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ORIGINAL ARTICLE

Development of ibuprofen microspheres using acetylated plantain starches as polymer for sustained release

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Abstract Ibuprofen has a short half-life (1–3 h) and is typically administered 3–4 times daily with subsequent adverse side effects. A good approach to reduce these effects is the preparation of sustained-release formulations of ibuprofen. Acetylated starches form water-insoluble, acid-resistant films that can substantially retard drug release. Ibuprofen microspheres were prepared using acetylated plantain starch as sustained-release polymer. Starch obtained from unripe plantain (*Musa Paradisiaca* normalis) were acetylated using acetic anhydride with pyridine (degrees of substitution, DS 1.5 ± 0.05 and 2.20 ± 0.10). The starches were characterized using morphology, crystallinity, swelling, density and flow properties. Ibuprofen microspheres were prepared by quasi-emulsion solvent diffusion method, using acetylated plantain starches DS 1.5 and 2.20 in comparison to Eudragit S100. Full 3^2 factorial experimental design was performed with polymer type (X_1), polymer: drug ratio (X_2) as independent factors; microsphere size, entrapment, and quantity of drug released in 12 h (Q_{12}) were dependent variables. The data from in vitro drug release were fitted to various kinetic models. Acetylation resulted in larger starch aggregates with disruption in crystalline order. Ibuprofen microspheres were spherical with size 5.50 ± 4.00–129.90 ± 12.97 µm. Drug entrapment was 43.92 ± 4.00–79.91 ± 6.15%. Values of Q_{12} ranged from 20.10 ± 0.55 to 54.00 ± 5.71%. Interaction between variables X_1 and X_2 had positive effects on size and entrapment. Drug release fitted zero order, first order and Baker-Lonsdale kinetic models. Acetylated starch of plantain with DS 2.20 was suitable as a polymer at polymer:drug ratio 4:1 for the formulation of ibuprofen microspheres with prolonged drug release.

Keywords Acetylation · Factorial design · Ibuprofen microspheres · Plantain starch

Introduction

Amongst the various approaches to controlling the delivery of therapeutic substances to the target sites, the use of microspheres as carriers for drugs has gained wide attention due to its prospects in various ways (Chen et al. 2008; Ramteke et al. 2012; Okunlola and Akindele 2016). Microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers which are biodegradable in nature and ideally having particle size 1–1000 µm. The microscopic size and spherical shape of the microspheres allow them to be injected into the body where they can disperse readily as well as provide a means of direct delivery to the blood stream. They offer reliable means to deliver drugs to the target site with specificity and maintain the desired concentration at the site of interest without untoward effects. The morphology of microspheres allows a controllable variability in degradation and drug release; thus, they may be employed to provide constant and prolonged therapeutic effect. This could lead to a reduction in dosing frequency resulting in improvement in patient compliance. It also provides for better delivery of drugs which may be destroyed by the body like proteineous drugs or those that have deleterious effect on the site of application such as non-steroidal anti-inflammatory drugs (NSAIDs) (Sahil et al. 2011; Saha et al. 2013).

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Starches are used as polymers for controlled release because they are biocompatible; readily available, relatively inexpensive, and capable of various chemical modifications. Although they have some inherent features that limit their use in pharmaceutical formulation, they also possess the capability of undergoing several reactions and processing techniques that modify the starch and yield a multitude of derivatives with desired properties for further processing and formulation (Ochubuijo and Rodrigues 2012). Acetylation of native starch is a modification technique that involves the esterification of the alpha-D-glucose units of the starch by nucleophilic reactions resulting in the substitution of the free proton of the hydrophilic hydroxyl groups on C_2, C_3 and C_6 with the hydrophobic acetyl group. The acetylated starch often shows an increase in solubility in non-aqueous solvents such as chloroform and acetone with increase in the degree of substitution (DS). This increased hydrophobicity of the modified starch is imparted by a reduction in hydrogen bonding between the modified starch and water molecules due to addition of the acetyl group (Babić et al. 2009; Ačkar et al. 2015). Acetylation also results in an increase in the shear strength and the heat and acid stability of the starch as well as decreasing the degree of cloudiness and syneresis of the modified starch in aqueous dispersions (Lui et al. 1997). The degree of change in chemical and functional properties of the acetylated starch depends on the extent of substitution, the reaction conditions and the distribution of the substituents in the starch molecule as well as the nature of the native starch. The number of acetyl groups that may be added or degree of substitution achieved, in turn, depends on the reactant concentration, reaction time, pH and presence of catalyst (Singh and Nath 2012; Neelam et al. 2012; Volkert et al. 2010; Ačkar et al. 2015). It has been reported that drug release rate slows down as DS for starch acetate increases and that starch acetates of specific DS are suitable for controlled release application because of their excellent bond-forming ability (Tuovinen et al. 2003; Pu et al. 2011; Okunlola et al. 2017). Ibuprofen is the oldest member of the propionic acid sub-group of the large family of NSAIDs, which is still widely used, all over the world. Among the major adverse effects of ibuprofen are gastrointestinal disturbance and ulceration (Fries et al. 1991). A good approach to reduce these unwanted effects is preparation of sustained-release formulations which reduce the exposure time of the gastrointestinal tract (GIT) to drug. Sustained-release formulations of ibuprofen could increase the efficacy by providing a more constant plasma concentration, and improve the patient compliance due to the less frequent administration, as well as the reduction in local irritant effect of the drug on GIT (Saravanan et al. 2003). The aim of the research was to modify starch obtained from the unripe fruits of plantain by acetylation (degrees of substitution, DS < 2; DS > 2) and then utilize the starches as polymer coatings in microspheres of ibuprofen. The process of acetylation was carried out using acetic anhydride with pyridine as a catalyst to achieve a high degree of substitution. Studies were carried out to develop and optimize the acetylated starch-based ibuprofen microspheres prepared by quasi emulsion solvent evaporation method. In this study, a full 3^2 factorial experimental design was performed using the Polymer type (X_1), ratio of polymer to drug (X_2) as the independent variables. The relationship between the dependent variables (particle size, entrapment and quantity of drug released in 12 h) and the independent variables was further elucidated using contour and response surface plots.

Materials and methods

Materials

Plantain (*Musa paradisiaca*) fruits were obtained from traders in Bodija market, Ibadan, Oyo State, Nigeria. The reagents used include Acetic Anhydride; Acetone (BDH Limited, Poole, England); Aerosil (Spectrum Chemicals, USA); Chloroform; Ethanol Absolute (Sigma-Aldrich GmbH); Eudragit S 100; Hydrochloric Acid 37% (BDH Limited, Poole, England); Potassium Dihydrogen Orthophosphate (Kermel); Potassium hydroxide (Riedel-de Haën, Germany); Sodium Dodecyl Sulphate (SDS) (Amresco®, Ohio, USA); Sodium Hydroxide (Sigma- Aldrich GmbH); Phenolphthalein; Pyridine (M.R.S Scientific LTD, UK) and ibuprofen (Nanjing Bangnuo Biotechnology Co. Ltd., China).

Methods

Starch extraction and modification

Plantain fruits were weighed, washed, peeled and the pulp diced into small cubes for easy maceration. This was followed by wet milling to obtain a slurry (about 25%w/v in distilled water). The slurry was filtered through a muslin cloth and the filtrate was left to stand to aid settling of the starch. The supernatant was decanted at 12 h-intervals and the starch slurry re- suspended in distilled water. Sodium meta-bisulphite was added to prevent colour change due to oxidation. The starch cake was collected after 72 h and dried in a hot air oven at 55 °C for 48 h. The dried mass was pulverised and then screened through a sieve of 250 µm and weighed (Kim et al. 1995).

Acetylation of the starch was done by dispersing 25 g of pre-gelatinized plantain starch in 200 g of pyridine inside a 1-liter round-bottom flask. One hundred grams of acetic anhydride was then added and the flask was fitted

to a rotary evaporator attached to a reflux condenser. The round-bottom flask was heated in an oil bath at 100 °C in a fume hood for 3.5 and 5 h. The reaction mixture was then cooled to room temperature and the product was precipitated from 1300 ml of ethanol under high shear homogenization. The precipitate was filtered, washed severally with ethanol to remove the pyridine odor and then filtered again. The crystalloids were dried in a forced convection hot-air oven and then screened using 125 µm sieve size (Singh and Nath 2012). The degree of substitution was determined as reported by Ogawa et al. (1999).

$$Acetylgroup(\%) = \frac{(Value\ of\ blank - Value\ of\ sample)(ml) \times Molarity\ of\ HCl \times 0.043 \times 100}{Sample\ weight\ (g)} \quad (1)$$

$$Degree\ of\ substitution = \frac{162 \times \%Acetyl\ group}{4300 - (42 \times \%Acetyl\ group)} \quad (2)$$

Characterization of native and acetylated plantain starches

Morphology

The shape and size of the native and acetylated starch granules were observed using an optical microscope and the scanning electron microscope (Hitachi SU8030 FE-SEM Tokyo, Japan).

FTIR analysis

The starches were analysed with the Fourier Transform-Infra Red spectroscopy using KBr in transmission mode. The transmission spectra were recorded using at least 32 scans with 8 cm^{-1} resolution in the spectral range of 4000−400 cm^{-1} (FTIR spectrum BX, PerkinElmer, Massachusetts, USA).

pH determination

The pH of 1% aqueous starch slurry was measured at 27.5 ± 0.5 °C (pH meter 720 A Thermo Electron Corporation, MA, USA.

Density determination

The bulk density of the natural plantain starch and acetylated plantain starch at zero pressure were determined by pouring 10 g of the powder at an angle of 45° through a funnel into a 50 ml glass measuring cylinder. The bulk density was measured as the ratio of mass to volume occupied by the starch. Determination was carried out in the triplicate. The tapped density was measured by applying 100 taps to 10 g of the starch sample in a graduated cylinder at a standardised rate of 38 taps per minute from a height of 2.54 cm (British Standard 1460). Determination were carried out in the triplicate. The particle densities of the starches were determined using the liquid pycnometer method with xylene as a non- solvent. A 50-ml capacity pycnometer was weighed empty (W) and then filled with non-solvent and the excess wiped off. The weight of the pycnometer with non-solvent was determined (W1). The difference in weight was calculated as W2. Two grams of the sample was weighed (W3) and quantitatively transferred into the pycnometer. The excess non-solvent was wiped off and the pycnometer was weighed again (W4). The particle density was calculated from the equation:

$$\frac{W2W3}{50(W3 - W4 + W)}\ g\ cm^{-1} \quad (3)$$

The determination was carried out in triplicate.

Determination of flow properties

The flowability of the starches were assessed using the Hausner's ratio and Carr's index:

$$Hausner's\ ratio = \frac{Tapped\ density}{Bulk\ density} \quad (4)$$

$$Carr's\ index = \frac{(Tapped\ density - Bulk\ density) \times 100}{Tapped\ density} \quad (5)$$

An open-ended cylinder was placed on a base of similar diameter. Starch powder (10 g) was allowed to flow freely through a funnel under gravity, to form a conical heap. The angle of repose was calculated from:

$$Tan\ \theta = \frac{h}{r} \quad (6)$$

where h is the height of the powder and r is the radius of the base of the cone. The angle of repose was calculated from the mean of three determinations.

Determination of swelling power

The swelling power at room temperature was determined and calculated as the ratio of volume of aqueous slurry of the starch after standing for 24 h in a 100 ml—measuring cylinder to the initial volume occupied by the dry starch powder. Determinations were done in triplicate.

Preformulation studies

Based on literature survey and trial batches conducted by varying critical product and process variables such as polymer concentration, polymer: drug ratio, stirring speed, temperature, curring time, etc, those variables that may have significant effect on the critical quality attributes of the formulation were identified. Some of the attributes observed for this initial screening were yield, appearance, size, entrapment and drug release. Preliminary optimization of polymer concentration, curring time, stirring speed, temperature was done by conducting the experiments at various levels. The polymer type and polymer: drug ratio were selected as independent variables and the response on particle size, entrapment and quantity of drug released in 12 h were investigated while keeping the other parameters constant.

Preparation of Ibuprofen microspheres containing acetylated plantain starches

The microspheres were prepared using the quasi-emulsion solvent diffusion method. Ibuprofen (0.5 g) and plantain starch acetate DS 1.5 (0.5 g) were dissolved completely in 50 ml of chloroform. In the drug- polymer mixture, 0.5 g aerosil was suspended uniformly under vigorous agitation. The resultant drug- polymer- aerosil suspension was poured into distilled water (750 ml) containing 0.08% of sodium dodecyl sulfate (SDS) (i.e., poor solvent) contained inside a 2-liter beaker and maintained under moderate agitation 450–750 rpm (Talboys Laboratory Stirrer Model No: 102 USA) and temperature of 38 °C (Maplelab Scientific Stirrer-Hotplate Model SHC-11).

The suspension was finely dispersed into quasi- emulsion droplets immediately under agitation, and the drug and polymers co- precipitated into the emulsion droplets. After agitating the system for 20 min, 750 ml of 0.08% SDS solution was added slowly to promote the diffusion of the chloroform from the emulsion droplets into the SDS, enhancing the solidification of quasi- emulsion droplets. Agitation was extended for 40 min until the translucent quasi- emulsion droplets turned into opaque microspheres. The solidified microspheres were recovered by filtration and washed with water, and the resultant products were dried in the Gallenkamp BS Oven 250 at 50 °C for 6 h.

The composition of the microspheres was varied using plantain starch acetate DS 2.2 and Eudragit S 100 as standard polymer, as well as varying the polymer to drug ratio from 1:1 described above to 2:1 and 4:1.

Experimental design

A full 3^2 factorial experimental design was performed using two factors, each at three levels as reported (Okunlola et al. 2017) giving nine possible combinations. The type of polymer (X_1) and drug-polymer ratio (X_2) were chosen as independent variables while the particle size, swelling, entrapment efficiency and quantity of dug released in 12 h (Q_{12}) were selected as dependent variables. Other process and formulation variables such as stirring speed, polymer concentration, concentration of dispersing agent and curing time were kept constant. The data were subjected to multiple regression analysis using statistical software (Minitab 17 Software USA). The 3-D response surface plots and contour plots for the variables particle size, entrapment and Q_{12} were generated.

Characterisation of microspheres

Size and morphology

The microspheres were sputtered with gold and their morphology and surface characteristics were analysed using scanning electron microscopy (SEM) Hitachi SU8030 FE-SEM Tokyo, Japan. The particle sizes of 100 microbeads were determined by optical microscopy.

FTIR analysis

The drug-polymer interaction and possible degradation of drug while processing was determined using FTIR (FTIR-Thermo Nicolet Nexus 870 Madison, WI, USA). Transmission spectra were recorded using at least 64 scans with 8 cm^{-1} resolution in the spectral range 4000–400 cm^{-1}.

Swelling index

Exactly 1 ml of microsphere bed was soaked in 5 ml phosphate buffer (pH 7.4) inside a 10-ml measuring cylinder for 12 h and swelling index was calculated as the ratio of the volume after 12 h to that of the original volume.

Entrapment efficiency

The quantity of microspheres containing 50 mg of drug was accurately weighed and crushed in a glass mortar with a pestle and the suspended in 50 ml of phosphate buffer, pH 7.4. After 24 h, the solution was filtered and the filtrate was analysed for drug using Spectrumlab 752s UV–Vis spectrophotometer at 275 nm. The drug entrapment efficiency, E (%) was calculated using the formula:

$$E(\%) = \frac{Practical\ drug\ content}{Theoretical\ drug\ content} \times 100 \quad (7)$$

Drug release study

The in vitro dissolution studies were carried out using the paddle method (USP XXI), rotated at 50 rpm in 900 ml of phosphate buffer, pH 7.4, maintained at 37 ± 0.5 °C. The microspheres (100 mg) were placed in the dissolution medium. Samples (5 ml) were withdrawn at different intervals and replaced with equal amounts of fresh medium. The amount of Ibuprofen released was determined at wavelength of 222 nm, using Spectrumlab 752 s UV–Vis spectrophotometer. Determinations were carried out in duplicates.

Kinetic modelling to determine mechanism of drug release

Data obtained from in vitro release studies were fitted to various kinetic equations to determine the kinetics and mechanisms of drug release from the microspheres. The results of the drug release for the formulations were fitted to zero order, first order, Higuchi (1961), Hixon and Crowell (1931), Korsemeyer et al. (1983), Baker and Lonsdale (1974), Weibull and Hopfenberg (1976) kinetic models. The model of best fit was identified by comparing the values of correlation coefficients.

Results

Characterization of native and acetylated plantain starches

The yield of starch from unripe plantain was 44.46%. The acetyl content of the modified plantain starch was 28.17 ± 0.70% with degree of substitution of 1.51 ± 0.05 and 39.10 ± 1.15% with degree of substitution DS 2.20 ± 0.10, respectively. FTIR spectra of the native and acetylated plantain starches are shown in Fig. 1 while the scanning electron micrographs (SEM) of the starches are shown in Fig. 2. The results of the starch granule sizes, pH, swelling, angle of repose and densities are presented in Table 1. From the bulk and tapped density values obtained, the Hausner's ratio and Carr's index were obtained and are also presented in Table 1.

Characterization of microspheres

The composition of the nine batches of ibuprofen microspheres containing acetylated starches DS 1.5 and 2.2 and Eudragit S100 are presented in Table 2. Figure 3 show the FTIR spectra of ibuprofen, acetylated plantain starch, ibuprofen microspheres containing the starch and SEM of Eudragit S100 and ibuprofen microspheres containing Eudragit, respectively. Figure 4 reveals the SEM images of the ibuprofen microspheres containing acetylated plantain starch and Eudragit. Figure 5 shows the dissolution profile of the nine batches of ibuprofen microspheres. From the plot of cumulative drug released vs time, the percent drug released after 12 h was determined for each batch.

Experimental design

The factorial design showing the levels of the variable, real values and the responses are presented in Table 3. The model incorporating first order polynomial terms was used to evaluate the responses and the fitted equation was $Y = b_0 + b_1X_1 + b_2X_2 + b_{12}\ X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2$. Y is the dependent variables, namely, size (μm), entrapment (%) and Q_{12} (%). Y indicates the quantitative effect of the independent variables X_1 and X_2; b_0 is the arithmetic mean response of the nine runs; b_1–b_5 are the coefficients of the term X. The main effects (X_1 and X_2) represent the average result of changing one factor at a time from its low to high value. The interaction term (X_1X_2) shows how the response changes when two factors are simultaneously changed. The polynomial terms (X_1^2 and X_2^2) are included to investigate non-linearity (Yadav and Sawant 2010). The simplified models were then utilized to produce three-dimensional response surface plots and contour plots to analyse the influence of independent variables. Regression parameters fitted the polynomial regression equation with correlation coefficient of ≥0.902 for almost all the dependent variables. The summary of the regression output of the factors for measured responses are shown in Table 4 while the results of the two-way ANOVA for the dependent variables are in Table 5. The 3-D response surface plots and contour plots for the variables particle size, entrapment and Q_{12} are shown in Fig. 6i and ii (a), (b) and (c), respectively. Figure 6(iii) shows the linear plots between observed and predicted values of the responses. Table 6 shows the comparison of experiment results with predicted responses of the ibuprofen microsphere formulations.

The correlation coefficient values obtained using different kinetic models for the batches of ibuprofen microspheres are presented in Table 7.

Discussion

Characterization of native and acetylated plantain starches

The native starch molecule is known to have three free hydroxyl groups with different levels of reactivity. Primary OH group located at position C6 is highly reactive and will be immediately react with the acetyl group when compared with the two secondary OH groups

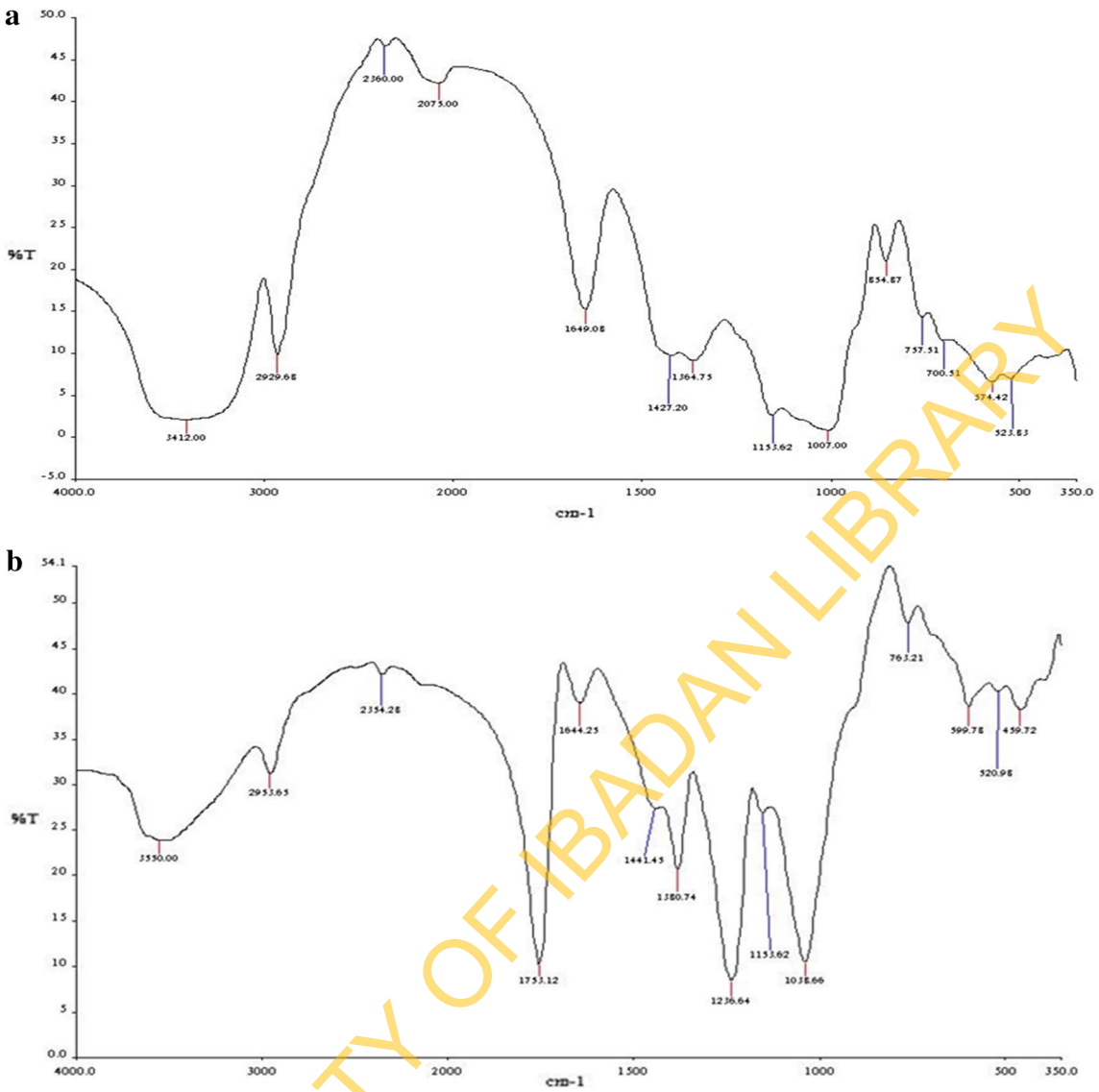

Fig. 1 FTIR spectra of **a** Native plantain starch; **b** acetylated plantain starch

at positions C2 and C3 as a result of steric hindrance (Kumoro et al. 2014). Acetylation of plantain starch was confirmed by the FTIR spectra in Fig. 1 where the peak of the carbonyl group, 1740 cm^{-1}appeared. The intensity of this peak increased with a decrease in the peak intensity of the hydroxyl groups at 3000–3600 cm^{-1}, indicating that the hydroxyl groups were replaced by the acetyl groups. FTIR spectra of the modified starch also showed some new absorption bands at 1435, 1375, 1240 cm^{-1} assigned to carbonyl C=O, CH_3 anti-symmetry deformation vibration, and CH_3 symmetry deformation vibration and carbonyl C–O stretch vibration, respectively (Chi et al. 2008). The absence of the peak in the region 1850–1760 cm^{-1} and the absence of absorption in the area of 1700 cm^{-1} for the carboxylic group implied that the product was free of unreacted acetic anhydride of acetic acid by-product respectively (Diop et al. 2011).

The SEM revealed that on acetylation, the oblong-shaped native starch granules became irregular, beehive-like, aggregates. The agglomeration takes place because of the hydrophilic acetyl groups introduced into starch molecules, which increases the number and strength of hydrogen bonding. This allows the starch molecules to come close to each other forming starch clusters which combine to form aggregates (Singh et al. 2004). Acetylation significantly ($p < 0.05$) increased the granule size of the starch, with acetylated starch DS 2.2 having the largest size. Acetylation also resulted in reduction in swelling of starches.

The pH of the acetylated starch starches increased slightly than the native starch. The order of ranking of the

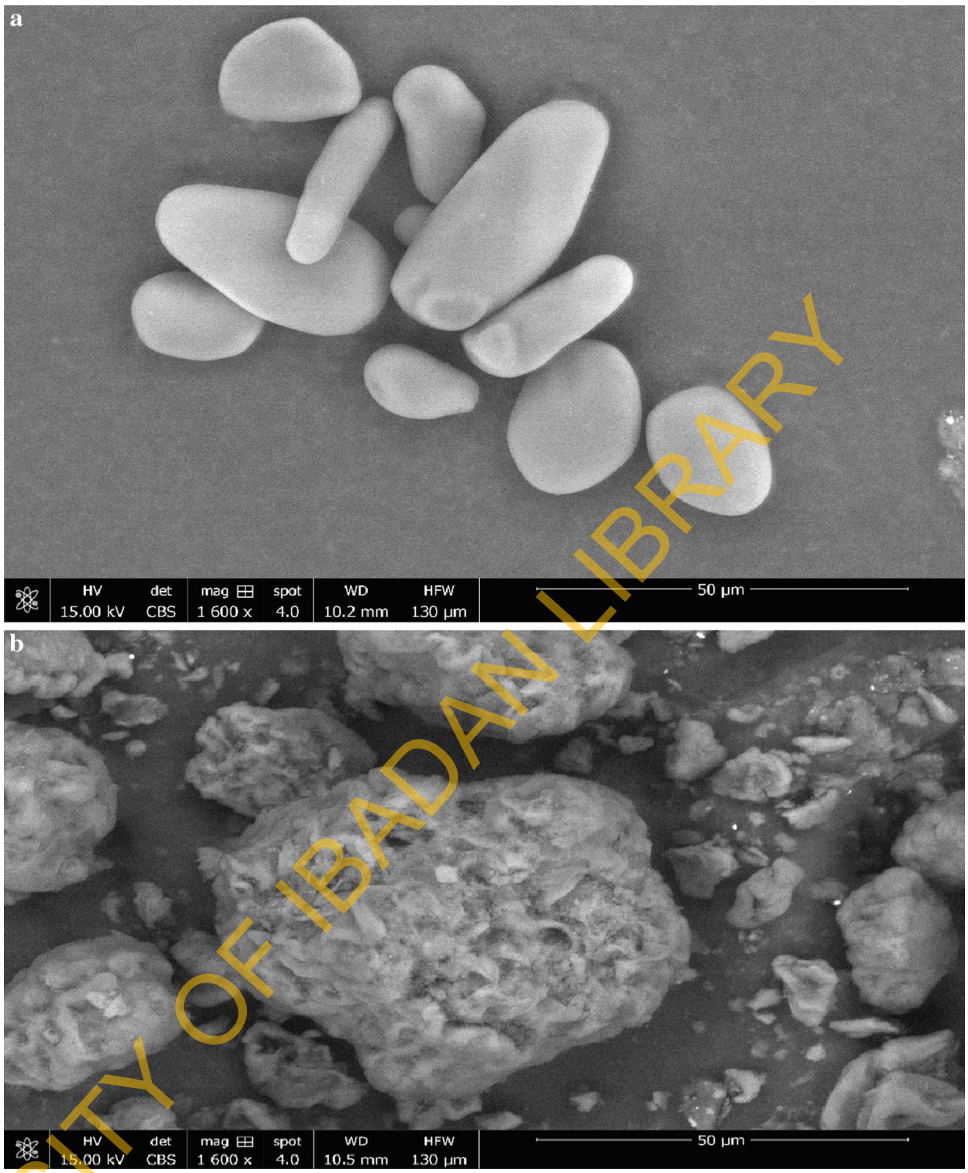

Fig. 2 SEM of **a** Native plantain starch; **b** acetylated plantain starch Mg ×600

particle, bulk and tapped density values was native > acetylated starch DS 2.2 > acetylated starch DS 1.5. From the bulk and tapped densities, Hausner's ratio and Carr's index were calculated. These values were in agreement with those of angle of repose indicating that the flow property of plantain starch was enhanced with acetylation, improving as the degree of acetylation increased from 1.5 to 2.2.

Characterization of microspheres

Polymeric microparticles have been reported to offer several advantages over the conventional dosage forms in controlled delivery and these include enhanced efficacy, reduction in frequency of drug administration and reduced toxicity (Haznedar and Dortunc 2003, Huh et al. 2010; Okunlola and Akindele 2016). The quasi-emulsion solvent evaporation method was chosen due to its simplicity and its suitability for drugs with poor aqueous solubility (Devrim and Canefe 2011). The scanning electron micrographs of the microspheres reveal that the microspheres of plantain starch acetate were near spherical in shape with slightly rough surface. The microspheres prepared were in the size range of 52.50 ± 4.00–129.90 ± 12.97 µm and the size was generally in the rank order of $B_7 > B_4 > B_1 > B_5 > B_8 > B_9 > B_2 > B_6 > B_3$. For better absorption of drug, minimum average size of microsphere particles is desired. The attainable particle size is dependent on the composition and polymer concentration (Kovacevic et al. 2011). Microspheres containing Eudragit S were observed to have the smallest size followed by those containing the acetylated plantain starch DS 2.2. while microspheres containing acetylated plantain starch DS 1.5 had the largest size. The

Table 1 Morphology and material properties of native and acetylated plantain starches (mean ± SD; n = 3)

Starch	Particle shape	Particle size (μm)	pH	Particle density ($g\,cm^{-3}$)	Bulk density ($g\,cm^{-3}$)	Tapped density ($g\,cm^{-3}$)	Hausner's ratio	Carr's index (%)	Swelling power	Angle of repose (degree)
Native plantain	Oblong	2.39 ± 0.03	5.81 ± 0.03	1.463 ± 8.00	0.371 ± 0.007	0.675 ± 0.024	1.82 ± 0.07	45.04 ± 2.25	1.02 ± 0.01	53.64 ± 0.66
Acetylated plantain DS 1.51	Irregular aggregates	26.11 ± 1.11	7.22 ± 0.04	1.353 ± 0.96	0.446 ± 0.004	0.630 ± 0.013	1.41 ± 0.01	29.21 ± 1.00	1.00 ± 0.02	33.47 ± 0.22
Acetylated plantain DS 2.20	Irregular, fibrous aggregates	41.56 ± 5.25	6.20 ± 0.04	1.375 ± 0.23	0.460 ± 0.004	0.662 ± 0.013	1.44 ± 0.03	30.51 ± 2.20	0.90 ± 0.01	30.20 ± 0.10

particle size was also observed to increase as polymer:drug ratio increased. The effect could be due to increased viscosity of the polymer-drug dispersion which produced larger droplets that led to the formation of larger particles. The FTIR spectra confirmed that new chemical entities were not formed indicating there was no interaction between the drug and the polymer. The FTIR absorption peaks observed in the spectra of ibuprofen were similar to those in that of the microspheres. Characteristic bands of C=O vibrations of the esterified carboxylic acid groups at 1730 cm^{-1} and of the carboxylic acid groups at 1705 cm^{-1} while C–N group, N=O group, C–H group and C–C group at 1069.71, 1326, 2895 and 1227 cm^{-1}, respectively were observed in both.

Entrapment efficiency was in the range of 43.92 ± 4.00–79.91 ± 6.15% and observed to increase with increase in amount of polymer. Ibuprofen microspheres containing acetylated plantain starch DS 2.2 had similar entrapment efficiency ($p > 0.05$) as those containing Eudragit S while both had significantly higher values than those containing acetylated plantain starch DS 1.5 ($p < 0.05$). Entrapment efficiency increased with increase in polymer: drug ratio.

The quantity of ibuprofen released from the microspheres after 12 h (Q_{12}) was in the range of 20.10 ± 0.55–54.00 ± 5.71%. The quantity of drug released was observed to decrease with increase in amount of acetylated starch. Acetylated plantain starch DS 1.5 gave the fastest dissolution with Q_{12} values being the highest at all drug-polymer ratios. Dissolution was most prolonged in those microspheres containing Eudragit S at drug: polymer 4:1. At this polymer: drug ratio, the quantity of ibuprofen released from microspheres containing Eudragit S was like those containing acetylated starch DS 2.2. The span of release of medicament from the microsphere formulations containing the starches was prolonged enough to justify the proposed polymer systems as drug release modulators for drug delivery systems.

Experimental design

From the results of the experimental design, mathematical models were generated for predicting the values of response parameters at selected ranges of the formulation factors. From the summary of regression outputs of significant factors for measured responses, regression values suggested good fit of the terms within the models. Results of the two-way ANOVA for the dependent variables show positive co-efficient values for independent variables X_1 and X_2 on particle size and entrapment, indicating that these variables increased as polymer type changed and as polymer: drug ratio increased. The results showed that minimum particle size with high entrapment efficiency was obtained by changing the polymer type from acetylated starch DS1.5 to

Table 2 Composition of ibuprofen microsphere formulations

Ingredients	Batches								
	B1	B2	B3	B4	B5	B6	B7	B8	B9
Ibuprofen (g)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Acetylated starch DS 1.5 (g)	0.5	1.0	2	–	–	–	–	–	–
Acetylated starch DS 2.2 (g)	–	–	–	0.5	1.0	2	–	–	–
Eudragit®S100 (g)	–	–	–	–	–	–	0.5	1.0	2
Aerosil (g)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Chloroform (ml)	50	50	50	50	50	50	50	50	50
0.08% w/v SDS solution (ml)	1500	1500	1500	1500	1500	1500	1500	1500	1500
Polymer: drug	1:1	2:1	4:1	1:1	2:1	4:1	1:1	2:1	4:1

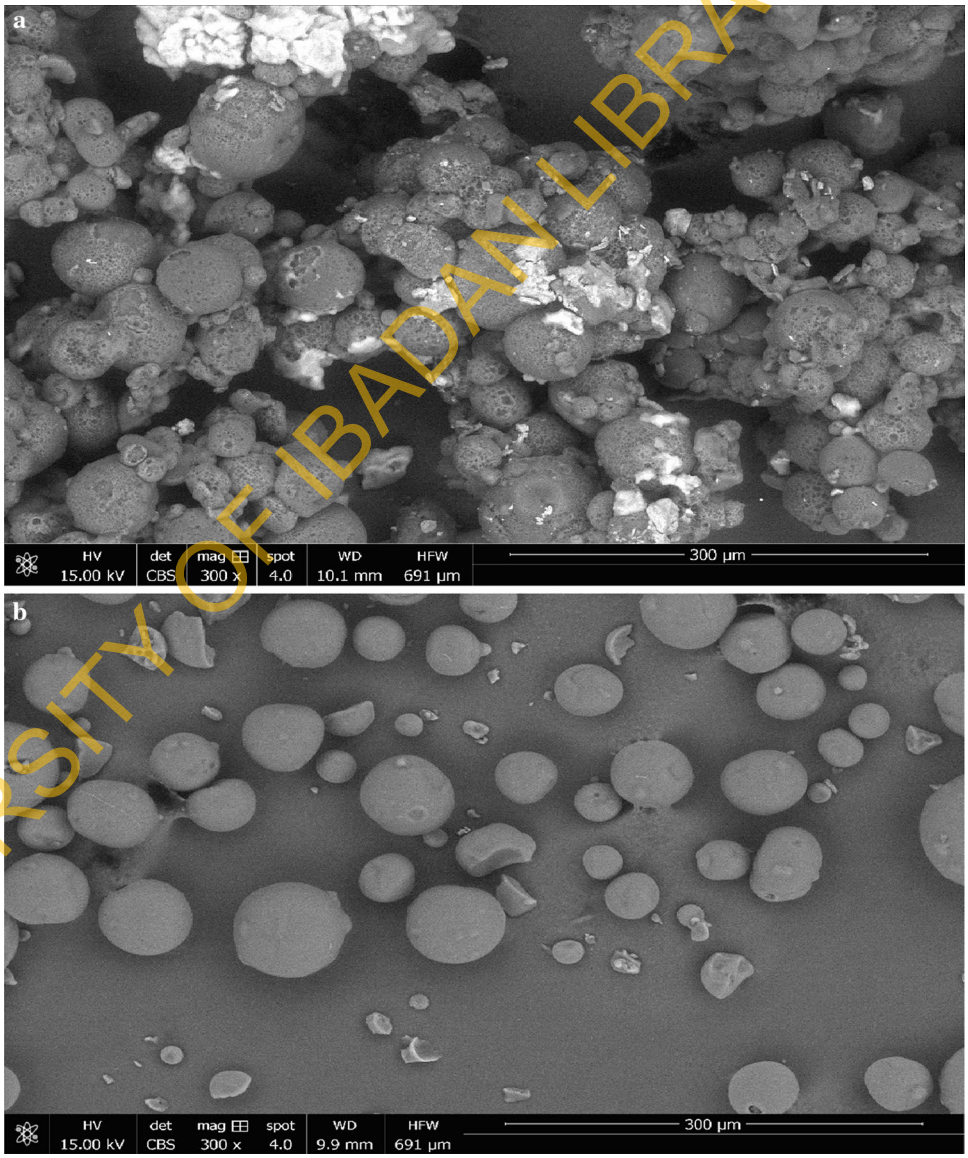

Fig. 3 SEM of ibuprofen-loaded microspheres containing **a** acetylated plantain starch and **b** Eudragit S100 at mg × 300

DS 2.2 and reducing the Polymer:drug ratio. On the other hand, the negative co-efficient values for both independent variables indicated that quantity of ibuprofen released in 12 h decreased as the polymer type was changed from acetylated starch DS1.5 to DS 2.2, from acetylated starch DS 2.2 to eudragit and by increasing the Polymer:drug ratio.

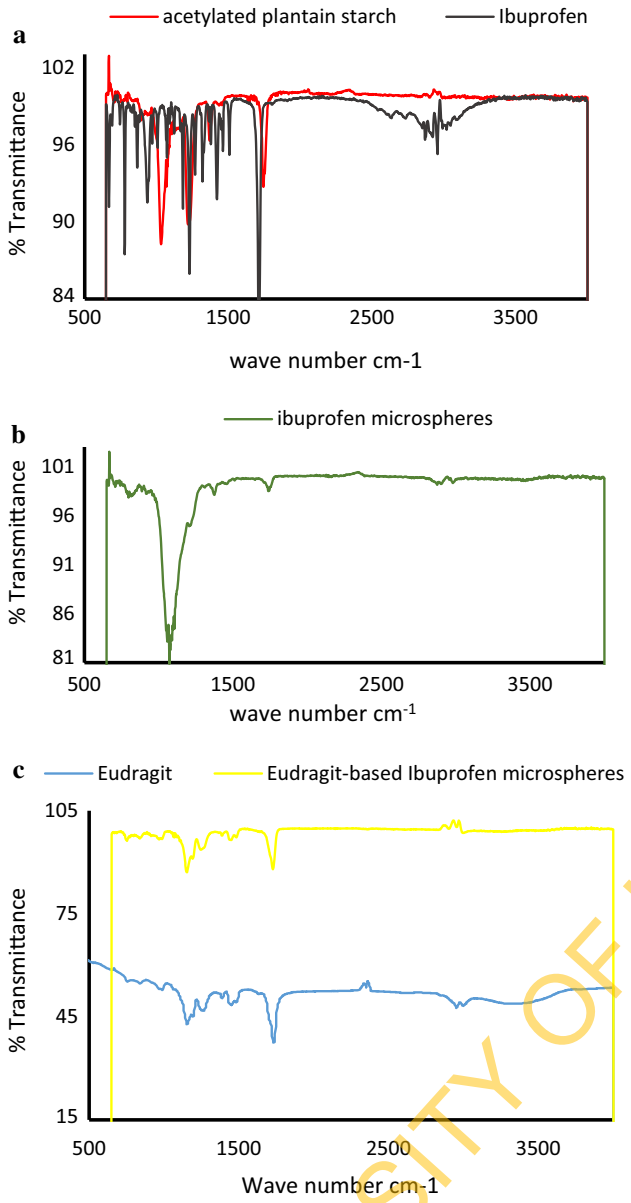

Fig. 4 FTIR spectra of **a** acetylated plantain starch and ibuprofen **b** ibuprofen microspheres containing acetylated plantain starch and **c** eudragit and ibuprofen microspheres containing eudragit

The results of the two-way ANOVA presented also revealed that the effect of drug: polymer ratio on all response variables of the microspheres was more significant ($p \leq 0.01$) than those of polymer type. The interactive term X_1 X_2 showed positive effects on all response variables studied indicating that their values increased when both factors were simultaneously increased.

Response surface plots, the graphic representations of the influence of the factors on the properties of the microspheres, were generated for each response parameters to study effect of each factor. Response surface plots are very useful to study the interaction effects, main effects and

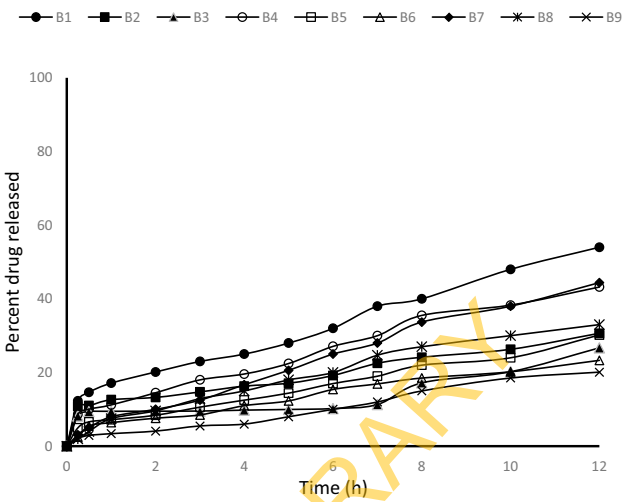

Fig. 5 Dissolution profile of ibuprofen microspheres

quadratic effects of the factors (independent variables) on the responses (dependent variables) at a time (Martinez et al. 2004). Figure 6i and ii(a) revealed that at constant drug:polymer ratio, changing polymer type from acetylated plantain starch DS 1.5 to acetylated starch DS 2.2 reduced particle size. The lowest particle size was observed with microspheres containing Eudragit. Figure 6i and ii(b) revealed an increase in entrapment with change in polymer type from acetylated plantain starch DS 1.5 to acetylated starch DS 2.2 with slightly higher entrapment obtained when Eudragit was the polymer. Figure 6i and ii(c) showed that at constant polymer: drug, there was a decline in quantity of drug released in 12 h as the polymer type was changed from acetylated starch DS 1.5 to DS 2.2. These values reduced in microspheres containing Eudragit as the polymer. Slow dissolution could be due to hydrophobicity of the modified starches with increasing degree of substitution. The poor wetting of the drug surface owing to the polymers' hydrophobic nature causes retardation in the release of drug.

Formulation optimization using desirability function

To optimize the formulation of ibuprofen microspheres, the levels of the factors that affect the selected responses were determined. The levels of the variables that can produce a robust product with characteristics of high quality were determined. Figure 6(iii) showed linear correlations plots between the predicted and observed values of size, entrapment and Q_{12}. The linear plots gave high values of R^2 (between 0.942 and 0.999) indicating excellent fitting of the models. The low magnitude of error and high values of R^2 indicate a high prognostic ability of the statistical models. Taking into consideration all responses, the following

Table 3 The 3^2 Factorial design for ibuprofen microspheres formulations

Batch	Variable levels		Real values		Response		
	X_1	X_2	X_1 (polymer type)	X_2 (polymer: drug)	Particle size (μm)	Entrapment (%)	Q_{12} (%)
B_1	−1	−1	Acetylated plantain DS 1.5	1:1	80.50 ± 5.00	43.92 ± 4.00	54.00 ± 5.71
B_2	−1	0	Acetylated plantain DS 1.5	2:1	96.40 ± 3.60	59.64 ± 3.55	30.65 ± 6.14
B_3	−1	+1	Acetylated plantain DS 1.5	4:1	129.90 ± 12.97	64.34 ± 5.15	26.70 ± 3.49
B_4	0	−1	Acetylated plantain DS 2.2	1:1	60.10 ± 2.15	55.30 ± 3.10	43.20 ± 0.42
B_5	0	0	Acetylated plantain DS 2.2	2:1	80.55 ± 14.20	61.27 ± 5.65	30.20 ± 0.44
B_6	0	+1	Acetylated plantain DS 2.2	4:1	105.40 ± 15.70	78.92 ± 6.05	23.30 ± 0.21
B_7	+1	−1	Eudragit S100	1:1	52.50 ± 4.00	52.35 ± 3.50	44.40 ± 4.34
B_8	+1	0	Eudragit S100	2:1	81.00 ± 7.10	68.56 ± 3.12	33.00 ± 0.40
B_9	+1	+1	Eudragit S100	4:1	93.10 ± 3.66	79.91 ± 6.15	20.10 ± 0.55

Table 4 Summary of regression outputs of significant factors for measured responses

Coefficients of parameters	Particle size (μm)	Entrapment (%)	Q_{12} (h)
b_o	86.61	62.69	35.96
b_1	13.37	5.49	−2.31
b_2	22.55	11.93	−11.92
b_{12}	2.44	1.56	1.64
b_{11}	−5.48	3.52	−1.41
b_{22}	9.25	7.67	−7.28
R^2	0.951	0.922	0.902

criteria were adopted: particle size ≤100 μm, entrapment efficiency ≥75% and $Q_{12} \leq 25\%$. The responses of factorial formulations suggested Polymer type of acetylated starch DS 2.2 and polymer:drug ratio of 4:1 (B_6) as the optimized ibuprofen microsphere formulations. The selected optimized formulation was prepared and the observed values were found to be comparable to the predicted values as shown in Table 6.

Kinetic models of drug release

Simulating the drug kinetics with different models, drug release for the microspheres generally fitted the first order kinetic models. This first order model can be used to describe the drug dissolution in pharmaceutical dosage forms such as those containing water-soluble drugs in porous matrices (Narashimhan et al. 1999). Batches B_6 and B_9 fitted the zero order while Batch B_2 and B_8 fitted the Baker–Londsale model. Those batches following zero order released the same amount of drug by unit of time and this zero order release is considered the ideal method of drug release in order to achieve a pharmacological prolonged action. The Baker–Londsale model was developed by Baker and Lonsdale (1974) from the

Table 5 Results of the two-way ANOVA for the dependent variables

Dependent variable	Source	Degree of freedom	Sum of squares	Mean square	F value	p value
Particle size	X_1	2	1166.77	583.38	19.89	0.008
	X_2	2	3052.76	1526.38	52.04	0.001
	Residual	4	117.33	29.33		
	Total	8	4336.85			
Entrapment efficiency	X_1	2	208.15	104.08	7.09	0.048
	X_2	2	855.41	427.70	29.13	0.004
	Residual	4	58.72	14.68		
	Total	8	1122.28			
Q_{12}	X_1	2	33.12	16.56	0.73	0.538
	X_2	2	810.60	405.30	17.82	0.010
	Residual	4	91.00	22.75		
	Total	8	934.72			

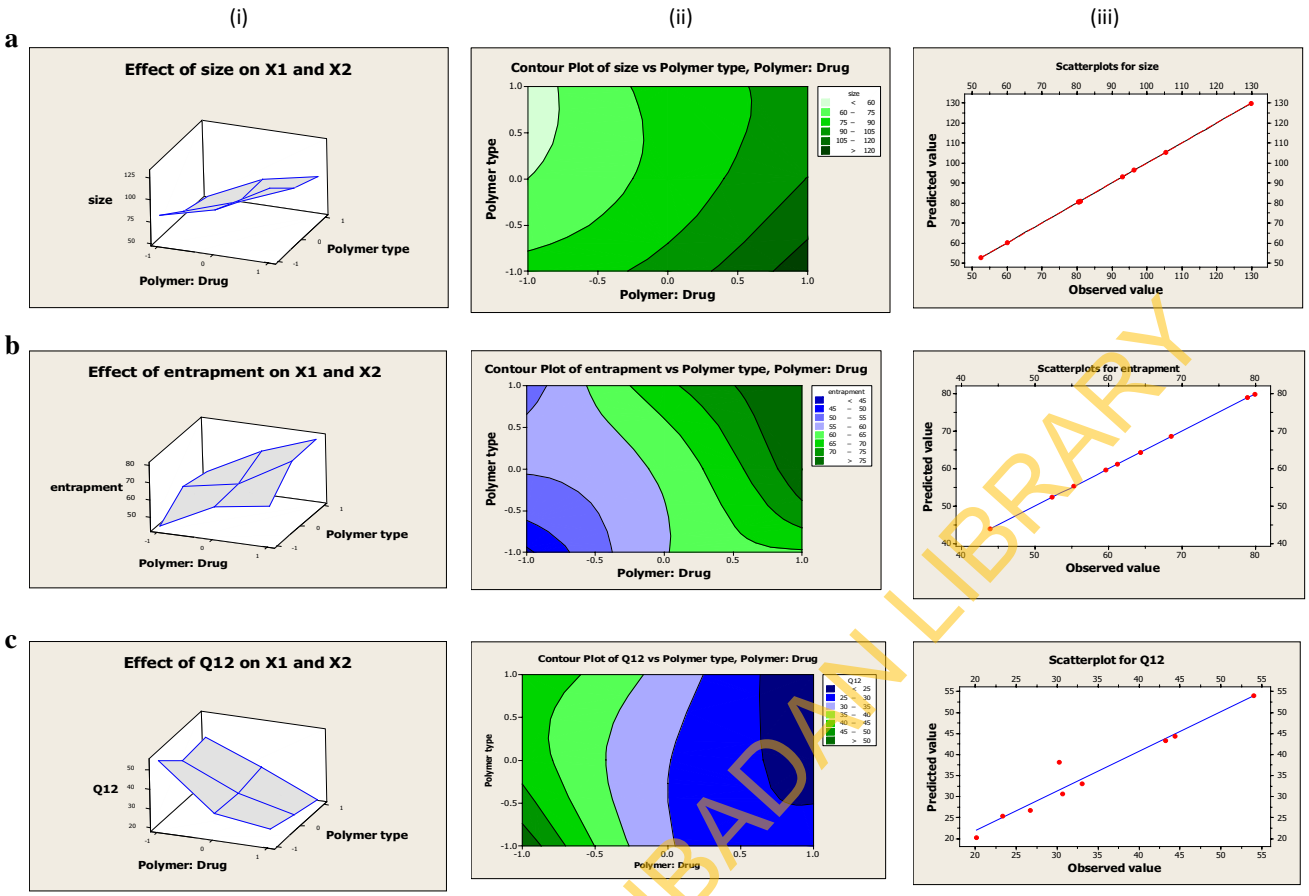

Fig. 6 (i) Response surface plots, (ii) contour plots showing effect of independent variables (X_1 and X_2) on **a** particle size, **b** entrapment and **c** Q_{12}, the quantity of drug (%) released in 12 h and (iii) linear plots between their observed and predicted values

Table 6 Comparison of experimental results and predicted responses of ibuprofen microsphere formulations

Optimized formula	Composition		Response					
	X_1 Polymer type	X_2 Polymer:drug	Predicted size (μm)	Experimental size (μm)	Predicted entrapment (%)	Experimental entrapment (%)	Predicted Q_{12} (%)	Experimental Q_{12} (%)
B_6	Acetylated starch DS 2.2	4:1	93.20	93.11	75.50	77.32	25.0	23.30

Higuchi model and describes drug release from spherical matrices (Seki et al. 1980). This equation has been used for the linearization of release data obtained for several formulations of microcapsules or microspheres (Polleto et al. 2007). This model indicates that swelling and dissolution are negligible in the systems of Batches B_2 and B_8 and their initial drug concentrations are much higher than drug solubility.

Conclusion

The study indicates that ibuprofen microspheres can be prepared using the simple, reproducible method, quasi-emulsion solvent evaporation. The in vitro drug release showed that optimized formulation contained acetylated plantain starches DS 2.2. and effectively prolonged release of the drug comparably to Eudragit S 100 at polymer: drug

Table 7 Correlation coefficients obtained for Ibuprofen microspheres using different kinetic models (n = 3)

Formulation	Zero order	First order	Higuchi	Hixson–Crowell	Korsmeyer		Baker–Lonsdale	Weibull	Hopfenberg
					R^2	n			
B_1	0.9506	0.9997[a]	0.9577	0.8853	0.9253	0.3672	0.9370	0.7147	0.9242
B_2	0.8858	0.9135	0.9283	0.8231	0.8547	0.2605	0.9540[a]	0.6651	0.9111
B_3	0.7817	0.7832[a]	0.7106	0.4656	0.4926	0.2113	0.7203	0.4316	0.5749
B_4	0.9642	0.9754[a]	0.967	0.9004	0.9369	0.4271	0.9530	0.7560	0.951
B_5	0.9743	0.9785[a]	0.9493	0.8834	0.9565	0.4442	0.9209	0.7484	0.9292
B_6	0.9918[a]	0.9717	0.9743	0.9412	0.9576	0.5178	0.9720	0.8099	0.9599
B_7	0.9716	0.9930[a]	0.9478	0.9413	0.9815	0.6757	0.9240	0.7656	0.9494
B_8	0.9791	0.9846	0.9790	0.9824	0.9896	0.6947	0.9950[a]	0.8420	0.9716
B_9	0.9788[a]	0.9762	0.8967	0.8513	0.9113	0.5818	0.8904	0.7101	0.8907

[a]Highest correlation coefficient of drug release kinetics

ratio 4:1. This study holds promise for the development of acetylated plantain starch as a cheaper alternative to synthetic polymers in sustaining drug release. Achieving this potential may require further research in this area of drug delivery.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Research involving human and animal participants This article does not contain any studies with human and animal subjects performed by any of the authors.

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