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Development of repaglinide microspheres using novel acetylated starches of bitter and Chinese yams as polymers

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ABSTRACT

Tropical starches from *Dioscorea dumetorum* (bitter) and *Dioscorea oppositifolia* (Chinese) yams were acetylated with acetic anhydride in pyridine medium and utilized as polymers for the delivery of repaglinide in microsphere formulations in comparison to ethyl cellulose. Acetylated starches of bitter and Chinese yams with degrees of substitution of 2.56 and 2.70 respectively were obtained. Acetylation was confirmed by FTIR, ^{1}H NMR spectroscopy. A 3^2 factorial experimental design was performed using polymer type and drug-polymer ratio as independent variables. Particle size, swelling, entrapment and time for 50% drug release (t_{50}) were dependent variables. Contour plots showed the relationship between the independent factors and the response variables. All variables except swelling increased with drug: polymer ratio. Entrapment efficiency was generally in the rank of Bitter yam > Ethyl cellulose > Chinese yam. Repaglinide microspheres had size 50 ± 4.00 to 350 ± 18.10 μm, entrapment efficiency 75.30 ± 3.03 to 93.10 ± 2.75% and t_{50} 3.20 ± 0.42 to 7.20 ± 0.55 h. Bitter yam starch gave longer dissolution times than Chinese yam starch at all drug-polymer ratios. Drug release fitted Korsmeyer-Peppas and Hopfenberg models. Acetylated bitter and Chinese yam starches were found suitable as polymers to prolong release of repaglinide in microsphere formulations.

1. Introduction

Advances in polymer science and drug carrier technologies have led to the development of novel drug carriers such as microspheres but challenges in this field of drug delivery include the search for newer polymers with the added attributes of being biodegradable, biocompatible, bioadhesive for specific cells or mucosa [1–3]. A renewable and almost unlimited resource, lately there has been a growing interest in starch as a carrier for target delivery of active drug substances through several routes [3,4–6]. This is due to the fact that starch is known to be relatively cheap, more abundant and produces lower toxicity products that are biodegradable and quite stable in the biological environment [7,8] relative to many other polymers. Many polymers and materials have been used in the past to develop microsphere delivery system, however, most are non-biodegradable. Examples include the studies of Adeyeye & Price and Vilivalam & Adeyeye [9,10]. Native starches can be modified to introduce new properties and/or remove certain inherent undesirable characteristics. Of the various methods of chemical modification of starches, acetylation can be performed with relative ease to improve significantly the physicochemical and functional properties of starch [11]. The free hydroxyl groups on C_2, C_3 and C_6 of the starch molecule are substituted with acetyl groups during acetylation and this modification of native starches improves their thermal stability, structural strength while reducing their swellability and water solubility. High acetyl substituted starch with a degree of substitution (DS) of >2 is of research interest because of their thermoplasticity [12,13]. As DS increases, the nature of the starch acetate changes from hydrophilic to hydrophobic and, simultaneously, the inter-particulate bonding capacity increases greatly [14]. It has been reported that drug release rate slows down as DS for starch acetate increases and starch acetates of specific DS are suitable for controlled release application because of their excellent bond-forming ability [15].

In most studies, official or proprietary starches such as corn, rice and potato have been used to modulate drug release from microspheres [16–20]. Modification of starches from botanical sources other than the official ones may yield starches that offer a wide

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range of functional properties permitting such applications. New, underutilized starches that could therefore be explored in drug delivery are the starches from bitter yam (also called trifoliate yam) and Chinese yam obtained from the tubers of *Dioscorea dumetorum* Pax and *Dioscorea oppositifolia* L (family Dioscoreaceae) respectively. The yams are known to possess high starch content [21,22]. Thus, the aim of the study is to modify bitter yam and Chinese yam starches by acetylation and then utilize the acetylated starches as polymers in microsphere formulations of repaglinide in comparison with ethyl cellulose, a water-insoluble polymer that is used as a coating material for sustained release microspheres. The model drug for this study is the antidiabetic agent, repaglinide. Repaglinide is a Biopharmaceutical Classification System (BCS) class II compound that is used in the treatment of Type II diabetes mellitus and differs from many other antidiabetic agents in its structure, binding profile, duration of action and mode of excretion [23]. Repaglinide is poorly soluble but highly permeable and exhibits bioavailability that is limited by its dissolution rate. Its low bioavailability is attributed to its extensive first pass metabolism and short half-life of about 1 h [24]. This necessitates it to be administered in several doses daily. From many patients' perspective, an ideal therapy for diabetes would have few steps involved in its administration, would be oral rather than injectable, and could be administered any time of the day and given once [25]. Repaglinide is known to have poor absorption in the upper intestinal tract [26]. The development of prolonged release dosage form of repaglinide would be a more convenient alternative to the conventional tablet dosage formulations, would lead to lower peak to trough plasma level fluctuations and, hence, reduce undesirable side effects. A release–retarding polymer will play a vital role in sustaining drug release from the microspheres. Acetylated starches of bitter and Chinese yams developed in our lab [27] will be utilized as polymers in repaglinide microspheres using the emulsification solvent-evaporation method. The microspheres formed will be characterized and the drug release properties of the microspheres will be evaluated by *in vitro* drug dissolution studies.

2. Materials and methods

2.1. Materials

Repaglinide was obtained from Hangzhou Danjang Chem Co Ltd, China and ethyl cellulose (ETHOCEL 20cps) was obtained from Colorcon, UK. Tubers of Chinese and bitter yams were obtained from local farmers in Ibadan, Nigeria and authenticated with voucher specimen with registry numbers FHI no 109673 and 109674 respectively. All other reagents were of analytical grade.

2.2. Methods

2.2.1. Acetylation of starches

Acetylation of the starch was done as reported [27]. Briefly, 25 g of pre-gelatinized starch was dispersed in 200 g of pyridine in a 1-l round-bottom flask followed by addition of 100 g of acetic anhydride. The flask was fitted to a rotary evaporator attached to a reflux condenser on the top with the round-bottom flask heated in an oil bath at 100 °C in a fume hood for 4 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 [13]. The degree of substitution was determined as reported by Ogawa et al. [28].

Table 1
Composition of repaglinide microsphere formulations.

Material	Drug:Polymer ratio		
	1:5	1:8	1:10
Repaglinide (g)	0.40	0.25	0.20
Starch acetate or Ethyl cellulose (g)	2.0	2.0	2.0
Chloroform (mL)	100	100	100
0.5% w/v SCMC solution (mL)	2000	2000	2000

2.2.2. Solid state characterization of starches

2.2.2.1. Morphology. The shape and size of the native and modified starch granules were observed using a scanning electron microscope (Hitachi SU8030 FE-SEM Tokyo, Japan) at an accelerating potential of 5.0 kV. All samples were sputter-coated with Au/Pd prior to examination.

2.2.2.2. ^1H NMR analysis. Starch (0.015 g) was dissolved in 1 ml of deuterated Dimethyl Sulphoxide (DMSO-d_6) for 6 h with stirring. The solution was centrifuged at 10,000 × *g* for 5 h and the supernatant was measured. ^1H NMR spectra were recorded on a NMR spectrometer (400 MHz Agilent DD MR-400 system equipped with Agilent 7600 96-sample auto sampler) [27].

2.2.2.3. FT-IR analysis. The native and acetylated starches were analyzed by FTIR (FTIR-Thermo Nicolet Nexus 870 Madison, WI, USA) in transmission mode. Transmission spectra were recorded using at least 64 scans with 8 cm^{-1} resolution in the spectral range 4000–400 cm^{-1}.

2.2.2.4. Swelling. Starch powder (5 g) was placed into a 100 ml measuring cylinder and the volume occupied was noted (V_1). Deionized water (90 ml) was added; the dispersion was shaken for 2 min and then made up to volume. The slurry was allowed to stand for 24 h before the sedimentation volume was read (V_2). The swelling index was calculated as V_2/V_1. Determinations were done in triplicates [27].

2.2.3. Preparation of microspheres

Several preformulation trials of the microspheres were initially prepared by varying the ratio of the modified starches to drug, concentration of dispersion agent, stirring speeds and curing times. Starch acetate (2 g) was dissolved in warm chloroform solvent (100 ml) to form a homogenous solution (2%w/v). Repaglinide (0.4 g), was added to the starch acetate solution and mixed thoroughly. The resulting mixture was added in a thin stream to 2 l of water containing 0.5% w/v sodium carboxylmethylcellulose as dispersing agent inside a 4-L beaker while stirring at 1000 rpm (Talboy mechanical stirrer model 103, USA) to emulsify the added dispersion as fine droplets. The solvent (chloroform) was then removed by continuous stirring at room temperature (28 °C) for 3 h to produce spherical microspheres, which were collected by vacuum filtration and washed repeatedly with water. The product was air dried to obtain discrete microspheres. The procedure was repeated using different starch acetate-drug ratios and ethyl cellulose as standard [29]. The detailed formulation contents of the optimized microspheres are presented in Table 1.

2.2.4. Experimental design

A full 3^2 factorial experimental design was performed using two factors, each at three levels as reported [30]. The nine possible combinations are shown in Table 2. 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 time taken for 50% drug release (t_{50}) were selected as dependent variables. Stirring speed, polymer concentration, concentration of

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dispersing agent and curing time were kept constant. The data were subjected to multiple regression analysis using statistical software (Minitab 17 Software USA.). The model incorporating first order polynomial terms was used to evaluate the responses and the fitted equation was $Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2$, where Y is the dependent variable, b_0 is the arithmetic mean response of the 9 runs and b_1 is the estimated coefficients for the related factor X_1. 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 the two factors change simultaneously. The polynomial terms (X_1^2 and X_2^2) are included to investigate non-linearity. Each experiment was conducted in triplicates and the means determined.

2.2.5. Characterization of microspheres

2.2.5.1. Scanning electron microscopy. The morphology and surface characteristics of the microspheres were observed using a scanning electron microscope (Hitachi SU8030 FE-SEM Tokyo, Japan) at an accelerating potential of 5.0 kV. All samples were sputter-coated with Osmium tetra oxide prior to examination using the Osmium plasma coater (OPC 60 X1, Japan).

2.2.5.2. FT-IR analysis. The physical mixture of the drug and polymer, drug-loaded microspheres, pristine drug and polymers were analyzed by FTIR (FTIR-Thermo Nicolet Nexus 870 Madison, WI, USA) in transmission mode. Transmission spectra were recorded using at least 64 scans with 8 cm^{-1} resolution in the spectral range 4000–400 cm^{-1}.

2.2.5.3. DSC analysis. DSC analysis of the drug-polymer mixture, drug-loaded microspheres, pristine drug and polymer was done using a differential scanning colorimeter (TA Instruments DSC 2920 Newcastle, Delaware, USA) in sealed stainless steel pans. The sample pan and the reference pan were heated from −20 to 250 °C at a scanning rate of 5 °C/min, held for 2 min at 250 °C and cooled to −20 °C at a rate of 20 °C/min.

2.2.5.4. X-ray diffraction analysis. The X-ray diffraction pattern was recorded for the samples with a copper anode x-ray tube (Cu-Kα_1 radiation) using an X-ray diffractometer (Rigaku D-max Tokyo, Japan). The samples were exposed to the X-ray beam at 40 kV and 20 mA. The scanning region of the diffraction angle (2θ) was from 5° to 60° at step size count of 2 s.

2.2.5.5. Swelling index. For estimating the swelling index, 1 ml of microsphere bed was soaked in 5 ml phosphate buffer (pH 7.4) in 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 (ml).

2.2.5.6. Entrapment efficiency. Drug-loaded microspheres (50 mg) were accurately weighed, crushed and suspended in 10 ml of phosphate buffer, pH 6.8. After 24 h, the solution was filtered and the filtrate was appropriately diluted using phosphate buffer, pH 6.8 and analyzed using UV/VIS spectrophotometer (Beckman Coulter DU 730 Life Science UV/Vis Spectrophotometer Fullerton, CA USA) at 282 nm. The drug entrapment efficiency (E) was calculated using the formula:

$$E(\%) = \frac{Actual\ drug\ content}{Theoretical\ drug\ content} \times 100$$

2.2.5.7. Drug release study. The *in vitro* dissolution studies were carried out using the paddle method (USP XXXVI), rotated at 50 rpm in 900 ml of phosphate buffer, pH 6.8, containing 2%w/v SDS and maintained at 37 ± 0.5 °C. The quantity of microspheres equivalent to 10 mg of repaglinide were estimated. Samples (5 ml) were withdrawn at different intervals and replaced with equal amounts of fresh medium. Each sample was diluted and the amount of repaglinide released was determined at wavelength of 282 nm, using a uv/visible spectrophotometer (Beckman Coulter DU 730 Life Science UV/vis Spectrophotometer USA**).** Determinations were done in triplicates.

2.2.6. Kinetic models and comparison of release profiles

Drug release data were fitted to zero order, first order, Higuchi [31], Hixon-Crowell [32], Korsemeyer – Peppas [33] and Hopfenberg equations [34]. The model of best fit was identified by comparing the values of correlation coefficients.

2.3. Data analysis

The differences between the formulations were determined by using the analysis of variance (ANOVA) in GraphPad Prism© 4 (Graphpad Software Inc. San Diego, CA). At the 95% confidence interval, *p* values, less than or equal to 0.05 were considered significant.

3. Results

3.1. Characterization of acetylated starches

The swelling index of native bitter and Chinese yam starches were 1.04 ± 0.04 and 1.17 ± 0.03 while those of the acetylated starches were respectively 0.94 ± 0.03 and 1.05 ± 0.05. The scanning electron micrographs of the yam starches comparing the morphology of the native and modified forms of the starches are presented in Fig. 1. ^{1}HNMR and FTIR spectra of the native and acetylated starches were measured to provide evidence of acetylation of the native starches and are presented in Figs. 2 and 3 respectively.

3.2. Characterization of microspheres

The composition of materials used in the various formulations of repaglinide microspheres is presented in Table 1. Scanning electron micrographs of microspheres containing acetylated yam starches and ethyl cellulose are presented in Fig. 4. Representative FTIR spectra of acetylated bitter yam starch, pristine drug and microspheres are presented in Fig. 5 while Fig. 6a and 6b show the DSC and XRD spectra for the assessment of thermal properties and crystallinity respectively. The values of particle size, swelling index and entrapment efficiency of the microspheres are shown in Table 2. The drug dissolution profiles of the microsphere formulations are shown in Fig. 7a. From the plots in Fig. 7a, the time taken for 50% drug release (t_{50}) were determined and are also presented in Table 2. The drug release of the microspheres were simulated with different kinetic models (first order, Higuchi, Hixon-Crowell, Korsemeyer – Peppas and Hopfenberg), and the values of their correlation coefficients are presented in Table 3. The drug release kinetics generally fitted the Korsemeyer – Peppas and Hopfenberg models and their plots are presented in Fig. 7b and c respectively. Results of the multiple regression analysis are summarized in Table 4. Surface plots were generated for the dependent variables, particle size, swelling, entrapment and t_{50}, and are shown in Fig. 8.

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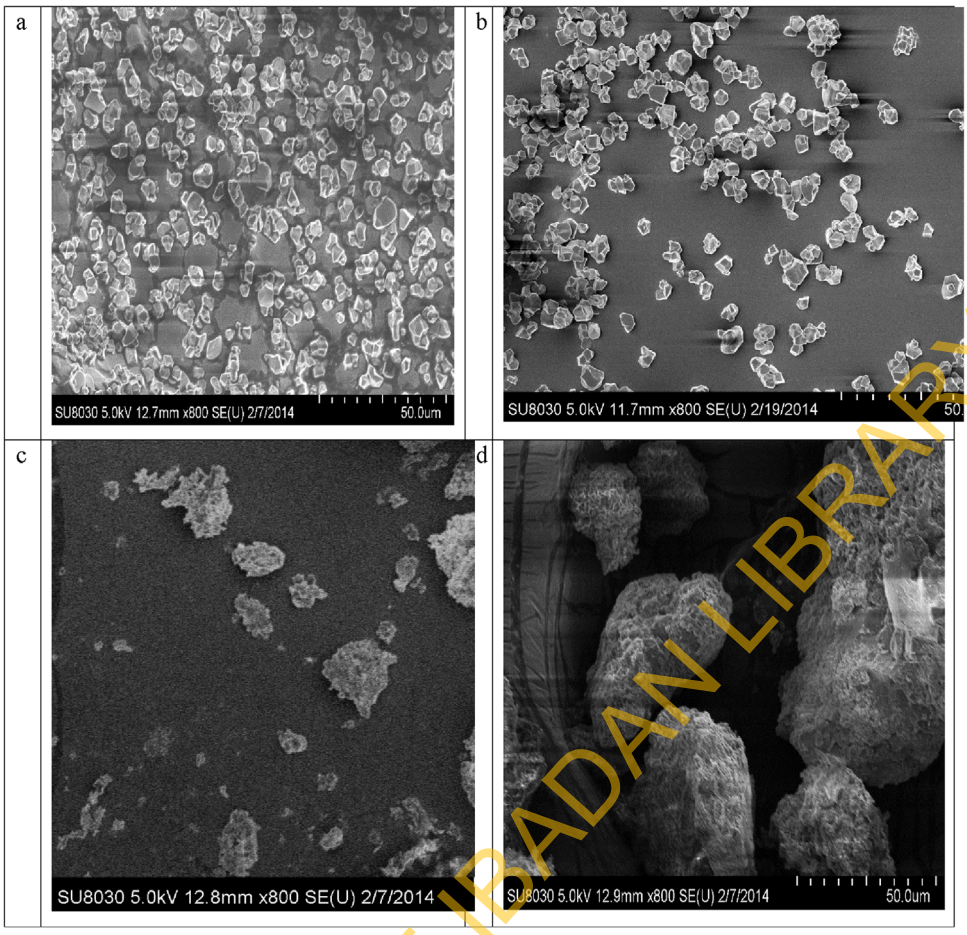

Fig. 1. SEM of: (a) native bitter yam starch; (b) native Chinese yam starch; (c) acetylated bitter yam starch; (d) acetylated Chinese yam starch Mg ×800.

Table 2
The 3^2 Factorial design for the repaglinide microspheres formulations.

Batchcode	Variable levels		Real values					
	X_1	X_2	X_1 (Polymer type)	X_2(Drug:Polymer ratio)	Particle size(μm)	Swelling	Entrapment (%)	t_{50} (h)
B_1	−1	−1	Acetylated Bitter yam starch	1:5	80 ± 5.00	1.20 ± 0.00	83.92 ± 4.00	4.00 ± 0.71
B_2	−1	0	Acetylated Bitter yam starch	1:8	120 ± 9.25	1.10 ± 0.01	89.64 ± 3.55	6.00 ± 0.14
B_3	−1	+1	Acetylated Bitter yam starch	1:10	280 ± 11.50	1.04 ± 0.01	92.63 ± 2.50	6.70 ± 0.49
B_4	0	−1	Acetylated Chinese yam starch	1:5	160± 4.15	1.40 ± 0.04	75.30 ± 3.03	3.20 ± 0.42
B_5	0	0	Acetylated Chinese yam starch	1:8	280 ± 14.20	1.14 ± 0.00	81.27 ± 2.65	4.20 ± 0.44
B_6	0	+1	Acetylated Chinese yam starch	1:10	350 ± 18.10	1.04 ± 0.01	88.92 ± 2.35	5.30 ± 0.21
B_7	+1	−1	Ethyl cellulose	1:5	50 ± 4.00	1.10 ± 0.01	82.35 ± 3.50	4.40 ± 0.34
B_8	+1	0	Ethyl cellulose	1:8	130 ± 10.12	1.04 ± 0.02	88.56 ± 3.12	6.00 ± 0.40
B_9	+1	+1	Ethyl cellulose	1:10	300 ± 24.00	1.01 ± 0.00	93.10 ± 2.75	7.20 ± 0.55

Table 3
Correlation coefficients obtained for repaglinide microspheres using different kinetic models (n = 3).

Formulation	Zero order	First order	Higuchi	Hixson-Crowell	Korsmeyer		Hopfenberg
					R^2	n	
B_1	0.909	0.837	0.817	0.774	0.925	0.295	[a]0.939
B_2	0.908	0.839	0.788	0.892	[a]0.982	0.556	0.945
B_3	0.926	0.869	0.770	0.910	[a]0.974	0.665	0.966
B_4	0.930	0.784	0.743	0.745	0.929	0.439	[a]0.992
B_5	0.924	0.789	0.813	0.932	[a]0.975	0.522	0.963
B_6	0.931	0.839	0.808	0.891	[a]0.996	0.455	0.970
B_7	0.864	0.763	0.782	0.901	[a]0.965	0.344	0.904
B_8	0.931	0.713	0.868	0.857	[a]0.953	0.454	0.919
B_9	0.949	0.800	0.766	0.898	[a]0.993	0.539	0.946

[a] Highest correlation coefficient of drug release kinetics.

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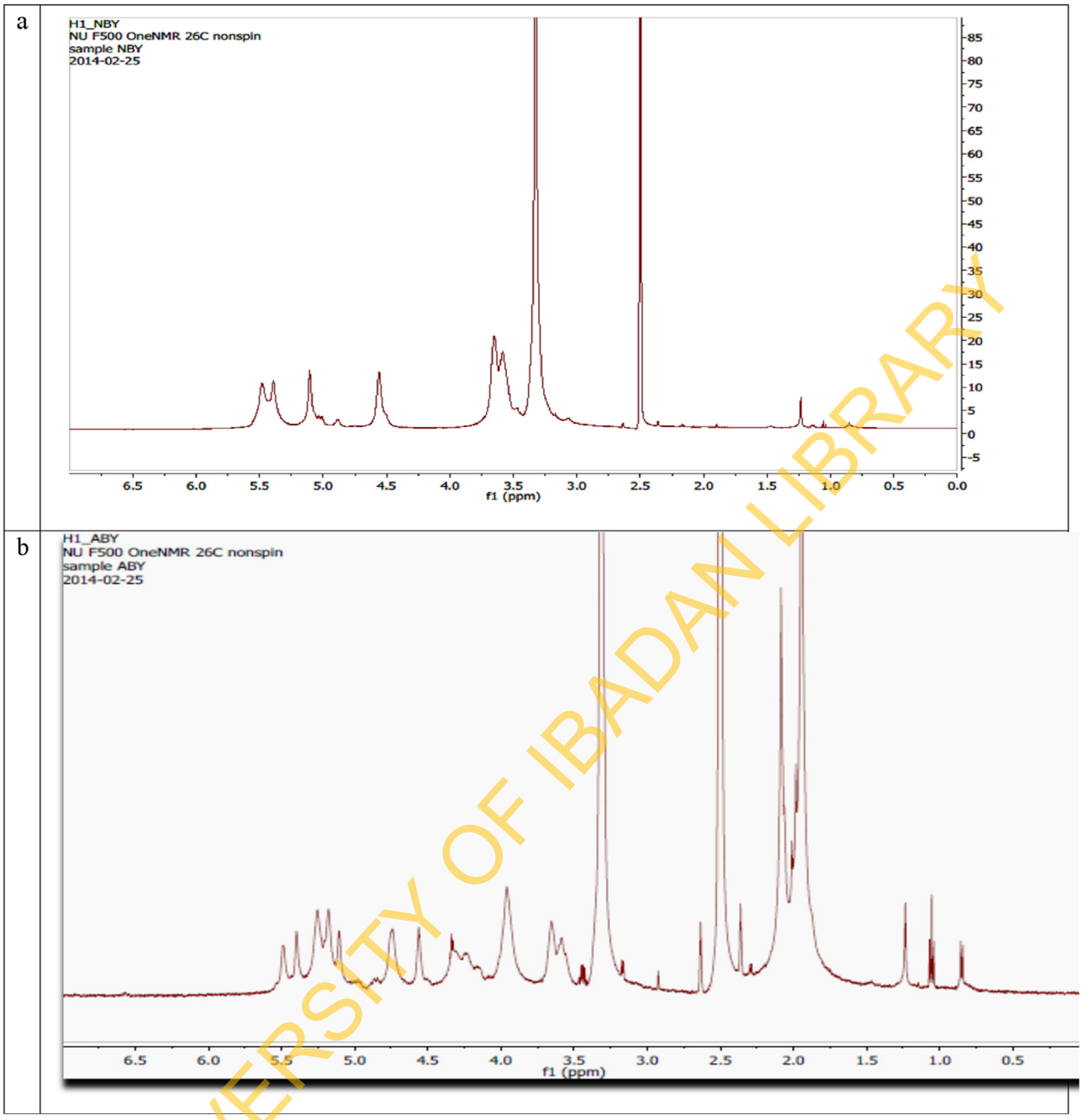

Fig. 2. NMR spectra of: (a) native bitter yam starch and (b) acetylated bitter yam starch.

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

Coefficients of parameters	Responses			
	Particle size(μm)	Swelling	Entrapment (%)	t_{50}(h)
b_o	194.40	1.12	86.19	5.22
b_1	0.00	−0.03	−0.36	0.15
b_2	1.07	−0.10	5.51	1.27
b_{12}	27.20	0.04	1.61	0.37
b_{11}	3.93	−2.75	3.42	3.45
b_{22}	22.90	0.05	1.04	0.21
R^2	0.960	0.818	0.972	0.979

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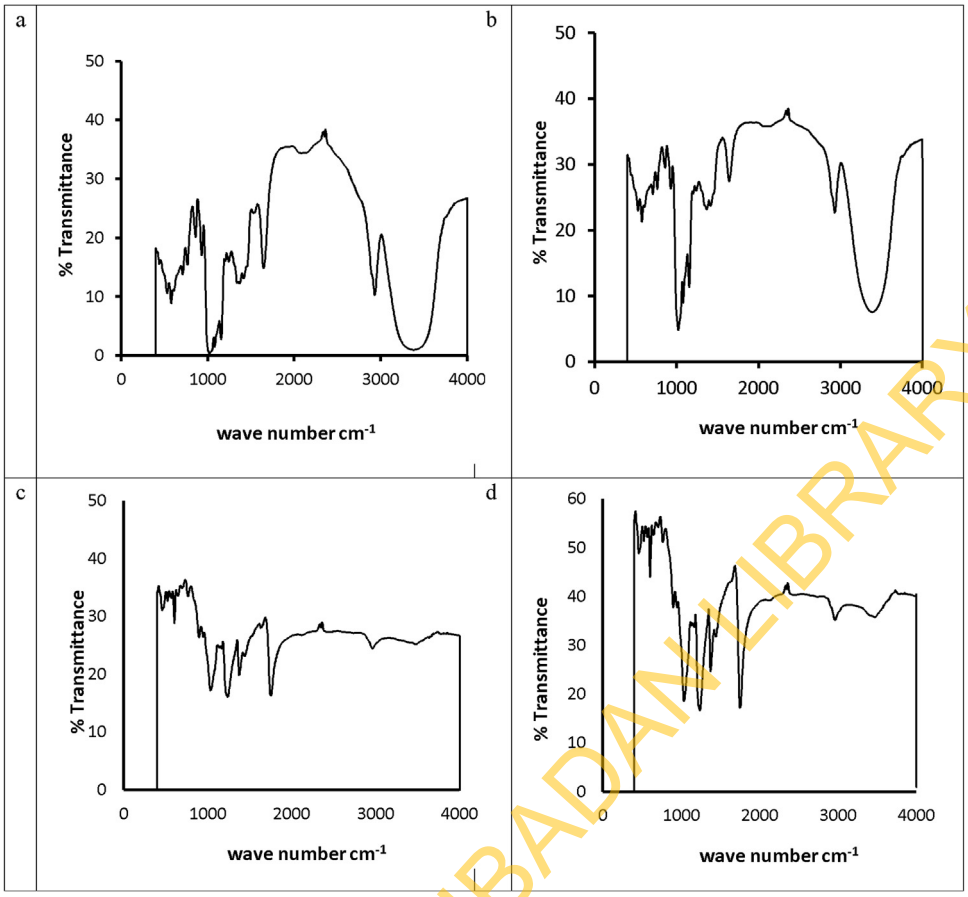

Fig. 3. FTIR spectra of: (a) native bitter yam starch; (b) native Chinese yam starch; (c) acetylated bitter yam starch; (d) acetylated Chinese yam starch.

4. Discussion

4.1. Characterization of starches

Acetylated bitter and Chinese yam starches with degrees of substitution of 2.56 and 2.70 respectively were obtained. During acetylation, free hydroxyl groups on C_2, C_3 and C_6 of the starch molecules were substituted with acetyl groups [13,27]. Acetylation resulted in reduction in swelling of starches. Differences observed in the swelling of the starches was probably due to the starch species and degree of macromolecular disorganization [27].

4.1.1. Scanning electron microscopy

The scanning electron micrographs showed bitter and Chinese yam in their native forms had polygonal granules which on acetylation formed larger, fibrous-like aggregates.

4.1.2. Proton nuclear magnetic resonance spectroscopy

The proton resonances of the anhydroglucose units showed changes in the acetylated yam starches when compared to their native forms. The starch backbone signals were at 3.6–5.5 ppm while those due to the methyl protons of acetate were at 1.9–2.1 ppm. The peaks at 1.05 ppm indicated that the hydrogen atoms of the hydroxyl groups in the native starches were substituted with acetyl groups [35].

4.1.3. FTIR spectra

In the FTIR spectra of the native starches, characteristic absorption bands were observed at 992, 929, 861, 765 and 575 cm^{-1}. New bands at 1700 cm^{-1}(Stretching C=O), 1375 cm^{-1}(Stretching C—CH3) were observed for the acetylated yam starches. FTIR bands at 3400 cm^{-1} (Stretching O—H) and 1083 cm^{-1} (C—O—C bond stretching) were weakened, confirming the replacement of the hydroxyl groups in the starch molecules with acetyl group [27,35].

4.2. Characterization of microspheres

The relatively high proportion of polymers in the drug: polymer ratios selected for the study was based on the consideration that repaglinide is a small dose-high potency drug which require the bulking effect of a polymer in its formulations. The microspheres of yam acetate were near spherical in shape with some porous surface. Repaglinide microspheres containing ethyl cellulose were spherical, discrete with microporous surfaces. In the XRD spectra, pure repaglinide showed a typical diffractogram of the crystalline substance, with intensive peaks between 8° and 30°. The degree of drug crystallinity decreased in the X-ray diffraction patterns of the repaglinide-loaded beads. This decrease could result from the drug distribution over the polymeric matrix. The DSC thermograms of the repaglinide-loaded microspheres had a less prominent peak during analyses, indicating no strong interactions between the polymer and drug. The FTIR spectra did not reveal the formation of new chemical entities. These results confirm the drug's chemical stability and its ability to retain its biological activity within the microspheres.

The size of microspheres obtained was in the range of 80 ± 5.00 to 350 ± 18.10 μm and was generally in the rank order of Chinese yam > ethyl cellulose > bitter yam. Repaglinide microspheres con-

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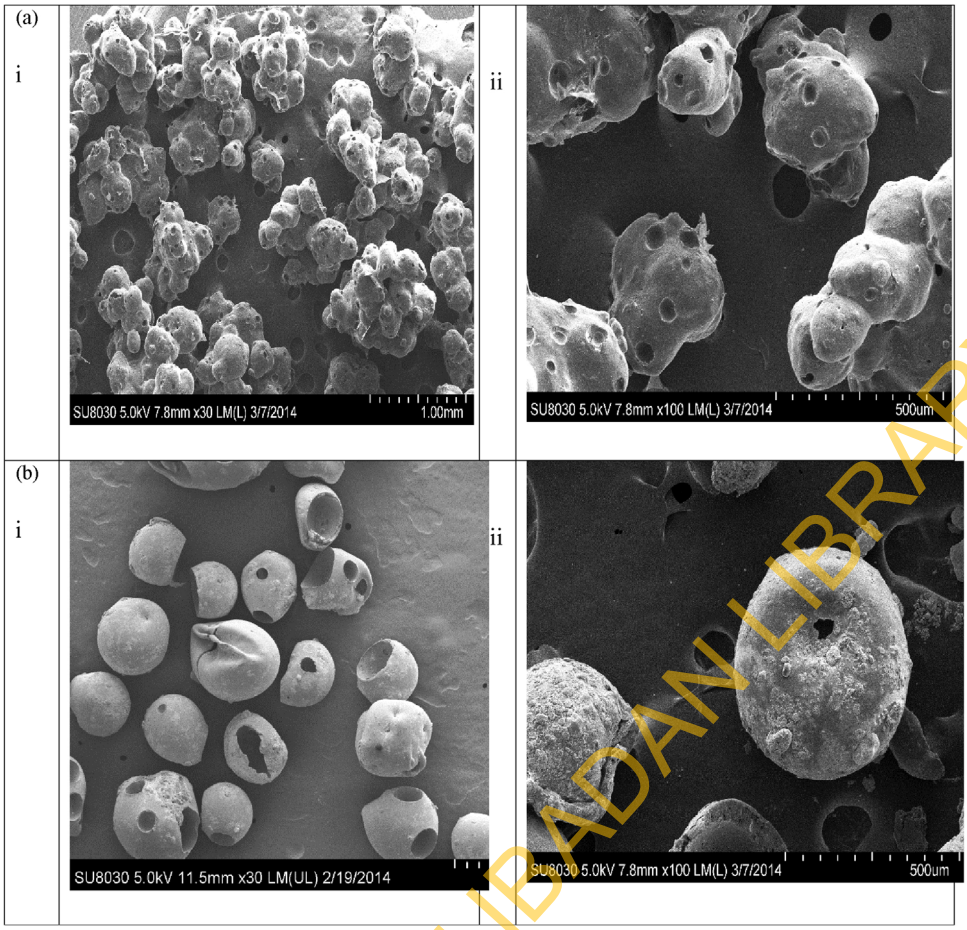

Fig. 4. SEM of repaglinide-loaded microspheres containing (a) Chinese yam starch (b) ethyl cellulose at magnification (i) ×30 and (ii) ×100 respectively.

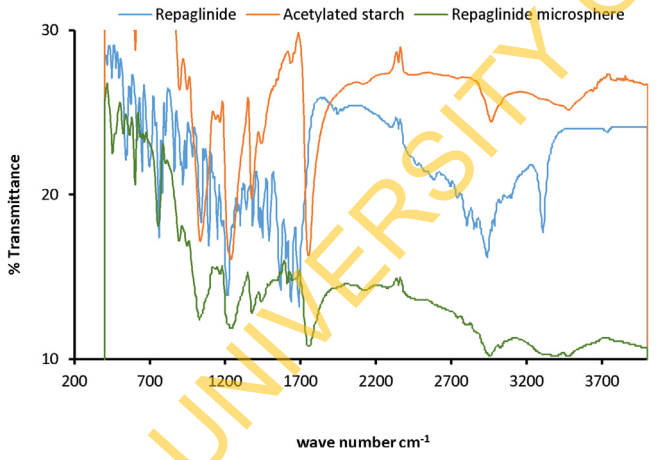

Fig. 5. FTIR spectra of: repaglinide; acetylated yam starch and repaglinide-loaded microsphere.

taining bitter yam had significantly higher entrapment efficiency ($p < 0.05$) than those containing Chinese yam but was not significantly different from those of ethyl cellulose ($p > 0.05$). Entrapment efficiency was in the range of 75.30 ± 3.03 to $93.10 \pm 2.75\%$ and was observed to increase with increase in amount of polymer. The relatively high entrapment of repaglinide could be attributed to its poor aqueous solubility in the dispersed phase.

Drug release from the microspheres was prolonged; involving a combination of erosion mechanism and diffusion through the hydrophobic acetylated starch. The values of the time taken for 50% drug release (t_{50}) was in the range of 3.20 ± 0.42 to 6.70 ± 0.49 h for the microspheres containing the acetylated yam starches. The dissolution time was observed to increase with increase in amount of acetylated starch. Bitter yam starch gave longer dissolution times than Chinese yam starch at all drug-polymer ratios. The microspheres containing ethyl cellulose at drug: polymer 1:10 gave the longest dissolution time of 7.20 ± 0.55 h. This may be attributed to the fact that the presence of starch rendered the gel matrix more porous than ethyl cellulose did, thereby facilitating drug release. The span of release of medicament from the microsphere formulations containing the starches was prolonged enough to justify the proposed polymer systems as potential drug release modulators for drug delivery systems.

4.3. Kinetic models of drug release

Simulating the drug kinetics with different models (first order, Higuchi, Hixon-Crowell, Korsemeyer – Peppas and Hopfenberg), drug release for the microspheres generally fitted the Korsmeyer-Peppas model, having the largest correlation coefficients ($R^2 \geq 0.95$). In this model, the value of n characterizes the release mechanism of drug. When $0.45 \leq n$, this corresponds to a Fickian mechanism; $0.45 < n < 0.89$ to non Fickian transport; $n = 0.89$ to case II (relaxational transport) and $n > 0.89$ to super case II transport [32,36]. From the values of the slopes, the drug release mechanism from the formulations containing the acetylated yam starches and ethyl cellulose is generally considered to

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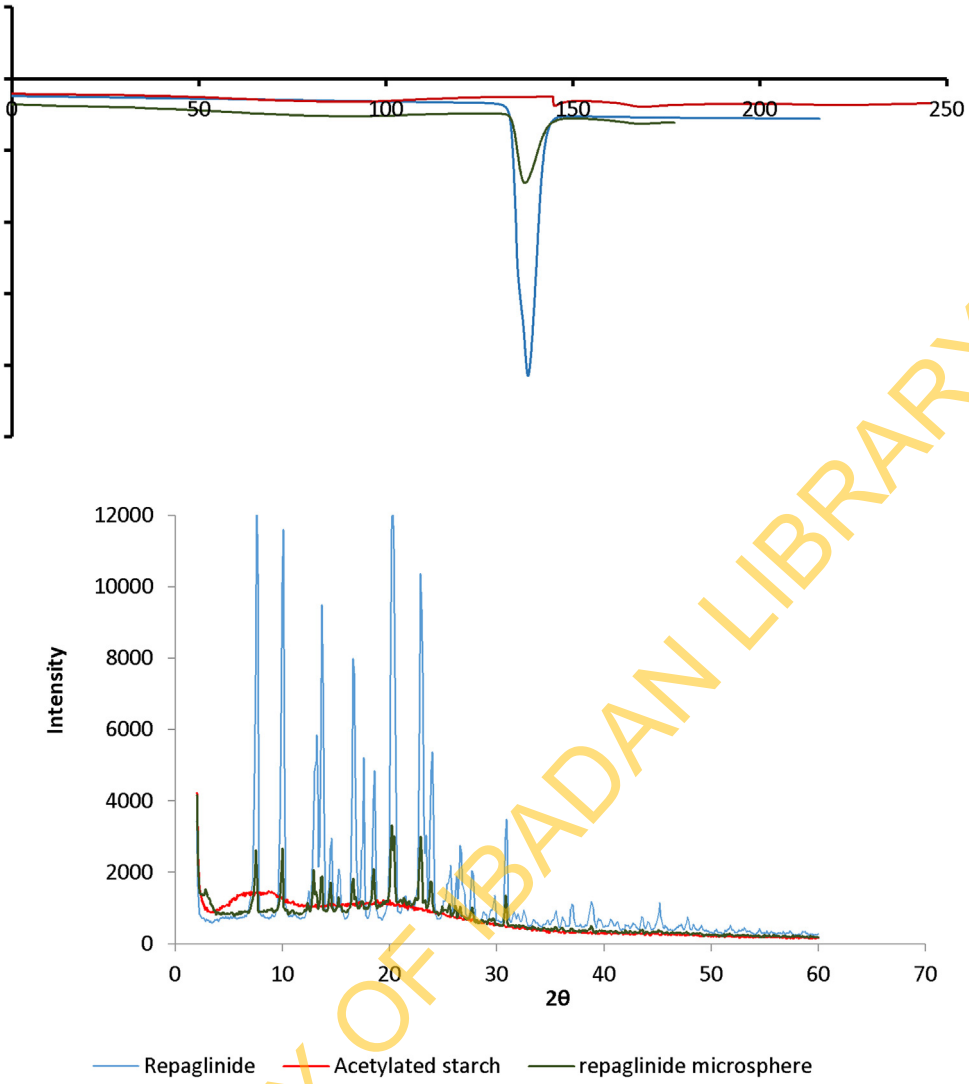

Fig. 6. (a) DSC endotherms and (b) XRD spectra of repaglinide; acetylated yam starch and repaglinide-loaded microsphere.

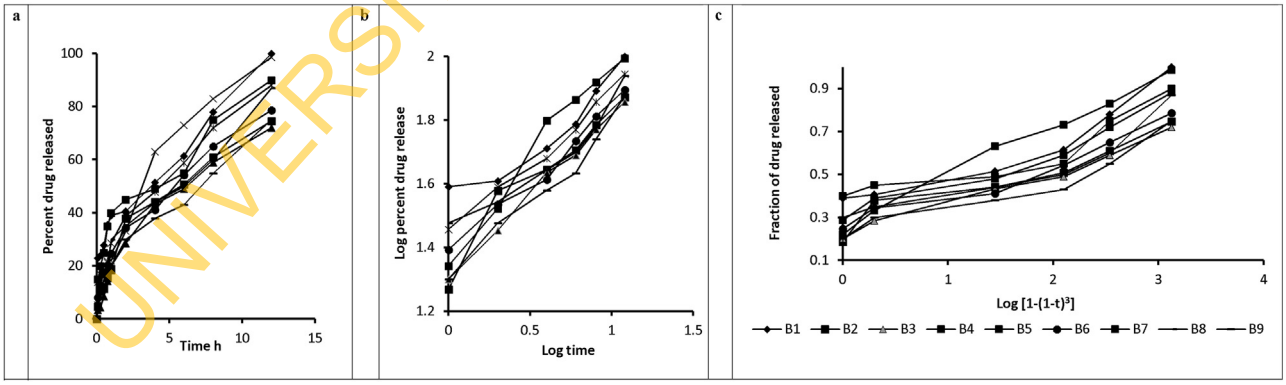

Fig. 7. (a) zero order; (b) Korsmeyer and (c) Hopfenberg plots for repaglinide microspheres.

be non-Fickian. However, the drug release for B_1 and B_4 (containing acetylated bitter and Chinese yams respectively at drug: polymer ratio of 1:5) fitted the Hopfenberg model with the correlation coefficient >0.93. The Hopfenberg model describes the release of drug from spherical formulations and is used to correlate the drug release from surface-eroding polymer so long as the surface remains constant during degradation process [34].

Regression parameters fitted the polynomial regression equation with correlation coefficient of ≥0.96 for almost all the dependent variables. The effects of particle size and dissolution time (t_{50}) on factors, X_1 and X_2 were positive, indicating that these variables increased with both change in polymer type from acetylated yam starches to ethyl cellulose and with increase in the amount of polymer. On the other hand, swelling and entrap-

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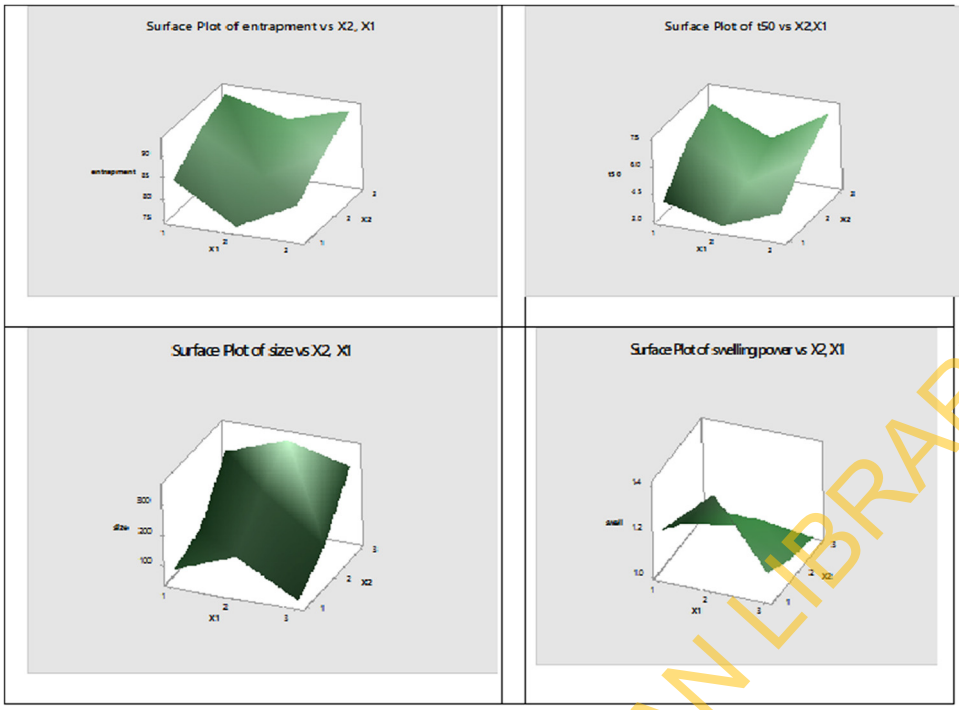

Fig. 8. Surface plots showing effect of independent variables (X_1 and X_2) on all the dependent variables.

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	21356	10677.80	11.31	0.023
	X_2	2	69689	34844.40	36.89	0.003
	Residual	4	3778	944.40		
	Total	8	94822			
Swelling	X_1		0.031	0.015	2.90	0.167
	X_2	2	0.065	0.033	0.08	0.061
	Residual	6	0.021	0.009		
	Total	8	0.117			
Entrapment efficiency	X_1	2	86.25	43.14	21.97	0.007
	X_2	2	182.79	91.40	46.57	0.002
	Residual	4	7.85	1.94		
	Total	8	276.89			
t_{50}	X_1	2	4.54	2.27	29.16	0.004
	X_2	2	9.77	4.88	62.80	0.001
	Residual	4	0.31	0.08		
	Total	8	14.62			

ment efficiency showed negative effects on factor X_1 indicating that changing the polymer type from yam starches to ethyl cellulose resulted in decrease in swelling and entrapment efficiencies of the microspheres. The effects of swelling on factor X_2 was also negative indicating that an increase in amount of polymer resulted in reduction in swelling. The effect of drug: polymer ratio on all response variables of the microspheres was significantly ($p < 0.01$) higher than those of polymer type. The interactive term $X_1 X_2$ showed positive effects on all response variables indicating that these increased when both factor were simultaneously increased.

The results of the two-way ANOVA presented in Table 5 indicate that the particle size, entrapment efficiency and dissolution time were significantly ($p < 0.05$) dependent on the type of polymer present in the formulation while the effects of polymer type was not statistically significant ($p > 0.05$) for swelling. The results also indicate that the drug: polymer ratio significantly ($p < 0.05$) affected all the microsphere properties except swelling. The positive value of X_2^2 show positive linearity in the effectiveness of polymers to increase all the microsphere variables as the amount is increased. Surface plots, the graphic representations of the influence of the factors on the properties of the microspheres, were generated for all dependent. Generally, the steeper the slope the stronger the interaction between the variables. The surface plots show that the polymer type and drug-polymer ratio interacted strongly to decrease swelling and size but increased entrapment and dissolution time of the microspheres.

5. Conclusion

Acetylated starches of bitter and Chinese yam starches were used as polymers in the formulation of repaglinide microspheres using the emulsion-solvent evaporation method. The method of preparation was simple and was found to be reproducible. Formulations containing bitter yam starch had significantly higher entrapment efficiency than those of Chinese yam but similar entrapment to those containing the standard, ethyl cellulose. The developed microsphere formulations containing the acetylated yam starches were found to be effective in providing prolonged

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release of repaglinide from the microspheres which showed a dependence on the amount of polymer present in the formulation. Drug release for the microspheres containing the acetylated yam starches and ethyl cellulose generally fitted the Korsmeyer-Peppas and Hopfenberg models. Acetylated bitter and Chinese yam starches could potentially serve as cheaper alternatives to other polymers in drug delivery.

Declaration of interest

The authors declare no conflict of interest.

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