

In vitro evaluation and application of Carbopol 940- tragacanth binary mixtures in the formulation of bio-adhesive hyoscine hydrobromide tablet.

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Abstract

Detachment of polymer coated glass beads / bio-adhesive tablets and measurement of force of adhesion of polymer-coated plate/bio-adhesive tablets were employed in the in vitro bio-adhesive assessment of carbopol 940, tragacanth and their binary mixtures on isolated mucin and on intestinal mucus. The effect of pH on the force of adhesion of these polymer dispersions on isolated mucin was also assessed. Systems containing tragacanth alone or its binary mixture with carbopol 940 exhibited lower bio-adhesive strength relative to carbopol 940. In systems where effect of pH on the force of adhesion was investigated, low pH and medium alkaline pH (pH 8-9) favoured strong bio-adhesive strength. The overall results indicate that effective and localized bio-adhesive delivery of hyoscine hydrobromide could be achieved with Carbopol 940 and with its binary mixture with tragacanth in the ratio of 3:2.

Introduction

In recent years considerable attention has been focused on developing delivery systems that utilize the principle of bio-adhesion for optimum drug delivery. Bio-adhesion has been defined as a phenomenon in which a biological substance is adhered to another substance, which may be either of biological or non-biological origin (1). When applied to drug delivery, the term is used to describe the adhesive phenomenon between two biological objects (2,3). Bio-adhesive drug delivery systems have been demonstrated as effective dosage forms for controlled delivery of various drugs using buccal, ocular, rectal, vaginal, nasal, sublingual and oral routes of administration (2,4). In addition, these delivery systems can be utilized to optimize either the systemic or local delivery of drug. Many polymeric materials, synthetic, semi-synthetic and plant hydrocolloids have been utilized as bio-adhesive materials for the delivery of drugs to certain regions of the gastrointestinal tract (GIT). In this regard, polymers with hydrophilic functional groups in their molecules such as carboxyl, Iryciroxyl, amide and sulphate groups which are now known to possess good bio-adhesive properties are employed. Among various polymers investigated, however, carboxymethylcellulose, Carbopols, hyaluronic acid, and polycarboxophil are regarded as substances with strong binding properties (5,6). A polymer can be used alone or in combination with other polymers to achieve either higher bio-adhesive strength, economy or both. Admixtures of two Carbopols and carboxymethylcellulose were recently evaluated as bio-adhesive system for metronidazole tablets (7). In the present study *in vitro* bio-adhesive properties of binary mixtures of a synthetic polymer, carbopol 940 and a plant gum, tragacanth, were evaluated and subsequently employed in the formation of bio-adhesive hyoscine hydrobromide tablets. The effects of pH on bio-adhesive strengths of these two polymers and one of their binary mixtures were also assessed. Prior to selecting a bio-adhesive compound to prepare a bio-adhesive delivery system, it is necessary to determine its adhesive capacity. In this regard, preliminary studies for *in vitro* screening are needed. This limits the number of formulations and or polymers that are going to be tested later *in vitro*

Materials and methods

Materials

The following materials were used as procured from their manufacturers: Carbopol 940 (G.F Goodrich, USA), tragacanth (Merck, Germany), hyoscine hydrobromide (S.H. Penick & Company, USA). Other chemicals used are of analytical grade. Simulated intestinal fluid (SIF) without pancreatin was freshly prepared according to BP 1980 specifications.

Methods

Preparation of Mucin: The ileum of a freshly killed Pig was excised open and the waste materials therein were rinsed with cold normal saline. The mucus surface was scrapped with a glass slide. Equal volume of distilled water was added to the mucin. This was homogenized for two hours and stored at 4°C for 48 hours. This mixture was centrifuged at 2,500 rpm for 30 minutes. The supernatant (S-mucin) and the precipitate (I-mucin) were recovered (8). The recovered I-mucin was stored at 4°C and was used for the bio-adhesive tests.

Measurement of viscosity of Polymer Dispersions

Table I: Polymer Compositions.

Carbopol 940	Tragacanth	Ratio	Percentage Composition
5.0	0.0	5:0	5.0
4.5	0.50	9:1	5.0
4.0	1.0	4:1	5.0
3.0	2.0 *	3:2	5.0
0.0	5.0	0:5	5.0

"Aqueous dispersions of 5%^w/w carbopol 940 and tragacanth and their binary mixtures were prepared according to the composition presented in Table I. Viscosities of these aqueous dispersions were measured using a universal torsion viscometer.

Triplicate determinations were made in each case. Additional 5%^{vv}/w dispersions of Carbopol 940 or tragacanth and their binary mixture of 3:2 were prepared in distilled water and their pH adjusted to 1.2, 3.0, 4.0, 8.0 and 9.0 with 0.1 N hydrochloric acid and 0.1 N Sodium hydroxide.

Measurements of bio-adhesive strength of polymer dispersions

Use of Tensiometer

The bio-adhesive strengths of the two polymers and their binary mixtures were determined using a tensiometer (A-Kruss, Germany, Model No. Nr. 3124) adapted to measure bio-adhesive strength. A 2-ml volume of the prepared I-mucin was placed in a watch glass which was placed on the platform of a properly zeroed tensiometer. A glass Plate of known dimensions was coated with the polymer dispersion to a uniform thickness of 2mm. The coated glass plate was placed on the lever arm of the tensiometer with the platform gradually moved to establish contact with the coated plate. A 5-minutes contact time between the polymer coat and the mucin was allowed to ensure proper mucin-polymer 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 coated glass plate from the surface of the mucin was read off from the microform balance in degrees and appropriate conversion of this to tension was obtained using Equation I:

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

Where, T is the tension equivalent to bio-adhesive strength, M is the mass required to return the lever pointer to its original position, L is the perimeter of the glass plate and F is a constant dependent on the perimeter of the plate and has a value of 0.94, and g is the acceleration due to gravity. The experiment was repeated three times for each polymer dispersion.

Bio-adhesion of coated beads on isolated intestinal mucus surface

The apparatus designed and used in this study has been presented and described in previous studies (7,9). It consists of a separating funnel clamped on a retort stand with a rubber tube attached at the end of the separating funnel. A metal support was used to position a plastic support at an angle of 30°. Freshly excised Pig ileum (5 cm by 20 cm) was pinned on the plastic support. A beaker was placed directly under the plastic support to collect detached beads. Glass beads of average diameter and weight 3 mm and 500 mg respectively, were thoroughly cleaned with distilled water and then with acetone to maximize the roughness factor (11,12). The beads were immersed in aqueous dispersions (5%w/v) of the two polymers or in their binary mixtures till a uniform coating was obtained for all the beads. Coated beads were air-dried and thereafter they were stored in a desiccator containing calcium chloride prior to use. The coated beads (10 from each batch) were placed on the exposed mucus surface of the pig ileum. The mucus - polymer coated beads interaction was allowed for a period of 5 minutes. A 250-ml simulated intestinal fluid (SIF) without pancreatin was allowed to flow over the beads at a rate of 30 ml minute⁻¹. The number of undetached beads was taken as a measure of bio-adhesive strength in each case (7).

Preparation of hyoscine hydrobromide bio-adhesive tablets and measurement of bio-adhesion

Hyoscine hydrobromide bio-adhesive tablets were prepared such that each tablet contained hyoscine hydrobromide, 5mg, 5%^{w/w} of either of the two polymers or their binary mixtures, and enough Avicel PH 101 to yield a 175mg tablet. Tablets were directly compressed in a hand operated Manesty tableting machine (Type 5B-175) fitted with 11/16-inch punches. Tablets were compressed to a low hardness of 3.5 ± 0.2 kgf to achieve normal release of the drug from the polymer matrix. The bio-adhesive test on the tablets was as described earlier under the use of tensiometer except that the metronidazole bio-adhesive tablets were attached to the glass plate by means of super glue.

Drug release testing

The dissolution profiles of the tablets were assessed using an Erweka dissolution apparatus (DT-D model) fitted with a paddle operated at 50 ± 1 rev. Minute⁻¹. The dissolution medium was freshly prepared 250ml SIF maintained at 37 ± 1°C. Samples were withdrawn at pre-determined time intervals and analysed spectrometrically at 260nm using a Milton Roy Digital Spectronic 1201

Results and discussion

The bio-adhesive strengths of the polymers and their binary mixtures on the basis of tensiometry are shown in Table 2. Carbopol 940 dispersion exhibited the highest bio-adhesive strength, while the least bio-adhesive strength occurred with 5%w/w tragacanth. Bio-adhesive strength of their binary mixtures generally decreased with increase in the proportion of tragacanth in the combinations. The higher bio-adhesive strength obtained with Carbopol 940 is probably due to the formation of elastic film between the mucin and the

polymer. The formation of this elastic film usually is a function of molecular size of the polymer. Optimum bio-adhesive strength is usually obtained with molecules having molecular weight greater than 1000,000 daltons (18). Carbopol 940 has a molecular weight of 4×10^6 daltons while tragacanth has a molecular weight of 8.4×10^5 daltons. This difference in molecular weight may have accounted for the wide difference in the bio-adhesive strength of Carbopol 940 and tragacanth on one hand and on the decreased bio-adhesive strength of the binary mixtures with greater proportion of tragacanth on the other hand. Mucus glycoproteins are believed to be responsible for the interaction with bio-adhesive polymers and Carbopols are known to interact with the glycoprotein component of the mucus gel to form strengthened network (3). Molecular interpenetration usually occurs during muco-adhesion and this leads to the strengthening of the interfacial layer of the muco-adhesive joint, which would help to retain the dosage form at the site of application (3). The process of muco-adhesion has been proposed to begin with the establishment of an intimate contact between the muco-adhesive polymer and the mucus gel (13). The second stage involves the penetration of the muco-adhesive polymer into the mucus gel network, followed by the formation of secondary chemical bonds between the mucus and the muco-adhesive material (3). Design of optimum bio-adhesive system, therefore, requires consideration of several properties of the bio-adhesive substances such as molecular weight of the polymer, its mobility and viscosity, the abundance of hydrophilic functional groups in the polymer and surface energy properties of the polymer. Factors like molecular weight of the polymer and its mobility in particular, determines the strength of the bio-adhesive bonds and the depth of interpenetration of the mucus and the bio-adhesive surface (5). The depth of penetration of adhesive material (l_p) into the substrate was given mathematically by Vasenin (9) as,

$$l_p = \frac{(\pi D_d t_c^{1/2})^{1/2}}{K_3} \dots\dots\dots (2)$$

where t_c is the time of contact between polymer and mucus surface, D_d is a constant which characterizes the mobility of the macromolecules, while k_3 is a constant which characterizes the stiffness, bond length a valence angles along the polymeric molecules. From equation 1, an equation was deduced which accounts for the number (N_c) of molecular chains crossing the phase boundary between the adhesive and substrate. The equation is given by

$$N_c = \frac{(2Ne)^{2/3}}{M} \dots\dots\dots (3)$$

Where N is the Avogadro's number, e is density of the polymer and m is the molecular weight of the polymer. Equation 3 indicates that polymer density and molecular weight can account for the degree and the strength of the interaction between polymers and mucus surfaces and could also count in part for differences in bio-adhesion of different polymers on biological tissue. The equation is however relevant in comparing the bio-adhesive performance of two distinct polymers, but may involve complex analysis when polymer combination or inter-polymer complex is involved. The reduction in bio-adhesive strength of the binary mixtures of the two polymers when the proportion of tragacanth was increased indicated probably a weak inter-polymer complexation between the two polymers. This is also evident from a reduction in the viscosity of the binary mixtures (Table 4). Inter-polymer complexation usually results in higher viscosity as is seen in inter-polymer complex formed between Carbopols and hydroxypropylcellulose(8). In addition, for maximum bio-adhesion to occur, the hydration of polymer coat to form a "tacky" film is necessary while formation of slippery film results in poor bio-adhesion (14). It is possible that hydration of tragacanth resulted in the formation of slippery film with consequent poor bio-adhesion.

The effect of pH on bio-adhesive strength of the polymers (Carbopol 940, tragacanth and their binary mixtures, ratio 3:2) is shown in Table 3. The single polymers and their combination (3:2) exhibited the highest bio-adhesive strength at pH 3.0 and at pH 8 or 9. This could be explained from the fact that low pH favours the adhesion of anionic polymers such as Carbopols and tragacanth to mucus surfaces (10). In addition, the gel-strengthening phenomenon of Carbopols is optimal at pH values around their pKa (4.75) (3). Carbopol 940 apparently exhibited its maximum viscosity at pH 8.0 while tragacanth and the binary mixtures of the two polymers exhibited their maximum viscosity at pH 9.0. This viscosity increase also favours bio-adhesion (5). The pH of the polymer-substrate interface is one of the environmental related factors that affect muco-adhesion (15). Based on the effect of pH on the bio-adhesive strength of the polymers and their binary mixture (3:2), the two polymers, either singly, or in combination could be effective in the localization of drugs either in the stomach or in the lower part of the small intestine. For effective localization of a dosage form, however, Carbopol 940 or its binary mixture with tragacanth (3:2) would be most suitable in this regard.

Bio-adhesive strengths of hyoscine hydrobromide bio-adhesive tablets based on tensiometry are also shown in Table 2. The bio-adhesive strengths of the tablets were higher than that obtained with the polymer dispersions. Highest bio-adhesion was obtained with tablets containing Carbopol and tragacanth in 3:2 ratio. This could be explained on the basis of the differences between an actual mucus surface adopted in the tablet bio-adhesive test and on the isolated and prepared 1-mucin slurry used for the polymer dispersions. Even though rate of hydration is bound to vary in the two systems, over-hydration of the polymers in the presence of the other, tablet excipients (which tend to absorb excess water) may have been minimal in the tablets. Such over hydration is known to give rise to slippery films which exhibit poor bio-adhesion. Tobyn et al (16) had earlier reported decreased bio-adhesion of Carbopols in the presence of microcrystalline cellulose. Their study was, however, carried out with a different method. The results of the present study therefore tend to suggest that true and optimum bio-adhesion of a polymer used in the formulation of bio-adhesive tablets could better be achieved by use of a direct interaction between the bio-adhesive tablet and the isolated mucus surface. Also results of interaction of polymer-tablets excipient with mucin may not correlate with eventual results obtained with compacts of such mixture.

The results of the detachment of coated beads and the tablets from the isolated mucus surface are shown in Figure 1 and 2 respectively. The bio-adhesive strength of the polymers based on the number of polymer-coated beads or tablets undetached followed the same trend as the bio-adhesive strength of the polymer dispersions which are shown in Table 2. With the polymer coated beads of the bio-adhesive tablets, bio-adhesive strength decreased with increased proportion of tragacanth in the combinations and was least with systems containing 5%/w tragacanth. Carbopol 940 contains carboxyl groups which ensures good hydration, and strong interaction which may require greater force to detach the polymer film from the mucus surface.

Drug Release Profiles of Bio-adhesive Tablets

The release of hyoscine from the bio-adhesive tablets is shown in Figure 3. A 4:1 binary mixture of Carbopol 940 and tragacanth gave the highest release of the drug within 20 minutes. Relatively slow release was obtained with tablets containing either of the two polymers or their other binary mixtures. Tablets containing Carbopol 940 or tragacanth or binary mixtures of these two polymers in the ratios of 9:1, and 3:2 exhibited a slow release pattern. The release pattern of the tablets, however, did not follow any particular pattern in relation to the proportion of tragacanth in the binary mixtures. This agrees with an earlier observation that polymer combinations can produce varied effects on drug release from bio-adhesive drug delivery systems (17). The drug release from the tablets started within few

Table 2: Bio-adhesive Strength of the Polymer Dispersion and Tab let based on Tensiometry

Polymer Composition	Bio-adhesive Strength (Nrrf ¹)	Tablet Compacts x 10 ²
Carbopol 940: Tragacanth	Polymer Dispersions x 10 ²	
5:0		
.9:1		13.20 +0.50
4 : 1		12.0 + 0.30
3:2		
0:5		

Table 3: Effect of pH on Bio-adhesive of Polymer Dispersions Tension Mm^m1 x 10²

PH	Carbopol 940:	Tragacanth	Carbopol: Tragacanth (3:2)
1.2	5.04 +0.60		
3.0			
4.0 "			
8.0	5.56 + 0.30		
9.0			

Table 4: Viscosity of Polymer Dispersions

Carbopol 940: Tragacanth	Viscosity (CP)
5:0	5700
9:1	5300
4: 1	5000
3:2	3700
0:5	3800

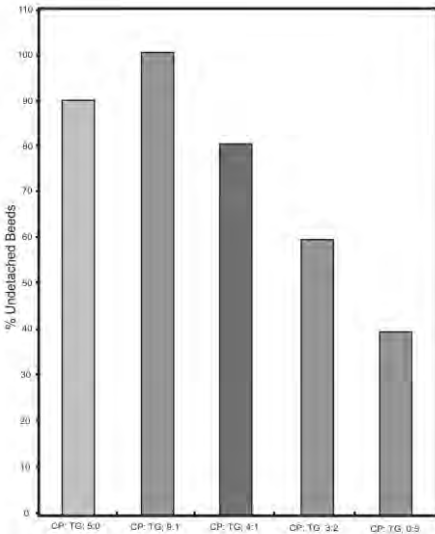


Fig 1: Bioadhesion of Hyoscine Hydrobromide bioadhesive Tablets on Isolated Intestinal Surface

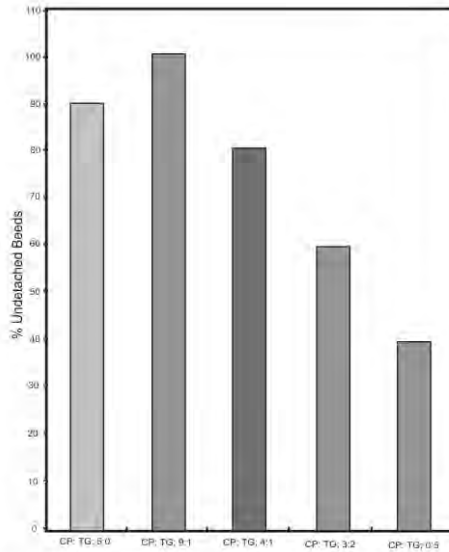
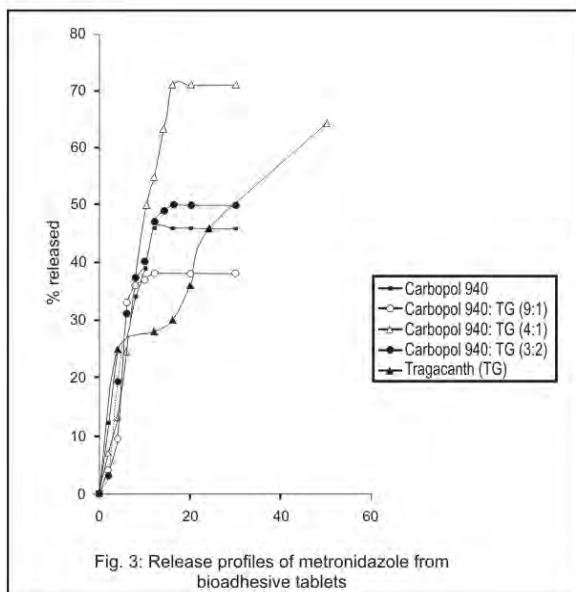


Fig 1: Bioadhesion of Coated Beads of Isolated Intestinal Surface



minutes and in some cases assumed a plateau state after about 10 to 20 minutes. This was considered desirable since hyoscine hydrobromide when given orally, is used in the treatment of certain disease conditions such as motion sickness, and tremor of paralysis (18) which require quick drug action. Presenting the drug in a bio-adhesive delivery system, therefore, will lead to a localized dosage form from which hyoscine hydrobromide will be released shortly after ingestion and in which the drug will be released gradually after sometime thereby ensuring adequate control of motion sickness or tremor of paralysis.

Conclusion

Carbopol 940 and its binary mixtures with tragacanth could be utilized in muco-adhesive drug delivery. Carbopols are relatively expensive while tragacanth is relatively cheap. Combination of these two polymers in certain proportions would offer economic advantage.

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