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Acetylated Starch of Ofada Rice as a Sustained Release Polymer in Microsphere Formulations of Repaglinide

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: Acetylated starches with degrees of substitution (DS) of > 2 have been found suitable for sustained release applications because of their hydrophobic nature and thermoplasticity. The short half-life and high dosing frequency of repaglinide make it an ideal candidate for sustained release.
Objectives: To formulate and evaluate repaglinide microspheres using acetylated starch of the indigenous rice species *Oryza glaberrima* Steud (Ofada) as polymer.
Materials and Methods: Ofada rice starch was acetylated with acetic anhydride in pyridine (DS 2.68) and characterized for morphology (Scanning electron microscope, SEM), Crystallinity (Fourier Transform Infra-Red spectroscopy, FTIR, and X-ray diffraction crystallography, XRD), density and swelling. Microspheres of repaglinide were prepared by emulsification solvent-evaporation method, varying the drug-polymer ratio (1:2, 1:4, 1:8 and 1:10) and polymer type (ethyl cellulose as standard). Microspheres were characterized for particle size, wall thickness, swelling, entrapment efficiency, time taken for 80% drug release (t_{80}) and permeability. Data obtained from *in-vitro* drug release studies were fitted to various kinetic models.
Results: Repaglinide microspheres were near spherical, discrete and of size range 23.45 ± 4.25 to 44.55±3.85 µm. FTIR spectra revealed the absence of drug–polymer interaction and complete drug entrapment. Particle size, swelling, entrapment and wall thickness increased with drug: polymer ratio and were generally higher in microspheres containing acetylated Ofada rice starch while t_{80} (195±6.60 - 395± 24.75 min) was lower. Drug release fitted the Hixson-Crowell kinetic model.
Conclusions: The acetylated starch of Ofada rice was found suitable as a polymer to sustain the release of repaglinide in microsphere formulations.
Keywords: Acetylation, Ofada rice starch, Repaglinide, Microsphere, Sustained release

INTRODUCTION

Sustained-release drug delivery systems are systems that prolong the duration of action of a drug by slowing its release (Shen *et al*, 2004). They offer numerous advantages over conventional dosage forms which include reduction in the fluctuation of drug level that diminishes untoward side effects of the drug while improving therapeutic outcome as the reduction in dosing frequency enhances patient compliance. Of the different dosage forms reported, nanoparticles and microparticles have attained much importance and occupy a unique position in sustained drug delivery technology (Singh *et al*, 2010). Pharmaceutical applications of microspheres require highly reproducible dosage as well as the controlled release of active agents which cannot be achieved with conventional powders and granules. Such microspheres can be manufactured from various natural and synthetic polymer materials and challenges in this field of drug delivery include the search for new polymers. To be successfully used in sustained drug delivery formulations, a polymer material must be chemically inert, should not invoke an inflammatory or toxic response, should be readily process-able and must have acceptable shelf life. In addition, the material should be capable of being metabolized in the body after fulfilling its purpose, leaving no trace.

A majority of investigations of natural polymers as matrices in drug delivery systems have focused on proteins and polysaccharides such as starch. In recent years, starches have been considered as new potential biomaterials for pharmaceutical applications because of their unique physicochemical and functional characteristics (Cristina *et al*, 2009; Freire *et al*, 2009; Okunlola *et al*, 2012). Improvement on the functional properties and applicability of starches has been achieved with various modifications. The process of starch modification involves

the de-structurization of the semi-crystalline starch granules and the effective dispersion of the component polymer. In this way, the reactive site (hydroxyl groups) of the amylopectin polymer becomes accessible to reactants (Rajan *et al*, 2008). There are a number of chemical modifications made to starch to produce many different functional characteristics and these include acetylation, acidification, etherification, oxidation, cationization, cross-linking and grafting of starches. Acetylated starches are distinguishable through their high levels of shear strength; they are particularly stable to heat, acid and form flexible, water insoluble films (Okunlola *et al*, 2015). As the degree of substitution increases, the nature of the starch acetate changes from hydrophilic to hydrophobic and simultaneously the inter-particulate bonding capacity increases greatly (Korhonen *et al*, 2002). Official starches such as potato starch have been modified by acetylation and were reported to substantially retard the release of drug, thus allowing sustained drug release (Tuovinen *et al*, 2003).

New underutilized starches that could be explored in sustained drug delivery are those obtained from the indigenous rice species *Oryza glaberrima* Steud (Ofada rice). Ofada rice is an important crop that has recently gained prominence in Nigeria and is fast gaining international attention (Danbaba *et al*, 2011). It has been cultivated and processed in many communities in Ogun state and some rice producing clusters in South West Nigeria (Danbaba *et al*, 2011; Ologbon *et al*, 2012). The high starch content of the crop makes it a cheaper source of starch that can be utilized in the pharmaceutical industries. Repaglinide is the first member of the group of meglitinides, a new class of insulin secretagogues anti-diabetic agent which was approved for clinical use by the FDA in 1998. These drugs modulate β-cell insulin release by regulating potassium efflux through the potassium channels. They have no direct effect on insulin exocytosis and can be indicated for use in type 2 diabetic individuals with sulfur or sulfonylurea allergy (Katzung, 2001). Repaglinide has a very fast onset of action, with a peak concentration and peak effect within approximately 1 hour after ingestion. Its low bioavailability is attributed to its short half-life of which necessitates it to be administered in several doses daily, thus reducing high level of patient acceptance and long term compliance. This makes it an ideal candidate for sustained release.

Thus, in this study, Ofada rice starch was modified by acetylation and then utilized as sustained-release polymer in microsphere formulations of repaglinide in comparison with standard ethyl cellulose, a water-insoluble polymer that is used as a coating material in microsphere formulations. The microspheres were formulated using the emulsification solvent-evaporation method.

MATERIALS AND METHODS

The materials used were Chloroform AR (PS Park Scientific Limited, Northampton, United Kingdom), ethyl cellulose (ETHOCEL 20cps) (Colorcon, UK) and repaglinide (purchased from Hangzhou Danjang Chem Co Ltd, China). Grains of Ofada rice were obtained from farmers in Shagbon village, Ogun State, Nigeria.

Starch Extraction

Starch was extracted from Ofada rice grains by soaking in distilled water. The mixture was blended to obtain slurry that was strained through muslin cloth followed by settling of the filtrate. The supernatant was decanted at 12 hours intervals and the starch slurry re-suspended in distilled water. The starch cake was collected after 72 hours and dried in a hot air oven at 60 ^{o}C for 48 hours. The dried mass was pulverized and then screened through a sieve of size 250 µm (Young, 1984).

Acetylation of Starch

Fifty grams of native starch was suspended in 550 ml of de-ionized water in a 1000 mL conical flask. The suspension was gelatinized by stirring below 100 ºC for 30 min over a hotplate. The gelatinized starch was precipitated with one Liter of anhydrous ethanol, stirring under a high shear homogenizer (Talboys Laboratory Stirrer LLC, Model No: 103 Troemner, USA). The precipitated material was filtered and the residue washed with acetone, filtered again and dried. The dried powder was screened (sieve size 125 µm).

Twenty five grams of the pregelatinized starch was dispersed in 200 g of pyridine in a 1 Liter round-bottom flask. One hundred grams of acetic anhydride was added to the dispersion. The flask was fitted to a rotary evaporator attached to a reflux condenser (Rotavapor R-100, Buchi, Switzerland) on the top. The round bottom flask was dipped into an oil bath and rotated at low speed inside a fume hood. The temperature was maintained at 100 ºC. The reaction was carried out for 4 hours with continuous stirring. After 4 hours, the reaction mixture was transferred to a beaker and cooled to room temperature. The product was precipitated from 1300 mL of ethanol under high shear homogenization. The precipitate was filtered, washed well with ethanol to remove the pyridine odor in the precipitate and then filtered again. It was dried in an oven and then screened using sieve size 125 µm (Singh and Nath, 2012).

Determination of degree of substitution

One gram of starch acetate and 50 mL of 75 % ethanol were mixed in a flask with a loose stopper. The mixture was stirred in a water bath at 50 ^{o}C for 30 min. After cooling to room temp, 40 mL of 0.5 N potassium hydroxide (KOH) solution was added to the mixture. The flask was fitted with a tight stopper and kept at room temperature with occasional shaking for 72 hours for complete saponification. An excess of alkali in solution was titrated with 0.5 N HCl solution using phenolphthalein as the indicator. A blank test was performed following the same procedure. The percent of acetyl group and degree of substitution (DS) were calculated as shown (Ogawa *et al*, 1999):

$$Acetyl\ group\ (\%) = \frac{(value\ of\ blank - value\ of\ sample)(ml)\ X\ Molarity\ of\ HCl\ X\ 0.043}{Sample\ weight\ (g)}\ X\ 100 \quad (1)$$

$$DS = \frac{162\ X\ \%\ Acetyl\ group}{4300 - (42\ X\ \%\ Acetyl\ group)} \quad (2)$$

Where 162 is the molecular weight of the anhydroglucose unit, 42 is the molecular weight of replaceable acetyl group and 4300 is the molecular weight of the acetyl group attached with 100 anhydroglucose unit.

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.

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}.

X-Ray Diffraction Analysis

The X-ray diffraction pattern was recorded with a copper anode x-ray tube (Cu-K$\acute{a}_1$ radiation) using an X-ray diffractometer (Rigaku D-max 2550 Tokyo, Japan). The scanning region of the diffraction angle (2θ) was from 5 ° to 60 ° at step size count of 2.

Determination of flow properties

Density measurement

A 50 mL capacity pycnometer was weighed empty (W), filled with the non-solvent (xylene) and the excess wiped off. The weight of the pycnometer with the non-solvent was determined (W_1). The difference in weight was calculated as W_2. A 2 g quantity of the sample was weighed (W_3) and quantitatively transferred into the pycnometer bottle. The excess non-solvent was wiped off and the pycnometer was weighed again (W_4).The particle density was calculated from the equation:

$$\frac{W2.W3}{50(W3-W4+W)gcm^{-3}} \quad (3)$$

The determinations were done in triplicate.

The bulk density of each starch powder at zero pressure (loose density) was determined by pouring 10 g of the powder at an angle of 45° through a funnel into a glass measuring cylinder with a volume of 50 mL. Determinations were done in triplicate.

The tapped density was measured by applying 100 taps to 10 g of starch sample in a graduated cylinder at a standardized rate of 38 taps per minute from a height of 2.54 cm (British standard 1460). Determinations were done in triplicate.

The flowability of the starches was assessed using the Hausner ratio and the Carr index.

Hausner's ratio =Tapped density/Bulk density.

$$Carr's\ Index = \frac{(Tapped\ density - Bulk\ density)}{Tapped\ density}\ X\ 100 \quad (4)$$

Angle of Repose

An open ended cylinder was placed on a base of similar diameter. Starch powder (5 g) was allowed to flow freely through a funnel under gravity, to form a conical heap. The angle of repose was calculated from:

$$Tan\ \theta = {}^{h}/_{r} \quad (5)$$

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.

Preliminary Formulation Studies

Pre-formulation studies were carried out in order to optimize the microsphere formulations. Several formulation trials were done with varying ratios of the polymer: drug, concentrations of dispersion agent, stirring speeds and curing times.

Preparation of Microspheres

Starch acetate (1 g) was dissolved in chloroform solvent (50 mL). Repaglinide (0.5 g) was added to the polymer solution and mixed thoroughly to form a homogenous blend. The resulting mixture was then added in a thin stream to 1 Liter of water containing 0.5 %w/v sodium carboxyl-methyl cellulose (SCMC) inside a 2 Liter beaker, while stirring at 1000 rpm with a mechanical stirrer (Talboy mechanical stirrer model 103, USA). The dispersion was emulsified as fine droplets. The solvent (chloroform) was then removed by continuous stirring at room temperature (28 ºC) for 2 hours to produce spherical microspheres which were collected by filtration and washed repeatedly with distilled water. The product was then air dried to obtain discrete microspheres. The procedure was repeated using polymer: drug ratios (4:1; 8:1 and 10:1) and ethyl cellulose as standard (Chowdary *et al*, 2010).

Characterization of Microspheres:

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. The microspheres were sputter-coated with Au/Pd prior to examination.

FT-IR Analysis

The drug-loaded microspheres, pristine drug and starch acetate polymer were analyzed by 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

For estimating the swelling index, 1 mL of microsphere bed was soaked in 5 mL phosphate buffer (pH 6.8) in a 10 ml measuring cylinder for 12 hours and swelling index was calculated as the ratio of the volume after 12 hours to that of the original volume.

Entrapment Efficiency

The quantity of microspheres that is equivalent to 50 mg of repaglinide drug content were accurately weighed, crushed

and suspended in 50 ml of phosphate buffer, pH 6.8. After 24 hours, the solution was filtered. The filtrate was appropriately diluted with phosphate buffer, pH 6.8 and analyzed using UV/VIS spectrophotometer (Veego UV-VIS Model 3 Spectrophotometer, Mumbai, India) at 240 nm. The drug entrapment efficiency (E) was calculated using the formula:

$$E\ (\%) = \frac{Practical\ drug\ content}{Theoretical\ drug\ content}\ X\ 100 \qquad (6)$$

Drug Release Study

The *in vitro* dissolution studies were carried out using the basket method (Veego tablet dissolution test apparatus, India) rotated at 50 rpm in 900 mL of phosphate buffer, pH 6.8, maintained at 37 ± 0.5 °C. The weighed quantity of microspheres was introduced into the basket to avoid floating. Samples (10 mL) were withdrawn at different intervals and replaced with equal amounts of fresh medium. The sample was diluted and the amount of repaglinide released was determined at wavelength of 240 nm, using a UV/visible spectrophotometer (Veego UV-VIS Spectrophotometer Model 3, Mumbai, India). Determinations were done in triplicate.

Permeability Coefficient

From the drug release data, the permeability coefficient (P_m) of the various microspheres was calculated using the equation described by Koida *et al*, (1986):

$$P_m = \frac{K_{app}\ X\ V\ X\ H}{A\ X\ C_s} \qquad (7)$$

where K_{app} = apparent dissolution rate constant calculated from the initial linear portion of the plot; V = volume of dissolution medium (cm^3); H = wall thickness of microspheres (cm); A = surface area of microspheres (cm^2) and Cs = solubility of the core in dissolution medium (mg).

$$H = {r(1-p)d_1}\Big/{3[(pd_2 + 1 - p)d_1]} \qquad (8)$$

where r = radius of microspheres

d_1 = density of drug = 1.137 gcm^{-3}

d_2 = density of polymer

p = proportion of medicament in microsphere

Kinetic Models and Comparison of Release Profiles

Data obtained from *in vitro* release studies were fitted to various kinetic equations to determine the kinetics and mechanism(s) of drug release from the microbeads. The results of the drug release for the formulations were fitted to:

First order ($\ln Qt = \ln Q_0 + K_1 t$), Higuchi ($Q = K_H\sqrt{t}$), Hixon-Crowell ($Q_0^{1/3} - Q_t^{1/3} = K\,t$) and Korsemeyer – Peppas ($Q_t / Q_\infty = K\,t^n$) kinetic equations (Hixson and Crowell, 1931; Higuchi, 1961; Korsmeyer *et al*, 1983). Q refers to quantity of drug released at time 0 (Q_0), time t (Q_t) or infinity (Q_∞). K is the release kinetics obtained from the slope of the plot while n refers to the number of samples. The model of best fit was identified by comparing the values of correlation coefficients.

Data Analysis

To compare the differences between the formulations, statistical analysis was carried out using the analysis of variance (ANOVA) using Graph Pad Prism© 4 (Graph pad Software Inc. San Diego, CA). At the 95 % confidence interval, *p* values, less than or equal to 0.05 were considered significant.

RESULTS AND DISCUSSION

Characterization of starches

Acetyl content and degree of substitution

The acetyl content of the modified starch was 41.93±0.90 % while the degree of substitution was 2.68 ±0.07. High acetyl substituted starch with a degree of substitution (DS) of > 2 is of research interest because of their thermo-plasticity and reduced swelling (Roper, 1996; Singh and Nath, 2012). As DS increases, the nature of the starch acetate changes from hydrophilic to hydrophobic and, simultaneously, the inter-particular bonding capacity increases greatly (Korhonen *et al*, 2002). This makes them suitable as polymers for sustained release.

Starch Morphology

Scanning electron images of the native and acetylated starches of Ofada rice are shown in Figure 1. The particle sizes of the starches are presented in Table 1. The Scanning electron micrographs (SEM) images of Ofada rice starch in their native forms showed polyhedral granules with mean particle sizes of 2.20±0.14 µm. The micrographs obtained for native and modified starches revealed that acetylation of starch disrupted the granular structure of the native starches. The acetylated starches showed significantly ($p<0.01$) larger, fibrous, irregular aggregates with mean size 17.80±1.25 µm. These observed shapes and morphology are consistent with those reported in literature (Korhonen *et al*, 2002; Singh and Nath, 2012).

FTIR analysis

The FTIR spectra of the native and modified starches are shown in Figure 2. The FTIR spectra of the native and modified starches showed broad bands at 3000-3600 cm^{-1}correspond to O-H stretching while the peaks at 2950 and 1647 cm^{-1}correspond to C-H stretching and δ (O-H) bending respectively. The spectra of the modified starches indicated the formation of amorphous structure resulting in decrease in the ordered structure of native starches. New bands at 1700 cm^{-1}(Stretching C=O), 1375 cm^{-1}(Stretching C-CH_3) were observed for the acetylated rice starches as had been previously reported (Harvey *et al*, 2012). 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 groups.

XRD analysis

The XRD spectra for the native and acetylated Ofada rice starches are shown in Figure 3. The native rice starch typically showed typical A-type reflection patterns with strong peaks at 2θ of between 13 ^{o} and 23 ^{o}. In contrast, the acetylated starches showed decrease in crystallinity when compared to native starches, with more peaks being disrupted, and a shift of peak to a lower 2θ of about 9 °. This correlated with FTIR observations.

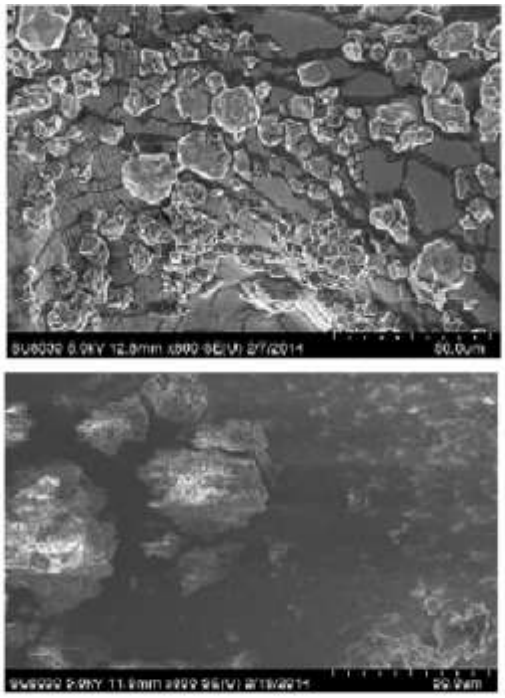

Figure 1: Scanning electron micrographs of native Ofada rice starch and acetylated Ofada rice starch Mg x 800

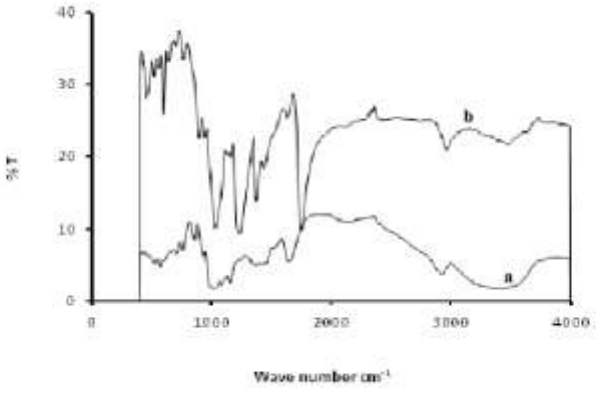

Figure 2: FTIR spectra for :(a) native Ofada rice starch and (b) acetylated Ofada rice starch

aterial properties of starches

The material properties of the starches are presented in Table 1. The Table includes values for swelling, density measurement and angle of repose. The swelling of acetylated starch significantly reduced when compared to that of the native form of the starch. Modification of starch granules by acetylation could limit the rate and extent of water absorption, thereby retard swelling. This is consistent with literature report (Harvey *et al*, 2012). From the values of the bulk and tapped densities, Hausner`s ratio and Carr`s Index were calculated. The Hausner's ratio (tapped to bulk density) provides an indication of the degree of densification and higher values of Hausner's ratio predict significant densification of powders. Generally, acetylation resulted in lower values of bulk density. The result suggests that the acetylated starches exhibited higher degree of densification with tapping. Carr's index is a measure of flowability and compressibility of a powder. The lower the Carr's index, the better the flowability but the poorer the compressibility (Carr, 1965). The result indicates that modification of Ofada rice starch resulted in improved flow. The angle of repose is another qualitative measure of the cohesiveness or the tendency of powdered materials to flow. Angle of 30 ° or below usually indicates that the powder is free flowing. Angles of repose of 40 ° or above indicate poor flow. The result of angle of repose further confirmed that acetylation improved the flow properties of the starch.

Formulations of repaglinide microspheres

Using emulsification-evaporation method, repaglinide-polymer solution in a water-immiscible solvent (chloroform) was emulsified into an aqueous solution containing a dispersing agent (SCMC). The subsequent evaporation of the solvent from the emulsion resulted in the formation of microspheres. Repaglinide is a small dose high potency drug which requires the bulking effect of a polymer in its formulations. Its low bioavailability is attributed to its extensive first pass metabolism and short half-life of about 1 hour. The development of sustained release dosage form of repaglinide would be a more convenient alternative to the conventional tablet dosage formulations with high dosing frequency. The composition of the various formulations of repaglinide microspheres containing the acetylated Ofada rice starch and ethyl cellulose at varied polymer:drug ratios are presented in Table 2.

Characterization of repaglinide microspheres

The properties of the microsphere were evaluated for particle size, swelling, wall thickness, entrapment efficiency, dissolution time and permeability constant. The results are presented in Table 3. The scanning electron micrographs of the repaglinide microspheres containing the acetylated starch as well as that containing ethyl cellulose are shown in Figure 4. The microspheres of acetylated Ofada rice starch were near spherical in shape and their surfaces appeared to be coated by the acetylated starches with some degree of porosity. Repaglinide microspheres containing ethyl cellulose were spherical, discrete but with smoother surfaces. Microspheres containing acetylated Ofada rice starch were larger at all polymers: drug ratio and their size was in the range of 23.45 ± 2.25 to 44.55 ± 3.85 µm while those of ethyl cellulose were 22.22 ± 0.12 to 28.47 ± 9.28 µm. Particle size appeared to increase with drug: polymer ratio as the amount of polymer content increased. The FTIR spectra of the pristine drug, acetylated Ofada rice starch and repaglinide microspheres containing the acetylated starch and ethyl cellulose are presented in Figure 5. The FTIR spectra indicate that that there was no interaction between repaglinide and the polymers and showed that the drug was well entrapped.

At all drug:polymer ratios, the swelling and entrapment efficiency were higher ($p<0.05$) in microspheres containing acetylated Ofada starch than those containing ethyl cellulose. The range of entrapment efficiency values was 80.55 ± 5.30 to 101.78 ± 6.15 %. An increase in the amount of polymer resulted in an increase in encapsulation efficiency. The wall thickness of the microspheres, which surrounds the core drug material was determined using the method of Luu *et al* (1973). Repaglinide microspheres containing acetylated Ofada rice starch had thicker wall coatings than ethyl cellulose at all drug: polymer ratios used. The wall thickness increased with increase in the amount of polymer. Permeability of the microspheres was calculated based on the release data as described by Koida *et al* (1986). Permeability appeared to decrease with increase in amount of polymer and wall thickness. At all ratios of drug: polymer it was observed that microspheres of the modified starch were less permeable than those of ethyl cellulose due to their thicker ($p<0.05$) coatings. The permeability of microspheres having porous surface such as those of the acetylated Ofada starch occurs when drug release is driven by osmotic pressure (Ozturk *et al*, 1990).

Drug Dissolution

The dissolution profiles of the various formulations are shown in Figure 6. The values of t_{80} (i.e. the time taken for 80 % of drug content to be released) were obtained from the plots and are presented in Table 3. The drug release from the Ofada starch-based microspheres was sustained over a period of time (t_{80} = 195 ± 6.60 to 395 ± 24.75 min) as a result of the hydrophobic polymer network of the acetylated starch. The dissolution time was observed to increase with increase in amount of polymer. At all drug: polymer ratios, the release rate was higher in microspheres containing Ofada rice starch. 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. Also, rate of release appeared to be dependent on wall thickness and permeability of the coating polymer. The microspheres containing ethyl cellulose at drug: polymer 1:10 gave the longest dissolution time of 580 ± 21.30 min. The prolonged release rate of ethyl cellulose could be related to its higher permeability coefficient at all drug: polymer ratios. In addition, the presence of pores in microspheres containing modified Ofada starch appears to have enhance drug

penetration and increased the rate of drug release in spite of their thicker wall coatings. The span of release of medicament from the microsphere formulations was prolonged enough to justify the proposed polymer systems as potential drug release modulators for sustained-release drug delivery systems.

Table 1: Physical and material properties of native and acetylated Ofada rice starches (mean ± sd, n = 3)

Sample	Particle shape	Particle size µm	Bulk density gcm^{-3}	Tapped density gcm^{-3}	Hausner`s ratio	Carr`s Index	True density gcm^{-3}	Angle of repose °	Swelling power
Native Ofada starch	Polyhedral	2.20 ± 0.14	0.42 ± .01	0.56 ± 0.01	1.33 ± 0.05	25.00 ± 4.05	1.48 ± 0.01	48.74 ± 3.75	1.70 ± 0.02
Acetylated Ofada starch	Irregular, fibrous	17.80 ± 1.25	0.49 ± .01	0.36 ± .04	1.21 ± 0 .01	17.65 ± 3.05	1.49 ± 0.00	39.99 ± 2.60	0.67± 0.05

Table 2: Composition for repaglinide microsphere formulations

Material	**Drug: Polymer ratio**			
	1:2	1:4	1:8	1:10
Repaglinide (g)	0.5	0.25	0.125	0.10
Polymer (acetylated Ofada starch or ethyl cellulose) (g)	1.0	1.0	1.0	1.0
Chloroform (mL)	50	50	50	50
0.5 % w/v SCMC solution (mL)	1000	1000	1000	1000

Table 3: Properties of repaglinide microsphere formulations

Formulation/ Batch		Particle size (µm)	Swelling	Entrapment (%)	Wall thickness (µm)	t_{80} (min)	Permeability constant cm^2/min
1:2 acetylated starch	B_1	23.45 ± 2.25	1.40±0.01	80.55± 5.30	2.24	195± 6.60	3.03
1:4 acetylated starch	B_2	31.05 ± 4.25	1.33± 0.00	86.49 ± 4.52	3.77	270± 16.10	2.79
1:8 acetylated starch	B_3	35.10 ± 5.33	1.32± 0.00	93.59 ± 6.83	4.94	360± 21.05	2.60
1:10 acetylated starch	B_4	44.55 ± 3.85	1.00± 0.02	101.78± 6.15	6.47	395± 24.75	1.99
1:2 ethyl cellulose	B_5	22.22 ± 2.25	1.35± 0.00	75.55 ± 4.50	2.13	200± 9.55	3.33
1:4 ethyl cellulose	B_6	26.98 ± 9.92	1.33± 0.01	80.26 ± 6.51	3.47	360± 14.20	3.15
1:8 ethyl cellulose	B_7	27.13 ± 0.12	1.0 ± 0.02	91.29 ± 6.66	3.82	480± 23.75	2.90
1:10 ethyl cellulose	B_8	28.47 ± 9.28	1.0 ± 0.01	95.71 ± 3.65	4.13	580± 21.30	2.57

Drug Release Kinetic models

The drug release kinetics was fitted to different models (first order, Higuchi, Hixson-Crowell and Korsemeyer – Peppas). The correlation coefficients obtained for each model are as presented in Table 4. The kinetic model showing highest correlation coefficient was considered as the most appropriate model for the dissolution data. Release of repaglinide from the microspheres containing acetylated Ofada rice starch (B_1 - B_4) and ethyl cellulose (B_6 and B_7) generally fitted the Hixson-Crowell model suggesting that the geometric shape of the microspheres diminished proportionally over a period of time**.** Hixson-Crowell introduced the concept of changing surface area during dissolution and derived the "cube-root law" to nullify the effect of changing surface area and linearize the dissolution curves.

The cube root equation is applicable to the dissolution of mono-disperse systems consisting of uniform sized particles (Hixson-Crowell, 1931**).** However, other microspheres containing ethyl cellulose had the zero order (B_7) and first order (B_8) release. In a first order system, drug release is dependent on the remaining concentration of drug in the bead while zero order release provides constant drug release over time irrespective of the formulation and environmental components.

The span of release of repaglinide from the microsphere formulations containing acetylated Ofada rice starch, though lower than ethyl cellulose, was prolonged enough to justify its use as potential sustained release polymer that is comparatively cost effective and can be a substitute to other synthetic polymers in drug delivery.

Table 4: Correlation coefficients obtained for repaglinide microspheres using different kinetic models (n = 3)

Batch	Zero order	First order	Higuchi	Hixson-Crowell	Korsmeyer	
					R^2	n
B_1	0.8569	0.8802	0.9585	*0.9715	0.9650	0.5991
B_2	0.9231	0.9798	0.9836	*0.9911	0.9779	0.636
B_3	0.9309	0.9790	0.9866	*0.9869	0.9469	0.7751
B_4	0.9172	0.7546	0.9745	*0.9787	0.9337	0.7272
B_5	0.8807	0.7907	0.9685	*0.9911	0.9763	0.7518
B_6	0.9718	0.9798	0.9764	*0.9836	0.9660	1.0004
B_7	*0.9841	0.9697	0.9564	0.9708	0.9578	1.0582
B_8	0.9811	*0.9902	0.9630	0.9751	0.9643	1.0490

*Highest correlation coefficient for batch

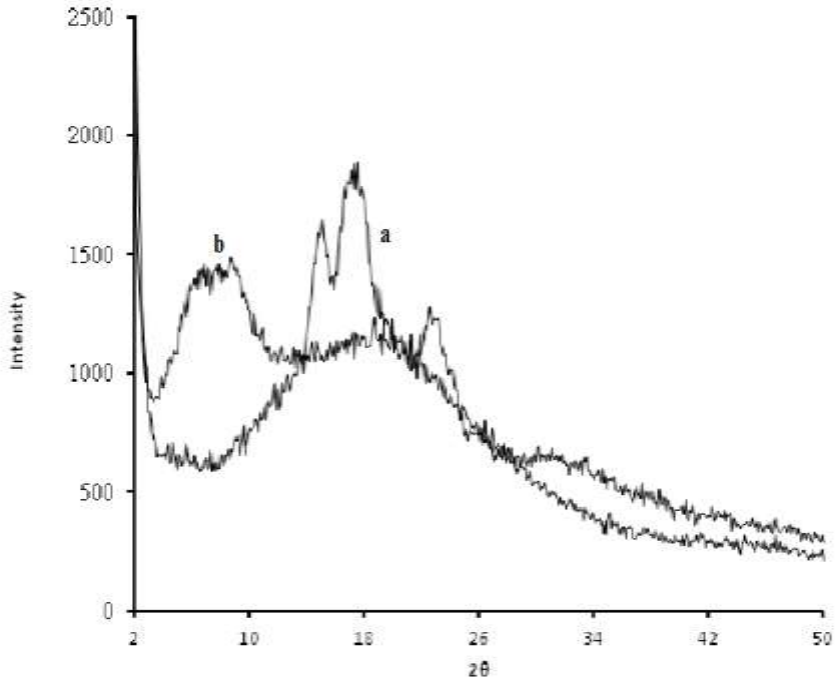

Figure 3: X-ray Diffraction (XRD) pattern of (a) native and (b) acetylated Ofada rice starch

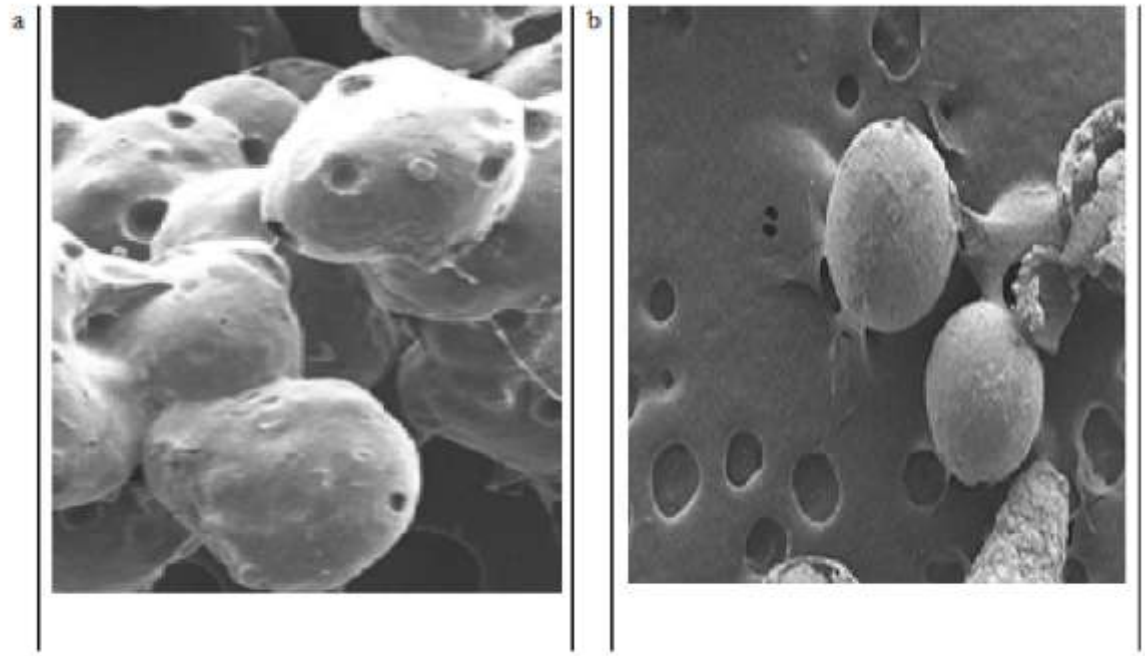

Figure 4: Scanning electron microscope (SEM) of repaglinide microspheres containing (a) acetylated Ofada rice starch and (b) ethyl cellulose Mg x 100

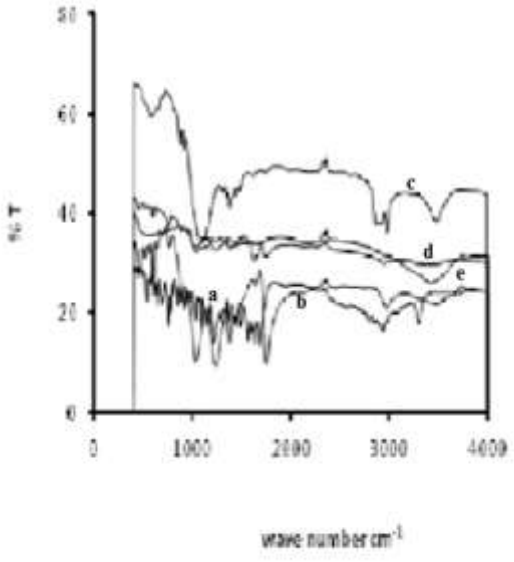

Figure 5: FTIR spectra of (a) repaglinide; (b) acetylated Ofada rice starch; (c) ethyl cellulose; (d) acetylated Ofada rice starch - based microspheres and (e) ethyl cellulose - based microspheres.

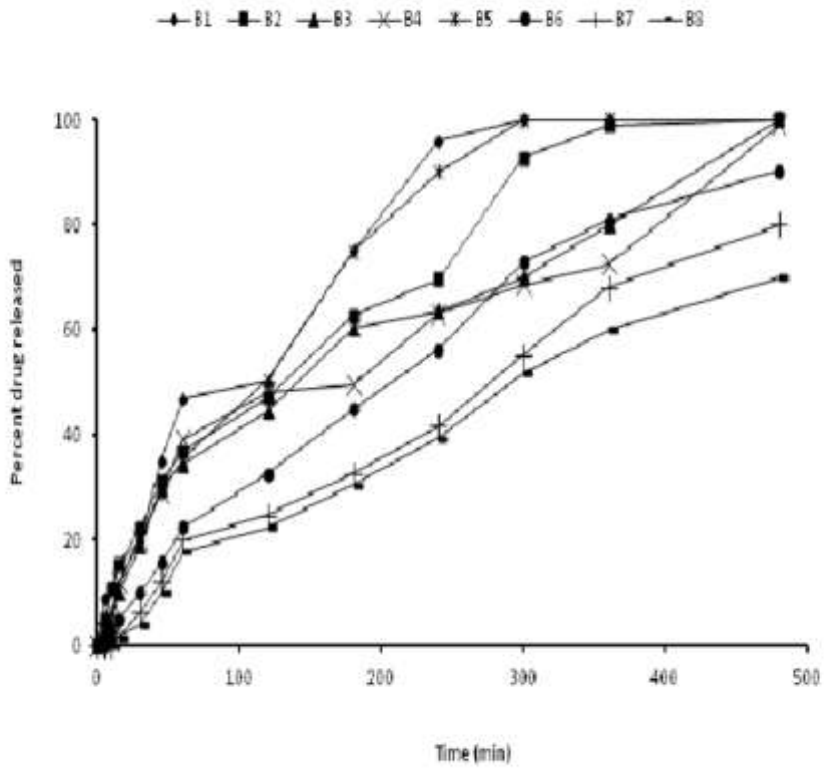

Figure 6: Dissolution profile of repaglinide microspheres

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