

## Studies on Bioadhesive Granules I: Granules Formulated with *Prosopis africana* (Prosopis) Gum

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**Prosopis gum (PG)** extracted from *Prosopis africana* was investigated for bioadhesive delivery of theophylline (TPL). Bioadhesive granules containing TPL were formulated and the bioadhesive properties evaluated using adhesion of the granules onto a porcine intestinal mucus surface. The bioadhesion of the gum dispersion was also evaluated using coated glass beads and the strength of the films formulated from the gums was also determined. The release properties of the TPL-containing granules were assessed by diffusion of TPL from the granules through porcine intestinal wall into a sink solution. Sodium carboxymethylcellulose (SCMC) was used as the standard bioadhesive polymer. Results indicated that PG is highly bioadhesive compared to SCMC. The result of the release studies also showed that PG could be used to deliver TPL in a bioadhesive dosage form.

**Key words** bioadhesion; granules; prosopis gum, sodium carboxymethylcellulose.

Most biological tissues contain mucosal epithelia covered by mucus.<sup>1)</sup> This makes it possible for bioadhesive polymers to interact with such tissues producing the phenomenon of bioadhesion. Glycoproteins present in mucus are believed to be responsible for interaction between mucus and biopolymers.<sup>2)</sup> However, many factors play a role in bioadhesion.<sup>3–5)</sup> Many *in vitro* methods are used to evaluate bioadhesion,<sup>6,7)</sup> but *in vivo* evaluation remains the most informative.<sup>8)</sup> Indeed, the method employed for evaluation of the bioadhesive properties of a polymer is generally formulation-specific and many biopolymers have been evaluated for bioadhesive delivery of drugs,<sup>9)</sup> however applicability depends on the target area and the physicochemical properties of the candidate polymer. In this study, prosopis gum (PG) obtained from the tropical plant, *Prosopis africana* (Fam. Mimosaceae) was used for bioadhesive-prolonged delivery of theophylline (TPL), an antiasthmatic drug whose physicochemical properties favour such a delivery system.<sup>10)</sup> *Prosopis africana* has been the subject of interest of many workers and has been assessed in many dosage forms.<sup>11–15)</sup> Gums are made up of highly branched polysaccharides with chain structure formed when monosaccharides condense with the elimination of water molecule(s). PG is a natural polysaccharide consisting chiefly of glucose, fructose, galactose and xylose as the monosaccharide units, as determined by thin layer chromatography and complete acid hydrolysis analysis.<sup>16)</sup>

### Materials and Methods

**Materials** Sodium carboxymethylcellulose (SCMC) (Aqualon); sodium chloride and hydrochloric acid (Merck); TPL (BDH); and acetone and ethanol (M and B) were used without further purification. All other reagent solvents were of analytical grade and were used as supplied. Distilled water was obtained from a glass still while PG was obtained from a batch processed in our laboratory.

**Preparation of PG** PG was extracted from the seeds of *Prosopis africana* using the method described in an earlier study.<sup>16)</sup>

**Evaluation of the Bioadhesive Strength of the Polymers Use of Coated Glass Beads** Increasing concentrations of PG and SCMC were used to coat glass beads with an average diameter of 3 mm and average weight 56 mg. The beads were coated to an average weight of 65 mg, by successive dipping in the polymer solution, air-drying and storage in a desiccator until use. Concentrations of polymers used for the study were 1.0, 2.0, 2.5, 5.0 and 10.0% w/v. The apparatus designed and used in this study is shown in Fig. 1, and consists 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 hog jejunum (1.7×15.0 cm) was pinned on the plastic support, and a beaker was placed directly under the plastic support to collect the detached beads. Before coating the glass beads, they were thoroughly cleaned with distilled water and then with acetone to maximize the roughness factor.<sup>17)</sup> Twenty coated beads were placed on the exposed mucus surface of the tissue (Fig. 1). Mucus-polymer interaction and polymer hydration was allowed to take place over a period of 15 min. Simulated intestinal fluid (SIF) without pepsin (250 ml) at pH 7.2, contained in the separating funnel, was allowed to flow over the beads at a rate of 30 ml per min. The number of undetached beads was noted and used as a measure of bioadhesion. The experiment was repeated five times and the average value recorded.

**Use of the Tensiometer** Preparation of Mucin: The mucin solution used for the study was prepared as described elsewhere.<sup>18)</sup>

**Bioadhesion Experiment:** This was performed using a tensiometer (A. Kruss, model No. Nr 3124, Germany) adapted to measure bioadhesive strength. The same polymer concentrations used in the coated bead experiment were used. A 2 ml volume of the prepared mucin solution was poured into a watch glass, which was placed on the platform of the zeroed tensiometer. The plate on which the aqueous polymer dispersion was coated to 2 mm thickness was dried for 5 h in a desiccator and then hung on the lever arm of the tensiometer and the platform gradually moved to establish contact with the coated plate. A 15 min contact time between the polymer coat and the mucin was allowed to ensure proper interaction. The glass plate was raised by means of a screw until it just detached from the surface of the mucin. The force required to remove the glass plate from the surface of the mucin was read off from the microform balance in degrees and conversion of this to tension was done using Eq. 1.<sup>19)</sup> In each case, an average of three determinations was taken.

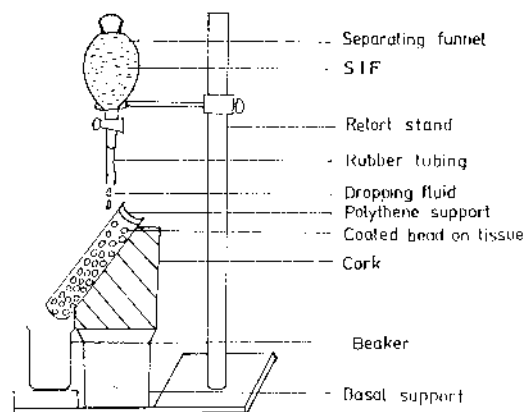


Fig. 1. Apparatus for Bioadhesion of Coated Glass Beads on the Tissue (Hog Jejunum)

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$$T = \frac{Mg}{2L} F \quad (1)$$

Where  $T$  is the tension equivalent to bioadhesive strength,  $M$  is the mass required to return the lever pointer to its original position,  $L$  is the perimeter of the plate,  $F$  is a constant dependent on the perimeter,<sup>19)</sup> and  $g$  is the acceleration due to gravity.

**Preparation of the Films** Films of equal thickness and diameter were prepared by casting a 10% w/v aqueous dispersion of PG and SCMC individually in petri dishes of 15 cm internal diameter. The casts were dried at 40°C for 10 h in an oven (Model 854, Memmert, Germany) and thereafter stored in a desiccator until required for use.

**Evaluation of the Films** The prepared films were microscopically and macroscopically examined for some physical parameters such as homogeneity, cracking tendency, etc. Average thicknesses of the films were also recorded.

**Evaluation of the Mechanical Properties of the Films** Films sectioned squarely (10 cm<sup>2</sup>) were padded with equal area of cellophane and held with a cyanoacrylate adhesive. One end of the film was attached to a clamped strong inelastic hook and to the other end was attached increasing weights. The load at which the film broke was noted for each film. An average of five determinations for each film was taken as the film strength.

**Preparation of Granules** Different batches of granules were prepared to contain 1:1, 2:1, 3:1 and 4:1 ratios of either PG or SCMC and TPL. 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. Granules without TPL were similarly prepared.

**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 SIF flow the undetached granules were recovered, dried and weighed. A similar experiment was run with the bland granules. The bioadhesion percent was evaluated by the equation below:

$$\% \text{ bioadhesion} = \frac{W_g^t - W_g^0}{W_0} \times \frac{100}{1} \quad (2)$$

where  $W_g^0$  is the recovered weight of granules without TPL,  $W_g^t$  is the recovered weight of the granules with TPL,  $W_0$  is the weight of the granules used for the bioadhesion test.

$$\% \text{ bioadhesion} = (W_g^0 - W_g^t) \times 100 \quad (3)$$

A 1 g quantity of granules was used throughout the experiment, hence Eq. 2 reduces to Eq. 3.

**Absolute Drug Content Measurement** A 1 g quantity of each batch of the granules was placed in a 100 ml volumetric flask. The flask was made up to volume with a decinormal solution of HCl, and allowed to hydrate for 24 h at 28°C. The solution was thereafter analysed spectrophotometrically at 272 nm using a spectrophotometer (SP 450, UV Vis, Pye Unicam). The drug concentration was calculated with reference to Beer's law plot for TPL.

**Release Studies** A 1 g quantity of each granule batch was introduced into a cut portion of jejunum (10 cm in length), which was tied at one end. A period of 15 min was allowed for bioadhesion to take place. A 10 ml quantity of SIF was introduced into the jejunum tube and the second end tied firmly. This set-up was introduced into a dissolution apparatus (DTD, Erweka, Germany) containing 500 ml of the dissolution medium (SIF) maintained at 37±1°C. At predetermined time intervals, samples of the dissolution medium were withdrawn and analyzed for TPL spectrophotometrically at 282 nm. An average of two absorbance readings at each time interval were recorded. The absorbance readings were thereafter converted to concentrations with reference to Beer's law plot.

## Results and Discussion

Table 1 shows the results of the bioadhesion test using coated glass beads. The results indicated that PG is more bioadhesive than SCMC at equivalent concentration. PG coated glass beads had maximum resistance to washing at a lower concentration (2.5% w/v) than SCMC (10%). This invariably leads to the conclusion that based on the bioadhesion of coated glass beads, PG is more bioadhesive than

Table 1. Bioadhesion of Coated Glass Beads on the Mucus Surface

Concentration (% w/v)	Percentage of glass beads undetached (%)	
	PG (mean±S.D.)	SCMC (mean±S.D.)
1.0	1.8±0.21	0.7±0.12
2.0	74.2±0.11	0.8±0.31
2.5	100.0	14.8±0.19
5.0	100.0	79.9±0.21
10.0	100.0	91.5±0.51

Table 2. Tensiometric Test of Polymers

Concentration (% w/v)	Bioadhesive strength (mN m <sup>-1</sup> )	
	PG (mean±S.D.)	SCMC (mean±S.D.)
1.0	19.24±0.11	1.29±0.19
2.0	22.81±0.71	4.44±0.11
2.5	38.51±0.31	7.25±0.12
5.0	40.78±0.25	16.22±0.41
10.0	41.81±0.29	19.41±0.21

SCMC. The differing values of resistance to washing of the coated glass beads may be due to differences in the strength of the gel network of the gum dispersions.<sup>13)</sup> There may be a greater interaction of the PG molecules in the gel and glycoprotein in the mucus, producing an interpolymer complex with greater bond strength than that produced by SCMC and mucus gel. The results of tensiometric determination of the bioadhesive interaction confirmed those of the bioadhesion assay using coated glass beads (Table 2). PG dispersions produced higher bioadhesive force than SCMC dispersions, possibly due to the reasons indicated above. It may also be due to the fact that PG gels were thicker and more adhesive than SCMC gels and thus adhered faster to the glass plate. The coat thickness of 2 mm was thus achieved faster with PG. Coating of the plate was very difficult with SCMC gels because of lower adhesive properties. This made the interaction with the mucus very weak and thus weaker bond strengths were developed, compared to PG gels.

Results of the macroscopic studies on the films indicated that the formed films were hard, brittle and opaque. Microscopic examination showed that all the films were homogeneous and had no marked difference in their porosities ( $p>0.05$ ). These properties may not be directly related to the bioadhesive properties of the polymers. However, they may give insight to how films deposited on glass beads or coated plates may behave prior to the bioadhesive experiments. Cracked films may lead to adhesive bond failure. Very hard films may prevent sorption of fluid which leads to swelling and bioadhesive interaction. Thus, bioadhesion may be delayed and the dosage form coated with polymer may pass through the gastrointestinal tract (GIT) without any gastro-adhesive process occurring. This could result in a significant fraction of the drug being wasted.<sup>20)</sup> The strength of the films however indicated that the films cannot withstand the shock of handling and transportation. The polymers had low values: PG (103.2±2.3 N) and SCMC (83.8±5.2 N). These indicate that the films of tablets coated with these polymers may crack and the objective of using these polymers to deliver a bioadhesive tablet formulation may not be achieved. This re-

Table 3. Bioadhesion Ability of the Granules

Polymer/Drug Ratio	Percentage of granules undetached (%)	
	PG (mean±S.D.)	SCMC (mean±S.D.)
1 : 1	85.1±0.2	58.7±0.7
2 : 1	81.9±0.4	55.9±0.4
3 : 1	81.7±0.6	54.9±0.2
4 : 1	79.2±0.1	41.8±0.8

sult is in agreement with earlier studies.<sup>21)</sup> The results of bioadhesion test and on formulated granules indicated that granules formulated with PG were more bioadhesive than those formulated with SCMC. They had a higher percentage of bioadhesion (Table 3). This result conforms to result of the bioadhesion of the coated glass bead on the everted jejunum. However, there was a decrease in the percentage bioadhesion with increase in the proportion of TPL in the granule batches. This may be due to a dilution effect on the part of the gum. Similar effects were reported by other research.<sup>22)</sup> These results show that TPL can be successfully delivered to jejunum, since bioadhesion, absorption and a possible prolonged effect on absorption could be achieved. It is possible for the bioadhesive granules to be encapsulated using capsule shells designed to withstand the acid environment of the stomach so that the shells could reach the ileum intact. Also, the formulated granules could be coated with pH sensitive polymers that are favoured by alkaline pH.<sup>23)</sup> Thus, the polymer hydrates after passing the stomach to allow the bioadhesive process to take place. This process is similar to the disintegration of the capsule shell above to liberate the bioadhesive formulation. Drugs that are sensitive to acidic pH, enzymatic attack in the stomach or those that cause unbearable gastric irritation or erosion are good candidates for this method of bioadhesive drug delivery. The physicochemical properties of the granulated drug must favour these conditions. The results of the release study corresponding to the diffusion of TPL through the jejunal membrane into the sink solution are shown in Figs. 2 and 3, and indicated a higher diffusion rate in granules containing a lower quantity of PG or SCMC. An increase in the quantity of gum retarded the diffusion of drug out of the granules. This is in accordance with the release of drugs from polymer matrices. Higher concentrations of polymers are known to retard drug release.<sup>24)</sup> This is because the gel barrier created by the polymer on swelling leads to entrapment of the drug molecules and an increase in tortuosity of the entire system. Similar results were obtained for both PG and SCMC, however, there was higher diffusion of TPL from granules prepared with PG than those prepared with SCMC. This may be due to a higher incidence of drug binding in SCMC than PG. Drug binding has been shown to retard drug release. It may also be due to the high swelling rate of PG.<sup>12)</sup>

## Conclusion

This study showed that TPL could be delivered in a bioadhesive dosage form formulated with PG, a natural polymer. This gum (PG) was found to be more bioadhesive than SCMC and could be harnessed in the formulation of bioadhesive dosage forms of some specialised drugs.

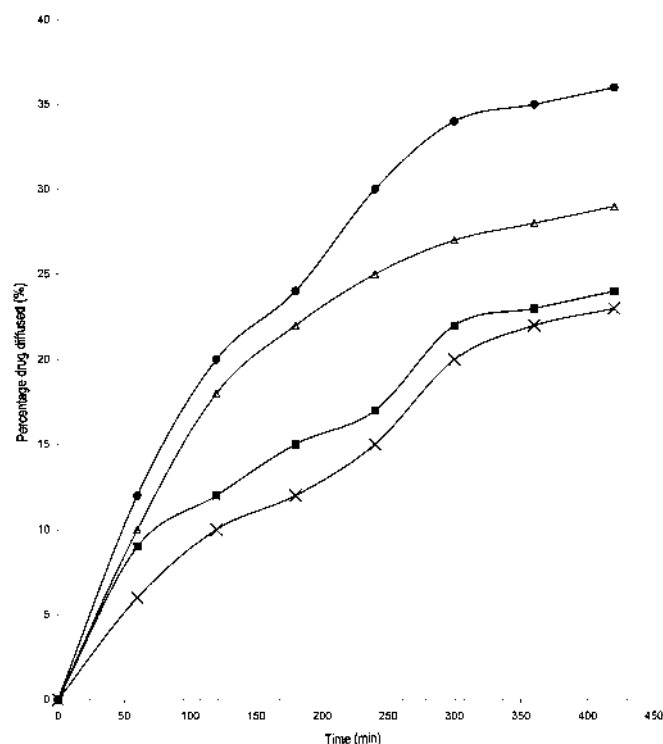


Fig. 2. Percent Theophylline Diffused from PG Granule Matrix as a Function of Time Using Hog Jejunum as Diffusion Barrier

●, 1 : 1 (PG : TPL); △, 2 : 1 (PG : TPL); ■, 3 : 1 (PG : TPL); X, 4 : 1 (PG : TPL).

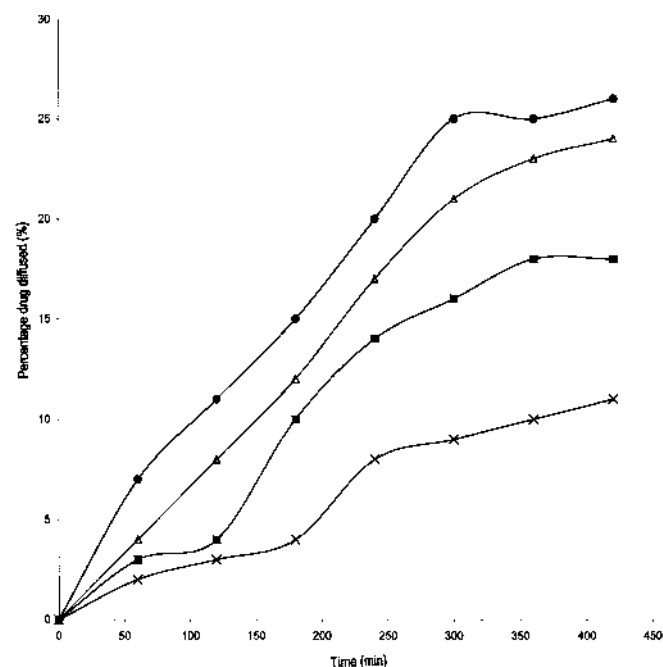


Fig. 3. Percent Theophylline Diffused from SCMC Granule Matrix as a Function of Time Using Hog Jejunum as Diffusion Barrier.

●, 1 : 1 (SCMC : TPL); △, 2 : 1 (SCMC : TPL); ■, 3 : 1 (SCMC : TPL); X, 4 : 1 (SCMC : TPL).

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