellor Sir, distinguished audience, you might be wondering why I have deliberately refused to divulge the structures of these new molecules; the document is ready to be submitted for a patent!

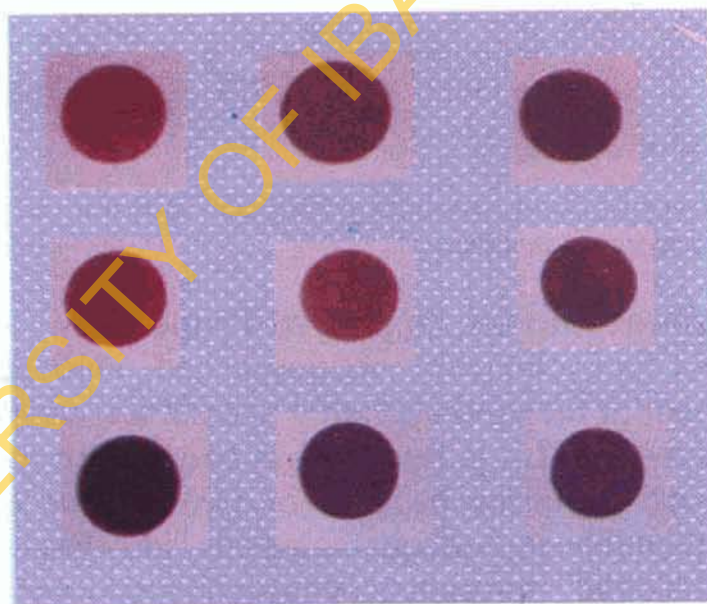

Fig. 25: KBr disks of the nine Novel Schiff bases with improved properties.

Colours Arising from Storage of Pharmaceutical Products

One of the professional duties of a Pharmacist is to provide medicines in a state of excellent physical, biopharmaceutical,

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microbiological and chemical status within the shelf life of the product. In many instances, a drug or its product is regarded as being stable if it maintains its integrity in terms of the aforementioned properties. Instability in pharmaceuticals manifest as caking of suspension, cracking of emulsions, mottling and colour separation of coloured tablets, excessive microbial contamination among other properties. Modern-day formulation efforts are aimed at providing optimal conditions for the stability of pharmaceuticals. Some major factors that contribute to instability of pharmaceutical products are chemistry of the drugs and excipients, light, humidity and oxygen. Two of the prominent factors - light and humidity - are extremely abundant in the tropical climate where Nigeria belongs. In the long run, instability leads to decomposition, and impurities may arise from decomposition or get carried through from raw materials.

My particular interest in impurities profiling stems from the colours produced during improper storage of pharmaceutical products, and this had led me to get involved in impurity profiling of marketed pharmaceutical products in Nigeria. I have adopted a combination of chromatographic and once again azo dye derivatization in the profiling of impurities in pharmaceutical products. The first procedure reported in this regard is a simple, precise and robust reversed phase liquid chromatographic (LC) method which was developed and validated for the quantitative determination of griseofulvin (GF) and its impurities in drug substances and tablet dosage forms (Kahsay, Adegoke et al. 2013). Robustness study was performed by means of an experimental design and multivariate analysis using Modde 5.0 software (Umetrics, Umeå, Sweden). Satisfactory results were obtained from the validation studies. The use of volatile mobile phases allowed for the identification of three main impurities present above the identification threshold using tandem mass spectrometry (MS). The chromatographic conditions were selected to accomplish optimal separation between griseofulvin its major impurities (fig. 26), and various parameters that could influence resolution of peaks were optimized (fig. 27).

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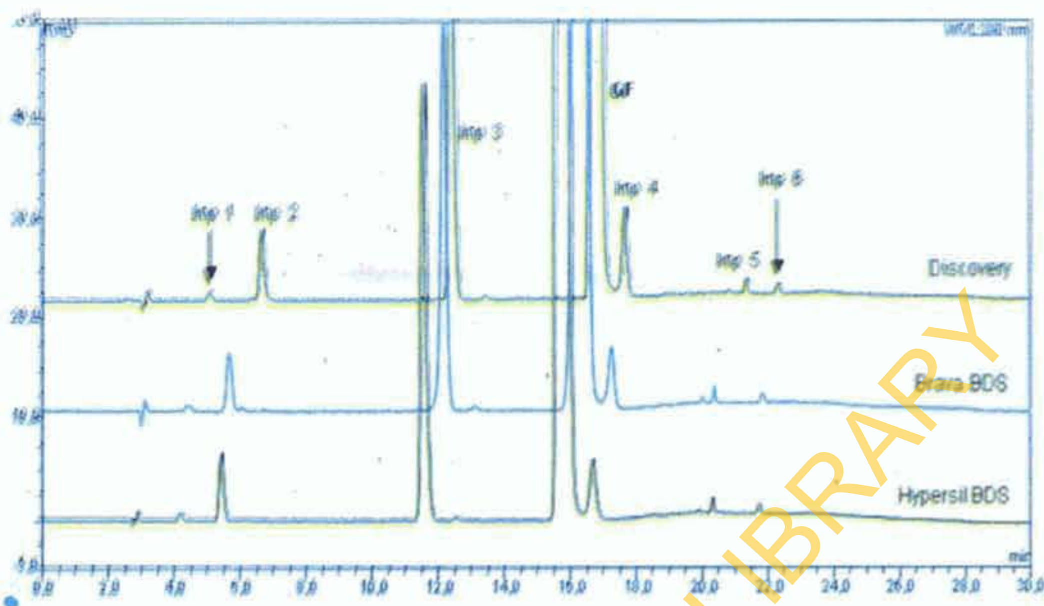
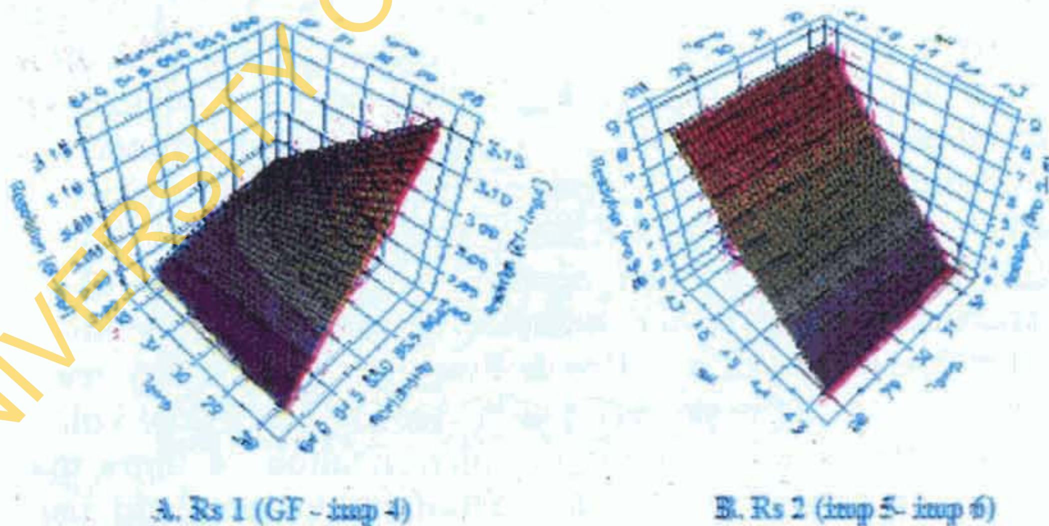


Fig. 26: Chromatograms obtained from the three C18 (250 mm x 4.6 mm, 5 µm) columns showing overall similar separations of GF and the impurities from a 0.5 mg/ml solution of griseofulvin drug substance using the final method. Chromatographic conditions: mobile phase A (Water – 0.1 % formic acid pH 4.5, 80:20 v/v) and B (ACN – water – 0.1 % formic acid pH 4.5, 65:15:20 v/v/v), flow rate 1 ml/min, column temperature 30 °C, injection volume 10 µl and UV detection at 290 nm. Imp: impurity and GF: Griseofulvin.



A. Rs 1 (GF - imp 4) B. Rs 2 (imp 5- imp 6)

Fig. 27: Response surface plots showing the influence of temperature and acetonitrile on Rs 1 (A) and pH and temperature on Rs 2 (B) for the determination of griseofulvin (GF) and impurities. The other parameters are kept constant at their central values.

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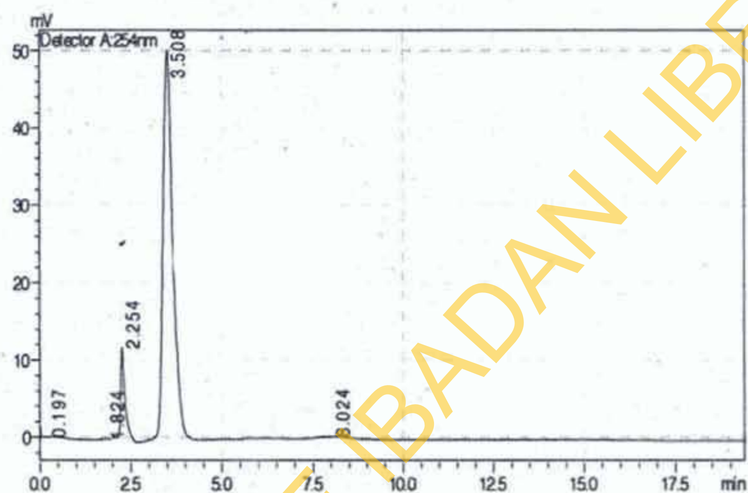
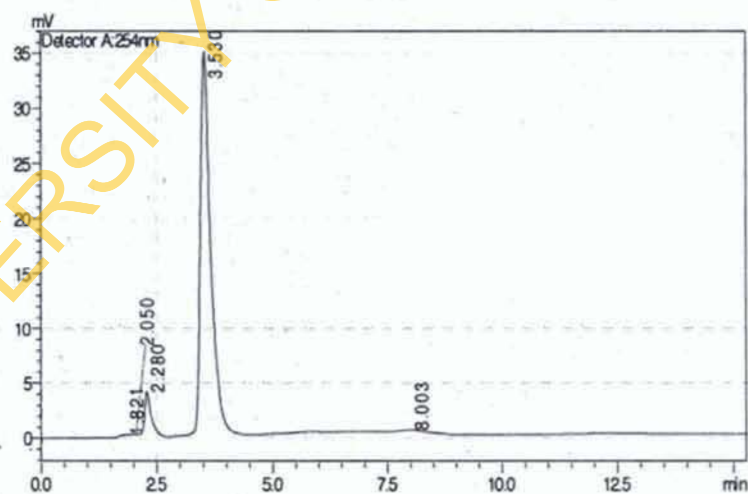
The strategy adopted in another study was to commence a comprehensive impurities profiling of some cephalosporins beginning with ceftriaxone. One expired brand and one brand still within its shelf life were investigated. The methodology consisted of a modification of the British Pharmacopoeia 2007 method (Adegoke 2013). The observed chromatograms are presented in figure 28. In the typical chromatograms, the relative area % of the main impurity in the old brand was 9.9 % relative to 7.0 % in the new brand.



Old brand of ceftriaxone



New brand of ceftriaxone

Fig. 28: Impurities profiling of ceftriaxone brands using isocratic high performance liquid chromatography.

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Mr. Vice-Chancellor Sir, one drug that has found regular use is paracetamol (PCM), which is a commonly used analgesic consumed by an average Nigerian daily. But the drug is subject to the presence of impurities, the major impurity is toxic to the body. *Para*-aminophenol (PAP) is a degradation product of paracetamol upon hydrolysis of the acetamido group to an amino. *Para*-aminophenol is the first intermediate in the industrial synthesis of paracetamol and a degradation product of paracetamol; thus, the main impurity in paracetamol. PAP is a metabolite of paracetamol and, like paracetamol, also induces hepatotoxicity. It is also nephrotoxic, being five times more potent than paracetamol (Harmon 2006). PAP could be carried over into paracetamol tablets as starting material or intermediate impurity; it could also be present as a degradation product. Therefore, its identification, quantification and control are an important part of drug quality assurance for PCM.

The presence of PAP gives a brownish tinge to paracetamol tablets. Recently, we designed a procedure which involves the diazotization of PAP and coupling with chromotropic acid. This project was executed by Agboola (2015). The method was found to be highly selective and sensitive and we were able to detect up to 0.14% PAP in commercial PCM tablets hawked in Bodija market of Ibadan metropolis. This level was found higher than recommended by the Pharmacopoeias (0.1%) pointing to the need for adequate regulation of drug distribution. The reaction pathway and the UV-Vis spectra produced for the reaction of PAP with chromotropic acid are presented in figure 29.

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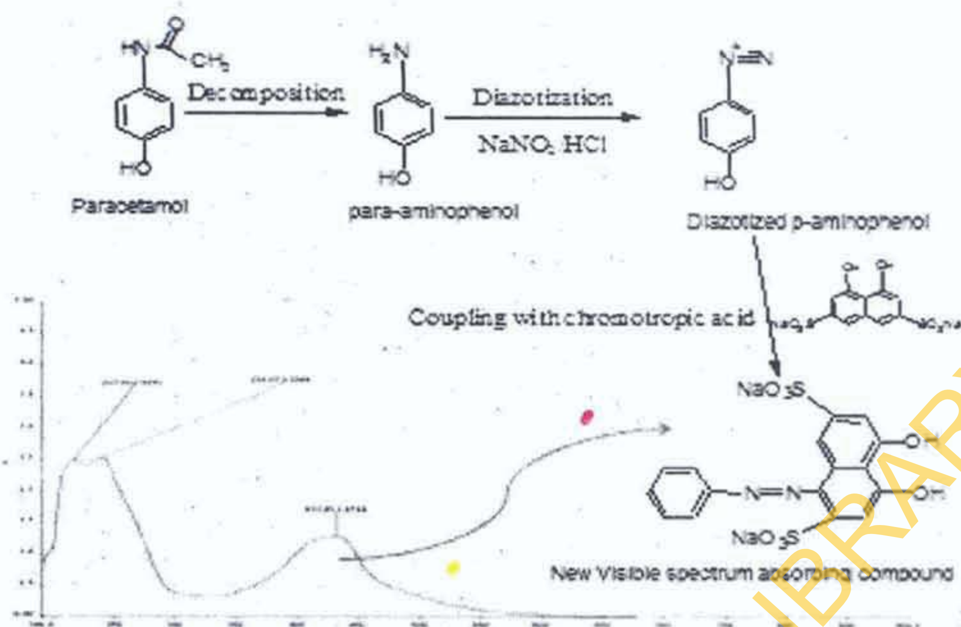

Fig. 29: Colorimetric micro-determination of PAP in Paracetamol

Collaborative Nanoparticle Research

Mr. Vice-Chancellor Sir, my interest in Nanoparticles Research emanated from two sides - the applications of nanoparticles in colours and the ecotoxicological profiles of these new compounds. Nanotechnology and its applications have gained momentum over the past decade. Possessing unique electro-mechanical and thermal properties, carbon nanotubes (CNT) and other nanoparticles have numerous applications in industrial and biomedical sectors. With my collaborators at the Laboratory of Genetic Toxicology, University of Calcutta, India, we are currently investigating cytotoxicity, genotoxicity and *in vitro* mechanistic-based binding studies of various nanoparticles using several tests and procedures.

Multi-Walled CNT (MWCNT) was examined in one of our studies (Ghosh et al. 2015). Over the past decade, a number of studies have investigated MWCNT-induced toxicity and potential carcinogenesis in mammalian systems. However, only limited information regarding the toxicity of MWCNT is available in the plant system. With few exceptions, most of these studies have addressed the effect on

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physiological parameters and germination rates. The study on *Allium cepa* root tips was designed to understand the toxic potential of MWCNT in the plant system with a major emphasis on cytotoxicity, genotoxicity, oxidative stress and global DNA methylation. Multiple cytotoxic (membrane integrity, mitochondrial dehydrogenase activity, mitochondrial membrane potential) and genotoxic (chromosome aberration, micronucleus formation, comet assay, DNA laddering and RAPD analysis) endpoints were evaluated to achieve a better understanding of MWCNT-induced toxicity in the plant system. The effects of MWCNT on oxidative stress and cell cycle progression were also evaluated. The results (figs. 30 and 31) revealed that MWCNT uptake in root cells significantly altered cellular morphology. Membrane integrity and mitochondrial function were also compromised. The nanotubes induced significant DNA damage, micronucleus formation and chromosome aberrations. DNA laddering assay revealed the formation of internucleosomal fragments, which is indicative of apoptotic cell death. This finding was confirmed by an accumulation of cells in the sub-G0 phase of the cell cycle. An increase in CpG methylation was observed using the isoschizomers MspI/HpaII. HPLC analysis of DNA samples revealed a significant increase in the levels of 5-methyl-deoxy-cytidine (5mdC). These results confirm the cyto-genotoxic effect of MWCNT in the plant system and simultaneously highlight the importance of this epigenetic study in nanoparticle toxicity.

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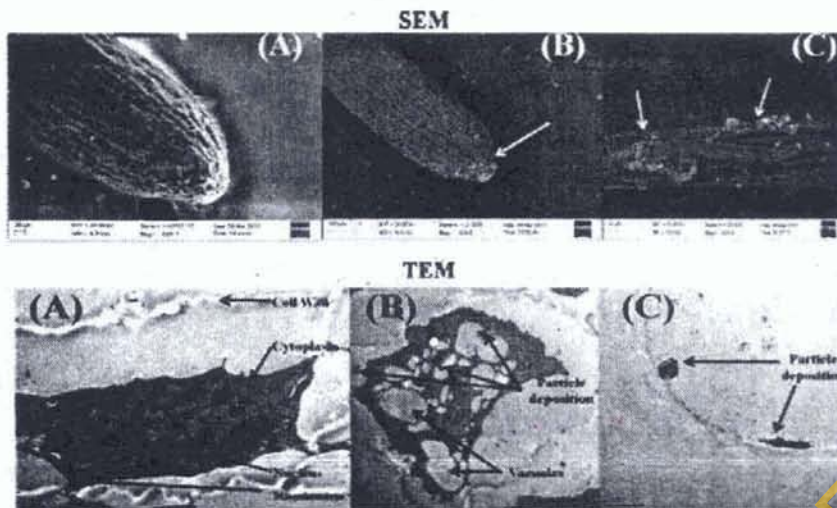

Fig. 30: SEM: Surface adhesion of MWCNT on *Allium cepa* root; (A) control roots showing the absence of particle adhesion to the surface, (B and C) showing MWCNT adhered to the root surface; TEM: TEM images of ultrathin sections of plant cells showing an effect of MWCNT treatment; (A) *A. cepa* root cells in the absence of treatment with normal cellular organization, well defined nuclear and organellar structures, (B and C) MWCNT treated *A. cepa* root cells with extensive vacuolation, loss of nuclear organization, ruptured plasma membrane and shrinkage of the protoplast, (C) showing particle deposition.

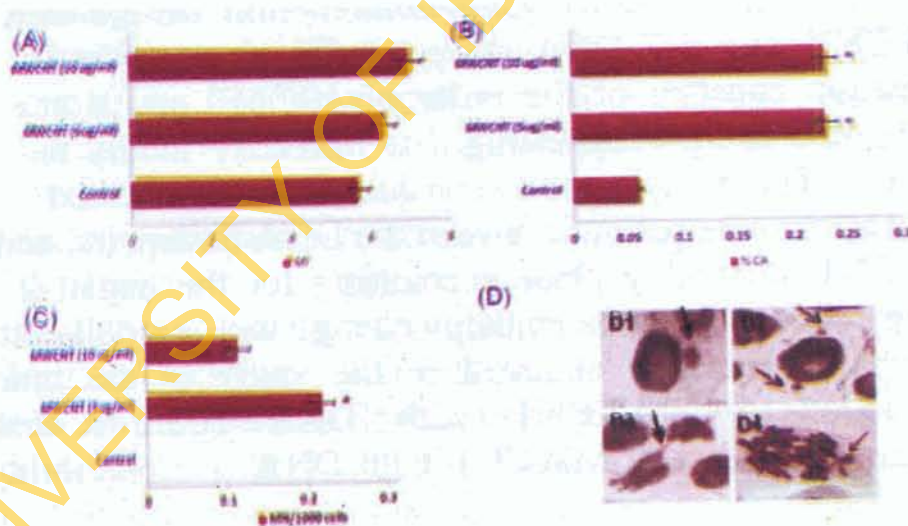

Fig. 31: Results of MWCNT-induced chromosome aberration and micronucleus assay-Allium test: bar graph showing the effect of MWCNT exposure on (A) mitotic index (MI),(B) % chromosome aberration (%CA), (C) micronucleus (MN) formation represented as MN/1000 cells; *P < 0.05; Figure in inset (D) shows representative images of aberrations scored- MN (D1-D2), anaphase bridge (D3) and early migration of chromosome in anaphase.

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The binding studies of MWCNT with calf-thymus DNA (Ghosh et al. 2016) were carried out to understand the mechanism by which specific DNA damage was produced in the *Allium cepa* root tip assay. MWCNT was found to produce structural changes in CT-DNA. The interaction or binding of MWCNT was investigated in order to discover if it brings about any significant changes of the DNA double helix using circular dichroism (CD) spectra of the CT-DNA at two concentration levels of MWCNT representing an increasing MWCNT/DNA molar ratio. In addition, spectrophotometric titrations between MWCNT and CT-DNA were carried out in order to utilize spectral changes as a means of detecting specific binding modes of either intercalation or degradation of DNA. Interactions of MWCNT induced significant changes in the CD spectra of the B-form of natural DNA.

The intensities of the positive CD band at 280 nm decreased significantly. This decrease was found to be concentration-dependent. Following spectrophotometric titrations, specific subtle conformational changes were observed with a molar ratio combination of 2:1 between MWCNT and CT-DNA; these were characterized by a formation constant of the order of 10^3 M^{-1} and a negative Gibbs free energy suggesting that MWCNT avidly binds to DNA. Thermodynamic considerations revealed that electrostatic interactions between the DNA base pairs, and the MWCNT are taking place accounting for the negative free energy change, positive enthalpy change with a small entropy change. The results obtained in the study of the binding interactions of MWCNT with DNA confirm that a cytogenetic effect of MWCNT with DNA is a possibility *in vivo*.

The Greens, The Reddish-brown, The Oil

One grand and glorious thing God has done for His creation is to give this world a variety of natural products and plants with diverse colours. While we make attempt to create colours, several plants have already been endowed with

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diverse colours. I got fascinated about the beauty of colourful plants in my first sojourn outside the shores of this country. At the burst of spring in Glasgow, Scotland several plants came out in most brilliant colours I have ever seen, following close to five months of extreme snowy winter and its storms.

I would not have been relevant in natural products research but for a major sacrifice back then in 1995. I started off as an Intern Pharmacist at the Jos University Teaching Hospital, and barely one month later I was specially invited as the pioneer Intern Pharmacist to the prestigious National Institute for Pharmaceutical Research and Development (NIPRD), Idu-Abuja. That meant a great pay cut as I earned ₦3,200 instead of ₦5,500 that colleagues in Teaching Hospitals were earning. I guessed the times of training were not yet over. However, not only did the sojourn at NIPRD yield two papers, I also encountered another great Chemist, Professor J. Okogun, of the Department of Medicinal Plant Research and Traditional Medicine, NIPRD (during my rotation in his department). He took me through the rudiments of chromatographic fractionation of natural products to the point of isolation of single components for spectroscopic characterization. I continue to thank God for that experience, which became a platform to assist friends and colleagues in the University of Ibadan. The skill on chromatographic fractionation and characterizations of isolated compounds has led to the successful completion of four Ph.D degrees in the University of Ibadan. Therefore, while I do not regard myself as a Natural Products Chemist, these collaborations have produced 26 publications. In one of such studies, Olayemi et al. (2010a, 2010b, 2011, 2013) provided the scientific basis for the folkloric uses of *Cnestis ferruginea* (de Candolle) Connaraceae by thorough studies of the haematological, acute and chronic toxicities and the reproductive effects of the brown crude methanolic extract and chromatographic fractions of the plant. In the collaborative studies with Oyedeji (2013a, 2013b), we established the scientific basis for the indigenous use of *Portulaca oleracea* through a

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variety of procedures among which are assessment of the haematological and biochemical parameters following oral daily dosing of the green-coloured crude extracts and chromatographic fractions to male albino rats, as well as anti-fertility and teratogenic effects on chromatographic pure fractions of the plant.

A landmark achievement in the collaborative efforts was the isolation, characterization and evaluation of the anti-TB properties of crude extracts and isolated compounds from the plants; *Eucalyptus camaldulensis* Dehnh. (Myrtaceae) and *Eucalyptus torelliana* F. Muell (Lawal et al. 2012). The plants are used in Nigerian traditional medicine for the treatment of cough associated with tuberculosis (TB) and other respiratory infections. The hexane, chloroform, methanol extracts, and isolated compounds of *E. camaldulensis* and *E. torelliana* were screened for activity against *Mycobacterium tuberculosis* H37Rv (MtbH37Rv) to authenticate the traditional use of these plants. The microplate alamar blue assay (MABA) method was used to investigate the anti-*M. tuberculosis* activities. Bioassay-guided fractionation of the hexane extract of *E. torelliana* leaf was performed, and isolated compounds were characterized by MS, 1D- and 2D-NMR. Spectroscopic characterization led to the identification of two compounds, hydroxymyristic acid methylester (1) and a substituted pyrenyl ester, a sterol (2).

Conclusions

Has there been any reward from the trawling? The search has been long; the pursuits toilsome, and the labour hard, with unfettered sacrifices. The search goes on and on. The soul has been held in a long perfusion, to apprehend an expected end. Pursuing hard on a tripodal stand, nay, not a stand, but a trawl... For wide was the search, tortuous the pursuit, traversing oceans and miles, far, far away from motherland. The comfort of the home to deny. Then, the Almighty God has always allowed torrents of grace and wisdom. Till......in a proclamation of the *Eureka* the Pharmacist longs. Then loo,

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it was found. A big fish? A big catch? Nay; several molecules of chemical and biological significances. But and then......The story continues, till more pearls get within the trawl. Onward the labour must be and will be. Guided by the same denominator. The denominator of grace and wisdom from the Almighty, *Who lifts the poor out of the dunghill and sets him among the princes of His people*. What a wonder God performs in righteousness. Who can query Him? Thanking you faithful Father *'for His mercies ay endure' 'Ever faithful, ever sure'*.

Recommendations

Mr. Vice-Chancellor Sir, I will be succinct and economical with my recommendations, and I will only mention two of them that are uppermost in my thoughts.

(1) I wish to recommend the need for us to be careful with our consumption of coloured and flavoured foods and other artificially-prepared consumer goods. Apart from the fact that majority of these coloured substances are deleterious in high doses, the ultimate effects on biological systems are yet to be adequately determined and reported. Worrisome is the conflicting daily admissible dose set by major world regulatory agencies on colourants across several regions of the globe.

(2) Mr. Vice-Chancellor Sir, I like to submit that the University of Ibadan is ripe for a functional (not less than 400 MHz) nuclear magnetic resonance spectrophotometer (NMR). In fact, the first University in Nigeria and one that still strives to be the best should be a hub for the spectroscopic characterizations of molecules of synthetic and natural origins. We look forward to a season in UI where our scientists will no longer need spectroscopic pilgrimage abroad. I know and I am persuaded that we are nearer that season as you accede to this demand, Mr. Vice-Chancellor Sir.

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Acknowledgments

My life is akin to a chemical product that has utilized several reagents, chemicals, solvents and reaction pathways to achieve a thoroughly pure crystalline end-product free from exaggerated levels of impurities. I have received benefits from several people, and I will only make attempts to mention as many as my finite mind can recollect, remember and recount. If you do not get mentioned by name and dispositions, just be assured that your labour of love has not been forgotten. Your specific labour of love on my life and career has been to the Lord of Harvest, who will surely grant you a glorious recompense for all your efforts.

I am a product of sacrificial tutoring and tutelage by my teachers, right from my nursery school days. Openly, I acknowledge the love and sacrifices of my teachers from Nursery, Primary, Secondary, Medical Laboratory Science School and University days. I particularly remember Miss Adegbite and Mr. Oduja (then in Primary school). I also acknowledge my teachers during the secondary school days - Mama Elizabeth Fadeji (*Mama Maths*); Mr. Oyatope and Mr. Alegi (my Chemistry teachers); Mrs. Nike Ajayi (Physics) and Mr. Abraham Olaniyan (Mathematics and Additional Mathematics). The contributions of Mr. Afolabi to my armory of hymns and praises are acknowledged. And to my Principal, Mr. Owolabi whose zeal, seriousness and commitment I cherish.

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have captured all principles of Pharmaceutical Chemistry and put them in great textbooks for all Pharmacy students across Africa to read. I thank God that I met you Sir, and all the impacts you and Mama have made in my life will not be forgotten. My gratitude goes to the Dean of Faculty of Pharmacy, Professor Chinedum P. Babalola, who has provided several platforms for my career advancements.

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BIODATA OF
PROFESSOR AREMU OLAJIRE ADEGOKE

Professor Aremu Olajire Adegoke was born on the 21 August, 1970 in Ogbomoso, Oyo State, Nigeria. He attended the Osupa Baptist Day Primary School, Ogbomoso and St. Joseph Primary School, Vom, Plateau State (1975-1981). After his primary education, he proceeded to the Baptist Secondary Grammar School, Ahoyaya, Ogbomoso (1981-1986) where he passed out as the overall best candidate (Eight Distinctions and a Credit) and as the best graduating student in Additional Mathematics, Chemistry, Economics and Geography; he also served as the Health and Laboratory Prefect.

For his higher education, he was at the Federal School of Medical Laboratory Technology, Jos, Plateau State (1987-1989). He was on the Distinction roll at the school throughout his studentship. He gained admission to study Pharmacy at the Faculty of Pharmaceutical Sciences, University of Jos in 1989 and graduated in 1995 with an overall Distinction, which due to the declassification of the Bachelor of Pharmacy degree, translates to a First Class degree. He was the best overall graduating student in the University of Jos in 1995, and the best student in Pharmaceutical Chemistry, Pharmaceutical Technology, Pharmaceutical Microbiology, Pharmacology, Pharmacognosy and Clinical Pharmacy. He had his internship training at the prestigious National Institute for Pharmaceutical Research and Development (NIPRD), Idu-Abuja, as the pioneer Intern Pharmacist (1995-1996), and the mandatory National Youth Service at the General Hospital, Dutse, Jigawa State (1996-1997).

He was awarded the M.Sc. in Pharmaceutical Chemistry at the University of Ibadan (1999) and Ph.D. in Pharmaceutical Analysis and Synthetic Chemistry (2005) from the same University. Professor Adegoke has been a recipient of many scholarships, prizes, fellowships, grants. He was a University of Jos Scholar from 1991-1995; Chemical and Allied Products Limited (1991-1994) and Mobil Producing Nigeria Unlimited (1991-1995) undergraduate

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scholarships awardee. He was awarded eight out of the nine convocation prizes from the University of Jos in 1996. During his Ph.D programme, he was awarded the John D. and Catherine T. MacArthur Foundation grant that enabled him to visit the Department of Pharmaceutical Sciences, University of Strathclyde, Glasgow, UK. He won four prizes (two each in 2007 and 2009) for publications of articles from his Ph.D Thesis, a scheme initiated by the Postgraduate School, University of Ibadan.

Professor Adegoke has been a recipient of many Postdoctoral Fellowships and Grants, including; TETFUND Overseas Conference grant to attend Drug Discovery and Therapy World congress in USA (2014); West African Research Association Travel Grant (2013); CV Raman International Fellowship for Senior African Researchers (2013); University of Ibadan Senate Research grants (2007 and 2012); COIMBRA Short Stay fellowship for Young African Researchers (2011); INSA JRD TATA Fellowship (2010), and CAS-TWAS Postdoctoral fellowship in China (2008).

He was appointed Graduate Assistant (1998-1999); Lecturer II in Pharmaceutical Chemistry in February, 2000; promoted to Lecturer I in October, 2003; Senior Lecturer in October, 2006 and Professor of Pharmaceutical Chemistry with effect from October 1, 2012. He has served as Sub Dean (Postgraduate), Faculty of Pharmacy, University of Ibadan, Ibadan (2006-2009); Member, University of Ibadan Zoological Garden Management Committee (2003-2007); Member, University of Ibadan Zoological Garden Finance Committee (2005-2007); Member, University-Private Sector Collaboration Sub-Committee, Centre for Entrepreneurship and Innovation, University of Ibadan, Ibadan (2007 to date); Acting Head, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Ibadan, Ibadan (2010 to 2012); Member, University Senate (2010 till date). He is also a member, Senate Curriculum Committee; Member, Administrative Committee for the Multidisciplinary Central Research Laboratory, University of Ibadan (July 2014 to

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date). He was Deputy Coordinator, Multidisciplinary Central Research Laboratory (MCRL), University of Ibadan (July 2014 to June 2015) and he is currently the Coordinator, MCRL, from July 5, 2015 to date.

He has served as External Examiner to the Departments of Pharmaceutical Chemistry, Obafemi Awolowo University, Ile-Ife and University of Lagos. He was a Visiting Senior Lecturer, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife (2013 to 2014). He is a Member, West African Postgraduate College of Pharmacists (2013 to date). He is a Lifetime Member, West African Research Association; Member, Pharmaceutical Society of Nigeria and Member, National Association of Pharmacists in Academia. He is an Expert reviewer for over 25 journals focusing on Spectroscopy, Organic Chemistry, Food Chemistry, Analytical Chemistry, Nanoparticles Research, Pharmaceutical Analysis and Natural Products Chemistry. Professor Adegoke has authored 94 publications (comprising of a co-edited book; a chapter in a book; 81 peer-reviewed journal articles and 11 conference papers). He is currently supervising three Ph.D students and has successfully supervised 17 B. Pharm. and over 30 PGD and MSc students. Professor Adegoke is an ordained Deacon at El-Shaddai Baptist Church, Ibadan. He is married to his friend, Adenike Esther Adegoke (nee Akanji), and the marriage is blessed with Ifeoluwa, Iyanuoluwa and Ibukunoluwa.

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NATIONAL ANTHEM

Arise, O compatriots
Nigeria's call obey
To serve our fatherland
With love and strength and faith
The labour of our heroes' past
Shall never be in vain
To serve with heart and might
One nation bound in freedom
Peace and unity

O God of creation
Direct our noble cause
Guide thou our leaders right
Help our youths the truth to know
In love and honesty to grow
And living just and true
Great lofty heights attain
To build a nation where peace
And justice shall reign

UNIVERSITY OF IBADAN ANTHEM

Unibadan, Fountainhead
Of true learning, deep and sound
Soothing spring for all who thirst
Bounds of knowledge to advance
Pledge to serve our cherished goals!
Self-reliance, unity
That our nation may with pride
Help to build a world that is truly free

Unibadan, first and best
Raise true minds for a noble cause
Social justice, equal chance
Greatness won with honest toil
Guide our people this to know
Wisdom's best to service turned
Help enshrine the right to learn
For a mind that knows is a mind that's free

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