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The Impact of Trimethyl Chitosan on In Vitro Mucoadhesive Properties of Pectinate Beads along Different Sections of Gastrointestinal Tract

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ABSTRACT Pectinate (PEC) beads are multiparticulate dosage forms which have been extensively investigated for oral drug delivery; however their mucoadhesive properties in various sections of GI tract have, not been yet reported. This work evaluated the in vitro mucoadhesive properties of PEC bead formulations, on rat everted gastrointestinal sections, either with or without trimethyl chitosan (TMC), an absorption-enhancing and fairly mucoadhesive derivative of chitosan. Reference Carbomer 934P (C934P) granules, as an established mucoadhesive polymer, and ethyl cellulose (EC)-coated pellets, as a nonmucoadhesive dosage form, were also used for comparison. Water uptake studies were also performed to further explain the effect of hydration on mucoadhesive properties. PEC beads showed mucoadhesion, which was in some cases comparable to C934P granules, towards the gastrointestinal tissues with following ranking: duodenum ≈ jejunum ≈ ileum > cecum > colon > stomach. In the dry state, the beads containing TMC were more mucoadhesive, while in the moist state simple PEC beads were shown to be more mucoadhesive. Over-hydration of TMC-containing beads may account for this observation. The results of this study suggest that in cases which prehydration can be avoided, such as when the beads are protected in a site-specific oral capsule, prior to reaching the target tissue, the incorporation of TMC into beads might be useful, as a means of increasing the mucoadhesive properties; However, further studies are needed to clarify their in vivo feasibility.

KEYWORDS Pectinate beads, Mucoadhesion, Trimethyl chitosan

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INTRODUCTION

Pectinate (PEC) beads are formulations, usually intended for oral drug delivery, with some distinct advantages such as safety of ingredients and being multiple-unit dosage forms, which have several benefits over single-unit dosage forms with regard to more predictable and reproducible gastrointestinal

transit time (Mathiowitz, 1999; Pillay & Fassihi, 1999). By careful selection of parameters, such as pectin and cross-linker types and their concentrations, cross-linking time and additional excipients and/or coatings, the beads can be targeted to different sections of the gastrointestinal (GI) tract. Numerous formulations of PEC beads have been investigated for drug delivery to different sections of the GI tract, ranging from stomach to colon (Munjeri et al, 1998; Musabayane et al., 2000; El Gibaly, 2002; Kim et al., 2003; Sriamornsak et al., 2005). However, mucoadhesive properties of dosage forms are also of utmost importance in its design. On the one hand, mucoadhesion causes increased residence time, which may be beneficial, especially for hydrophilic drugs and for drugs with a high molecular weight such as peptides and proteins. On the other hand, if a mucoadhesive dosage form is to be targeted to specific parts of GI tract, the nonspecific adhesion to other sections prevents it from reaching the target area.

Some research groups have studied mucoadhesive properties of pectin. Liu et al. (2005) have investigated the interaction of various pectin gels with porcine colonic tissues. Nafee et al. (2004) have evaluated mucoadhesive properties of pectin and other polymers for buccal tablet formulations. Schmidgall & Hensel (2002) tested the bioadhesive properties of polygalacturonides, including pectins, using ex vivo colon tissue. However, the assessment of mucoadhesive properties of PEC beads in various sections of GI tract has not yet been reported.

Moreover, different types of beads have been proposed for oral delivery of relatively high-molecular and/or water-soluble drugs, such as peptides and proteins (Musabayane et al., 2000; Kim et al., 2003, 2005; Rasmussen et al., 2003). However, successful delivery and absorption of such drugs from GI tract needs to be coupled with inclusion of absorption enhancers in the dosage form. N, N, N-trimethyl chitosan chloride (TMC) has been shown to possess absorption enhancing properties and at the same time to be fairly mucoadhesive (Thanou et al., 2000; Snyman et al., 2003). Therefore, TMC was also included in the PEC beads and its reciprocal effect on properties of PEC beads was assessed.

This preliminary study was designed to determine in vitro mucoadhesive properties of PEC beads either with or without TMC, along the different sections of GI tract. Reference Carbomer 934P (C934P) granules, as an established mucoadhesive polymer, and ethyl cellulose (EC)-coated pellets, as a nonmucoadhesive dosage form, were also used for comparison. Some simple measures, such as water uptake studies, and microscopic images, were also taken to partly explain the possible mechanisms underlying the beads mucoadhesive properties. This initial report might also be useful in determining whether there is need to protect the PEC beads from adhesion to upper GI sections, when their targeting to lower GI sections is in mind.

MATERIALS

Low-methoxy amidated pectin type GENU® pectin LM-104 AS-FS (PEC) (D.M. = 28%, D.A. = 20%) was purchased from CPKelco (Denmark). Zinc acetate (Zn acetate) was obtained from Merck (Germany). Carbomer 934P was purchased from Noveon (Belgium). Ethyl cellulose-coated pellets were obtained from Shahr Darou Pharmaceutical Co. (Iran), using extrusion-spheronization and pan coating, in which cores of Avicel PH101 (FMC BioPolymers, Philadelphia, Pennsylvania, USA), α-D-lactose monohydrate (Merck, Germany) and potato starch, were coated with ethyl cellulose (Ethocel 45) (Dow, USA). However because of the company's regulations it is not permitted to disclose and publish the details of manufacturing process. All other chemicals used were of reagent grade.

TMC with degree of quaternization of 65% was synthesized according to the method by Sieval et al. with slight modifications as reported before (Sieval et al., 1998; Atyabi et al., 2005).

As a result of co-precipitation of some NaCl with TMC chloride, in the last step of TMC synthesis, a 7% (w/v) solution of TMC chloride was dialyzed for 2 days against distilled water to remove the excess NaCl. The resulting solution was dried under vacuum and used in the formulations.

METHODS

Preparation of Formulations

In a previous study conducted by our research group (Atyabi et al., 2005), bead formulations were optimized with regard to their morphological characteristics, release and swelling behavior; Therefore in this study, the same optimized parameters were used

for preparation of beads. PEC (5% w/v), either without or with TMC (0.83 or 1.25% w/v, equivalent to PEC–TMC weight ratio of 6:1 or 4:1) (**PEC–TMC6:1** and **PEC–TMC4:1** formulations, respectively), were dissolved in distilled water. The gel mixture was completely dearated under vacuum (Fast Vac™ vacuum pump, J/B Industries, Warren, Ohio, USA) and used to prepare the beads by ionotropic gelation using an electrostatic bead generator (Nisco encapsulator VAR V-1, Switzerland) equipped with a syringe pump (Kd Scientific, Holliston, Massachusetts, USA). The mixture was applied drop-wise into a cross-linking solution of Zn acetate (0.15 M) at the rate of 10 mL/h. The dropping distance was 5 cm. The volume of the cross-linking solution was 25 mL for every 10 mL of the gel mixture. The prepared beads were stirred for 2 hr in the cross-linking solution, then washed twice with distilled water and dried overnight at room temperature.

Carbomer 934P and ethylcellulose have been established respectively as mucoadhesive polymer and non-adhesive polymers along the gastrointestinal tract (Fu et al., 2002). Therefore, in our study, Carbomer 934P was used as a mucoadhesive polymer and positive control, and ethylcellulose, as a negative control, and for the purpose of comparison to PEC beads.

Carbomer 934P **(C934P)** granules were prepared by dry granulation. Briefly, a preweighed amount of C934P was pressed with a tablet press. The produced discs was crushed and passed through a 16-mesh-size sieve. The particles were then pressed again and the mentioned procedures were repeated twice more to obtain hard granules.

The beads, granules and ethyl cellulose-coated pellets **(EC)** were sieved through sieves of 18 and 25 mesh sizes, and the particles of 710–980 μm size were used throughout this study. The size and size distribution of the microparticles were determined by inspection of 100 particles using a light microscope, equipped with a micrometer.

Water Uptake Studies

The hydration theory of mucoadhesion has long been recognized (Lee et al., 2000; Jasti et al., 2003). In order to further clarify the possible role of hydration mechanisms in adhesive properties of the microparticles in this study, beads moistened for 2 min in 700 μL of the respective buffer solution, were also tested for mucoadhesion. Moreover, water uptake studies were also performed by placing 30 microparticles of determined weight in small containers and moistened with 700 μL of the corresponding buffer for 2 min. Then the buffer media was completely removed with a micropipette. The beads were immediately blotted on a filter paper and weighed. The percentage of water uptake (WU) was calculated using equation 1, where W_d and W_m are the dry and moist weights of the beads, respectively:

$$\text{WU}\ (\%) = [(W_m - W_d)/\text{W}_d] \times 100 \qquad (1)$$

Mucoadhesion Studies

The everted sac technique and determination of adhesion number was used to assess the mucoadhesive properties of microparticles. The everted sac technique is an established method, with proven correlation to CAHN microbalance bioadhesion assay, and an indicator of mucoadhesion (Santos et al., 1999; Chowdary & Rao, 2004). The mucoadhesion studies were performed based on a previously reported method (Miyazaki et al., 2003), using the GI sections removed from fasted rats (300–400g, male, Sprague-Dawley strain, Tehran University of Medical Sciences), sacrificed by an overdose of ether. Antrum region of stomach, cecum, and colon, which are anatomically distinct sections, were excised. The first 1/20th of the intestinal section was considered as duodenum. The 2/5th and 3/5th of the remaining sections were considered as jejunum and ileum respectively. The sections were flushed with ice-cold saline and cut into segments equal to approximately 9 cm^2, which were then everted carefully using a glass rod, and tied at both ends. A total of 30 microparticles were scattered, as uniformly as possible, on the everted rat tissues. The everted sacs were then transferred to vials filled with 10 mL of the respective buffer media and agitated horizontally in a shaking water bath (37°C) for 15 or 60 min. A USP acidic buffer medium (pH 1.5) and phosphate buffered saline (PBS; pH 7.4 and 6.8) were used for stomach, small intestine, and large intestine, respectively.

Thereafter, the sacs were removed from the buffer and the number of microparticles still adhering to the tissue was determined. Each experiment was performed four times. The adhesion number of microparticles was calculated using Eq. (2), where N_a is the

number of adhered microparticles and N_t is the total number of applied particles:

$$\text{A.N.}(\%) = (N_a / N_t) \times 100 \quad (2)$$

Scanning Electron Microscopy Pictures

In order to clarify the surface characteristics of the microparticles, SEM micrographs were prepared. This might shed some light on the mechanisms underlying the adhesion of the microparticles. Microparticles were coated with gold to a thickness of about 30 nm in vacuum evaporator. Morphological examination of the bead surface was performed using scanning electron microscope (MV2300, Camscan, UK) at 10 kV.

Statistical Analysis

Statistical analysis was performed with the Student's unpaired *t*-test. A difference was considered to be statistically significant when the *p*-value was less than 0.05.

RESULTS AND DISCUSSION

Morphology and Size of the Microparticles

The beads were spherical immediately after preparation; however upon drying they shrank significantly and became somewhat flat on the surface that they dried. The beads containing TMC were more spherical upon drying, in macroscopic observation. The sizes of the all bead formulations were in the same range and approximately 850 (±150) μm., from which the beads sieved through sieves of 18 and 25 mesh sizes, (710–980 μm), were used. In case of C934P granules, because of dry granulation process, multimodal size distribution ranging from very fine particles (approximately 100 μm) to as large as 1 mm were obtained and the granules sieved through sieves of 18 and 25 mesh sizes, (710–980 μm), were used in the study. The EC-coated pellets were spherical to elliptical. Pellets of 710–980 μm were used as well.

Water Uptake Studies

By referring to results of water uptake studies (Table 1), it can be deduced that inclusion of TMC has caused 2- to 10-fold increase in hydration in PEC–TMC6:1 and PEC–TMC4:1 formulations, compared to PEC formulation. This rapid overhydration results in the formation of a wet slippery mucilage with decreased adhesion (Lee et al., 2000). On the other hand, hydration and water uptake were not significant in PEC formulation (Table 1). C934P moist granules exhibited the highest extent of water uptake and hydration.

TABLE 1 **Water Uptake (%) of Microparticles After 2 min Incubation With Different Buffer Media (Mean ± SD, *n* = 4)**

	Water uptake (%)		
	pH value simulating GI sections		
Formulation	1.5	7.4	6.8
PEC–TMC4:1	31.13 ± 0.97	29.89 ± 12.01	25.85 ± 7.23
PEC–TMC6:1	19.42 ± 0.35	8.06 ± 3.01	9.09 ± 1.71
PEC	9.13 ± 5.47	5.33 ± 1.97	2.12 ± 1.84
C934P	321.5 ± 30.41	420 ± 73.43	485 ± 50.7
EC	15.7 ± 2.68	13.3 ± 3.65	5.08 ± 3.86

Water absorption of a dry mucoadhesive device upon contact with mucus and hydration of dry polymers play san important role in mucoadhesion. The hydration theory of mucoadhesion has long been recognized (Lee et al., 2000; Jasti et al., 2003). Some studies have used mucoadhesion studies in both dry and prehydrated states and water uptake studies, to clarify the mechanisms of mucoadhesion and to assess the effect of prehydration on mucoadhesive properties of devices. Mortazavi (2002) investigated the effect of prehydration and water uptake rate of various polymeric discs on their mucoadhesive properties on rat intestinal mucosa. Llabot et al. (2002) correlated the mucoadhesive properties of oral carbomer–HPMC tablets with their water uptake properties. The mechanism by which mucoadhesion takes place has been said to have two stages, the contact–wetting and hydration stage followed by the consolidation stage–the establishment of the adhesive interactions. Therefore, the polymer hydration and swelling are properties related to the mucoadhesion of the system (Llabot et al., 2002). Moreover it has also been observed that higher amounts of water, absorbed by some polymers, resulted in a more loosely packed polymer structure. This extensive uncoiling of the polymer chain led to a reduction in the degree of mechanical entanglement and penetration and hence mucoadhesion (Cleary et al., 2004).

Therefore, the water uptake studies together with mucoadhesive studies in both dry and prehydrated states, and correlating the results with each other, can explain whether the mucoadhesion in a specific polymeric system is dominated by the first stage of wetting or hydration, or by other mechanisms. Additionally, the investigation on dry particles gives some basic insights into the application of these systems, in cases which prehydration can be avoided, such as buccal application or when the beads are protected in a site-specific oral capsule, prior to reaching the target tissue.

Mucoadhesion Studies and the Impact of TMC on Mucoadhesive Properties

Tables 2 and 3 depict the mucoadhesive properties of the microparticulate formulations, determined by the adhesion number (AN) on rat everted GI sections. The determination of AN of the microparticles was done macroscopically, since the beads were milky- to brown-colored, the EC-coated pellets were white and easily visible on the tissues. C934P granules were prepared from discs with a very high hardness and although they became hydrated upon exposure to buffers, but were still visible until 20 min. However, they lacked the desired cohesive properties and completely hydrated to transparent gels and dissolved in 30 min and further determination of their A.N. was not possible.

The AN of all PEC bead formulations, either with or without TMC, in both dry and moist states, exhibited the following trend:

$$\text{duodenum} \approx \text{jejunum} \approx \text{ileum} > \text{cecum} > \text{colon} > \text{stomach}$$

The observed difference of beads mucoadhesion to different GI sections, might be both because of the inherent characteristics of different mucosa (Rubinstein & Tirosh, 1994) and/or to the different pH values of the simulated gastrointestinal fluids and their effects on the functional groups of the polymers in each formulation.

TABLE 2 Adhesion Number (%) of Dry Microparticles in Different GI Sections of Rat Determined by Everted Sac Method (Mean ± SD, $n = 4$)

	Adhesion number (%)					
	Part of the GI tract					
Formulation	Stomach	Duodenum	Jejunum	Ileum	Cecum	Colon
PEC–TMC4:1	16.67± 3.84	93.33 ± 9.42	95 ± 3.33	96.67 ± 3.84	81.67 ± 8.38*	80 ± 5.44
PEC–TMC6:1	13.33 ± 5.44*	91.67 ± 8.38	93.3 ± 13.3	96.67 ± 3.84*	68.33 ± 3.33*	50 ± 13.9
PEC	10 ± 3.84*	86.7 ± 12.1	86.7 ± 12.2	85 ± 14.7	46.67 ± 19.63*	30 ± 8.61
C934P	46.67 ± 6.66*	80 ± 0	84.4 ± 10.1	88.8 ± 10.1	95.56 ± 3.85*	95.56 ± 3.85
EC	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

*$p < 0.05$.

TABLE 3 Adhesion Number of Moist Beads in Different GI Sections of Rat Determined by Everted Sac Method (Mean ± SD, $n = 4$)

	Adhesion number (%)					
	Part of the GI tract					
Formulation	Stomach	Duodenum	Jejunum	Ileum	Cecum	Colon
PEC–TMC4:1*	2.22 ± 3.84*	62.22 ± 3.84*	51.11 ± 3.85	53.3 ± 6.67	26.67 ± 6.66	8.88 ± 3.84
PEC–TMC6:1*	0 ± 0*	75.56 ± 3.85*	66.67 ± 6.67*	68.9 ± 3.85	37.78 ± 3.84	17.78 ± 10.18
PEC*	4.44 ± 3.84	93.3 ± 6.67	84.44 ± 10.18	88.9± 10.2	40 ± 6.66	37.78 ± 7.69
C934P	13.33 ± 0	82.22 ± 3.84	77.78 ± 3.849	75.6 ± 10.2	86.67 ± 6.66	84.44 ± 3.84
EC	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

*$p < 0.05$.

Schmidgall & Hensel (2002) have shown that pectin with low degree of esterification and linear oligogalacturonide segments–which is somewhat similar to the pectin used in our study–possesses significant mucoadhesion against the GI mucous membranes. Liu et al. (2005) have stated that at physiological pH (pH 7.2), most of the carboxyl groups in pectin and the sialic acids of mucin are ionized and negatively charged. Therefore hydrogen bonding between pectin and mucin should be limited by electrostatic repulsion, whereas molecular entanglements should be favored caused by the expansion of coils. Hence, with increased pH values, expanded pectin chains on the surface of the beads, seem to readily penetrate into the mucin networks. This explanation might account for improved mucoadhesion of the beads in intestinal sections and higher pH values, compared to gastric tissues, and also colonic tissue, to a lesser extent. In contrast, in gastric sections, in which acidic pH values are encountered, caused by relatively neutral electrical nature of pectin, the mobility of coiled pectin chains and molecular entanglement are hindered and less mucoadhesion is observed.

With regard to the effect of inclusion of TMC in PEC beads, on the mucoadhesive properties of these formulations, it was observed that in the dry state, increasing the amount of TMC in PEC–TMC4:1 and PEC–TMC6:1 formulations led to higher mucoadhesion compared to simple PEC beads (Table 2), particularly in cecum and colon ($p < 0.05$). However, this difference in formulations was not remarkable in intestinal sections, i.e., duodenum, jejunum, and ileum. This might be because of the fact that the mucoadhesion of PEC formulation in the small intestinal tissues was already high and therefore any further possible increase in AN by addition of TMC to the formulation could not be detected. It has been shown that electrostatic interaction is a major factor in the adhesion of cationic chitosan to purified anionic pig mucin, by the negative charge carried by the moieties on mucin glycans, and fixed positive charge of the TMC (Snyman et al., 2003). Moreover, it is reported that TMC polymers with relatively high degrees of quaternization–which show an increased absorption-enhancing effect–possess sufficient mucoadhesive properties for inclusion into mucoadhesive drug-delivery systems (Keely et al., 2005).

However, in the moist state, the mucoadhesive trend observed for dry formulations was reversed and inclusion of TMC in the formulations resulted in reduced mucoadhesive properties compared to PEC formulation, especially in intestinal sections ($p < 0.05$; Table 3). However the AN of simple PEC beads, were not much altered in the moist state compared to dry state (Tables 2 and 3). As mentioned earlier in the section pertaining to results of water uptake studies (Table 1), inclusion of TMC has caused 2- to 10-fold increase in hydration in PEC–TMC6:1 and PEC–TMC4:1 formulations, compared to PEC formulation. This rapid overhydration in TMC-containing formulations, leads to formation of wet slippery gel layer, and consequently decreased adhesion (Lee et al., 2000). However, hydration and water uptake were not significant in PEC formulation (Table 1).

The concept of integrating both the carboxylic acid groups and cationic amine groups in mucoadhesive polymeric systems has also been suggested by other research groups. Such an approach exploits the interpenetrating and hydrogen-bonding characteristics of carboxylic moieties, together with ionic interactions of cationic polymers, with mucosa. Liu et al. (2005) synthesized a pectin derivative carrying side chain primary amine groups and assessed its mucoadhesive properties. Similar to TMC, the primary amine groups of this pectin derivative are mostly protonized at physiological pH (pH 7.2). Hence, electrostatic attractions between the polymer and anionic mucus produce ionic bonding, followed by facilitated hydrogen bonding via other functional groups on the polymeric chain, such as hydroxyl groups of pectin, and chain entanglement. Consequently, the incorporation of TMC in the PEC beads also makes use of the same concept. Furthermore, the absorption-enhancing effects of TMC in broad pH ranges, might also justify its use in PEC bead formulations.

Mucoadhesive properties of C934P granules, used as an established mucoadhesive polymer and positive control, both in dry and moist states, were highest in cecum and colon, closely followed by intestinal segments, and finally lowest in stomach. The ANs of C934P granules were significantly higher than PEC formulations in both dry and moist state in stomach, cecum and colon ($p < 0.05$). Nevertheless, in dry state and in intestinal sections, no difference was observed between the C934P granules and the PEC beads. C934P moist granules exhibited the highest extent of water uptake and hydration. Nevertheless their

mucoadhesive properties were not much altered in the moist state. In general, in moist state, the ANs of C934P were significantly higher than all pectin formulations, in intestinal sections ($p < 0.05$). Therefore, it seems that in the case of C934P, the wetting and hydration stage is not the predominant mechanism of mucoadhesion.

Rubinstein & Tirosh (1994) have reported that the thickness of mucus gel is significantly higher in stomach, compared to cecum; and mucoadhesion of polycarbophil discs is highest in cecum. Our data on C934P granules, which have a molecular structure similar to polycarbophil, are in accordance with the results in the mentioned study. According to their data, the mucoadhesion of polycarbophil is more pronounced in lower pH values, where the carboxylate moieties are protonated, and in lower GI sections, where mucus layer thickness is lowest. Therefore, it seems that for polycarbophil, and possibly for carbomer, the decreased mucus layer thickness and hydrogen bonding accounts for mucoadhesive properties (Lee et al., 2000).

Several studies have reported conflicting results regarding the mucoadhesive properties of polymers with carboxylate and/or hydroxyl moieties–such as pectin and carbomer, which are used in this study–on GI tissues (Durrer et al., 1994; Nielsen et al., 1998; Chary et al., 1999; Solomonidou, 2001). These reports, point out that complex interrelation of several parameters such as flexibility of polymer chains, intra- and inter-molecular hydrogen bondings, ionic interactions, coiled or uncoiled configuration of polymer chains and optimal degree of hydration, together with tissue characteristics are responsible for mucoadhesive properties.

EC-coated pellets were used as a negative control in this study, were shown not to have any mucoadhesive tendency towards the GI tissues. All bead formulations were proven to be significantly more mucoadhesive in intestinal and ceccal and colonic segments, in both dry and moist states compared to EC-coated pellets ($p < 0.05$).

The AN of the microparticles was also determined after 60 min, which was almost the same as the values obtained for 15-min incubation (data not shown to avoid repetition). Hence, our results suggest that the PEC beads retain their mucoadhesive properties during a period which can be expected for mucus turnover time (Lehr et al., 1991).

The mentioned mechanisms might shed some light on the mucoadhesive trend observed by us for PEC beads. Furthermore, it should be born in mind that since carboxylate groups on pectin chains are only partially cross-linked and the cross-linking cations on the surface of the beads are displaceable upon exposure to the acidic medium or in presence of cation-complexing species such as phosphate in phosphate buffers, free functional groups are present on the surface of the beads and thus it might be possible to apply the reports on the mucoadhesive properties of pectins and TMC to interpret the results obtained with PEC beads (Fundueanu et al., 1999; Pillay & Fassihi, 1999). However, further studies on the possible interaction of pectin with zinc and/or TMC and their stoichiometry are needed to clearly define the mechanisms of bead mucoadhesion.

Role of Surface Morphology on Mucoadhesive Properties

Some researchers suggested that microparticles with smooth surfaces might have decreased mucoadhesive properties (Peppas & Buri, 1985; Chowdary & Rao, 2004). As seen in Fig. 1, C934P granules had a rough and relatively porous surface, which might to some part, contribute to better mucoadhesive properties of these granules. EC coated pellets showed an uneven surface and in higher magnifications seemed smooth, which might partly account to their lack of mucoadhesive properties. PEC beads had some cracks on them. No obvious difference was observed for beads with or without TMC. These SEM images revealed less with respect to impact of surface smoothness on mucoadhesion mechanisms of the beads in our study and, seemingly other aforementioned factors play more important role on mucoadhesive properties of investigated beads.

CONCLUSION

This work briefly reports the in vitro mucoadhesive properties of zinc PEC formulations and the effect of inclusion of TMC, as an absorption-enhancer and fairly mucoadhesive ingredient. PEC bead formulations were shown to be mucoadhesive towards rat everted intestine, in an extent comparable to C934P granules, and to some extent towards ceccal and

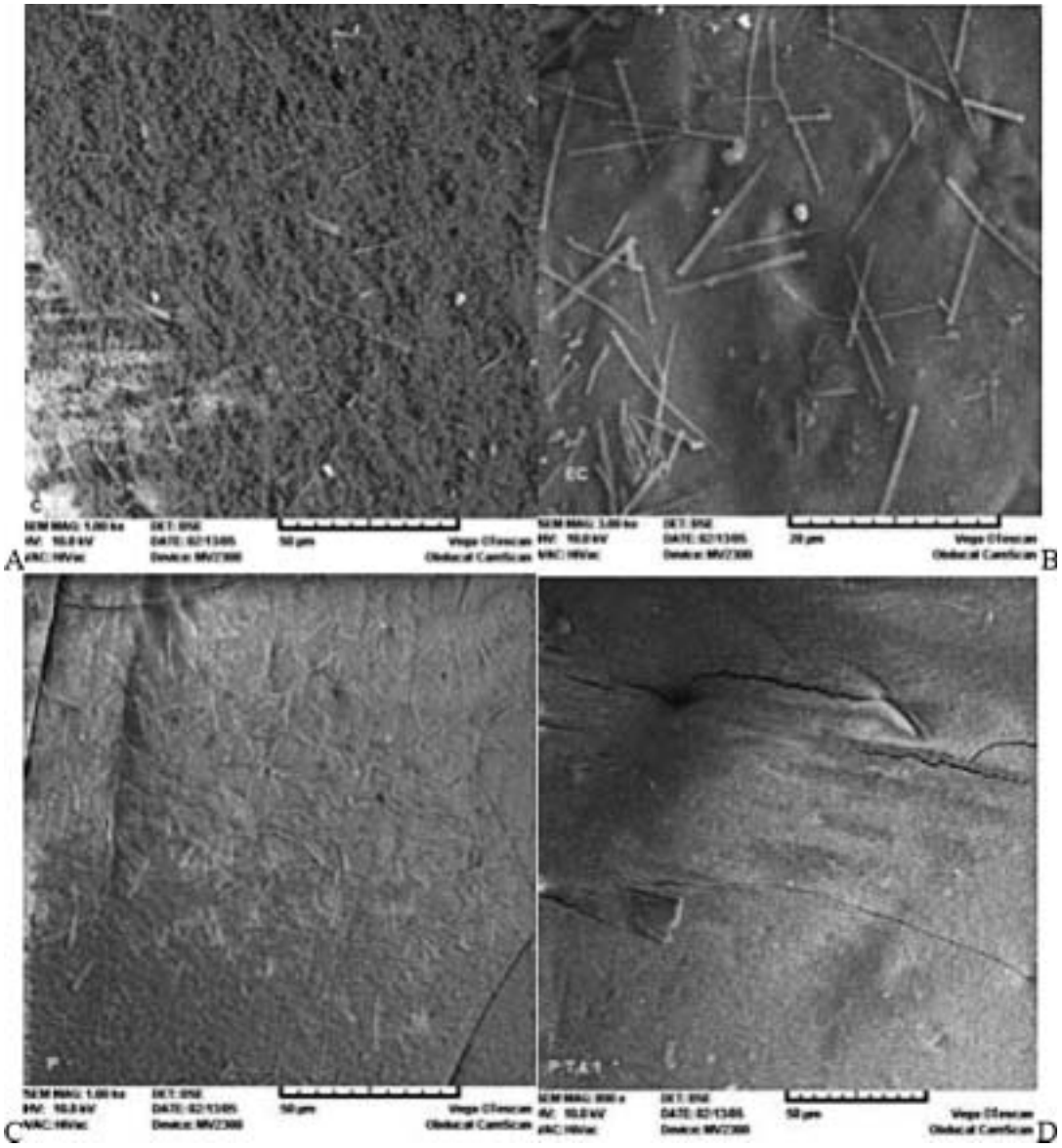

FIGURE 1 **SEM Micrographs of Microparticles. (a) Carbomer 934P granules; (b) Ethylcellulose-coated pellets; (c) Pectinate (PEC) beads; (d) Pectin–TMC with 4:1 ratio (PEC–TMC4:1) beads.**

colonic mucosa. However, they did not exhibit marked mucoadhesion in the stomach compared to C934P. In the dry state, inclusion of TMC in the beads increased their mucoadhesion to 2- to 3-fold in cecum and colon compared to simple PEC beads, which was somewhat comparable to C934P granules. Nevertheless, in the moist state the simple PEC beads exhibited higher mucoadhesion compared to TMC-containing formulations, which might be caused by the rapid over-hydration of beads arising from presence of TMC. The results of this preliminary study suggest some possibilities which researchers should bear in mind when studying the PEC beads; nonetheless further studies are needed to determine the correlation of these results with in vivo conditions. Based on the observation that PEC beads do not adhere to gastric mucosa, if intended as nonmucoadhesive formulations for intestinal delivery of drugs, the beads can be probably administered by the oral route without further modification, provided that their release characteristics are optimized. Moreover, PEC beads, either with or without additional mucoadhesive or absorption-enhancing polymers, might be feasible as mucoadhesive formulations. However, on the one hand, if they are to be targeted to the small intestine as a mucoadhesive formulation, they probably have to be coated, so they would reach the small intestine without being over-hydrated in the stomach.

On the other hand, when targeting the PEC beads to cecum or colon, it might be imperative to protect the formulations from adherence and over-hydration in upper intestinal sections by some means, such as coating with nonadhesive polymers. Incorporation of TMC into the beads might be useful, as a means of increasing the mucoadhesive properties, in cases which prehydration can be avoided, such as when the beads are protected in a site-specific oral capsule, prior to reaching the target tissue, or basically other applications such as buccal, or vaginal routes of administration, Moreover, it should be noted that that even in the moist state, TMC-containing formulations, still are considerably mucoadhesive compared to the nonadhesive polymers, especially in the intestinal sections. Furthermore, although not yet investigated, but the basic concept of the absorption-enhancing effects of TMC in broad pH ranges, might also justify its use in PEC bead formulations. However, this needs to be investigated in further research studies.

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