Impact of degree of substitution of acetylated Ofada rice starch polymer on the release properties of nimesulide microspheres .

Adenike Okunlola*, Temitope Owojori

Department of Pharmaceutics and Industrial Pharmacy, University of Ibadan, Ibadan, Nigeria

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Original Article

ABSTRACT

Nimesulide microspheres were prepared by the quasi-emulsion solvent diffusion method, using acetylated starches of the indigenous Ofada rice (*Oryza glaberrima* Steud) with degrees of substitution (DS) 1.42 and 2.62. A full 2^3 factorial experimental design was performed using DS (X_1), drug:polymer ratio (X_2) and polymer concentration (X_3) as independent factors; size, entrapment, swelling and time taken for 80% drug release (t_{80}) were the dependent variables. Contour plots were generated and data from the *in vitro* release studies were fitted to various kinetic models. Nimesulide microspheres were near-spherical, sizes varying from 50.91±16.22 to 74.24±24.73 μm for microspheres containing starch DS 1.42 and from 21.05±4.25 to 46.10±3.85 μm for starch DS 2.62. Drug entrapment was 56.75±0.45 to 98.28±2.30%. DS had the greatest effect on the size, swelling and dissolution time ($p = 0.01$) which was confirmed by the contour plots. The interaction between factors DS and drug:polymer ratio (X_1X_2) had the greatest effect on the microsphere properties ($p = 0.04$). Drug release was fitted into the First Order, Higuchi and Korsmeyer models. Acetylated starch of Ofada rice DS 2.62 was found more suitable for the formulation of microspheres because of reduced size and swelling, higher entrapment and prolonged drug release.

KEY WORDS: Acetylation, degree of substitution, factorial design, Nimesulide, Ofada rice starch

INTRODUCTION

The coating polymer materials used for micro-encapsulation determines the physical and chemical properties of the resultant microspheres. Such polymers should be chemically compatible, non-reactive with the core material and provide the desired coating properties such as strength, flexibility, impermeability, optical properties and stability (1). The use of natural biodegradable polymers as coating materials in microsphere formulations has received considerable attention (1, 2). They remain attractive primarily because they are readily available, relatively inexpensive, biodegradable and capable of a large number of chemical modifications. A

*Corresponding author: Adenike Okunlola, Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria, Tel: 234 8023351064, E-mails: nabidogun@yahoo.com, adenike.okunlola@mail.ui.edu.ng

majority of investigations of natural polymers as matrices in drug delivery systems have centered on proteins and polysaccharides including starches (2). Unmodified native starches, irrespective of their source, are undesirable for many applications because of their inability to withstand processing such as extreme temperature, extreme pH, high shear rate or freeze/thaw variation. To overcome this, modifications are usually carried out to introduce desirable properties, to repress inherent properties that are undesirable or to impart new properties to meet a requirement for a specific application. The three common approaches for modifying starches are chemical, physical or enzymatic modification.

Chemical modification is generally achieved by etherification, esterification, crosslinking, oxidation, cationization or grafting of starches. In the acetylation reaction, the number of acetyl groups incorporated onto the starch molecule, and the rate and efficiency, depends on the reagent type and concentration, pH, presence of catalyst, reaction time, botanical origin of the starch and on the size and structural characteristics of the native granule (3, 4). The acetylation of starch results in a considerably more hydrophobic material which has been shown to have better retention of tensile properties in aqueous environments (5). When starch reacts with acid, it loses the hydroxyl group and the free hydroxyl groups on C_2, C_3 and C_6 of the starch molecule are substituted with acetyl groups. As the degree of substitution (DS) increases, the nature of the acetylated starch changes to hydrophobic and interparticular bonding capacity increases (6). They form flexible, water insoluble films that are acid and heat-resistant and can substantially retard drug release (7).

Oryza glaberrima Steud (family Poaceae) also known as Ofada rice is a particular delicacy in Nigeria where it is largely cultivated and processed in the southwestern rice producing communities (8). The rice grain's high starch content makes it a less expensive source of starch which render it a cost effective pharmaceutical excipient. The modification of Ofada rice starch by acetylation with varying degrees of substitution (DS 1.42; DS 2.62) was carried out to produce polymers which were then used to formulate nimesulide.

Nimesulide (4'-nitro-2'-phenoxy methane sulfonanilide) was introduced in 1985 as one of a number of potent nonsteroidal anti-inflammatory drugs (NSAIDs) useful for treating various inflammatory conditions (9). It is a weakly acidic drug and differs from other nonsteroidal anti-inflammatory drugs (NSAIDs) in that its chemical structure contains a sulfonanilide moiety, rather than a carboxylic group, as the acidic group. However, as with other non-steroidal anti-inflammatory drugs, nimesulide is fairly insoluble in water (≈ 0.01 mg/ml). This low aqueous solubility and wettability of the drug makes it more challenging to formulate for oral or parenteral pharmaceutical dosage forms, which may result in variable bioavailability. To overcome some of these drawbacks, in this study nimesulide was encapsulated and formulated into microspheres using acetylated Ofada starch with DS 1.42 and DS 2.62 for sustained drug release. The microspheres were prepared by the quasi-emulsion solvent diffusion method. A full 2^3 factorial experimental design was also performed using the degree of substitution of starch (X_1), ratio of drug to polymer (X_2) and polymer concentration (X_3) as the independent variables. The effects of variation of these factors on some properties of nimesulide microspheres were then determined.

MATERIALS AND METHODS

Starch extraction and acetylation

Starch was extracted from the Ofada rice grains according to a procedure previously reported by Okunlola *et. al.*, (10) and the modification

was carried out by acetylation according to the method by Singh and Nath, 2012 (11). Briefly, 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 at a temperature above 65°C, which is the gelatinization temperature of Ofada starch, for 30 minutes on a hotplate (MS 400 Hot Plate Magnetic Stirrer, LIDA Instrument, Shanhgai China). The gelatinized starch was precipitated using 1 litre of anhydrous ethanol. The precipitated material was filtered and the residue washed with acetone, filtered again and dried at 50°C (GallenKamp Moisture Extraction Oven-Model: BS 250, GallenKamp Co., *UK*)

The dried powder was screened (sieve size 125 μm). Twentyfive grams of the pregelatinized starch was dispersed in 200 grams of pyridine in a 1-liter round-bottom flask. One hundred grams of acetic anhydride was added to the dispersion. The flask was placed into an oil bath and maintained at 100°C rotating at low speed inside a fume hood. To prepare acetylated starches with DS 1.42 and 2.62, the reaction was carried out for 3 and 4.5 hours respectively with continuous stirring. At the end of the reaction, the mixture was transferred to a beaker and cooled to room temperature and the product was precipitated from 1300 ml of ethanol under high shear homogenization. The precipitate was filtered, washed thoroughly with ethanol to remove the residual pyridine in the precipitate and, then filtered again, before drying in an oven at 40°C (GallenKamp Moisture Extraction Oven-Model: BS 250 GallenKamp Co., *UK*) (12).

Determination of degree of substitution

One gram of acetylated starch 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°C for 30 minutes. After cooling to room temperature, 40 ml of 0.5 N potassium hydroxide (KOH) solution was added. The flask was fitted with a tight stopper and kept at room temperature for 72 hours shaking occasionally for complete saponification. The excess of alkali in the 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 using Equations 1 and 2 (13):

$$\text{Acetyl group (\%)} = \frac{V_b - V_s \times M \times 0.043 \times 100}{S_w} \qquad \text{Eq. 1}$$

where, V_b is the blank titration volume in ml, V_s is the sample titration volume in ml, M is the molarity of HCl and S_w is the sample weight in grams.

$$\text{Degree of Substitution} = \frac{162 \times \text{\% Acetyl group}}{4300 - (42 \times \text{\% Acetyl group})} \qquad \text{Eq. 2}$$

where, 162 is the molecular weight of the anhydroglucose unit, 42 is the molecular weight of the replaceable acetyl group and 4300 is the molecular weight of the acetyl groups attached to 100 anhydroglucose units.

Morphology

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

Fourier Transform Infrared Spectroscopy Analysis

Starch powder (1% of the KBr amount) was mixed with the KBr powder and trituration was carried out using an agate mortar for 5 minutes. The die-set was assembled and the powder-

mixture was transferred into it. The powder was pressed for 2 minutes (Thermo Qwik Handi-Press P/N 0016-125) to form a thin and transparent KBr disc. The starches were analyzed using a Fourier Transform Infrared Spectroscopy (FTIR) (FT-IR-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}.

Preparation of the nimesulide microspheres

The nimesulide microspheres were prepared using the quasi-emulsion solvent diffusion method of spherical crystallization. One gram of nimesulide and 2 grams of acetylated Ofada starch were dissolved completely in 100 ml of chloroform. In the drug-polymer mixture, 1 gram of Aerosil was suspended uniformly by vigorous agitation. The resultant drug-polymer-Aerosil suspension was poured into distilled water (750 ml) containing 0.08% of Sodium Dodecyl Sulphate contained in a 2-liter beaker under moderate agitation 450-750 RPM (Talboys Laboratory Stirrer Model No: 102 USA) and thermally controlled at 38°C (Maplelab Scientific Stirrer-Hotplate SHC-11, UK). Using agitation the suspension was immediately dispersed into fine quasi-emulsion droplets and the drug and polymers co-precipitated in the emulsion droplets. After agitating the system for 20 minutes, 750 ml of SDS was added slowly to promote the diffusion of the chloroform from emulsion droplets into SDS thereby enhancing the solidification of the quasi-emulsion droplets. Agitation was continued for another 40 minutes until the translucent quasi-emulsion droplets turned into opaque microspheres. The solidified microspheres were recovered by filtration and washed with water, and the resultant products were dried in a Gallenkamp BS Oven 250 at 50°C for 6 hours. This procedure produced microspheres with a varying ratio of drug to polymer and polymer concentrations (1:2 and 1:4; 2 and 4 % w/v respectively). The various batches and their composition are presented in Table 1.

Table 1 Composition of nimesulide microsphere formulations

INGREDIENTS	B_1	B_2	B_3	B_4	B_5	B_6	B_7	B_8
DRUG (g)	1.0	0.5	2.0	1.0	0.5	1.0	2.0	1.0
ACETYLATED STARCH DS 1.42 (g)	2.0	2.0	4.0	4.0	-	-	-	-
ACETYLATED STARCH DS 2.62 (g)	-	-	-	-	2.0	2.0	4.0	4.0
AEROSIL (g)	1.0	0.5	2.0	1.0	0.5	1.0	2.0	1.0
CHLOROFORM (ml)	100	100	100	100	100	100	100	100
0.08 %$^w/_v$ SDS SOLUTION (ml)	1500	1500	1500	1500	1500	1500	1500	1500
DRUG:POLYMER	1:2	1:4	1:2	1:4	1:4	1:2	1:2	1:4
POLYMER CONC (% w/v)	2	2	4	4	2	2	4	4

Experimental design

A full 2^3 factorial experimental design was performed using three factors, each at two levels. The eight possible combinations are shown in Table 2. The degree of substitution DS (X_1), ratio of drug to polymer (X_2) and polymer concentration (X_3) were chosen as independent variables while microsphere size, swelling, entrapment and time taken for 80% drug release (t_{80}) were selected as dependent variables. The main effects (X_1, X_2 and X_3) represent the average result of changing one factor at a time from its low to high value.

Characterization of Microspheres

Size and morphology

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 the samples were sputter-coated with Osmium tetra oxide prior to examination using the Osmium plasma coater (OPC 60 X1, Japan). The particle sizes of 100 microbeads were determined by optical microscopy.

Swelling index

Exactly 1 ml of microsphere bed was soaked in phosphate buffer (pH 7.4) inside a 10 ml measuring cylinder for 12 hours and the swel-

Table 2 Factorial Design for the formulation and evaluation of batches

BATCH CODE	CODED LEVELS			REAL VALUES			Particle size (μm)	Swelling (v/v)	Entrapment (%)	t_{80} (h)
	X_1	X_2	X_3	X_1 Degree of substitution	X_2 Drug : Polymer	X_3 Polymer concentration % w/v				
B_1	-1	-1	-1	1.42	1:2	2	50.91 ± 16.22	1.35 ± 0.05	56.75 ± 0.45	2.00 ± 0.21
B_2	-1	+1	-1	1.42	1:4	2	57.98 ± 20.15	1.30 ± 0.02	71.60 ± 2.36	3.10 ± 0.30
B_3	-1	-1	+1	1.42	1:2	4	54.59 ± 19.67	1.42 ± 0.00	76.75 ± 0.45	3.40 ± 0.18
B_4	-1	+1	+1	1.42	1:4	4	74.24 ± 24.73	1.20 ± 0.00	88.02 ± 3.01	4.60 ± 0.36
B_5	+1	+1	-1	2.62	1:4	2	21.05 ± 4.25	1.15 ± 0.01	65.48 ± 4.80	5.00 ± 0.50
B_6	+1	-1	-1	2.62	1:2	2	30.10 ± 5.33	1.10 ± 0.05	95.80 ± 2.58	5.40 ± 0.40
B_7	+1	-1	+1	2.62	1:2	4	40.55 ± 3.85	1.22 ± 0.00	96.65 ± 0.07	7.00 ± 0.45
B_8	+1	+1	+1	2.62	1:4	4	46.10 ± 3.85	1.20 ± 0.03	98.28 ± 2.30	7.10 ± 0.96

ling index was calculated as the ratio of the volume to the original volume after 12 hours.

Entrapment Efficiency

A quantity of microspheres containing 50 mg of drug was accurately weighed and crushed using a glass mortar and pestle and then suspended in 50 ml of phosphate buffer, pH 7.4. After 24 hours, the solution was filtered and the filtrate was analyzed for drug using a Spectrumlab 752s UV-VIS spectrophotometer (Ningbo Biocotek Scientific Instruments Co., Ltd, Zheijang, China) at 295 nm. The drug entrapment efficiency (E) was calculated using Equation 3:

$$E\ (\%) = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \times 100 \qquad \text{Eq. 3}$$

Drug release study

The *in vitro* dissolution studies were carried out using the paddle method (USP XXI), rotated at 50 RPM in 900 ml of phosphate buffer, pH 7.4, maintained at 37 ± 0.5°C. The microspheres (containing 100 mg drug) were placed in the dissolution medium. Samples (5 ml) were withdrawn at different intervals and replaced with equal amounts of fresh medium. The amount of nimesulide released was determined at wavelength of 295 nm, using the Spectrumlab 752s UV-VIS spectrophotometer. Determinations were done in triplicate.

Modelling and comparison of release profiles

Data obtained from the *in vitro* release studies were fitted to various kinetic equations to determine the kinetics and mechanisms of drug release from the microspheres. The results of the drug release from the formulations were fitted to Zero Order, First Order, Higuchi (14), Hixon-Crowell (15) and Korsemeyer-Peppas models (16). The model of best fit was identified by comparing the values of correlation coefficients.

RESULTS AND DISCUSSIONS

Characterization of starches

Acetylated Ofada starches with degrees of substitution of 1.42 ± 0.02 and 2.62 ± 0.07 were obtained. The acetyl content of the modified starches were 27.52 ± 0.23% and 41.93 ± 0.90 %, respectively.

Scanning Electron Microscopy

The scanning electron micrographs are shown in Figure 1. Native Ofada starch in its native form had polyhedral granules with mean

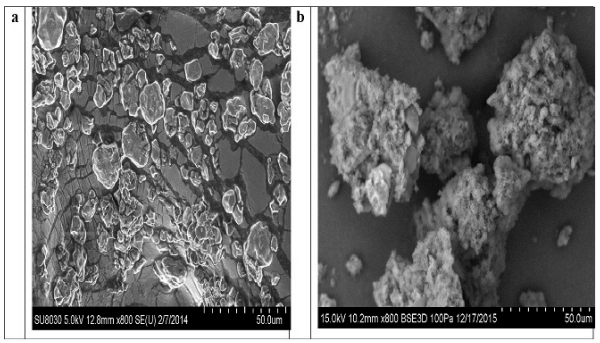

Figure 1 Scanning electron micrographs of (a) native Ofada rice starch and (b) acetylated Ofada rice starch Mg x 800.

particle sizes of 2.20 ± 0.05 μm. Acetylation of starch disrupted the granular structure of the native starches and the acetylated starches showed significantly ($p<0.01$) larger, fibrous, irregular aggregates with a mean size of 17.80 ± 0.75 μm.

FTIR analysis

The FTIR spectra of native and acetylated starches are presented in Figure 2. FTIR spectroscopic analysis showed that the characteristic absorption intensities of esterified starch increased with increase in the degree of substitution, and the characterized peak of hydroxyl group almost disappeared in the spectrum of DS 2.62 acetylated starch. New bands at 1700 cm^{-1} (Stretching C=O), 1375 cm^{-1}(Stretching C-CH3) were observed for the acetylated starches. FTIR bands at 3400cm^{-1} (Stretching O–H) and 1083cm^{-1} (C-O-C bond stretching) were weakened, confirming the replacement of the hydroxyl groups in the starch molecules with acetyl group (10, 17).

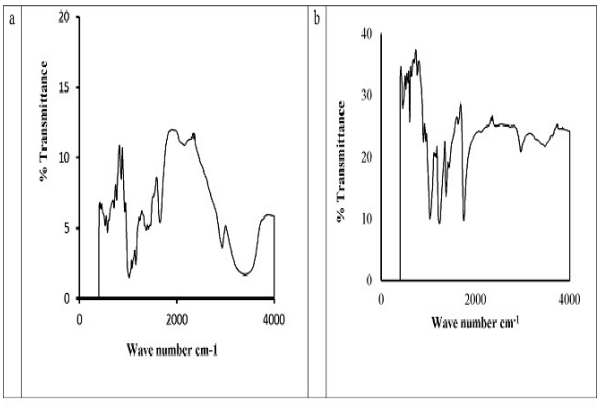

Figure 2 FTIR spectra for (a) native and (b) acetylated Ofada rice starches.

Characterization of microspheres

Microspheres of nimesulide were formulated using the acetylated Ofada rice starch as polymers by the quasi-emulsion solvent diffusion method. Compared with the solvent evaporation method for microsphere preparation, the solidification of the liquid droplet in this process is much faster. Furthermore, the method did not require the use of antiadhesion agents, such as talc, which are often used in the evaporation method. The size of the micospheres, the entrapment, and release characteristics of drugs have been reported to be dependent on the method used to prepare the microspheres (18). The scanning electron micrographs of the nimesulide microspheres containing the acetylated starch

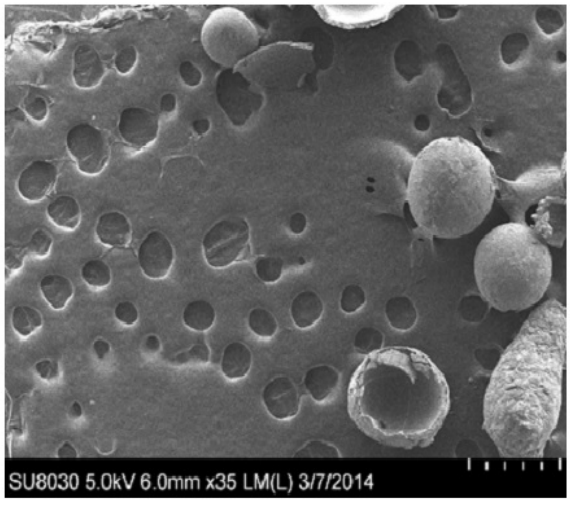

Figure 3 Scanning Electron Micrograph of nimesulide microspheres containing acetylated Ofada rice starch Mg x 500.

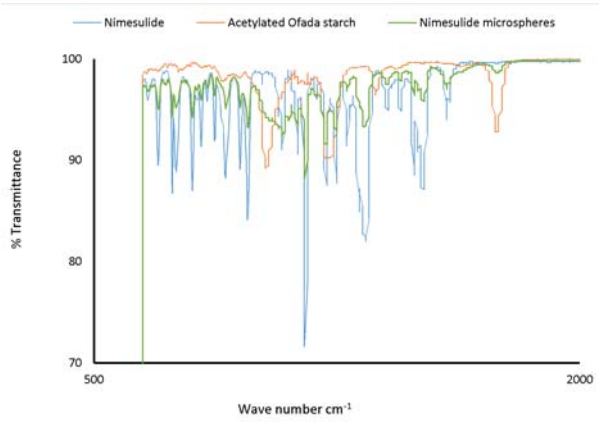

Figure 4 FTIR spectra of nimesulide, acetylated Ofada rice starch and acetylated starch based nimesulide microspheres.

are shown in Figure 3. The microspheres were discrete particles that were near-spherical in shape and no drug crystals were observed. The particle size analysis revealed that the mean diameter of the microspheres containing starch DS 1.42 was larger than that of DS 2.62. (50.91±16.22 to 74.24±24.73 μm and 21.05±4.25 to 46.10±3.85 μm, respectively). Furthermore, as the concentration of starch increased, the size of the microspheres also increased. Formulations with higher concentrations of starch produce viscous polymer solutions which may be difficult to breakdown into smaller droplets, thus resulting in larger microspheres. The observed increase in surface smoothness with decreasing polymer concentration could be attributed to the rapid diffusion of the solvents from the formulations having low polymer concentration (19). The FTIR spectra shown in Figure 4 showed that the drug was well entrapped into the polymers.

The characteristic peaks for microspheres in the FTIR spectra was found to be super-imposable to that of the pure drug. There were no extra peaks, band shift and broadening compared to the pure drug and polymers, which showed that there was no drug polymer interaction.

Experimental design

The 2^3 full factorial experimental design was carried out using Minitab 16 Statistical Software (Minitab Inc., USA). The experimental design provides a clear indication of the quantitative effects of the three parameters, the degree of substitution of starch polymer (X_1), drug: polymer ratio (X_2) and polymer concentration (X_3) on particle size, swelling, entrapment efficiency and dissolution time. This allows the three factors to be varied simultaneously, thus enabling evaluation of the effects of each variable at each level as well as showing the interrelationships among them (20). The coded and real values of the factors and response variables are presented in Table 2 while the values of the individual and interaction coefficients are presented in Table 3.

Table 3 Summary of the individual and interaction coefficients of the variables on the particle size, swelling, entrapment and dissolution time of nimesulide microspheres

FACTOR	COEFFICIENT	PARTICLE SIZE (μm)	SWELLING	ENTRAPMENT (%)	t_{80} (h)
X_1	Effect	-12.49	-0.08	7.89	1.43
	p-value	0.01	0.33	0.33	0.02
X_2	Effect	2.90	-0.03	0.32	0.25
	p-value	0.06	0.25	0.95	0.13
X_3	Effect	6.93	0.02	8.76	0.85
	p-value	0.02	0.40	0.30	0.04
X_1X_2	Effect	-3.78	0.04	-6.85	0.33
	p-value	0.04	0.21	0.37	0.10
X_1X_3	Effect	1.94	0.03	-0.35	0.10
	p-value	0.08	0.30	0.95	0.29
X_2X_3	Effect	3.40	-0.03	3.55	0.08
	p-value	0.05	0.25	0.57	0.37

The relative magnitudes of the effects of the factors on these variables can be observed either as a positive effect which shows an increase in magnitudes of the response value or a negative effect that shows a decrease.

Particle size

The size of microspheres strongly affects the rate of drug release. As the size decreases, the surface area-to-volume ratio of the particle increases. Thus, for a given rate of drug diffusion through the microsphere, the rate of flux of drug out of the microsphere, per mass of formulation, will increase with decreasing particle size (21).

The results show that the coefficient value was negative for the influence of DS (X_1) on particle size indicating that changing the degree of substitution from 1.42 to 2.62 resulted in a decrease in the size of the microspheres. The coefficients that influence the drug:polymer ratio (X_2) and polymer concentration (X_3) for particle size were positive, indicating that an increase in these factors resulted in increase in the sizes of microspheres produced. The ranking of the coefficients for particle size was $X_1 > X_3 > X_2$ showing that DS was the most significant variable ($p = 0.01$) that influenced the size of the formulated microspheres.

The values of the interaction coefficients for particle size were $X_1X_2 > X_2X_3 > X_1X_3$. This indicates that the interaction between DS and drug:polymer ratio (X_1X_2) had the greatest effect ($p = 0.04$) on particle size. The value of the interaction coefficient X_1X_2 was negative indicating that both DS and drug:polymer polymer ratios interacted to produce microspheres of reduced size.

Swelling

The negative coefficient values of X_1 and X_2 on swelling indicated that changing the DS from 1.42 to 2.62 and increasing the drug:polymer ratio from 1:2 to 1:4 produced microspheres with reduced swelling. The results also showed a positive value for X_3 suggesting that an increase in the polymer concentration increased the swelling of the nimesulide microspheres. This could be due to the high water sorption capacity of the starch indicating the effectiveness of the Ofada starch to increase swelling with increasing concentrations of polymer (22). The order of the coefficients for swelling was $X_1 > X_2 > X_3$ suggesting that DS was also the variable that influenced the swelling behavior of the microspheres the most.

The values of the interaction coefficients on swelling was $X_1X_2 > X_1X_3 = X_2X_3$, indicating that the interaction between DS and drug: polymer ratio (X_1X_2) had the greatest effect on the swelling of the microspheres. The positive value of the interaction coefficient indicates that the variables interacted causing an increase in the swelling of the microspheres.

Entrapment

Entrapment efficiency for all the formulations varied from 56.75 ± 0.45 to $98.28 \pm 2.30\%$, showing a dependence on the degree of substitution of the starch, ratio of the drug: polymer, as well as, polymer concentration. Entrapment increased with increasing DS, polymer ratio and polymer concentrations (23, 24). It was observed that B_1-B_4 containing lower DS showed lower values of entrapment than those for B_5-B_8, due to the relatively less hydrophobic nature of the starch DS 1.42.

The coefficients value were positive for the influence of the three factors on entrapment, suggesting that entrapment efficiency increased with increase in these factors. The ranking of the coefficients on entrapment was $X_3 > X_1 > X_2$ suggesting that the polymer concentration had the greatest effect on the entrapment of the drug in the microspheres containing the acetylated starches. The ranking of the

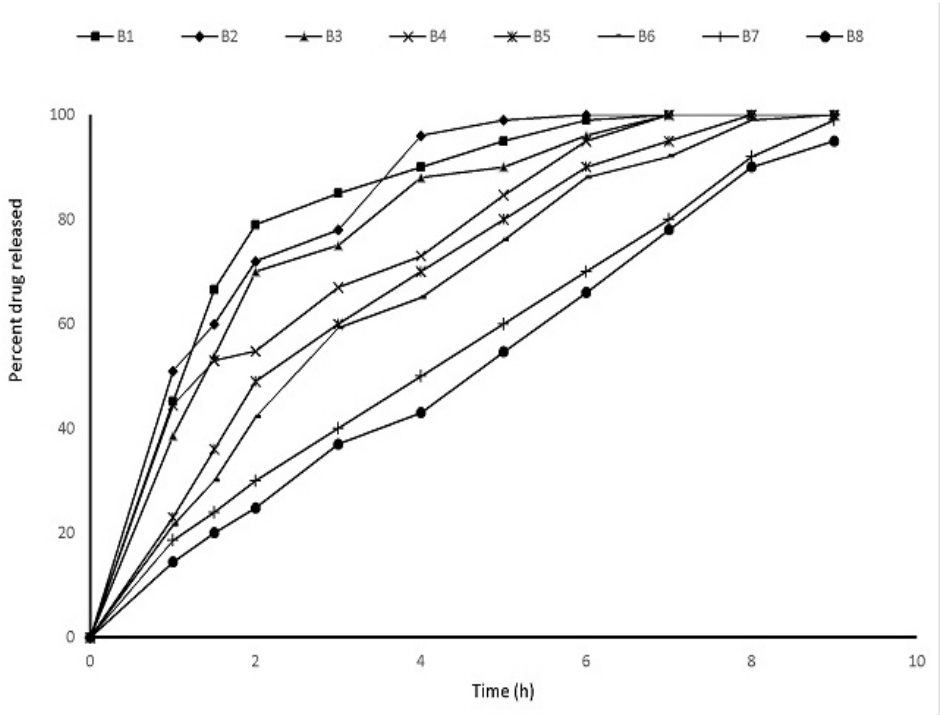

Figure 5 Dissolution profile of nimesulide microspheres.

interaction coefficients on entrapment was $X_1X_2>X_2X_3>X_1X_3$. The interaction between DS and drug:polymer ratio (X_1X_2) had the greatest effect on the percentage entrapment of nimesulide in the microspheres.

Dissolution

The *in vitro* release of nimesulide from the starch-based microspheres was carried out in a pH 6.8 phosphate buffer media to simulate the physiological condition. The dissolution profile for all the batches are shown in Figure 5. The percentage cumulative release was lower for the B_5-B_8 containing starch DS 2.62 than for the B_1-B_4 containing starch DS 1.42. Microspheres containing higher polymer ratio and higher polymer concentration showed a similar trend of cumulative release.

The coefficient values of X_1, X_2 and X_3 on the dissolution time (t_{80}) were positive indicating the increase in the three factors that produced microspheres with longer dissolution times. The ranking of the coefficients on t_{80} was $X_1>X_3>X_2$ indicating that the DS had the greatest effect ($p = 0.02$) on the time of the release of nimesulide from the microspheres. The values of the interaction coefficients for t_{80} were ranked as $X_1X_2>X_1X_3>X_2X_3$ showing that DS and drug:polymer ratio (X_1X_2) interacted synergistically to prolong the time taken for the release of nimesulide from the microspheres.

Contour plots were developed by coding the values of the degree of substitution and drug: polymer ratio and are shown in Figure 6. A contour plot is a graphical representation of the relationships among three numeric variables in two dimensions (25). The contour plots can be used to predict the relevant responses as they reveal the relative effects of the variables on the responses. Figures 6 (a), (b), (c) and (d) show the influence of the DS and the drug:polymer

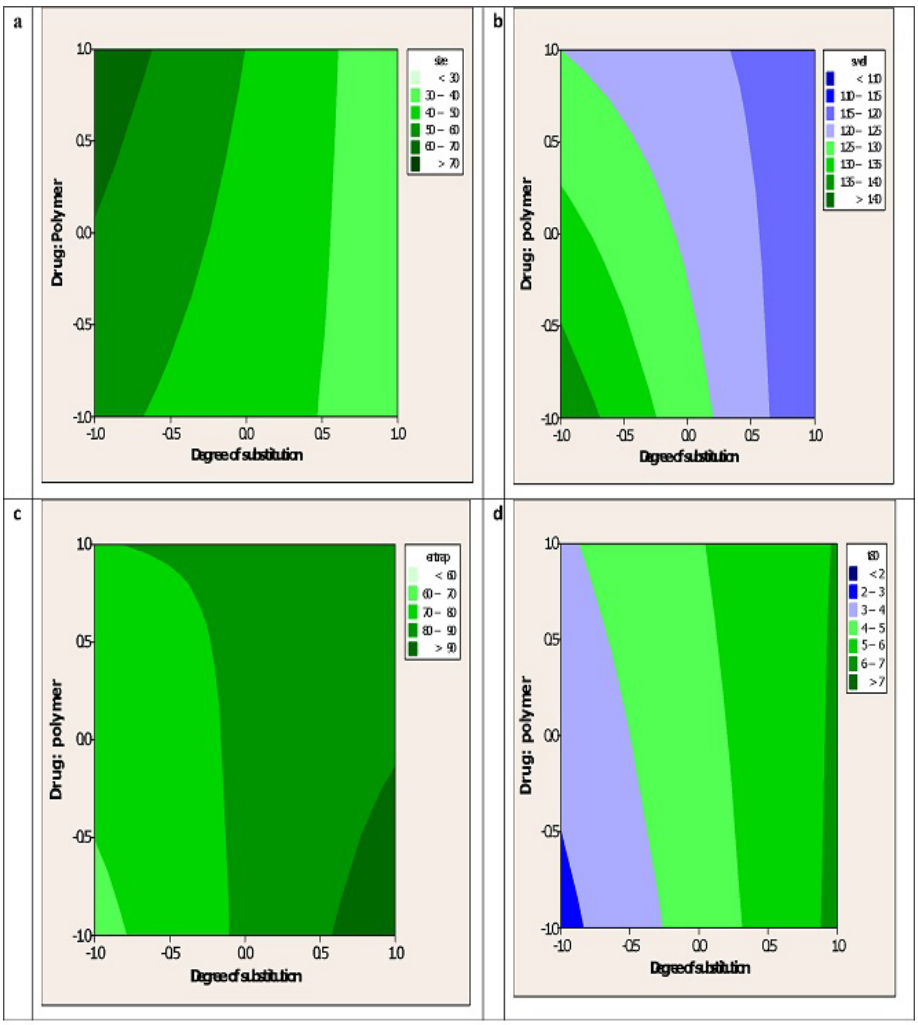

Figure 6 Contour plots of (a) size, (b) swelling, (c) entrapment and (d) dissolution time (t_{80}) if nimesulide microspheres as a function of the degree of substitution of starch and the concentration of starch.

ratio on the size, swelling, entrapment and t_{80} respectively. The darker region of the plots shows that the degree of substitution exerted a higher effect on the entrapment and dissolution time than drug:polymer ratio in the acetylated Ofada starch-based nimesulide microsphere formulations. The contour plots show that the acetylated Ofada rice starch with DS 2.62 was suitable for the microsphere formulations because they were capable of high entrapment efficiency and sustained release.

Drug Release Kinetics and Models

Drug release from polymeric microspheres can occur in 3 phases. First, through rapid release (burst release) that is attributed to drug that is adhered to the wall of the microspheres. Second, through slow release, which depends on the degradation kinetics of the polymer used in the microspheres and third through accelerated delayed release, which depends on the diameter of the microsphere (18, 19). The two main factors that influence drug release

from polymeric microspheres are the degradation of the polymer matrix and diffusion, both of which are influenced by the polymer morphology (18).

Acetylated Ofada rice starch used in this study is an amorphous and biodegradable material in which acetylation of the starch reduces its enzymatic degradation, thereby improving its potential as a carrier system. The biodegradation of acetylated starch is known to be inversely proportional to its degree of substitution (26). At the initial stage of drug release from the starch-based microspheres, nimesulide loosely bound on the surface of the microspheres is released by a mechanism of diffusion through the surface due to water uptake immediately after exposure. At the later stage, the drug releases more slowly, the rate being determined by the diffusion through the polymer matrix. The diffusion of the nimesulide occurs through the amorphous region of the starch polymer. Simulating the drug kinetics using different models (Zero order, First order, Higuchi, Hixon-Crowell and Korsemeyer-Peppas) as presented in Table 4, it was observed that generally, drug release for the microspheres containing acetylated Ofada rice starch with DS 1.42 (Formulations B_1, B_2 and B_3) fitted the First Order kinetic model with the exception of B_4 which fitted the Korsmeyer-Peppas model (n= 0.3997).

Table 4 Correlation coefficients obtained for release of nimesulide from microspheres using different kinetic models (n = 3)

FORMULATION	ZERO ORDER	FIRST ORDER	HIGUCHI	HIXSON-CROWELL	KORSMEYER	
					R^2	n
B_1	0.6461	0.9785	0.8768	0.8722	0.8472	0.3116
B_2	0.6786	0.9296	0.8990	0.9258	0.9203	0.3187
B_3	0.7547	0.9812	0.9389	0.9356	0.9152	0.4048
B_4	0.8407	0.9176	0.9783	0.9783	0.9823	0.3997
B_5	0.9130	0.9644	0.9829	0.9846	0.9652	0.6446
B_6	0.9363	0.9718	0.9793	0.9905	0.9775	0.2727
B_7	0.9936	0.9006	0.9540	0.9848	0.9975	0.7727
B_8	0.9961	0.8846	0.9315	0.9821	0.9964	0.8716

In a First Order model, drug release is dependent on the remaining concentration of drug in the microspheres. The microspheres containing acetylated Ofada rice starch with the DS 2.62 in B_5 and B_6 fitted the Higuchi models as indicated by their correlation coefficients. Higuchi developed theoretical models to study the release of highly and minimally water-soluble drugs incorporated in an insoluble matrix. According to this model, drug release was a diffusion process that was dependent on the square root of time. Higuchi proposed this model of drug release based on the assumptions that the initial concentration of drug in the matrix is much higher than the drug solubility; matrix swelling and dissolution are negligible (14). Formulations B_7 and B_8 showed similar drug release kinetics to B_4 i.e., the Korsmeyer-Peppas model (n = 0.7727 and 0.8716 respectively). In this model, the value of n characterizes the release mechanism of the 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 (16, 27). From the values of the slopes, the drug release mechanism from the formulation B_4 containing the acetylated Ofada starch DS 1.42 is considered Fickian while those of B_7 and B_8 are non-Fickian.

CONCLUSION

Acetylated Ofada starches with degrees of substitution of 1.42 ± 0.02 and 2.62 ± 0.07 were used in the formulation of nimesulide microspheres at varying drug:polymer ratios and polymer concentrations. Microspheres containing acetylated Ofada rice starch DS 2.62 showed significantly smaller particle size, reduced swelling, higher entrapment and longer dissolution times. The degree of substitution was the most significant variable that influenced the size, swelling and dissolution time of nimesulide microspheres while polymer concentration had the most influence on entrapment. The drug release fitted mainly into the Korsmeyer kinetic models, as well as, First

Order and Higuchi kinetic models. This study suggests the usefulness of acetylated Ofada rice starches with the DS 2.62 for the formulation of microspheres with high entrapment and prolonged drug release which can serve as a suitable substitute to synthetic polymers in drug delivery.

DECLARATION OF CONFLICT OF INTEREST

The authors hereby declare that there is no conflict of interest.

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