Formulation and *in vitro* release studies of pegylated mucin based matrix tablets

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Abstract: The effects of polymer concentration on the flow properties of granules and *in-vitro* release profiles from matrix tablets of three model drugs formulated from pegylated mucin base was investigated. Mucin was extracted from the African giant snail and in combination with PEG was used to produce a copolymer matrix base, which was mixed with the model drugs using wet granulation method. The granules and tablets were evaluated according to official and unofficial requirements. Results showed best flow with Acetylsalicylic acid (ASA) and Chloroquine Phosphate (CQ) granules with Hausner ratio of 1.04-1.2, Carr's index of 4.2-17.5% and angle of repose between 19°-26°. The tablets met B.P specifications with respect to tablet weights, friability and drug content. The release profiles showed faster release of the drug with high content of PEG and a slower release with high concentration of mucin. Pegylated mucin base will find useful application in the development of a wide range of formulations.

Keywords: Polyethylene glycol, mucin, matrix tablet, *in vitro* release.

INTRODUCTION

Pegylation is described as the molecular attachment of polyethylene glycols (PEGs) of different molecular weights to active drug molecules or surface treatment of drug-bearing particles with PEGs. It is one of the most promising strategies with the goal of improving the pharmacokinetic behaviour of therapeutic drugs (Abuchowski *et al*., 1977). The main pharmacokinetic outcomes of pegylation are summarized as changes occurring in overall circulation lifespan, tissue distribution pattern and elimination pathway of the parent drug/particle (Fee, 2003). Based on these favourable pharmacokinetic consequences leading to desired pharmacodynamic outcomes, a variety of small molecule drugs as well as peptides/protein have been pegylated and evaluated successfully (Graham, 2003; Levy *et al*., 1988; Bailon *et al*., 2001; Wang *et al*., 2002). Pegylation can also increase therapeutic efficacy by enabling increased drug concentration, improved biodistribution and longer dwelling time at the site of action (Kodera *et al*., 1998). As a result, therapeutic drug concentration can be achieved with less frequent dosing, a significant benefit to patients who are taking injected drugs and long-time medication such as in diabetes and hypertension management. Mucin is ubiquitous in many human and animal tissues, it can be found in the intestine, eye, ovaries and salivary glands etc. Its negative charge makes it a good candidate for drug delivery as it can be conjugated to positively charged drug molecules and targeted to various tissues. It is highly biocompatible, non-toxic and easily biodegradable. Mucin is often used for drug modelling of bioadhesive systems. The interaction of various polymers at the mucin-polymer interface is often used to explain the mechanism of mucoadhesion. The molecular bridges which result between mucin-polymer interaction account for the adhesive strength. Apart from these bridges, the electronic properties of mucin help in mucoadhesion. Mucin, therefore has a high potential as a pharmaceutical excipient (Adikwu, 2009).

The African giant snail is one of the animal sources of mucin. The snail secretes a large amount of slime that covers its entire fleshy body. The slime is composed entirely of mucin. This source of mucin has an added advantage because the bye product (fleshy body of the snail) of the mucin extraction is also used as food.

The objective of this work was to investigate the effect(s) of polymer concentration on the flow properties of polymeric matrix granules and *in vitro* release profiles of matrix tablets of three model drugs formulated from pegylated mucin base.

MATERIALS AND METHOD

Materials

Polyethylene glycol 4000, acetone, magnesium stearate, talc, hydrochloric acid and ferric chloride were all from BDH Chemicals, UK. Mucin was extracted from snail bought at a local market in Benin City, Nigeria. Acetylsalicylic acid, Paracetamol and Chloroquine phosphate powder were gifts from Nomagbon Pharmaceuticals Ltd, Benin City, Nigeria. All other chemicals used were of reagent grade.

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Extraction of Mucin

Adult snails (*Archachantina marginata*) were washed and their shells cracked open. The fleshy body was pulled out from which the excretory materials were removed. The fleshy parts were then placed in 250mL of water and washed until the mucin was completely washed off. These washings (about 1L) were pooled together and precipitated using 1L of chilled acetone. The precipitate was lyophilized in a lyophilizer (Baird and Tatlock, Q. No. MF 45, England). The brownish lyophilized flakes of the snail mucin were pulverized into fine powder using an end runner mill (Pascall, England) and stored in an airtight container until used.

Preparation of Granules

Wet granulation method was used for the preparation of the polymeric matrix granules for paracetamol and chloroquine phosphate. The formula for the preparation of different batches of paracetamol and chloroquine phosphate is shown in table 1. Various amounts of copolymers of polyethylene glycol (PEG) and mucin were mixed together in a beaker and dissolved in 10mL of water. The polymers were incubated at room temperature for 24h. The drugs were incorporated and stirred for 30 min until a uniform consistency was formed. The damp mass was spread on a tray and dried at room temperature for 3 days. It was passed through a 2 mm sieve and to this was added lactose and a blend of calculated amounts of lubricant and glidant.

Table 1: Formula for the preparation of the different batches of Paracetamol, Chloroquine phosphate and Acetylsalicylic acid polymeric matrix granules

Batch Codes	PEG	Mucin	Drug
P1	50	25	500
P2	37.5	37.5	500
P3	25	50	500
C1	100	50	250
C2	75	75	250
C3	50	100	250
A1	100	50	300
A2	75	75	300
A3	50	100	300

NB: All weights are in mg units
P=Paracetamol, C=Chloroquine phosphate, A=Acetylsalicylic acid

Dry granulation method was used to formulate the acetylsalicylic acid granules. The various batches (table 1) were prepared by blending the dried copolymers directly with the drug. The blended mixture was then passed through a 710µm sieve. Thereafter, lactose (100mg) was added as a filler and talc (5% of the total weight of granules) and magnesium stearate (2% of the total weight of granules) were added. The resulting granules from both the wet and dry granulation processes were stored in a desiccator containing dry silica gel until evaluation. One hundred tablets were prepared per batch.

Evaluation of the prepared granules

The granules were subjected to the following evaluation; angle of repose, bulk and tapped densities according to official requirements/specifications. Compressibility (Carr's index) and Hausner factor were thereafter calculated.

Compression of granules

Batches of the granules were compressed into tablets using a single punch tableting machine (F-3 Manesty Machines, UK). For paracetamol and acetylsalicylic acid granules, the compression pressure was maintained at 30 arbitrary units and 28 for chloroquine phosphate. The die volume was adjusted to compress tablets of uniform weight by using granules weighing 675mg for paracetamol, 500mg for chloroquine phosphate, and 550 mg for acetylsalicylic acid. The tablets made were then kept in airtight containers and stored in desiccators until evaluation.

Evaluation of matrix tablets

The following tests were carried out on the compressed tablets using standard procedures: tablet weight uniformity, hardness and friability (BP, 2003).

Tablet weight uniformity

The weight of each of 20 tablets was determined from each batch using an electronic balance (College B154, Mettler Toledo, Switzerland) and the mean weight and standard deviation were computed.

Tablets hardness

The hardness of each of ten tablets per batch was determined (Campbell Electronics, Model HT-30/50, UK). The mean hardness was calculated.

Tablet friability test

The weight of ten tablets was determined on the electronic balance. The tablets were then placed in the drum of a friabilator (Roche Friabilator, UK) revolving at 25 rpm which exposes the tablets to rolling and repeated shock resulting from free fall within the apparatus. After four minutes, the tablets were brought out, dusted and reweighed. The weight was then recorded and friability calculated as percentage loss in weight.

Dissolution studies

The dissolution profiles were carried out using the BP basket method for the various batches of the tablets. A dissolution medium of 800mL of 0.1M HCl solution maintained at 37±0.5°C with a basket revolution of 50 rpm was used. A 5mL volume of leaching fluid was withdrawn at various intervals and replaced with an equivalent volume maintained at same temperature

Table 2: Flow properties of Paracetamol (PCM), Chloroquine phosphate (CHQ) and Acetylsalicylic acid (ASA) granules

Drug	Batch	Bulk Density (g/cm^3)	Tapped Density (g/cm^3)	Carr's Index (%)	Hausner Factor	Angle of Repose (°)
PCM	P1	0.3836	0.5967	35.71	1.5556	69.87
	P2	0.4067	0.6100	33.32	1.4999	55.84
	P3	0.4351	0.6192	29.73	1.4999	52.65
CHQ	C1	0.6250	0.7580	17.50	1.2130	26.44
	C2	0.6470	0.7590	14..80	1.1730	20.63
	C3	0.6615	0.7590	12.80	1.1470	19.80
ASA	A1	0.6430	0.8180	15..40	1.2720	27.80
	A2	0.6040	0.7030	14.00	1.1640	24.43
	A3	0.6700	0.7000	04.20	1.0450	19.03

Table 3: Some physical characteristics of the various matrix tablets studied

Drug	Batch	Tablet Weight (g±SD)	Tablet Hardness (KgF±SD)	Friability (%±SD)
Paracetamol	P1	0.6743±0.005	1.05±0.024	0.11±0.007
	P2	0.6763±0.003	1.95±0.045	0.09±0.003
	P3	0.6980±0.004	2.95±0.120	0.05±0.001
Chloroquine Phosphate	C1	0.3940±0.022	1.50±0.401	0.83±0.050
	C2	0.4010±0.003	243±0.102	0.15±0.002
	C3	0.4030±0.001	3.01±0.201	0.04±0.001
Acetylsalicylic Acid	A1	0.5490±0.021	5.40±0.428	0.15±0.002
	A2	0.5480±0.002	4.30±0.340	0.07±0.001
	A3	0.5540±0.002	5.83±0.420	0.03±0.001

Table 4: r^2 values for different release models

Batch	Zero Order	First Order	Higuchi	Korsmeyer Peppas
P1	0.801	0.931	0.986	0.769
P2	0.715	0.959	0.984	0.701
P3	0.829	0.970	0.988	0.754
C1	0.756	0.983	0.969	0.789
C2	0.522	0.983	0.969	0.723
C3	0.764	0.959	0.962	0.761
A1	0.855	0.990	0.983	0.788
A2	0.864	0.956	0.970	0.756
A3	0.726	0.938	0.988	0.786

(37±0.5°C) of the dissolution medium. The samples were filtered and diluted with an equal volume of 0.1M HCl. This was continued for 60 min. The absorbances of the resulting solutions were measured at λ_{max} of 245 nm for paracetamol, 340 nm for chloroquine phosphate and 540 nm for acetylsalicylic acid using a UV/Visible spectrophotometer (PG Instrument, T70, USA). The 5mL withdrawn aliquot for acetylsalicylic acid was first hydrolyzed by heating at 90°C for 2 h and 1mL of 5% ferric chloride added and allowed to stand for 5 min. The concentration and the percentage of drug released at each time interval was determined using the equation from the relevant standard calibration plot obtained for the three drugs. A minimum of triplicate determinations were carried out for all experiments and the results were reported as mean ±SD.

Kinetic modelling of drug dissolution profiles

The dissolution data was fitted to Zero order, First order, Higuchi and Korsmeyer-Peppas to ascertain the kinetic modeling of the drug release. The method was adopted for deciding the most appropriate model (Mumtaz and Ch'ng, 1995; Rao *et al*., 2007).

RESULTS

Flow properties of granules

Results in Table 2 show that the bulk and tapped densities of the batches, P1, C1 and A1 containing high concentrations of PEG gave values with a significant difference ($P<0.05$) among the other batches of the three drugs indicating a high degree of porosity while values for P3, C3 and A3 containing high concentrations of

mucin were not significantly different (P>0.05). The values of the compressibility index (table 2). Show good compressibility with all the batches of acetylsalicylic acid and chloroquine phosphate while paracetamol was the poorest.

Also the Hausner's ratios from the table show a highest value of 1.2 for acetylsalicylic acid and chloroquine phosphate indicating free flowing powder/granules while a value range of 1.49-1.55 for paracetamol granules indicates cohesive powder/granules (Hausner, 1967). The angle of repose values from table 2 of greater than 50° for paracetamol granules implies that the granules had unsatisfactory flow properties, while acetylsalicylic acid and chloroquine phosphate granules with values below 25° had very good flow properties.

Physical characteristic of the matrix tablets

Table 3 shows the mean weight of the matrix tablets prepared for the various batches of the active drugs. The weights of all the tablets met the British Pharmacopoeia (2003) specification, i.e., that not more than two of the individual weights should deviate from the average weight by more than ±5% and none should deviate by more than ±10%.

The hardness test is not an official test. However, a minimum hardness of 4 KgF is desirable or satisfactory (Rudnic and Schwartz, 2000). From the results in table 3, only three batches of acetylsalicylic acid tablets gave a satisfactory result for hardness, having values of 5.40 KgF (Batch A1), 4.30 KgF (Batch A2) and 5.83 KgF (Batch A3). Table 3 also shows the values of the friability of the matrix tablets. The friability values of less than 1% for all the tablets produced were satisfactory.

Drug release profiles

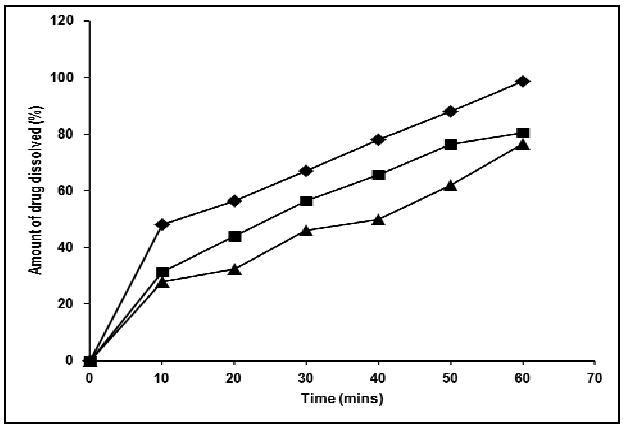
Figures 1 a, b and c show the release profiles of the drugs from the various tablets. From the dissolution plots of the three different drugs under study it was observed that the matrix tablets formulated from batches with higher concentrations of mucin and a lower concentration of PEG showed a slower release of the active medicament from tablets when compared with those made from batches with lower concentrations of mucin and higher concentrations of PEG.

Drug release kinetics and mechanism

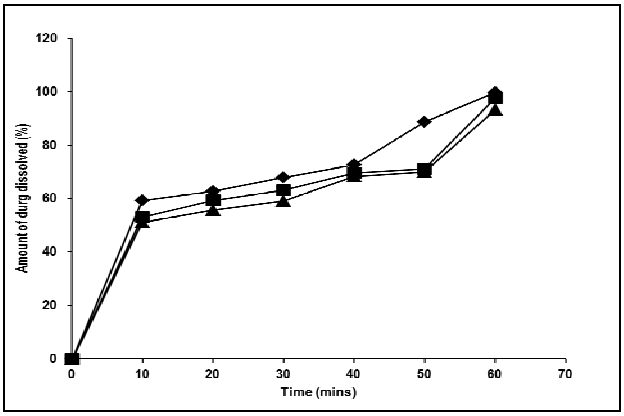
To ascertain the release mechanism and kinetics of the drugs from the matrix tablets, the release data of the three drugs were fitted into mathematical models and R^2 values for zero order. First order, Higuchi and Korsmeyer-Peppas models were determined as shown in table 4. The higher R^2 values for First order and Higuchi suggest that the release of the three drugs follows a first order kinetics with diffusion mechanism.

DISCUSSION

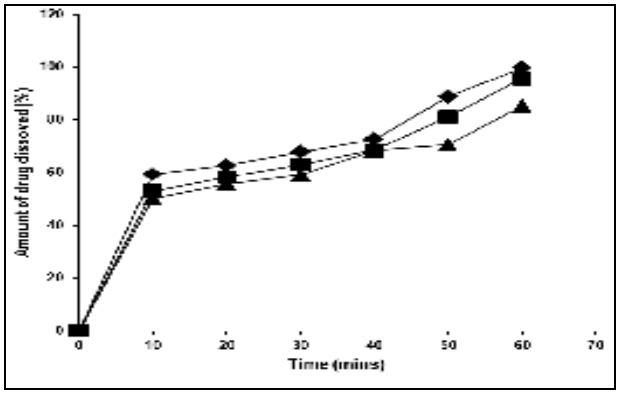
The greater difference in the bulk and tapped densities of the batches, P1, C1 and A1 containing high concentrations of PEG was attributable to the hydrophilicity of PEG, absorbing moisture and making those batches more porous than the others and mucin exhibiting its bioadhesive properties leaves little or no void space for moisture or air.



A



B



C

Fig. 1 (a, b and c): Dissolution of Paracetamol (a), Chloroquine phosphate (b), and Acetylsalicylic acid (c) from the different batches of the matrix tablets. (Key: P1, C1, A1 (◆), P2, C2, A2 (■), P3, C3, A3 (▲))

The compressibility index values for paracetamol indicates a fluid granule with P3 value showing granules with passable flow properties (very fluid granules) while P2 and P1 values show poor granule flow properties (fluid cohesive granules).

The high hardness value of A3 was as a result of the high concentration of mucin used in that batch. It was observed that the friability values decreased as the mucin concentration was increased from batches 1-3, possibly due to the bioadhesive nature of mucin. Although the release profiles of the drugs from the various tablets do not vary significantly when looking at the drugs individually, a modified release is evident in the batches of the drugs with a higher concentration of mucin. This modification of release could be due to several factors; the amount of drug in the tablet, the solubility of the drug in the matrix, the additives and probably the eluting solvent. Desai *et al*., (1966), also enumerated these factors as probable determinants affecting drug release profile. Another possible cause for the release modification is the fact that the polymeric matrix tablets of the various drugs are not whole hydrophilic.

In a typical hydrophilic matrix, the tablets swell in contact with moisture. Bamba *et al*. (1979) noted that the solvent will penetrate and gel the matrix, the active substance will dissolve and diffuse out through the gel in front of it. According to Higuchi, (1961), molecular size of the drug and water permeation, gelation rate, dissolution rate of the drug in the penetrating water, diffusion rate of the drug in the gel and the Higuchi porous penetration, are the several factors that could determine the rate of drug release from hydrophilic matrices. PEG, a hydrophilic synthetic polymer will facilitate water permeation of the matrix, gelation and dissolution of the drug while on the other hand mucin, a natural polymer that is water insoluble will hinder the above mentioned parameters.

Furthermore, from the R^2 values of the matrix tablets, it follows that the relationship between drug release and time is not linear suggesting a slow release kinetic and the release mechanism is a diffusion-controlled mechanism. This is in line with Higuchi (1963), who in analyzing the mechanism of drug release from matrices, postulated two processes that usually prevail; dissolution controlled and diffusion controlled mechanisms. Mucin in the matrix tablets studied will retard dissolution of the drug in the matrix mainly by retarding water permeation and hence controlling dissolution and secondly, it will also control the gelation rate of the matrix and the diffusion of the drug through the gel. Matrix tablets made with mucin will require a considerable time to release their medicament, hence, the modification of drug release.

CONCLUSION

The study shows that pegylated mucin base is a suitable base in the formulation of drug of different physical properties, be it a neutral, a basic, or an acidic drug. This base also shows some promise in modifying drug release from a matrix tablet. Matrix system formulated with mucin can therefore be used in situations where optimum therapeutic level with minimum toxic effect from frequent administration is desired.

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