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Formulation and evaluation the bioadhesive properties of drug delivery system based on PEGylated mucin matrices

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ABSTRACT

Objective: To formulate and evaluate the application of PEGylated mucin in oral bioadhesive drug (OBD) delivery system. **Methods:** The bioadhesive strength of different formulation ratios of polyethylene glycol (PEG) and mucin was evaluated by tensiometry. Co polymer matrices containing mucin and PEG (PEGylated mucin) in the following ratios of 1:1 (A), 2:1 (B), 1:2 (C), 0:1 (D) and 1:0 (E) were prepared by co-precipitation. Microparticles were prepared from the matrices using a size reduction technique. Five different samples were prepared using direct mixtures in the appropriate solvenfs having mucin and PEG ratios of 1:1, 2:1 , 1:2, 0:1 , 1:0 and labeled as (A-E). The matrices were evaluated for their flow properties and the *in vitro* bioadhesion characteristics of the samples were examined. **Results:** The range of flow rates of the matrices was 1.35-2.23 g/sec. The angle of repose was in the range of 37.3-39.9 degree. The bulk and tapped densities were within the ranges of 0.41- 0.49. The Hausner's quotient (HQ) were 1.12, 1.24, 1.14, 1.25 and 1.2 for matrix batches A-E respectively, The bioadhesive strength of polymer matrices appeared to be directly related to amount of mucin. The order of bioadhesive strength is 2:1 > 1:1>1:0> 1:2> 0:1 of PEG: mucin in both simulated intestine fluid (SIF) and simulated gastrointestinal fluid (SGF). The physical properties of the micrometrics properties of the matrices were within the accepted values. **Conclusions:** OBD preparation containing PEGylated mucin can be prepared by direct compression and be used in drug delivery to the oral cavity.

1. Introduction

Drug-delivery applications utilize bioadhesive macromolecules to localize treatment to a specific area of the body, thereby increasing the residence time and improving the oral bioavailability[1]. For intravascular applications, a targeting agent such as a ligand is incorporated into the drug-delivery system, creating adhesion through the ligand-receptor interaction present at the endothelium surface. Bioadhesion in this instance is governed by (i) the shear stress caused by the hemodynamic force exerted over the cell/ particle, (ii) the loading rate which is affected by the viscosity of the biological environment and (iii) the ligand/receptor density ratio which can be controlled during the fabrication of the system[2]. For oral drug-delivery systems, mucoadhesion is the specific type of bioadhesion responsible for localizing the system at the mucous gel layer, which lines the absorptive regions of the alimentary canal. Polymers that have typically been utilized in the development of mucoadhesive controlled release formulations include hydrophilic macromolecules containing numerous hydrogen bonding groups such as polymethacrylic acid[3]. Dosage forms have been engineered so that they take advantage of the abilities to molecularly design polymer networks to impart specific structural characteristics so that polymers comprising the dosage form are multifunctional. This new generation of bioadhesives has employed hybrid materials such as lectins, fimbrial proteins, or ligands which have specific interaction sites within the body. It was discovered that there was no report on the effect of polyethylene glycol (PEG) on mucin in any form either as drug deliver or on the bioadhesive property; hence this study was carried out to evaluate the effect of PEG on the bioadhesive property of mucin as a drug delivery system

2. Materials and methods

2.1. Extraction of snail mucin (slime)

After procurement, the shells of the giant African land snails were knocked open at the apex and a spirally coiled

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rod inserted to remove the fleshy body from where the excretory parts were removed. The fleshy parts were then placed in 250 mL of water and washed several times until the (slime) mucin was completely washed off. These washings were pooled together in a plastic bucket, precipitated with chilled acetone and lyophilized in a lyophilizer. The greyish-brown lyophilized flakes of the snail mucin were pulverized into fine powder using a mortar and pestle and stored in an airtight container until use.

2.2. Preparation of PEGylated-mucin

Mucin and PEG were mixed in the following ratios: 1:1, 2:1, 1:2, 0:1, 1:0 to form batch (A-E). A known quantity of mucin and polyethylene glycol were weighed and put in a separate 100 mL baker. 20 mL of solvent (water) were added into the sample and allowed to stand for 72 h, these is to allowed complete interaction of the solvent and the different polymer. The baker that contained polyethylenglycol was later mixed with the hydrated mucin in a baker and allowed to stand for another 72 h to form a single polymer after allowing for molecular interaction and bonding formation to take place. The mixture was precipitated with chilled acetone. The precipitate were collected, dried, pulvirised and kept in a tight container until used. The same method was applied to all the batches.

2.3. Physicochemical and organoleptic properties of snail mucin

A small quantity of the powdered mucin was subjected to physiochemical tests using established standard procedures for the determination of proteins, carbohydrates, fats and oil[4]. The physical characterisation of the mucin was carried out using a small quantity of the mucin sample as described in our previous studied[5].

2.4. Preparation of drug bioadhesive tablets

Batches of *Vernonia amygdalina* aqueous extract tablets were produced using the different ratios of the PEG: mucin as polymers. Wet granulation method of tablet production was employed and the granules compressed in a tablet press (F3 Manesty) with a force of 48 kgf. The ratio of polymers used for the different batches are stated in Table 1, together with the amount of drug used.

Table 1
Ratios and quantities of the polymers used in tableting (mg/kg).

Batches	Ratios	Amount of polymer		Drug
		Polyethylene glycol	Snail mucin	
A	1:1	25	25	2.5
B	2:1	50	25	2.5
C	1:2	25	50	2.5
D	0:1	0	25	2.5
E	1:0	25	0	2.5

2.5. Determination of the matrix micromeretics

2.5.1. Flow rate and angle of repose

The funnel method described by Carstesen and Chan[6] was employed to measure the flow rate of the matrices. A 5.0 g quantity of the respective matrices was introduced into a plastic funnel with the following parameters: efflux tube length of 5.5 cm, funnel diameter measured from bottom of efflux tube of 7 cm, diameter of funnel at top 5.8 cm and diameter of efflux tube 0.6 cm.

The matrices were allowed to fall freely into weighed clean pieces of papers that serve as collector, whose areas have been established. The flow times were noted and the resulting height of the heap (h), were measure using meter rule. The diameter (d), of the base of the heap was also measured. The flow rate was calculated using the following equation:

$$F = \frac{Mass(g)}{Flow\ times(s)} \quad \cdots\cdots \text{Eqn.1}$$

The angle of repose, Ø, was calculated from Eqn. 2

$$Tan\ Ø = \frac{h}{0.5d} \quad \cdots\cdots \text{Eqn.2}$$

2.5.2. Bulk and tapped densities

A 5 g quantity of the granules was introduced into a clean 25 mL calibrated measuring cylinder calibrated in cm^3. The volume of the matrices was read directly from the calibrated cylinder without tapping. The cylinder was then tapped from a constant height 25 times and the tapped volume was then read. The bulk (D_b) and tapped densities (D_t) were expressed in g/mL. The compressibility index of the matrices was also determined.

$$D_b = \frac{Mass}{Flow\ volume} \ (kg{\cdot}m^{-3}) \quad \cdots\cdots \text{Eqn.3}$$

$$D_t = \frac{Mass}{Flow\ volume} \ (kg{\cdot}m^{-3}) \quad \cdots\cdots \text{Eqn.4}$$

2.2.7.3 Hausner's quotients

Hausner's quotients (HQ) and percentage compressibility index (PC) were calculated (V_b = bulk volume and V_t = tapped volume) using the following equations:

$$HQ = \frac{D_t}{D_b} \quad \cdots\cdots \text{Eqn.5}$$

$$PC = \frac{V_b - V_t}{V_b} \times 100 \quad \cdots\cdots \text{Eqn.6}$$

2.5.3. Tensiometric bioadhesive test

A tensiometer (Lecomte Du Nuoy Tensiometer, Model Nr 3124, A. Kruss Germany) was used for the study. Hog ileum of about 5 cm long and 2 cm wide was longitudinally slit to expose the mucus surface. The ileum was pinned on a cork placed on the metal support of the tensiometer. A flexible constantan wire on which a plastic plate at width of 2 cm was attached and hung at the place meant for it on the lever. The plastic plate was made to gently touch the intestinal mucus surface. The plate was thereafter raised by means of a screw until it just detached from the surface of the mucus. The tension required for this was read off. Some weight was used to return the lever back to zero and the weight determined.

One tablet from each batch was glued to the plastic plate of the tensiometer using a cyanocrylate adhesive. The plate with the tablet was then hung on the lever which was then

zeroed. The hog ileum on the metal support was raised to establish contact with the glued tablet. A time interval of 5 min was allowed for tablet mucus interaction. Thereafter, the plate was raised by means of a screw until the tablet just detached from the surface of the mucus layer. The tension required for the tablets removal was read off from the tensiometer in degrees. An average of three determinations was recorded. The procedure was repeated for all the tablet batches. The respective averages in degrees were thereafter converted to tension equivalent of bioadhesive strength using the formula below:

$$T = \frac{mg}{2L} \times F \cdots\cdots\cdots\cdots\cdots\cdots \text{Eqn.7}$$

Where T = tension, m = weight in kg, g = acceleration due to gravity (10 m/s^2), L= perimeter of the plastic plate, F= constant =0.94.

3. Results

The results of the studies are shown in Tables and Figure 1 below. The characterisation carried out on snail mucin shows that carbohydrate, protein and fats were the predominant composition of the mucin as presented in Table 2, while the bulk volume, tapped volume, bulk density and true density of the PEGylated matrices were determined. Other characterisation such as angle of repose, Hausner's quotients, flow rate and % compressibility were similarly assessed. All tests were done in triplicates. The bioadhesive properties of the PEGylated matrices on SIF and SGF are shown in (Figure 1)

Table 2
Physicochemical properties of snail mucin.

Test	Observation	Inference
Carbohydrate	+ + + + +	Present
Protein	+ + +	Present
Fats	+	Present

+ Present in trace amount; + + + + Copiously present

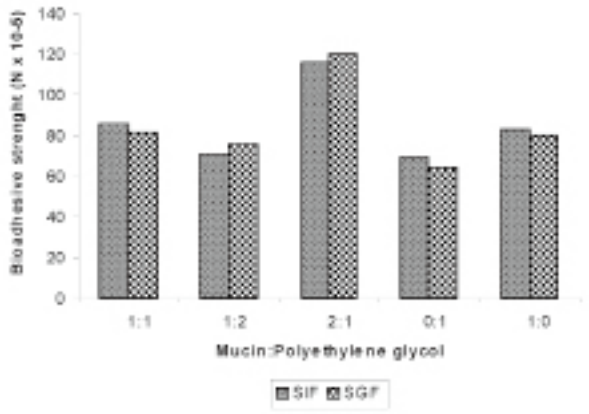

Figure 1. Bioadhesive strength of mucin / polyethylene glycol matrices in SIF and SGF.

Table 3
Physical properties of the matrices are shown below.

Properties	Ratios of mucin : polyethylene glycol				
	1:1	2:1	1:2	0:1	1:0
Bulk vol. (cm^3)	10.00± 0.70	10.80±0.10	11.50±0.50	10.90±1.20	12.20±1.50
Tapped vol. (cm^3)	8.90±20.50	8.80±20.50	10.20±2 0.50	8.80±20.75	10.20±2.50
Bulk density (g/ cm^3)	0.50±21.50	0.46±20.25	0.43±21.20	0.49±2 0.75	0.41±0.50
True density (g/ cm^3)	0.56±20.55	0.57±21.50	0.49±22.10	0.61±21.50	0.49±2.00
Compressibility (%)	11.00±22.50	18.50±22.50	11.30±2 0.35	19.20±21.50	16.40±20.25
Angle of repose Ø	35	31	30	33	33
Flow rate (sec)	3.00±20.00	3.00±20.10	2.0±21.10	4.00±22.50	3.50±20.50
Hausner's Quotients	1.12±22.00	1.24±20.25	1.14±21.50	1.25±20.75	1.20±20.35

Note: Results are the means of 3 measure±0.5 SD.

4. Discussion

Results of some physiochemical tests performed on the snail mucin shows that carbohydrates, proteins and trace amounts of fats were predominant composition of the mucin. Physical characterisation of mucin both wet and dry states shows that mucin is light-brownish in colour, almost tasteless and has a pleasant meaty odour.

The physical properties of the co-polymer matrices are presented in the result part. The range of flow rates of the matrices was 1.35-2.23 g/sec. The angle of repose, which indirectly quantifies powder flowability and relates to inter-particles cohesion[7] was in the range of 37.3-39.9 degree. This obviously indicated that the matrices have good flowability properties. The bulk and tapped densities were within the ranges of 0.41-0.49. The Hausner's quotient (HQ) were 1.12, 1.24, 1.14, 1.25 and 1.2 for matrix batches A-E respectively. These show that A, C and E were within the accepted range for Hausner's quotients(HQ), while B and D were slightly above the range. Studies have shown that values approximate to 1.2 indicate good characteristics for a powder[8]. The percentage compressibility (PC) values of the co-polymer matrices A- E were 11.0, 18.5, 11.3, 19.2 and 16.4 respectively. Studies have proved that material having PC values of 5-15, 12-16 and 18-21% have excellent, very good and fair flow behaviours respectively[9]. The result obtained in this characterisation is an indication that, matrices A and C have an excellent properties, E has very good while B and D have fair flow behaviours.

The main aim of this bioadhesive study was to evaluate the effect of the co-polymer matrices on the mucosal wall and to see the impact of mucin on the bioadhesive property

of PEG as compared to the individual polymer on the fluid environment (SGF and SIF). The result showed that the bioadhesive strength of the tablets formed with the various PEG and mucin ratio increased as the mucin concentration increased in the formulations, for all the media used. The highest mucoadhesive strength of 120.0×10^{-5} N was observed in the formulations that contained mucin : PEG (2:1) of 120.0×10^{-5} N in SGF followed by 116.0×10^{-5} N in SIF, but was least for tablets prepared with polyethylene glycol (0:1). The order of the bioadhesive strengths was as follows: 2:1 > 1:1>1:0> 1:2> 0:1 in both SIF and SGF. For bioadhesion to occur, a succession of phenomena, whose role depends on the nature of the bioadhesive material, is required. The first stage involves an intimate contact between a bioadhesive and a membrane, either from a good wetting of the bioadhesive surface, or from the swelling of the bioadhesive. In the second stage, after contact is established, penetration of the bioadhesive into the crevices of the tissue surface or inter penetration of the chains of the bioadhesive with those of the mucus takes place. Low chemical bonds can then settle. On a molecular level, mucoadhesion can be explained based on molecular interactions. The interaction between two molecules is composed of attraction and repulsion. Attractive interactions arise from Van der walls forces, electrostatic attractions, hydrogen bonding and hydrophobic interactions. Repulsive interactions occur because of electrostatic and steric repulsion[10]. For mucoadhesion to occur, the attractive interaction should be larger than non -specific repulsion[11]. Study has shown that mucoadhesive dosage forms that can stick to the site of application/absorption have attracted considerable interest since the idea was first introduced early in the 1980s[12]. The advantages of mucoadhesive formulations include, prolonged residence time at the site of drug absorption and better contact with the underlying mucosa so that the diffusional path of the drug to the epithelium is shorter. Furthermore, some mucoadhesive polymers can modulate the permeability of epithelial cells by partially opening tight junctions[13]. Recent researche have shown that nanoparticles coated with low molecular weight (MW) PEG possess hydrophilic and near neutrally-charged surfaces that minimize mucoadhesion by reducing hydrophobic or electrostatic interactions[14]. These coatings were inspired by viruses with similar surface properties that are capable of moving rapidly through human mucus[15]. However, a vast amount of literature has shown that PEG can be strongly bioadhesive, presumably by interpenetrating polymer network (IPN) effects between PEG chains and the mucus mesh[16] and/or hydrogen bonding between ether oxygen atoms in PEG and sugars on glycosylated mucin[17]. Research has shown that PEG is a biocompatible polymer that has enjoyed widespread use in drug delivery technology, it is considered adhesive toward mucosal tissue, Here the author described a simple approach to enhancing mucoadhession of PEG polymers through end group functionalisation with the amino acid 3,4-dihydroxyphenyl-L- alanine (DOPA) using a variety of surface analytical methods[18]. The finding from our study is in line with the above as, our PEG-mucin showed a better bioadhesion than when the polymer were used individually in both SIF and SGF. The study here suggested that the PEGylated-mucin matrices may be a promising candidate for oral controlled drug delivery system because of its bioadhesive forming ability and sustaining the release of drug in contact with the mucosa wall of the intestine.

Conflict of interest statement

We declare that we have no conflict of interest.

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