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-structuring 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-particle 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 Danjiang 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 ºC 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 Trennner, 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 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 ºC 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):

\[
\text{Acetyl group (\%) = \left(\frac{\text{value of blank} - \text{value of sample} \times \text{Molarity of HCl} \times 0.041}{\text{Sample weight (g)} \times 200}\right) \times 100}
\]

\[
\text{DS = } \frac{162 \times \% \text{ Acetyl group}}{4300 - (42.8 \times \% \text{ Acetyl group})}
\]
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-Kα 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 (W₁). The difference in weight was calculated as W₂. A 2 g quantity of the sample was weighed (W₃) and quantitatively transferred into the pycnometer bottle. The excess non-solvent was wiped off and the pycnometer was weighed again (W₄). The particle density was calculated from the equation:

\[
\frac{W_2.W_3}{50(W_3-W_4+W)} \text{gcm}^{-3}
\]  

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 = (Tapped density - Bulk density)/Tapped density x 100**

\[
\tan \theta = \frac{h}{r}
\]

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.

**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:

\[
\tan \theta = \frac{h}{r}
\]

**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 °C) 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:

\[
E(\%) = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \times 100
\]  

(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 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
formula described by Koida et al. (1986):

\[
P_m = \frac{K_{app} \times V \times X \times H}{A \times C_s}
\]  

(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 \(C_s\) = solubility of the core in dissolution medium (mg).

\[
H = r(1-\rho)\frac{d_t}{3[(pd_s^2 + 1 - \rho)d_t]}
\]  

(8)

where \(r\) = radius of microspheres
\(d_t\) = density of drug = 1.137 g cm\(^{-3}\)
\(d_s\) = density of polymer
\(\rho\) = 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 \(Q_t\) = ln \(Q_0\) + \(K_t\) \(t\)), Higuchi (\(Q = K_{Higuchi} \sqrt{t}\)),
Hixon-Crowell (\(Q = K_{HC} t^{1/3}\)), \(Q = K t\) and Korsemeyer –
Peppas (\(Q = K r^n\)) kinetic equations (Hixon and
Crowell, 1931; Higuchi, 1961; Korsemeyer et al., 1983). \(Q\) refers to quantity of drug released at time \(t\) (\(Q_0\)), time \(t\)
(\(Q_t\) or infinity (\(Q_\infty\)). \(K\) 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)
> 2 is of research interest because of their thermoplasticity 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 \(\delta\) (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° and 23°. 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
Characterization 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 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 microsphere 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 t80 (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 (t80 = 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)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Particle shape</th>
<th>Particle size µm</th>
<th>Bulk density g/cm³</th>
<th>Tapped density g/cm³</th>
<th>Hausner’s ratio</th>
<th>Carr’s Index</th>
<th>True density g/cm³</th>
<th>Angle of repose °</th>
<th>Swelling power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native Ofada starch</td>
<td>Polyhedral</td>
<td>2.20 ± 0.14</td>
<td>0.42 ± 0.01</td>
<td>0.56 ± 0.01</td>
<td>1.33 ± 0.05</td>
<td>25.00 ± 4.05</td>
<td>1.46 ± 0.01</td>
<td>48.74 ± 3.75</td>
<td>1.70 ± 0.02</td>
</tr>
<tr>
<td>Acetylated Ofada starch</td>
<td>Irregular, fibrous</td>
<td>17.80 ± 1.25</td>
<td>0.49 ± 0.01</td>
<td>0.36 ± 0.04</td>
<td>1.21 ± 0.01</td>
<td>17.65 ± 3.05</td>
<td>1.49 ± 0.00</td>
<td>39.99 ± 2.60</td>
<td>0.67 ± 0.05</td>
</tr>
</tbody>
</table>

Table 2: Composition for repaglinide microsphere formulations

<table>
<thead>
<tr>
<th>Material</th>
<th>Drug: Polymer ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1:2</td>
</tr>
<tr>
<td>Repaglinide (g)</td>
<td>0.5</td>
</tr>
<tr>
<td>Polymer (acetylated Ofada starch or ethyl cellulose) (g)</td>
<td>1.0</td>
</tr>
<tr>
<td>Chloroform (mL)</td>
<td>50</td>
</tr>
<tr>
<td>0.5 % w/v SCMC solution (mL)</td>
<td>1000</td>
</tr>
</tbody>
</table>
Table 3: Properties of repaglinide microsphere formulations

<table>
<thead>
<tr>
<th>Formulation/ Batch</th>
<th>Particle size (µm)</th>
<th>Swelling</th>
<th>Entrapment (%)</th>
<th>Wall thickness (µm)</th>
<th>t_{50} (min)</th>
<th>Permeability constant cm²/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:2 acetylated starch B₁</td>
<td>23.45 ± 2.25</td>
<td>1.40±0.01</td>
<td>80.55± 5.30</td>
<td>2.24</td>
<td>195± 6.60</td>
<td>3.03</td>
</tr>
<tr>
<td>1:4 acetylated starch B₂</td>
<td>31.05 ± 4.25</td>
<td>1.33± 0.00</td>
<td>86.49 ± 4.52</td>
<td>3.77</td>
<td>270± 16.10</td>
<td>2.79</td>
</tr>
<tr>
<td>1:8 acetylated starch B₃</td>
<td>35.10 ± 5.33</td>
<td>1.32± 0.00</td>
<td>93.59 ± 6.83</td>
<td>4.94</td>
<td>360± 21.05</td>
<td>2.60</td>
</tr>
<tr>
<td>1:10 acetylated starch B₄</td>
<td>44.55 ± 3.85</td>
<td>1.00± 0.02</td>
<td>101.78± 6.15</td>
<td>6.47</td>
<td>395± 24.75</td>
<td>1.99</td>
</tr>
<tr>
<td>1:2 ethyl cellulose B₅</td>
<td>22.22 ± 2.25</td>
<td>1.35± 0.00</td>
<td>75.55 ± 4.50</td>
<td>2.13</td>
<td>200± 9.55</td>
<td>3.33</td>
</tr>
<tr>
<td>1:4 ethyl cellulose B₆</td>
<td>26.98 ± 9.92</td>
<td>1.33± 0.01</td>
<td>80.26 ± 6.51</td>
<td>3.47</td>
<td>360± 14.20</td>
<td>3.15</td>
</tr>
<tr>
<td>1:8 ethyl cellulose B₇</td>
<td>27.13 ± 0.12</td>
<td>1.0 ± 0.02</td>
<td>91.29 ± 6.66</td>
<td>3.82</td>
<td>480± 23.75</td>
<td>2.90</td>
</tr>
<tr>
<td>1:10 ethyl cellulose B₈</td>
<td>28.47 ± 9.28</td>
<td>1.0 ± 0.01</td>
<td>95.71 ± 3.65</td>
<td>4.13</td>
<td>580± 21.30</td>
<td>2.57</td>
</tr>
</tbody>
</table>

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 (B₁ - B₄) and ethyl cellulose (B₆ and B₇) 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 (B₇) and first order (B₈) 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)

<table>
<thead>
<tr>
<th>Batch</th>
<th>Zero order</th>
<th>First order</th>
<th>Higuchi</th>
<th>Hixson-Crowell</th>
<th>Korsmeyer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>R²</td>
<td></td>
<td>n</td>
</tr>
<tr>
<td>B₁</td>
<td>0.8569</td>
<td>0.8802</td>
<td>0.9585</td>
<td>*0.9715</td>
<td>0.9650</td>
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<tr>
<td>B₂</td>
<td>0.9231</td>
<td>0.9798</td>
<td>0.9836</td>
<td>*0.9911</td>
<td>0.9779</td>
</tr>
<tr>
<td>B₃</td>
<td>0.9309</td>
<td>0.9790</td>
<td>0.9866</td>
<td>*0.9869</td>
<td>0.9469</td>
</tr>
<tr>
<td>B₄</td>
<td>0.9172</td>
<td>0.7546</td>
<td>0.9745</td>
<td>*0.9787</td>
<td>0.9337</td>
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<tr>
<td>B₅</td>
<td>0.8807</td>
<td>0.7907</td>
<td>0.9685</td>
<td>*0.9911</td>
<td>0.9763</td>
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<tr>
<td>B₆</td>
<td>0.9718</td>
<td>0.9798</td>
<td>0.9764</td>
<td>*0.9836</td>
<td>0.9660</td>
</tr>
<tr>
<td>B₇</td>
<td>*0.9841</td>
<td>0.9697</td>
<td>0.9564</td>
<td>0.9708</td>
<td>0.9578</td>
</tr>
<tr>
<td>B₈</td>
<td>0.9811</td>
<td>*0.9902</td>
<td>0.9630</td>
<td>0.9751</td>
<td>0.9643</td>
</tr>
</tbody>
</table>

*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.
REFERENCES


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