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COMMUNICATION

In Vitro and In Vivo Adhesion Testing of Mucoadhesive Drug Delivery Systems

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ABSTRACT

Bioadhesive tablets were prepared by physical mixing of polymers and drug, then granulating and compressing into a tablet. The mucoadhesion was evaluated by shear stress measurement, detachment force measurement, and X-ray photography of the rabbit gastrointestinal tract. The strong interaction between the polymer and the mucous lining of the tissue helps increase contact time and permit localization. Polymers like hydroxypropyl methylcellulose K4M (HPMC K4M), hydroxypropyl methylcellulose 100 cps (HPMC 100 cps), carbopol-934, sodium carboxy methylcellulose (Na CMC), guar gum, and polyvinylpyrrolidone (PVP) were tested by shear stress measurement and detachment force measurement methods. HPMC K4M, showing maximum bioadhesion, was used in further studies. Adhesion was maximum between pH 5 and pH 6. Maximum adhesion was observed in the duodenum, followed by the jejunum and ileum. Barium sulfate ($BaSO_4$) matrix tablets containing polymer and drug were subjected to X-ray studies in rabbits, and it was found that the tablet was mucoadhesive even after 8 hr. Enteric coating did not show any effect on mucoadhesion after passing from the stomach.

Key Words: *Design; Bioadhesive; Delivery systems; Detachment force; Evaluation; Mucoadhesion; Polymers; Shear stress; Testing methods; X-ray method.*

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INTRODUCTION

The first step in the selection of a bioadhesive for a particular application is to determine whether its properties are suitable for the intended application. Testing is essential for the development, quantification, processing, and proper use of bioadhesives. Since there are a large number of bioadhesives in different physical forms and biological substrates of different natures, evaluation of bioadhesive properties is inherently complex and diverse. Evaluation and comparison of the properties of various bioadhesives can be obtained only if all the conditions of the test and the experimental procedures are kept constant (1).

EXPERIMENTAL

Materials

Hydroxypropyl methylcellulose K4M (HPMC K4M) (Trident Pharmaceuticals, Hyderabad, India); polyvinylpyrrolidone (PVP), molecular weight 44,000 (BDH Chemicals, England); Carbopol-934 (Wilson Laboratories, Bombay, India); HPMC 100 cps (Loba Chemie, Bombay, India); guar gum (Trident Pharmaceuticals, Hyderabad, India); and sodium carboxy methylcellulose (Na CMC) (Wilson Laboratories, Bombay, India) were used in this study.

Methods

In Vitro Adhesion Testing

The in vitro adhesion testing of different polymers was done based on the principles of shear stress measurement and the detachment force measurement.

Shear Stress Measurement Method

Two smooth, polished glass blocks were selected, one block was fixed with the adhesive Araldite onto a glass plate that was fixed on a table. The level was adjusted with a spirit lamp. The upper block was passed down through a pulley by a thread, the end of which was tied to a pan. The assembly is shown in Fig. 1. Solutions of different polymers (2%) were prepared using water as a solvent, and solutions of different pH (pH 2–4: acid, phthalate buffer; pH 6: neutralized phthalate buffer; pH 6–8: phosphate buffers) (2) were prepared with same concentrations of polymers. One drop of the polymer was kept on the center of the fixed block, and then onto it the second block was placed and pressed with some pressure

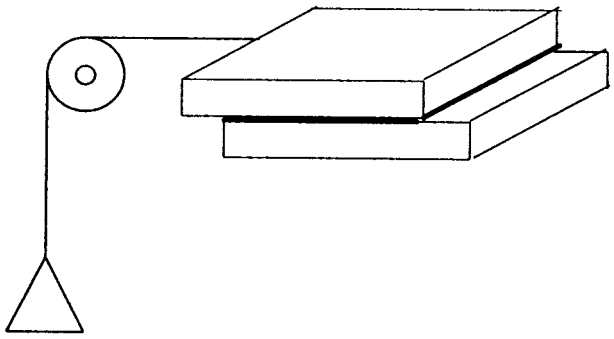


Figure 1. The assembly used in the shear stress measurement method.

(100 g). After keeping it for fixed time intervals of 5, 10, 15, 20, and 30 min, the weights were added to the pan. The weights required to pull the block or to make it slide down from the base block represent the adhesion strength, that is, the shear stress required to indicate the adhesion strength.

Detachment Force Measurement Method

This is the method used to measure in vitro mucoadhesive capacity of different mucoadhesive polymers. It is the modified method developed by Martti Marvola (3) to assess the tendency of mucoadhesive materials to adhere to the esophagus. The assembly is shown in Fig. 2. Immediately after slaughter, different parts of intestine were removed from sheep and transported to the laboratory in Tyrode solution kept at 4°C. The composition of Tyrode solution (g/L) is NaCl, 8; KCl, 0.2; $CaCl_2$ $2H_2O$, 0.134; $NaHCO_3$, 1.0; sodium dihydrogen phosphate, 0.05; glucose-H_2O, 1.0. During the experiment, the solution was aerated with pure oxygen and kept at 37°C. Segments 6–7 cm long were cut from the intestine. The lower end of each intestinal segment was tied off, and this was then tied to the aerator tube, with the upper end tied around a 15-mm diameter glass tube. Different parts of sheep gastrointestinal tract (duodenum, jejunum, and ileum) were taken to study the effect of pH variation in the gastrointestinal tract on detachment force.

The adherence can be recorded using the 8-mm tablets of plain chlorpheniramine maleate and chlorpheniramine maleate plus polymer matrix (1:10). A hole was drilled in the tablets to be tested. A thread was passed through the tablet and tied around it; this tablet was placed, using a plastic tube as an applicator, in the intestinal preparation for a fixed time, and on the other end of the glass rod a pan was attached in which a beaker was placed.

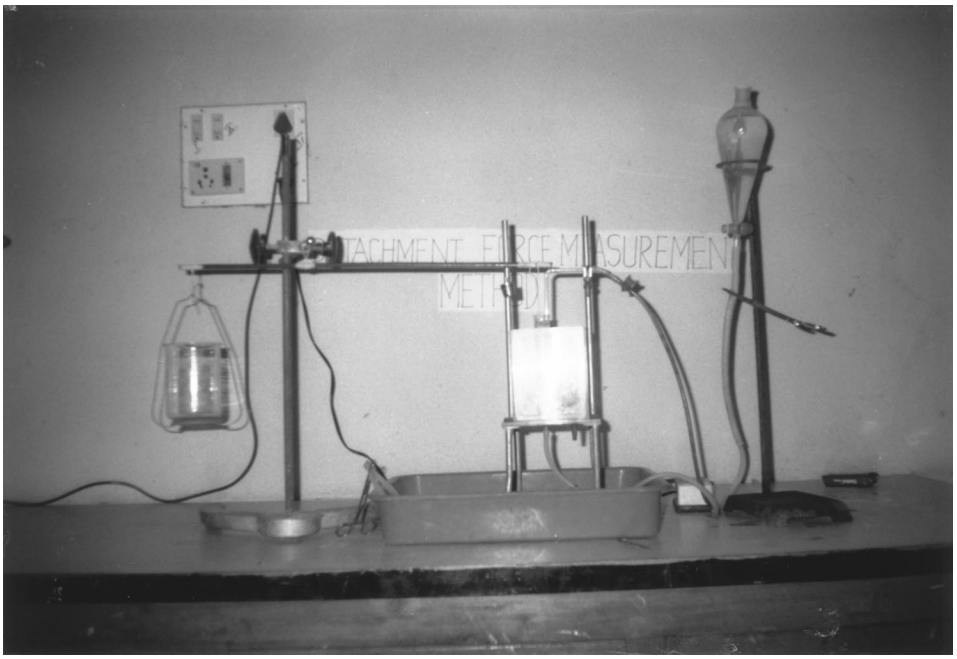


Figure 2. Assembly used in detachment force measurement method.

After keeping the preparation for a fixed time (i.e., 30 min and 1 hr). The water was added with a burette dropwise to the beaker. The force needed to detach the tablet was measured using a modified prescription balance. This force was used as a parameter for adherence. The force F in newtons was calculated by the equation

$$F = 0.00981\ W/2$$

where W is the amount of water in the beaker in grams.

In Vivo Bioadhesion Testing: X-Ray Studies

The 6-mm diameter $BaSO_4$ tablets (weight 120 mg) were prepared in three different types of formulations: conventional tablets, $BaSO_4$ and polymer in a 5:7 ratio matrix tablets, and enteric-coated tablets of $BaSO_4$ matrix tablets. The polymer selected for the study was HPMC K4M.

Nine healthy rabbits of the same age and weight were the subjects. They fasted overnight, and on the morning of the next day, the three types of tablets were administered each to three rabbits followed by giving 25 ml of water and at different intervals of 2,4,6 and 10 hrs. The rabbits were X-ray photographed and observed for the position of the tablet up to 10 hr after administration. In between the study, the rabbits were fed after 5 hr of tablet administration, and the effect of food was also observed.

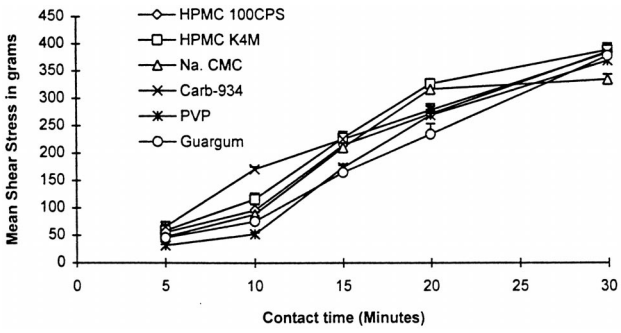


Figure 3. Effect of contact time on adhesion strength of polymers.

RESULTS AND DISCUSSION

The results of the shear stress measurement for the weight required to break the adhesion recorded with mean and standard deviation for various polymers with different contact times are shown in Fig. 3. The experiments were performed six times for each polymer, and the average values were taken. From Fig. 3 it can be seen that increasing contact time for adhesion increased the force required in terms of weight for all the polymers. Increasing the time of contact increases the adhesion

strength, allowing greater adhesion (4). Adhesion was reported to be affected by hydration, favoring optimal hydration (5). Increasing contact times might also be reduce hydration due to evaporation. Care was taken to prevent evaporation, thus longer contact times facilitated greater adhesion.

In the present work, the polymers tested were HPMC 100 cps, HPMC K4M, Na CMC, Carbopol-934, PVP, and guar gum, all at 2% strength. Good reproducibility was seen in the shear stress measurements for most polymers. From the results in Fig. 3, it can be seen that, of all the polymers, HPMC K4M was found to have greater adhesion; with increasing contact time, adhesion increased. The maximum increase in adhesion with contact time was observed with HPMC K4M. The order of polymers by increasing adhesion strength are Na CMC, PVP, guar gum, HPMC 100 cps, Carbopol-934, and HPMC K4M. In general, results are in agreement with earlier reports. HPMC possesses a large number of carboxyl of hydroxyl groups that are responsible for adhesion.

From the Fig. 4, it can be seen that the maximum adhesion occurs between pH 5 and pH 6. After pH 6, the adhesion was decreased. Similar results were obtained by earlier workers, and it was reported that maximum adhesion was seen at pH 5–6 for most of the polymers checked (6). The charge density of the polymers is influenced by the pH, which in turn affects adhesion.

To confirm whether the polymers exhibit the same strength in contact with the mucous membranes and formulations, they were further checked for the bioadhesive strength of tablets based on detachment force measurement.

Hydration of the mucoadhesive polymer is essential to initiate the mucoadhesive bonding process. In the case of tablets applied in the dehydrated state, which is most

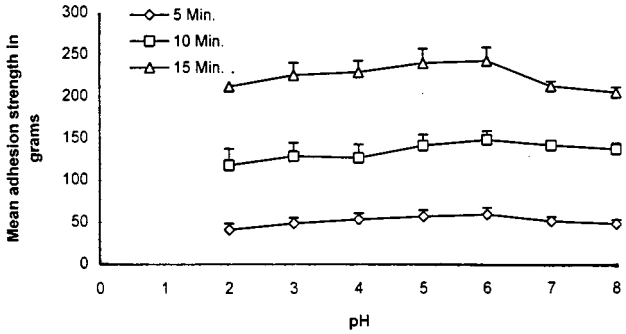


Figure 4. Effect of pH on adhesion strength of HPMC K4M.

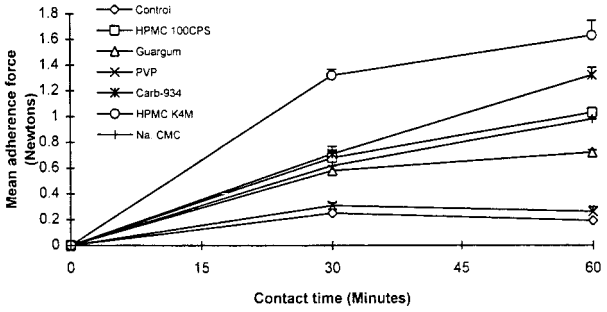


Figure 5. Detachment force measurement method.

convenient, it is essential that sufficient water is available so that rapid hydration takes place, and a flexible rubbery state occurs. The capillary attraction force arising when water from the space between the mucosa and the polymer is taken up by a dry system may be considerable (7). It is of great importance that the mucoadhesive material will develop a bond with only a minimum applied force. Once the bond is formed, reduction in rate of swelling due to water uptake from the tissue surface may only prolong the association of the tablet with the mucosa. Removal of water from the underlying mucous layer by the hydrating polymer may increase the cohesive forces of mucous; this plays a vital role in the establishment of an effective mucoadhesive bond.

The detachment force in newtons and the corresponding weight required to detach the tablets adhered to sheep intestine are shown in Fig. 5. Increasing contact time enhanced the force required for all polymers tested. The matrix tablets were prepared with polymers and drug substances by physical mixing, granulating, and then compressing. The highest mucoadhesion, as observed from

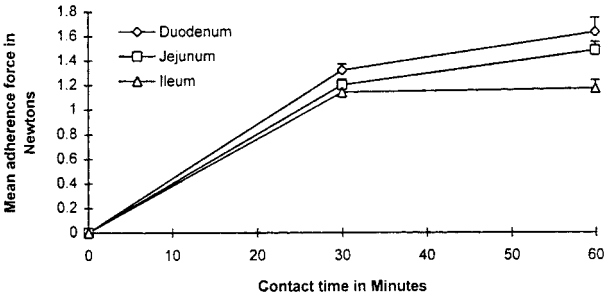
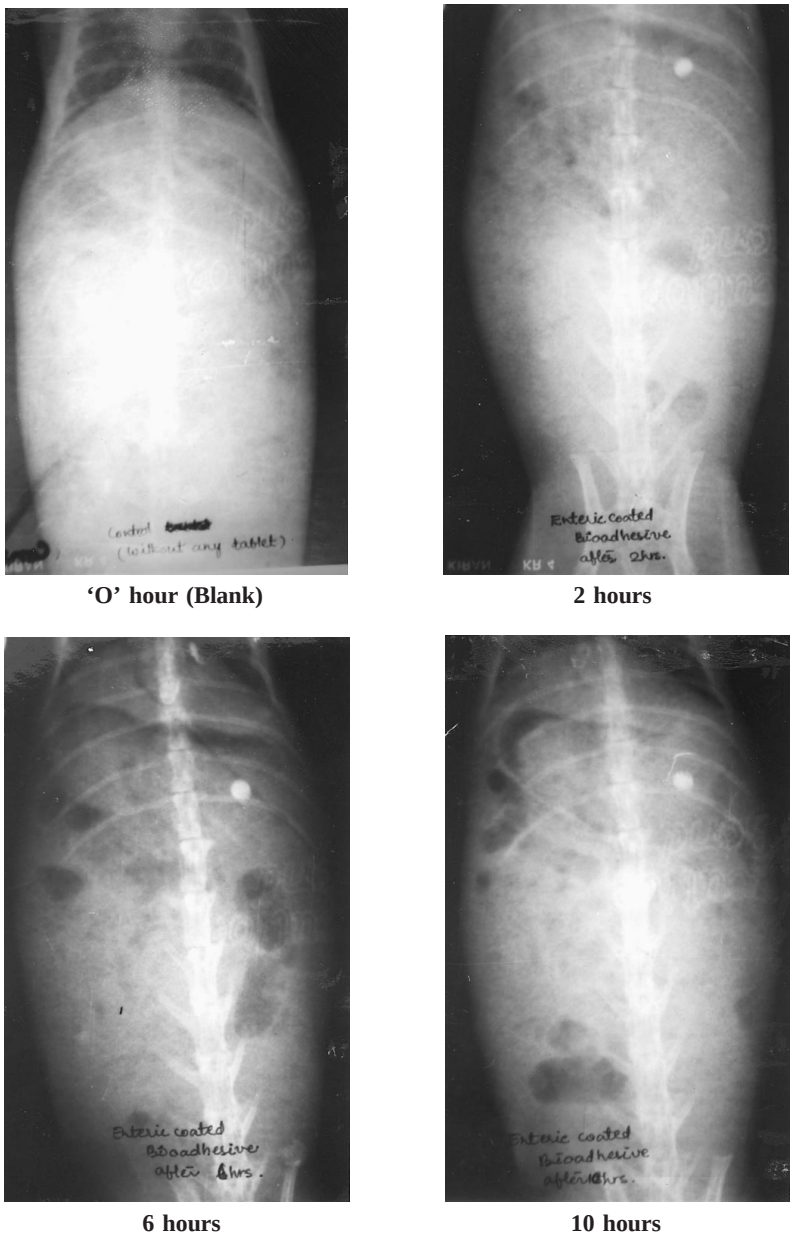


Figure 6. Detachment force measurement at different parts of small intestine of sheep.

the highest force required for detachment, was seen with HPMC K4M, followed by Carbopol-934 and HPMC 100 cps. The results were in agreement with general principles and also with our results obtained by the shear stress measurement method.

Based on the results obtained from these two experiments, the polymer HPMC K4M was selected, formulated into matrix tablets, and subjected to detachment force method at different parts of the sheep intestine to find the adhering force of these tablets to different parts of the intestine (viz., duodenum, jejunum, ileum).

From Fig. 6, it was found that the maximum adherence force was at the duodenum, followed by the jejunum and then ileum. This may be due to pH variation throughout



'O' hour (Blank) **2 hours**

6 hours **10 hours**

Figure 7. X-ray photographs taken after administering the enteric-coated bioadhesive matrix tablet.

the gastrointestinal tract. The pH varies from pH 5 in the duodenum to pH 7 in the ileum. In simple adhesion testing, it also was found that the adhering force was greater between pH 5 and pH 6. The results correlate well.

The results of the in vivo mucoadhesion testing by X-ray studies in rabbits were shown in photos for blank tablets (control tablet of $BaSO_4$), HPMC K4M matrix tablets of $BaSO_4$ and drug, and enteric-coated HPMC K4M matrix tablets of $BaSO_4$ and drug. The control tablets disintegrated within 1 hr and could not be traced in the X-rays exposed at the second hour. Mucoadhesion with matrix tablets was strong, and the tablets adhered in the stomach and duodenum, as seen from the X-ray photograph. The matrix tablets adhered in the stomach, and enteric-coated tablets adhered in the duodenum (Fig. 7) after passing out of the stomach. The mucoadhesion was so strong that they could be seen at almost the same place even 10 hr after administration. There was no significant effect of food in the transit of the tablets with mucoadhesive polymer.

CONCLUSIONS

Adhesion was observed to decrease in the order HPMC K4M, HPMC 100 cps, Carbopol-934, PVP, guar gum, and Na CMC. Enhanced contact time increased adhesion in all the polymers. Between pH 5 and pH 6, the adhesion was found to be maximum in all polymers. To study the tendency of adherence of the tablet dosage forms to the gastrointestinal membrane by a modified Martti Marvola method, HPMC K4M was found to have maximum mucoadhesion. The adherence was maximum in the duodenum, followed by the jejunum and ileum. Enteric coating did not show any effect on mucoadhesion after passing from the stomach. X-ray photographs of rabbits reveal that good bioadhesion was achieved with HPMC K4M for 8 to 10 hr. An enteric-coated tablet reached the duodenum and was seen even after 10 hr.

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