Evaluation of Snail Mucin as Bioadhesive Agent for the Delivery of Chlorpropamide

M. U. Adikwu., P. O. Nnamani and A. A. Attama
Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Nigeria

Corresponding author: Adikwu, M. U. Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Nigeria

Abstract

Snail mucin was extracted from the giant African snail, Archachatina marginata, Fam. Arionidae, by washing with distilled water and precipitation with chilled acetone and lyophilization of the precipitate. The bioadhesive properties of the snail mucin dispersions were evaluated using a tensiometer and coated glass beads. Granules containing admixtures of snail mucin and Carbopol Ultrez-10 with chlorpropamide as the model drug were formulated, evaluated and their bioadhesiveness also determined. The release properties of chlorpropamide from the granules were studied in simulated intestinal and gastric fluids. The snail mucin dispersion had high bioadhesive strength, and the strength was found to be maximal when simulated gastric fluid was used as the washing fluid. The formulated granules also had high bioadhesive strengths and possessed good compressibility properties. The release of chlorpropamide from the granules varied depending on the proportion of the snail mucin/Carbopol Ultrez-10 admixtures. Analysis of the release mechanism indicated that diffusion was the predominant mechanism of release. From the result of the study, snail mucin could prove to be a potential pharmaceutical excipient either alone or in combination with other polymers for bioadhesive formulations.

Key Words: - Snail mucin, bioadhesive agent, delivery, chlorpropamide.

Introduction

Bioadhesion is a state in which two materials at least, one of which is of a biological nature, are held together for extended period of time by interfacial forces (Longer and Robinson, 1986). For drug delivery purposes, the term bioadhesion implies attachment of a drug carrier system to a specific biological location, which can be the epithelial tissue or the mucous coat on a tissue surface (Khanna *et al*, 1998), thus increasing drug absorption and overall bioavailability (Mortazavi, and Smart, 1994; Maggi *et al*, 1994; Caramella, *et al*, 1994). There are several types of controlled release bioadhesive dosage forms in use, some of which include: oral, buccal and nasal bioadhesive controlled release devices (Ishida *et al*, 1983). Bioadhesive dosage forms are targeted at particular sites such as nasal, buccal, gastrointestinal tract (GIT), cervical, vaginal and dermal regions to reduce toxic side effects and increase the therapeutic efficacy of drugs (Attama *et al*, 2003).

Polymer – mucus bioadhesion have been extensively explained by the following theories: - the electronic, adsorption, wetting, diffusion and fracture theories (Taylor, 1986). However, due to the diversity in mechanism involved in this interaction, no single theory has explained fully all the phenomena observed in bioadhesion. The electronic theory of Derjagium and Smilga (1969) states that upon contact of two materials with different electronic structures, there is electron transfer, which leads to the formation of a double layer of electrical charges at the adhesive interface. The attractive forces across the double layer are thus responsible for adhesion.

The adsorption theory states that the materials adhere to each other because of van der Waals, hydrogen bonding and other similar forces (Taylor, 1986; Kinloch, 1988; Bskin, *et al*, 1985). The wetting theory is largely applicable to liquid adhesives. This is related to interfacial tensions between the liquid bioadhesive materials to displace other materials that have been present in the stomach (Krammer, 1983). Such bioadhesive materials on contact with the surface must form a zero or near zero contact angles, a relatively low viscosity and should make an intimate contact that excludes air entrapment (Vogutskii, 1963). The surface tension, of the bioadhesive material and tissue will influence the extent of wetting. For a bioadhesive material to displace the gastric content and adhere spontaneously on the tissue, the spreading coefficient must be positive (Vogutskii, 1963).

The interpenetration of macromolecular chains at the polymer-polymer interface at a temperature higher than the glass-transition temperature is the basis of the diffusion theory of polymer adhesion proposed by Voyutski (1963). The molecular bridges which result from polymer self-diffusion account for the adhesive strength (Krammer, 1983).

The fracture theory of adhesion can be applied to polymers in contact with soft tissue and relates the difficulty of separation of two surfaces after adhesion due to the adhesive bond strength [13]. The characteristic feature of the mucus itself plays a very important role in the adhesion of polymers onto the mucus membranes. Lehr *et al.* (1991) noted that drug delivery through the mucosa of the GIT is rate limited by mucus-turnover rather than by tightness of polymer adherence to the mucus lining. The mucus lining exists in different forms along the GIT, respiratory tract and reproductive system. It also varies at different site within the same tract or system. The site at which the attachment is strongest is regarded as the optimal site for bioadhesion. This site is also expected to have low mucus turnover rate and low sensitivity to stimuli that might enhance mucus secretion (Rubinstein and Tiresh, 1993). Increase in the thickness of mucus decreases bioadhesion. Hence, the adhesion of polymer to the areas with thinner mucus layer e.g. caecum is always stronger than those with thick mucus layers e.g. stomach. The polymer-mucus layer interaction in the caecum for example is greater than the polymer-polymer molecule interaction. In other words, the force of adhesion between a polymer and thinner mucus level is greater than the force of cohesion of molecules. The pH of both the mucus and the polymer affect the adhesion of the polymer.

Mucus has adherent quality that makes it adhere tightly to the food or other particles (Guyton, 1981). The process of mucoadhesion has been proposed to begin with the establishment of an intimate contact between the mucoadhesive polymer and the mucus gel (Khalid *et al*, 1997). The importance of surface energy thermodynamics otherwise known as the work required in increasing the surface area by 1 m^2 in mucoadhesion has been recognised as an important factor in establishing an intimate contact (Smart et al, 1984). This explains the physical or mechanical bond that results in deposition and inclusion of an adhesive material in the crevices of the tissues. Subsequently, the penetration of mucoadhesive polymer into the mucus gel network followed by formation of secondary chemical bonds between the mucoadhesive materials are influenced by several factors, which include the ionic charge of the polymer and the strength of the hydrogen bonding between the polymers (Bamba *et al*, 1979; Genarro, 1995).

The hydrogen bond, which is a secondary chemical bond, is formed by compounds containing hydrophilic functional groups such as hydroxyl, carboxyl, sulphate and amino groups (-OH, COOH, $-SO_4$, $-NH_2$) respectively (Taylor, 1986). Van der Waal interactions are classified based on the Debye forces due to permanent dipole-induced dipole interactions, Keesom forces due to permanent dipole-permanent dipole interaction and London forces due to induced dipole-induced dipole interactions.

Mucus gel is a thick secretion composed mainly of water, electrolyte and a mixture of several glycoproteins, which themselves are composed of large polysaccharides (Guyton, 1981). This mucus gel is held together by either primary disulfide bonds or secondary bonds (electrostatic and hydrophobic interactions) (Taylor, 1986). These cohesive mucin-mucin forces are the rate-limiting step in bioadhesion of several polymers (Khalid *et al*, 1997). Therefore, the bioadhesive bond depends on the strength of the mechanical bonds within the mucus. Hence, the higher the mucin-mucin cohesive forces, the lower the bioadhesive bond force.

Investigation on the effect of molecular weight of four viscosity grades of sodium carboxymethylcellulose (SCMC) by Smart *et al* [1984] showed that molecular weight affects bioadhesion and that the optimum is 8, 600 Daltons. Polymers with molecular weight greater than 100,000 are said to exhibit maximum adhesion (Guyton, 1981).

Mucus is composed of among other things, water [14] and in the presence of mucus adhesive material absorbs water from the mucus. Equilibrium should occur between the adhesive material and the mucus for bioadhesion to take place otherwise slippery mucilage leads to loss of adhesion. Tacky film gives maximum adhesion than hydration to form slippery mucilage (Chen and Cyr, 1970).

Considering the fact that mucus is negatively charged, any mucoadhesive material with a net positive charge e.g. gelatin will produce a relatively high degree of bioadhesion of longer duration, while a mucoadhesive polymer such as carboxymethylcellulose (CMC) with a net negative charge will produce a relatively low degree and duration of bioadhesion (Chen and Cyr, 1970). Different parts of the body secret mucus with different degrees of negativity.

Park and Robinson (1984) studied the binding of various polymers to the mucin and epithelial cell surface and noted the importance of ionizable groups. They found that polymers with ionizable groups were generally most adhesive.

Provided the time allowed for interaction between the mucus and the mucoadhesive material does not exceed certain limit, the contact time is directly proportional to the strength of the force of adhesion. Over-hydration during this period should be prevented as noted by Chen and Cyr (1970) to avoid loss of adhesion.

Mucus is a thick secretion from the mucus gland located on the surface of epithelium in most parts of the GIT (Guyton, 1981). It is

composed mainly of water, electrolytes, and mixtures of several glycoproteins, which themselves are composed of large polysaccharides bound with much smaller quantities of protein. Mucus has been described as a gel or high viscous solution, which adheres to the luminar surface of the GIT as reported by Herman (1987). Mucus is also a highly viscous product, which forms a coat over the lining of hollow organs in contact with the external media (Taylor, 1986). Mucus is slightly different in different parts of the GIT, but everywhere, it has several important characteristics that make it both an excellent lubricant and a protectant for the wall of the gut. This slight difference is as a result of variation in the composition of the mucus from different origins. The mucus secretion with its main component as the glycoprotein fraction is responsible for the gel-like characteristic (Taylor, 1986). Each glycoprotein consists of approximately 20 % protein and 80 % sugar with an average molecular weight of about 2.14 x 10^6 Daltons (Sha, 1995).

The mucus gel is held together by either primary (intra-chain disulfide bond) or secondary (electrostatic and hydrophobic interaction). These glycoprotein molecules aggregate or associate with each other by means of non-covalent interaction forming the gel matrix responsible for the rheological properties of the mucus (Taylor, 1986). This rheological property makes it possible for bioadhesive polymers to interact well with the mucus leading to effective bioadhesion.

Polymers are substances of high molecular weight, consisting of repeating monomer units (Martin *et al*, 1995). The chemical and physical properties depend among other things, on their sizes, symmetry and arrangement of the monomer units. The monomer units may be branched as in amylopectin or linear as in amylose while some substances such as starch consist of both linear and branched monomer units. Polymers exist in different forms and in pharmacy, polymer of natural, semi-synthetic and synthetic sources are employed. These can be classified either as water-soluble or water-insoluble polymers. The former has an ability to increase the viscosity of solvents at low concentration to swell or change shape in solution and to absorb unto surfaces (Florence and Attwood, 1988). It is the combination of slow solution rate and the formation of viscous surface layer that make hydrophilic polymers useful in controlling the release rate of soluble drugs. The latter (water-insoluble polymers) has a low rate of solution hence are used as membrane for dialysis or filtration, to form thin film coating materials, and as packaging materials or to form matrices for enveloping drugs to control their release properties (Adamson, 1982). Examples include polyethylene, polyvinyl chloride, methylacrylate-methacrylate co-polymers and ethyl cellulose.

Three major categories have been utilized successfully as bioadhesives (Taylor, 1986).

1. Carboxyl-containing polymers
2. Hydroxyl-containing polymers
3. Polymers with charged species polymers

Park and Robinson (1984) found that cationic polymers exhibited low values of binding potential while anionic polymers exhibited high values. Also, among the anionic polymers, it was found that those with carboxyl groups showed the highest binding potentials. Several neutral but hydroxyl-containing polymers also exhibited high binding potentials. Introduction of amphiphilicity property to a low molecular mass polymer by incorporating a hydrophilic moiety into the molecule has been reported to improve the bioadhesive properties (Florence and Attwood, 1988). This was observed to be due to increase in surface activity and secondary bond forming capacity due to the increase in molecular chain length. Numerous polymers have been investigated for bioadhesive properties and they include polysaccharides – acacia gum (gum arabic), tragacanth, alginates, water-soluble celluloses – methylcellulose (Celacol), hydroxymethylcellulose (Natrosol 250), sodium carboxymethylcellulose (SCMC), hydrated silicates and magnesium aluminium silicate (Veegum) (Anonymous, 1986).

Carboxypolymethylene (Carbopol) is a high molecular weight polymer up to about 3,000,000 Daltons. It is a synthetic product with a cross-linked polymer of acrylic acid co-polymerized with approximately 0.75-2 % of allyl sucrose containing a high proportion of carboxyl groups (USP, 1970). Its aqueous solution is acidic. When neutralized, the solution becomes very viscous with maximum viscosity between pH 6 and 11. Carbopols are anionic and electrolytes reduce the viscosity of their system, thus high concentration of the polymer has to be employed in vehicles where ionizable drugs are present. They loose their viscosity on exposure to sunlight and this can be prevented or minimized by the addition of antioxidants.

Carbopols are used as suspending agents in pharmaceutical preparation and as binders in tablets. They are used in the formulation of prolonged acting tablets or sustained release tablet. Carbopol exist in various types such as 940, 941 and 943. There is also Carbopol Ultrez-10. The first two are white fluffy, acidic and hygroscopic powder with slight characteristic odour (USP, 1970). The difference in the molecular weight of Carbopol accounts for these different forms. For instance, Carbopol 941 has a molecular weight of 4 x 10^6 Daltons while that of Carbopol 940 is 1 x 10^6 Daltons (USP, 1970). They are soluble in

water, and solutions of sodium hydroxide, potassium hydroxide, borax, amino acids, tetraethylalcohol, and lauryl and stearyl amines

Some chemicals like benzoic acid, sodium benzoate and benzalkonium chloride decrease the viscosity of Carbopol dispersion. Carbopol 940 and 941 are incompatible with phenol, cationic polymers, strong acids and maximum concentration of electrolytes. It is discoloured by resorcinol and microorganisms grow well in an unpreserved aqueous dispersion.

In this study, mucin from snail is used as mucoadhesive materials as it is thought to possess some or a combination of the properties of many of the polymers used in pharmaceutical formulations and is a biomaterial that is histocompatible.

Materials and Methods

Materials: The following materials were procured from their local suppliers and used without further purification: gelatin, sodium carboxymethylcellulose (SCMC) (Aqualon Co. USA), Carbopol-940 and Carbopol Ultrez 10 polymer (B.F. Goodrich, USA); Simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were prepared following the compendium (USP XX) (USP, 1970) specification. All other reagents and solvents used were of analytical grade and were used as such.

Snails: The giant African land snails used were procured from Nkwo Ibagwa local market in Enugu State, Nigeria and kept under standardized laboratory conditions before used for the extraction of mucin.

Extraction of snail mucin: After procurement, the shells of the giant African land snails were knocked open at the apex and a spirally coiled 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 each time until the mucin was completely washed off. These washings were pooled together and precipitated using chilled acetone in a plastic bucket and lyophilized. The greyish-brown lyophilized flakes of the snail mucin were pulverized into fine powder using a mortar and pestle and stored in an air-tight container until used.

Molecular weight determination by gel permeation chromatography on sephadex G-200: The sephadex G-200 was allowed to swell in excess buffer (0.05 M Tris buffer, pH 7.5) for the recommended time of three days at room temperature, in order to obtain satisfactory flow rates through the gel and good separation. The column was poured and equilibrated with the buffer. The void volume was established with blue dextran (V_o) (5 mg/ml; weight average-molecular weight 2 x 10^6; read at 625 mμ). The sample (snail mucin dispersion) was applied. The volume at which the active fraction snail mucin eluted from the column was determined (V_e). Four standards (10 mg/ml) were applied to the column in runs of two standards per run to determine the elution volumes (V_e) of the standards. The standards used were methyl red, bovine serum albumin (BSA), ribonuclease and ovalbumin. The Kav for the active fraction (snail mucin) and the standards were calculated using Eqn. 1

$$K_{av} = \frac{V_e - V_o}{V_t - V_o} \quad \ldots \qquad \text{Eqn. 1}$$

where V_e is elution volume of the (active) material, V_o is elution volume of blue dextran, V_t is the total volume of gel bed ($\cong \pi r^2 h$), r is radius of the column and h is height of the column.

To prepare the standard curve, K_{av} of the standards was plotted against log. molecular weight. From the K_{av} of the active material (snail mucin), which was unknown, the molecular weight was determined from the standard curve.

Determination of snail mucin isoelectric point: Seven buffer solutions of different pH values ranging from 3.2 – 5.7 were made in 7 test tubes (Table 1). A 0.5 ml volume of 2 % snail mucin (protein solution) was added to each test tube and the contents mixed. The test tubes were shaken and noted for the appearance of a cloudy solution. A 2 ml quantity of ethanol was added to each of these test tubes and visual estimation of the solution degree of clouding was determined.

Tensiometric determination of bioadhesive strength of snail mucin

Preparation of snail mucin stock: The respective quantities of 1 g, 2 g, 3 g, 4 g and 5 g of powdered snail mucin were weighed using a weighing balance (August Sauter, KG-D 7470, Germany) and each dispersed in 25 ml of water contained in volumetric flasks. These dispersions were stirred for 15 min and allowed to hydrate completely for 24 h to give 4, 8, 12, 16 and 20 % w/v dispersions respectively.

The bioadhesion test: A tensiometer (Lecomte Du Nuoy Tensiometer, Model Nr 3124, A. Kruss Germany) was used for this study. A smooth polythene support was secured on a platform (a component of the tensiometer) with an adhesive. A freshly excised pig jejunum was thoroughly washed of its waste material. A small portion of the pig jejunum with diameter 2 cm and length 5 cm was used. The mucus surface of the intestines was exposed and used immediately for the test. The tissue was pinned unto the

polythene support of the bioadhesive instrument placed on a metal support. The instrument was zeroed and the bioadhesion of the clean glass plate determined in degrees by gradually raising the platform such that the plate on the lever arm of the tensiometer was in contact with the tissue and 7 min contact time allowed for interaction to take place. The glass plate was raised by means of screw until it just detached from the surface of the tissue. The force required to remove the glass plate from the surface of the tissue was read off from the microform balance in degrees.

The glass plate was washed, dried and subsequently coated to a thickness of 2 mm with the different aqueous dispersions of the snail mucin concentrations and allowed to adhere for 15 min. This was repeated six times for each concentration of the snail mucin aqueous dispersion and the forces required to detach the glass plate from the tissue were recorded in degrees and conversions of these to tension were done using Eq. 2 (Kinloch, 1980):

$$T = \frac{2Mg}{2L} \cdot F \quad \ldots \quad \ldots \quad 2$$

where T is tension equivalent to the bioadhesive strength, M is the mass required to return the lever to zero position after each bioadhesive experiment, L is the area of the bioadhesive interface, F is the instrument constant (0.94) and g is acceleration due to gravity.

Evaluation of the bioadhesive strength of the snail mucin using coated glass beads

Preparation of simulated gastric fluid (SGF) without pepsin: A 2.0 g quantity of sodium chloride was dissolved in sufficient distilled water and 7.0 ml of hydrochloric acid added, and the solution was made up to 1000 ml with distilled water. The pH of the solution was adjusted to 1.2 using a pH meter (model 290 MK, Pye Unicam).

Preparation of simulated intestinal fluid (SIF) without pancreatin: A 6.8 g quantity of monobasic potassium phosphate (K_2HPO_4) was dissolved in 250 ml of water and 190 ml of 0.2 N sodium hydroxide (NaOH) and 400 ml of water were added. The resulting solution was adjusted to a pH of 7.5 ± 0.1 with 0.2 N NaOH, and later diluted with water to 1000 ml.

Coating of glass beads: Glass beads of diameter 3.0 mm with an average weight of 56 mg were cleansed with water and acetone thoroughly to maximize the roughness factor. They were allowed to dry. Different concentrations of the snail mucin dispersions prepared as in section 2.2.15.1 were used to coat the glass beads to an average weight of 65 mg, by successive dipping in the mucin dispersion, air drying and storage in a desiccator until used.

The bioadhesion test: The apparatus designed and used in this study consisted of a separating funnel clamped to a retort stand with a rubber tube attached at the end of the funnel. A metal support was used to position a plastic support at an angle of 30 °. Freshly excised pig jejunum (1.7 x 15.0 cm) was pinned on the plastic support, and a beaker was placed directly under the plastic support to collect the detached beads and the detaching solvent. Fifteen coated glass beads were placed on the exposed mucin surface of the tissue. Mucus-polymer interaction and polymer hydration was allowed to take place over a period of 7 min.

Simulated gastric fluid (SGF) without pepsin (250 ml, pH 1.2) contained in separating funnels, was allowed to flow over the coated glass beads at a rate of 30 ml per min. The number of undetached beads were noted and used as a measure of bioadhesion. The experiment was repeated five times for each concentration of the polymer and the average value recorded and analyzed statistically. This procedure was repeated for simulated intestinal fluid (SIF) without pancreatin (250 ml, pH 7.5 ± 0.1).

Bioadhesion of snail mucin granules

Preparation of granules: Different batches of granules were prepared to contain 1:1, 1:2, 2:1, 0:1 and 1:0 ratios of binary combinations of Carbopol ultrez-10 and snail mucin, and chlorpropamide. The granules were prepared by wet granulation, as in tablet production. The dried granules were sized and those falling within a size range of 1 – 2 mm were used for the bioadhesion study.

Bioadhesion test on the granules: The apparatus designed for the coated bead experiment above was used. In this instance however, a 1 g quantity of the granules was uniformly spread on the everted tissue. At the end of the SGF flow the undetached granules were recovered, dried and weighed. The bioadhesion percent was evaluated. This was repeated five times for all the batches.

Determination of the granule micromeritics

Flow rate and angle of repose: The funnel method described by Carstensen and Chan (1977) was employed to measure the flow rate. A weighed quantity was introduced into a plastic funnel with the following dimensions:

- efflux tube length 6 cm.
- diameter of funnel measured from bottom of efflux tube 8 cm.
- diameter of funnel at top 6.2 cm.
- diameter of efflux tube 0.80 cm.

A 10 g quantity of the granule was allowed to fall freely onto a weighed piece of paper whose area had also been determined. The time of flow was noted. The resulting heap height, h, was measured with a meter rule. The diameter, d, of the heap base was also measured. The flow rate, F, was computed from Eqn. 3.

$$F = \frac{\text{Mass(g)}}{\text{Flow time(s)}} \quad \ldots \quad \ldots \quad \text{Eqn. 3}$$

The angle of repose, θ, was calculated from Eqn. 3.

$$\theta = \tan^{-1}\left(\frac{h}{d/2}\right) \quad \ldots \quad \ldots \quad \text{Eqn. 4}$$

Average of five determinations was taken.

Bulk and tapped densities: A 10 g quantity of granules were weighed and gently introduced into a clean, dry 50 cc measuring cylinder calibrated in cm^3. The volume of the granules was read without tapping. The bulk density (D_b) was determined from the bulk volume by the following relationship:

$$D_b = \frac{\text{Mass}}{\text{Flow Volume}} \ (Kgm^{-3}) \ldots \quad \text{Eqn. 5}$$

The tapped density (D_t) was determined by uniformly tapping the cylinder on a flat firm surface until there was no change in volume (that is consolidated bulk density and so gives higher value than the bulk density).

$$D_t = \frac{\text{Mass}}{\text{Flow volume}} \quad (Kgm^{-3}) \ \ldots \text{Eqn. 6}$$

An average of three determinations was taken.

Hausner's quotient and percentage compressibility were calculated using the following equations:

$$\text{Hausner's Quotient} = \frac{D_t}{D_b} \quad \ldots \text{Eqn. 7}$$

$$\text{Percentage Compressibility} = \frac{D_t - D_b}{D_t} \times 100 \text{ or } \left(\frac{1 - V_t}{V_b}\right) \times 100 \quad \text{Eqn. 8}$$

where V_t = tapped volume, V_b = bulk volume.

Absolute drug content: For each granule batch, 0.5 g of the formulated granule was weighed in a balance and placed differently in a 100 ml volumetric flask. A 70 ml volume of SIF (pH 7.5 ± 0.1) was poured into each of these volumetric flasks and the granules allowed to hydrate for 24 h. The contents were thereafter filtered and 0.25 ml of each filtrate made up to 25 ml with the SIF. The absorbances were read off using a spectrophotometer (UNICO-UV 2010 PC) at 229.7 nm. This was repeated five times and was done for all the batches. The drug contents were calculated with reference to the Beer's plot for chlorpropamide in SIF

Release studies: The release of the drug was assessed in a magnetic stirrer hot plate assembly (Model SR. No. IUM 52188, Remi Equipment, India) using 500 ml of SIF (pH 7.5 ± 0.1) as the dissolution medium. The medium was maintained at 37 ± 0.1 °C throughout the test period. At zero time, 0.5 g of the formulated granule was put inside the dissolution medium. At predetermined times of 5, 15, 30, 45, 60, 90, 120 and 180 min, 5 ml of the dissolution medium was withdrawn, adequately diluted, assayed spectrophotometrically. Each amount of dissolution medium withdrawn was immediately replaced with equivalent amount of fresh dissolution medium. The concentration of chlorpropamide released during each period was determined with reference to Beer's plot in SIF. The data generated was further analyzed graphically. The t_{50} and the t_{20}, which are the time for 50% and 20 % of the drug to be released, were extrapolated from the drug release profiles. The release was further analyzed using the Higuchi's square root model (Higuchi, 1963) as well as the Fick's model (Peppas, 1985) for the amount of drug released.

Results and Discussion

Molecular weight determination of the snail mucin by gel permeation chromatography on sephadex G-200: Gel filtration behaviour of proteins of known molecular weight – methyl red, bovine serum albumin (BSA), ribonuclease and ovalbumin were chosen as reference proteins and their elution volumes measured for each of the columns used. In addition, the void volume (V_o) of each column (elution volume of substances completely excluded from the gel pores) was measured in experiment with blue dextran plus reference proteins. The molecular weight of the snail mucin was estimated from the calibration curve of the standards to be 4, 281.14 Daltons. The useful working range of Sephadex G-200 depends on the extent to which the gel has swollen, and evidently varies also from lot to lot. The lower molecular-weight limit for useful fractionation of polypeptides and proteins is approximately 5000 Daltons, whereas the upper limit may be within 500,000 to 1,000,000 Daltons or even higher, depending on the gel. The lower molecular weight limit for complete exclusion from the pores of Sephadex G-200 will also depend on the gel.

Isoelectric point (IEP) determination of the snail mucin: The Isoelectric point is the pH at which there is no net electric charge on a protein. At this pH, the electrophoretic mobility is zero because Z in the equation below is zero:

$$V = \frac{EZ}{f} \quad \ldots \quad \ldots \text{Eqn...9}$$

V is velocity of migration of a protein in an electric field; E is the strength of the electric field; Z is the

net charge on the protein while f is the frictional resistance, which is a function of size and shape. The isoelectric point of the snail mucin was found to be 3.4. Below the isoelectric point pH, it is expected that the snail mucin is positively charged and above, it is negatively charged.

Tensiometric determination of bioadhesive strength: The result of bioadhesive strength of aqueous dispersion of the snail mucin determined by tensiometry is shown in Fig. 1. The bioadhesive tension observed was due to the formation of an elastic film between the hog mucin and the snail mucin showing a good interaction and the formation of highly elastic polymer coat. The tension produced increases with the increase in the concentration of the mucin extract. For maximum bioadhesion to occur according to Chen and Cyr (1970), the hydration of polymer coat has to form "tacky" film otherwise slipperiness affects bioadhesion.

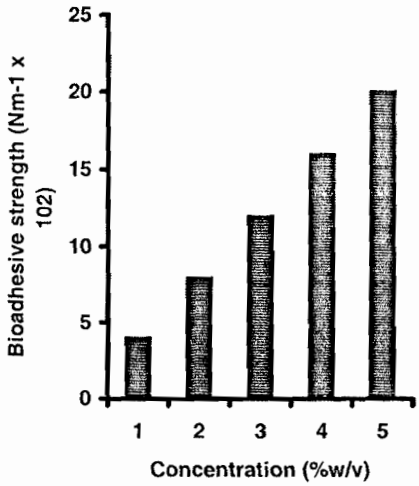


Fig. 1: Effect of concentration on the bioadhesive strength of the snail mucin dispersions: 1 = 4 %w/v; 2 = 8 %w/v; 3 = 12 %w/v; 4 = 16 %w/v; 5 = 20 %w/v

Evaluation of bioadhesive strength using coated glass beads: Fig. 2 shows the result of the bioadhesive test using coated glass beads. Different solvents have varying effects on the percentage of glass beads detached from the layer. This gives an indication that different detaching solvents with different pH values have ideal regions in the mucosal cavity where drugs could be targeted. pH determines the solubility, stability and viscosity of a given material, and these affect the ease of absorption of drugs from the gastrointestinal tract into the blood system.

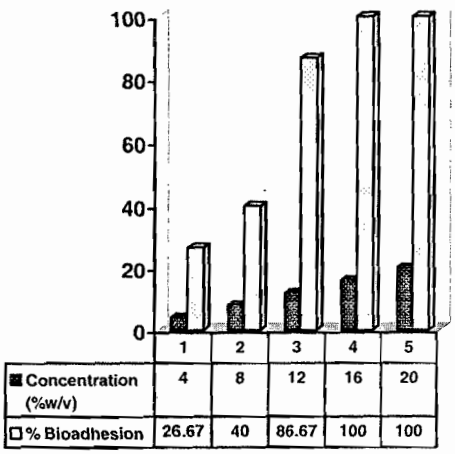


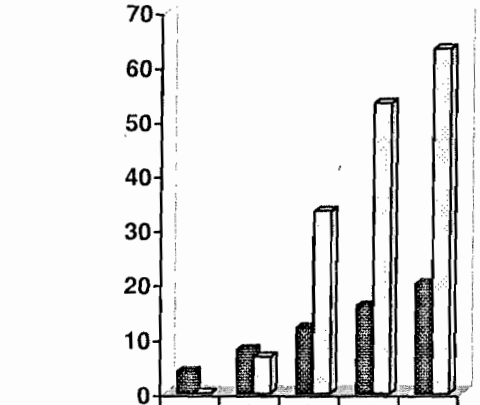
Fig. 2. Effect of concentration of snail mucin on the bioadhesion of coated beads using SGF as the detaching solvent

The SGF used in this study has a pH of 1.2 while the SIF has a pH of 7.5.

The figure revealed high bioadhesive strength as shown by the 16 %w/v and 20 % w/v concentrations of the mucin, which gave 100 % bioadhesion. The bioadhesive strength was very much higher when SGF was used as washing solvent implying that the snail mucin could be useful in formulating drugs that release primarily in the gastric mucosal region. This is further supported by the observation made by Smart *et al* (1984) during a study of a 1 % gelatin gel at various pH values, where they concluded that low pH favours bioadhesion.

Invariably, it was inferred that the SIF with a pH of 7.5 ± 0.1 weakened the bioadhesion of the snail mucin-coated glass beads by not offering much resistance to washing even at the highest concentration Fig. 3. At the highest concentration of 20 % w/v, SIF gave a bioadhesive value of 66.33 %, which is much less than the bioadhesion (86.67 %) offered by the 12 % w/v concentration of the mucin using SGF as the washing fluid. Therefore, it is meaningless to structure a dosage formulation with snail mucin to target entero (intestinal) adhesion or retentivity since mucoadhesion at that site is not favoured.

Bioadhesive test on the granules: The result of the bioadhesion so far supports the use of SGF as the detaching solvent, which indicates that low pH favours mucoadhesion. SGF was thus made use of in the case of granule studies. Moreover, chlorpropamide used in the formulation is shown to dissolve in dilute solutions of alkali hydroxides so that the SGF also favoured its use in the study. The result is presented in Fig. 4.



	1	2	3	4	5
■ Concentration (%w/v)	4	8	12	16	20
□ % Bioadhesion	0	6.67	33.33	53.33	63.33

Fig. 3: Effect of concentration of snail mucin on the bioadhesion of coated glass beads using SIF as the detaching solvent

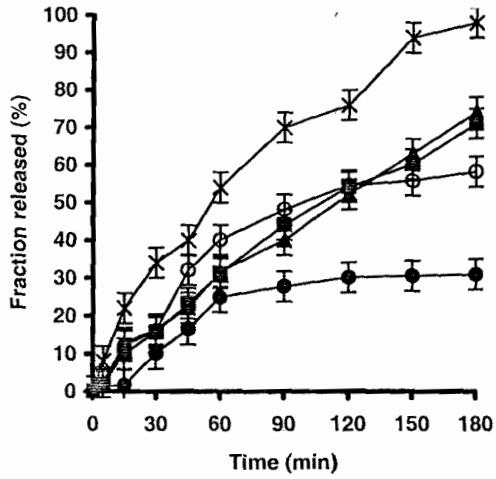


Fig. 4. Release profile of chlopropamide from the granules in SIF (pH 7.5) containing Carbopol Ultrez-10/snail mucin combinations

—■— Batch 1 (1:1) —●— Batch 2 (1:2)
—▲— Batch 3 (2:1) —×— Batch 4 (0:1)
—○— Batch 5 (1:0)

The result of bioadhesion on the formulated granules indicated that granules formulated with snail mucin alone that is, batch 4 were more bioadhesive than those formulated with Carbopol Ultrez-10 polymer alone (batch 5) and thus had a higher percentage of bioadhesion. However, there was optimum bioadhesion at equal combination of the Carbopol Ultrez-10 and snail mucin as in (batch 1). Maximum percentage bioadhesion was also achieved with Carbopol Ultrez-10 and snail mucin at a ratio of 2:1 (batch 3), which gave exactly the same percentage of bioadhesion as in 1:1 ratio (batch 1). These results show that chlorpropamide can be successfully delivered to the stomach by mucoadhesive formulations using the polymers studied, since bioadhesion, absorption and a possible prolonged effect on absorption can be achieved.

Granule micromeritics: The properties of the formulated granules are presented in Table 1. The range of granule flow rate was 1.43 – 2.50 g/sec. The angle of repose, which indirectly quantifies powder flowability, relates to inter-particle cohesion (Aulton, 1998) and was in the range of 39.47 – 41.05°. This implies good flow properties. The bulk and tapped densities were within the ranges of 0.50 – 0.59 and 0.63 – 0.71 respectively. The Hausner's quotient was within the range of 1.20 – 1.26. An increase in consolidated bulk densities is advantageous in tableting (Aulton, 1998). For Hausner's quotient, values approximately 1.2 indicate good flowability. The percentage compressibility values of the granules lie between 16.90 and 19.40 %. Materials that have values of 5 – 15, 12 – 16 and 18 – 21 % possibly have excellent, very good and fair flow behaviours respectively (Aulton, 1998). Those of values 23 – 35 % compressibility indicate poor flow. Therefore, the granules had excellent and good flow behaviour.

Release studies: The release profile of chlorpropamide from the granules is shown in Fig. 5. The release was fastest from the granules produced with small proportion of snail mucin alone as evident from batch 4 (0:1), which gave a maximum percentage release of 98.00 % at 180 min. Batch 5 (1:0) granules which had Carbopol Ultrez-10 but no snail mucin gave a much less percentage release of 58.17 at 180 min. However, optimum combination of snail mucin and Carbopol Ultrez-10, shown by their bioadhesive strengths, gave the least percentage release of 30.92 % (batch 2). Besides, the percentage release when Carbopol Ultrez-10 and snail mucin were in combinations of 1:1 (batch 1) and 2:1 (batch 3) were reasonably high giving values of 71.06 % and 74.15 % respectively. Polymers are known to retard drug release due to increase in tortuosity on gelling (Tongiven and Bintinf, 1998) or due to drug binding. This tortuosity increases the path length available for drug to diffuse out from the gel matrix. All the granule batches retarded the release of chlorpropamide achieving a maximum release of less than 100 %. The t_{50} for the release of chlorpropamide was achieved in 60 min for granule batch 4 (0:1), 120 min for batches 1

Table 1: Granule micromeritics

S/No.	Polymer-drug ratio	Flow Rate (g/s)	Angle of Repose (degrees)	Bulk density (g/ml)	Tapped density (g/ml)	Hausner's quotient (HQ)	Percentage compressibility (%)
1	1:1	1.88	39.47	0.50	0.63	1.26	20.63
2	1:2	2.31	39.75	0.54	0.67	1.24	19.40
3	2:1	2.50	39.77	0.55	0.67	1.22	17.91
4	0:1	2.50	40.02	0.59	0.71	1.20	16.90
5	1:0	1.43	41.05	0.51	0.63	1.24	19.05

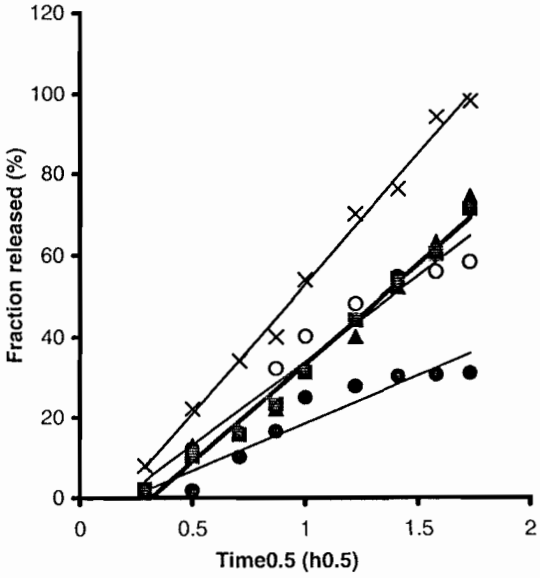


Fig. 5. Higuchi's plot of the percentage of chlorpropamide released from granules containing Carbopol Ultrez-10/snail mucin combinations

■ Batch 1 (1:1) ● Batch 2 (1:2)
▲ Batch 3 (2:1) × Batch 4 (0:1)
○ Batch 5 (1:0)

(1:1), batch 3 (2:1) and batch 5 (1:0). This was not achieved for the granule batch 2 (1:2). A more useful comparative approach was t_{20}, that is the time taken to release 20 % of chlorpropamide from the granules. The time taken to release 20 % of the drug was 15 min for batch 4, 45 min for batches 1, 3 and 5 then 60 min for batch 2. These prolonged periods of release of active ingredient from the granules further attests to the usefulness of these mucoadhesive materials (Carbopol Ultrez-10 and snail mucin combinations) for the formulation of chlorpropamide as a prolonged release dosage form.

The release result was further analysed using Higuchi's square root model (Higuchi, 1963). A plot of the amount of drug released against the square root of time when linear, indicates that diffusion is the predominant process of release. The entire granule batches showed linear plots except batch 2 granules due to high quantity of snail mucin in it (Fig. 6).

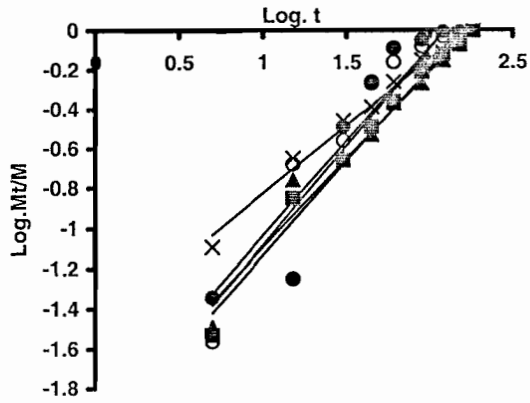


Fig. 6. Fick's plot of chlopropamide released from the granules containing Carbopol Ultrez-10/snail mucin combinations

■ Batch 1 (1:1) ● Batch 2 (1:2)
▲ Batch 3 (2:1) × Batch 4 (0:1)
○ Batch 5 (1:0)

This showed biphasic linear lines signifying that at initial time intervals, the release of drug was due to peripheral granules with improperly embedded drug giving an initial straight line but difficulty in penetration of the SIF accounted for the break in linearity and the second linear segment was due to the release as a result of total penetration of the SIF.

The release of chlorpropamide from the granule dosage form was also analysed using Fickian diffusion model to determine the mechanism of release of chlorpropamide from the granules (Peppas, 1985). To understand the release mechanism of chlorpropamide from the granules, the release rate was described with the following equations:

$$\frac{M_t}{M} = Kt^n \quad \ldots \quad \ldots \quad \ldots \quad \text{Eqn. 10}$$

$$Log\, M_t/M = \log K + n \log t \quad \text{Eqn. 11}$$

M_t/M is the fraction of released drug at time *t*, *K* is a characteristic constant that incorporates the structure and geometric characteristics of the release device and n is the release exponent indicative of the mechanism of release. As the K

value becomes higher, the drug is released faster. The n value of 1 corresponds to zero-order release kinetics. $0.5 < n < 1$ means a non-Fickian (anomalous) release model and n = 0.5 indicates Fickian diffusion [33]. From the plot of Log M_t/M versus log t (Fig. 6), the kinetic parameters, *n* and *k* were calculated and presented in Table 2.

Table 2: Buffer composition and solution pH for isoelectric point determination

Test tube number	Buffer mixture 0.2 M CH_3COOH	Composition (ml) 0.2 M CH_3COONa	Solution pH
1	1.95	0.05	3.2
2	1.90	0.10	3.4
3	1.80	0.20	3.8
4	1.40	0.60	4.4
5	1.00	1.00	4.7
6	0.60	1.40	5.1
7	0.20	1.80	5.7

This shows that the n values of all the batches lay between 0.5 and 1 thus indicated that release of chlorpropamide from the bioadhesive granules followed the non-Fickian diffusion model (anomalous behaviour). However, n values for batches 1, 2, 3 and 5 were approaching unity and could be said to have exhibited almost zero-order kinetics. The *K* values for batch 4 was higher than others indicating faster release in that batch. This is understandable since it contained a trace amount of snail mucin (0:1) only.

Conclusion: It has been shown in this study that snail mucin has the potential of being used as bioadhesive agent for the gastric delivery of chlorpropamide. It is highly bioadhesive especially at a low pH 1.2. The granules showed good release profiles, as well as acceptable micromeritic properties. The batches with low amounts of snail mucin gave better release of chlorpropamide.

Acknowledgment

This research was supported by the International Foundation for Science, Stockholm, Sweden, through a grant to Dr. M. U. Adikwu. He is grateful to the authorities of the International Foundation for Science, Sweden, for granting him the research award used for this studies (Grantee No. F/3560-1). The author is also grateful to God who created the animal that produces the mucin.

References

Adamson, A. W. (1982). Physical Chemistry of Surface". 4th ed., Wailey New York, 120-135.

Anonymous (1986) Handbook of Pharmaceutical Excipients, American Pharmaceutical Association, Washington D. C. and Pharmaceutical association of Great Britain London.

Attama, A. A., Adikwu, M. U. and Nnamani, P. O. (2003). Delivery of diclofenac sodium via non-disintegrating bioadhesive tablets of paraffin wax. *S.T.P. Pharma. Sci.*, 13 (2), 147-150.

Aulton, M. E. (1998). The Science of Dosage Form Design. Churchill Livingstone, U. K. pp. 17-31; 75-80; 154-161.

Bamba, M., Piusiux, F., Marty, J. P. and Cartensen, J. T. (1979). Release mechanism in gel forming sustained release preparation. *Int. J. Pharm.* 2, 229-231.

Baskin, T. M., Barisov, O. V., Karach, A. K. and Zhuhiria, E. B. (1985). Absorption of polypeptides on a solid surface adsorption and B-structure-coil transmission. BW-polymers 20, 24, 27.

Caramella, C., Bonferoni, M. C., Rossi, S. and Ferrari, F. (1994). Rheological and tensile tests for the assessment of polymer-mucin interaction. *Eur. J. Pharm. Biopharm.*, 40, 213-217.

Carstensen, J. T., and Chan, P. C. (1977). "Flow rates and repose angles of wet-processed granulations". *J. Pharm. Sci.*, 66, 1235-1238.

Chen, J. H. and Cyr. G. N. (1970). Adhesion in biological system. Academic Press London. 163-181.

Derjagiun, B. V. and Smilga, V. R. (1969). Adhesion: Fundamentals and Practice, Wolaren, London.

Florence, A. T. and Attwood, D. (1988). "Physicochemical Principles of Pharmacy". 2nd ed. Macmillian Press Ltd.

Genarro, A. R. (ed.)(1995). Remington The Science and Practice of Pharmacy. 19th ed. Vol. II Mack Publishing Company, Exton, Penysylvania, 8992.

Guyton, A. C. (1981). Textbook of medical physiology, sixth ed. Philadelphia, W. B. Saunders Company London, 801-802.

Hermann, L. S. (1987). The formation and activities of glycoproteins. *Int. J. Pharm.* 35, 55-60.

Higuchi T., (1963). Mechanism of sustained action medication: theoretical analysis of the rate of release of solid drug dispersed in solid matrices. *J. Pharm. Sci.* 52, 1145-1149.

Ishida, M. M., Nambu, N. and Nagai, T. (1983). Mucosal dosage form of Lidocaine for toothache using hydroxypropyl cellulose and Carbopol. *Chem. Pharm. Bull.*, 30, 986.

Khalid, M. T., Hung-Seng Ch'ng and S. Bai (1997). Effect of bioadhesive polymer on phenol red absorption in normal and ulcer rats. *Int. J. Pharm.* 156, 59-63.

Khanna, R., Agarwal, S. P. and Ahuja, A. (1998). Mucoadhesion buccal drug delivery: a potential alternative to conventional therapy. *Indian J. Pharm. Sci.*, 60 (1). 1 – 11.

Kinloch, A. J. (1980). The Science of adhesion surface and interfacial aspects. *J. Mater Science*, 15, 2141.

Krammer, H. W. (1983). Adhesion between polymers. *Acta Polymers*, 34, 112.

Lehr. C. M., Poetma, F.G.P., Junginger, H.E. and Tukker, J. J. (1991). An estimate of turnover time of intestinal mucus gel layer in the rat *in-situ* loop. *Int. J. Pharm.*70, 235-240.

Longer, M. A. and Robinson, J. R. (1986). Fundamental aspects of bioadhesion. *Pharm. Int.* 7, 114-117.

Maggi, L., Carena, E., Torre, M. L., Guinchedip, P. and Conte, U. (1994). In vitro/in vivo methods for the evaluation of bioadhesive polymers: a preliminary study. *STP Pharma. Sci.*, 4, 343-348.

Mattim, L., Attwood, D., Collett, J. H. and D'Emmanuele, A. (1995). The bioadhesive properties of a triblock co-polymer of ∈-caprolactone and ethylene oxide. *Int. J. Pharm.* 113, 223-225.

Mortazavi, S. A. and Smart, J. D. (1994). An in vitro method for assessing the duration of mucoadhesion. *J. Control. Rel.*, 31, 207-212.

Park, K. and Robinson, J. R. (1984). Bioadhesive polymer as platform for controlled drug delivery method to study bioadhesion. *Int. J. Pharm.* 19, 109-127.

Peppas, N. A. (1985). Analysis of Fickian and non-Fickian drug release polymer. *Pharm. Acta Helv.* 60, 110-111.

Rubinstein, A. and Tiresh, B. (1993). Mucus gel thickness and turnover in the gastrointestinal tract of the rat: response to cholinergic stimulus and implication for mucoadhesive. *Pharm. Research* 11 (6), 794-799.

Shah, A. C. (1995). Oral sustained release formulation. Avraham, Y. and Eva, H.W.P.(eds.) 34.

Smart, J. D., Kelaway, I. W. and Worthington, E. C. (1984). An in vitro investigation of mucosal-adhesive materials for use in controlled drug delivery. *J. Pharm. Pharmacol.*, 36, 295-299.

Taylor, D. C. (1986). Sites and mechanism of intestinal drug absorption. *Int. J. Pharm.* 17, 9.

Tongiven X. and Bintin H. (1998). Mechanism of sustained drug release in diffusion controlled polymer matrix – application of the percolation theory. *Int. J. Pharm.* 170, 139-149.

United States Pharmacopoeia XX ed., 1970 p. 1105.

Vogutskii, S. S. (1963). Autohesion and adhesion of high polymers, Wiley, New York.