Research Paper

Pectin-based microspheres for colon-specific delivery of vancomycin

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Abstract

Objectives The aim of this study was to describe a colon-specific delivery system based on pectin hydrogels formed by complexation with chitosan.

Methods Hydrogels were prepared at different weight ratios (4 : 1, 7 : 1, 10 : 1; pectin/chitosan), loaded with vancomycin hydrochloride (2 : 1, 4 : 1; polymer/drug weight ratio) and collected by spray-drying. The microspheres obtained were characterized in terms of morphology, swelling behaviour, mucoadhesive properties and drug loading efficiency. The influence of different pectin/chitosan hydrogels on the release behaviour of microspheres at pH 2.0, 5.5 and 7.4 were evaluated *in vitro* with and without pectinolytic enzyme.

Key findings The results showed that water uptake was increased by raising the environmental pH (from 2.0 to 7.4) and the pectin/chitosan weight ratio, while drug availability was increased by raising the environmental pH (from 2.0 to 7.4) and decreased by raising the pectin/chitosan weight ratio. In the presence of pectinase, the glycoside bonds of pectin were degraded and a considerable amount of drug was released in a short time.

Conclusions This study suggested that pectin/chitosan microspheres were able to limit the release of vancomycin under acidic conditions and release it under simulated colonic conditions, confirming their potential for a colon-specific drug delivery system.

Keywords chitosan; colon-specific delivery; hydrogel; mucoadhesion; pectin

Introduction

Colonic drug delivery offers interesting possibilities not just as absorption site of drugs but also for the treatment of local diseases such as irritable bowel syndrome and inflammatory bowel disease.[1] The colon offers different favourable properties as a site for drug delivery such as a neutral pH, long transit time, great sensitivity to absorption enhancers, reduced digestive enzymatic activity and the presence of large amounts of enzymes for polysaccharides (e.g. β-D-glucosidase, β-D-galactosidase, amylase, pectinase, dextranase, etc.), which are secreted by a large number and variety of colonic bacteria. An interesting approach for colon-specific drug delivery is based on the use of polysaccharides (e.g. pectin, dextran, inulin, etc.) specifically degraded by colonic bacteria (enzymatically controlled delivery systems).[2–4] Pectin is an anionic polysaccharide present in the cell wall of most plants, consisting mainly of D-galacturonic acid and its methyl ester linked via $\alpha(1–4)$ glycosidic bonds. This polymer shows interesting biological properties, including biocompatibility and mucoadhesivity. Moreover it is almost totally degraded by colonic bacteria and is not digested by gastric or intestinal enzymes. However, because of its water solubility, several approaches have been tried to inhibit pectin solubility in the aqueous fluids of the gastro-intestinal tract.[5] In some of these approaches, pectin was mixed with synthetic or natural polycations to form polyelectrolyte complexes, the swelling and release profiles of which can be modulated by appropriate preparative conditions.[6] The aim of this study was to describe a colon-specific delivery system based on pectin hydrogels formed by complexation with chitosan and loaded with vancomycin. Chitosan is a natural derivative of chitin consisting of glucosamine and *N*-acetylglucosamine that, like pectin, shows good properties of biocompatibility, biodegradability and mucoadhesivity.[7–10] Vancomycin hydrochloride is a large (MW 1485.73 g/mol) and water soluble (50 mg/ml)

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drug with an isoelectric point of 7.2. Vancomycin is indicated for the treatment of serious, life-threatening infections by Gram-positive bacteria that are unresponsive to other less toxic antibiotics, and in the treatment of pseudomembranous colitis it must be given orally to reach the site of infection. Hydrogels were prepared at different pectin/chitosan weight ratios and collected by spray-drying to obtain microspheres. In-vitro swelling, mucoadhesion and release tests with and without pectinolytic enzyme were performed to investigate the influence of pectin/chitosan hydrogels on the release behaviour of vancomycin in the gastro-intestinal tract.

Materials and Methods

Materials

Pectin from apples (M_r 30 000–100 000; degree of esterification 70–75%), chitosan low-viscous (viscosity $\leq$ 200 mPa s at C = 1% in 1% acetic acid, T = 20°C; deacetylation degree 97%), vancomycin hydrochloride and mucin (Type II: crude, from porcine stomach) used for this study were obtained commercially from Fluka (Milan, Italy). Pectinase from *Aspergillus aculeatus* (activity $\geq$ 9500 U/ml) was obtained from Sigma (Milan, Italy). All other chemicals and solvents were of analytical grade and purchased from Carlo Erba (Milan, Italy). Swelling, mucoadhesion and release studies were carried out in aqueous buffers with the following compositions (mM): 65.0 NaOH, 30.6 $C_6H_8O_7H_2O$, 68.8 HCl 37% for buffer solution pH 2.0; 4.2 $Na_2HPO_4.10H_2O$, 100.0 KH_2PO_4, 45.5 NaCl for buffer solution pH 5.5; 8.4 $Na_2HPO_4.10H_2O$, 7.4 KH_2PO_4, 94.0 NaCl for buffer solution pH 6.8; 6.7 $Na_2HPO_4.10H_2O$, 1.4 KH_2PO_4, 136.9 NaCl for buffer solution pH 7.4.

Preparation of microspheres

Pectin (0.500 g; 0.875 g; 1.250 g) was dissolved in 250 ml of de-ionized water and supplemented with chitosan (0.125 g) to obtain different pectin/chitosan weight ratios: 4 : 1 (Pec_4-Ch_1), 7 : 1 (Pec_7-Ch_1), 10 : 1 (Pec_{10}-Ch_1). The solution was stirred at room temperature for 24 h. The suspension obtained from the precipitation of the pectin/chitosan interaction product was homogenized at 17 500 rev/min for 5 min (Ultra-Turrax, T 25 basic homogenizer; IKA, Dresden, Germany) and then was spray-dried (Buchi Mini Spray Dryed, B-191, Switzerland) after appropriate dilution. The drying conditions were as follows: inlet temperature, 110°C; outlet temperature, 49°C; air flow rate, 600 Nl/h. The obtained microspheres were spherical with an average diameter of 3 μm (Nikon Eclipse E400 Microscope; Tokyo, Japan). The drug-loaded microspheres were prepared by dissolving vancomycin hydrochloride in the pectin/chitosan reaction mixture before the spray-drying process (2 : 1, 4 : 1; polymer/drug weight ratio).

Characterization by Fourier transform infrared spectrometry

Infrared spectra of pectin, chitosan and pectin/chitosan microspheres obtained by spray-drying were recorded with a Jasco FT-IR-410 spectrophotometer (Jasco, Lecco, Italy).

The samples were prepared by processing compressed KBr disks.

Scanning electron microscopic studies

The morphology of microspheres was analysed by scanning electron microscopy (SEM). The microspheres were fixed on supports and coated with gold–palladium under an argon atmosphere using a gold sputter module in a high-vacuum evaporator. Samples were then observed with LEO 420 (LEO Electron Microscopy Ltd, Cambridge, UK) at 15 kV.

Swelling studies

To quantify the swelling of pectin/chitosan microspheres in acidic and alkaline environments, disks approximately 200 mg in weight were prepared by a punch press working at 1 ton/cm² (Specac manual hydraulic press, Orpington, UK). The disks were immersed in 20 ml pH 2.0, 5.5 or 7.4 aqueous buffers at 37°C and weighed after each hour for 6 h. Water uptake was determined according to the following equation:

$$\text{Water uptake (\%)} = [(W_h - W_d) \times 100]/W_d \tag{1}$$

where W_h is the weight of the hydrated disks and W_d is the initial weight of the dry disks.

Mucoadhesion properties

In-vitro mucoadhesion studies were performed by adapting a method of Nakamura.[11] Twenty grams of a hot agar/mucin solution (1 and 2%, w/w, respectively, in pH 7.4 buffer) was cast on a Petri dish (10 cm in diameter) and left to gel at 4–8°C for 3 h. Disks were prepared by direct compression of microspheres (100 mg) with a punch press working at 1 ton/cm² (Specac manual hydraulic press, Orpington, UK). Disks were placed on top of the gel and after 10 min the plate was attached to the disintegration test apparatus (Eur. Pharm.) and moved up and down in pH 2.0, 5.5 or 7.4 buffers at 25°C. The adhesion potential was directly related to the residence time of the disks on the plate.

Determination of drug loading

An amount of 10.0 mg of microspheres was weighed, transferred into a 10.0-ml volumetric flask and brought to volume with pH 7.4 phosphate buffer. The suspension thus obtained was sonicated for 30 min, left at room temperature for 5 h and then ultracentrifuged at 10 000 rev/min for 10 min (ALC 4239R centrifuge; Milan Italy). The supernatant was finally diluted in water and analysed by HPLC method.[12,13] The drug loading was calculated according to the following equation:

$$\text{Drug loading (\% w/w)} = (\text{mass of drug in microspheres/mass of microspheres}) \times 100 \tag{2}$$

In-vitro release studies

To detect the amount of free drug available from the microspheres, 60 mg of microspheres with or without drug were introduced into a donor cell containing 5 ml of phosphate buffer at pH 2.0, 5.5 and 7.4, respectively, separated by a dialysis membrane (MW cut-off = 12 000–14 000 Daltons;

Delchimica Scientific Glassware, Milan, Italy) from a receiving compartment containing 40 ml of the same aqueous buffer. The system was thermostatted at 37°C and the drug was detected in the receiving phase by HPLC method for 6 h.[12,13] The release studies were also performed in pH 5.5 phosphate buffer for 10 h with pectinase (2 ml/l). The pH was chosen as a compromise between the mean pH of the colon and the optimum pH of the pectinolytic enzymes. Finally, to simulate gastro-intestinal transit conditions, release studies were also performed in pH 2.0 phosphate buffer for 2 h and subsequently in pH 5.5 for 1 h, in pH 6.8 for 2 h and in pH 7.4 with pectinase (2 ml/l) for up to 10 h.

Statistical analysis

All of the experiments were done in triplicate. One-way analysis of variance was performed to assess the significance of the differences among data. Tukey–Kramer post-test was used to compare the means of different treatment data. $P < 0.05$ was considered statistically significant.

Results

Characterization by Fourier transform infrared spectrometry

To confirm the interaction of pectin with chitosan, samples were analysed by Fourier transform infrared spectrometry (FT-IR) spectroscopy. Figure 1 shows the FT-IR spectra of pectin, chitosan and pectin/chitosan microspheres formed at different pectin/chitosan weight ratios. Pectin showed the typical $\nu_{C=O}$ band of methyl ester group at 1747 cm^{-1} and $\nu_{C=O}$ band of carboxyl group at 1615 cm^{-1}. Chitosan showed the characteristic $\nu_{C=O}$ band of amide at 1654 cm^{-1} and δ_{N-H} band of amine at 1594 cm^{-1}. The shift in amine band to 1635 cm^{-1} in the spectrum of pectin/chitosan microspheres indicates a change in environment of this group through its interaction with pectin.[14] Moreover, the spectra obtained by increasing the polymer ratio from Pec$_4$-Ch$_1$ to Pec$_{10}$-Ch$_1$ did not show any difference.
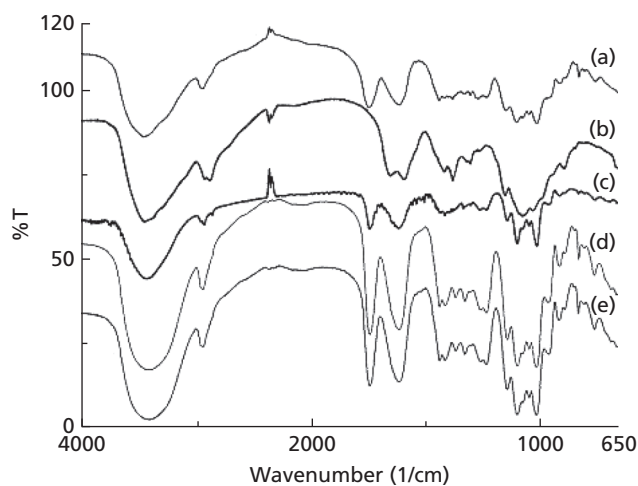

Figure 1 FT-IR spectra of pectin (a), chitosan (b) and pectin/chitosan microspheres, Pec$_{10}$-Ch$_1$ (c), Pec$_7$-Ch$_1$ (d) and Pec$_4$-Ch$_1$ (e).

Scanning electron microscopic studies

SEM photomicrographs of loaded (2 : 1, polymer/drug weight ratio) microspheres (Pec$_4$-Ch$_1$ chosen as an example) are reported in Figure 2. Drug-loaded microspheres showed a regular shape and smooth surface; moreover, no free drug was present. Photomicrographs obtained with drug-unloaded microspheres did not show any difference (data not shown).

Swelling studies

Figure 3 shows the results of the swelling studies of pectin/chitosan hydrogels at different pH values to simulate gastro-intestinal conditions. All the hydrogels presented an excess of pectin and at pH 5.5 and 7.4 the majority of carboxylic groups will be ionized, producing the highest swelling degree. At pH 2.0, pectin showed a higher amount of the un-ionized carboxylic groups, resulting in less swelling. Moreover, under low pH conditions (2.0), the hydrogels needed only 1 h equilibration to reach the highest swelling degree. At the higher pH values (5.5 and 7.4), it is possible to observe long-term swelling behaviour and the hydrogels needed 6 h equilibration to reach the highest swelling degree. Finally, hydrogels provided different swelling behaviour as a function of pectin/chitosan weight ratio (4 : 1, 7 : 1, 10 : 1) according to the different amount of free negative charges. Highest pectin/chitosan weight ratios provided highest water uptake.
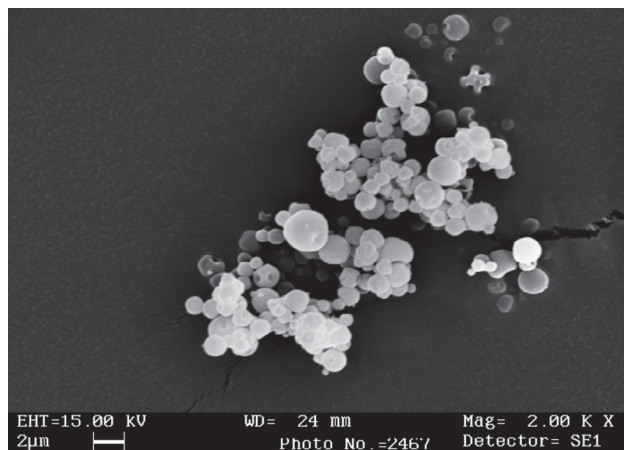

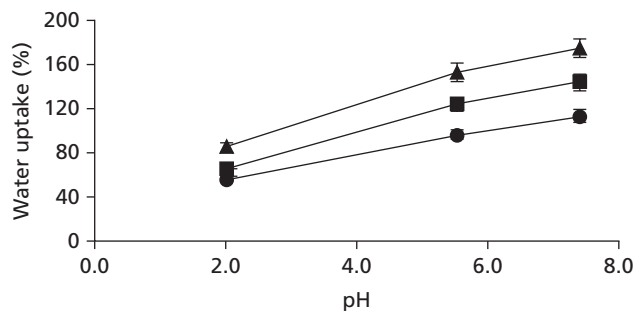**Figure 2** SEM images of loaded (2 : 1; polymer/drug weight ratio) microspheres (Pec$_4$-Ch$_1$).

Figure 3 Effect of pH on swelling degree of Pec$_4$-Ch$_1$ (●), Pec$_7$-Ch$_1$ (■) and Pec$_{10}$-Ch$_1$ (▲) after 6 h. Data represent the mean of three determinations ± SD.

Mucoadhesion properties

Figure 4 shows the mucoadhesion properties of pectin/chitosan hydrogels at different pH values (2.0, 5.5 and 7.4) to simulate gastro-intestinal conditions. All the hydrogels showed good mucoadhesive capacity (expressed as residence time of the pectin/chitosan disk on the agar/mucin plate) at all pH values considered and stronger mucoadhesive forces at pH 5.5 and 7.4 despite the presence of negative charges on the polymer chains and the mucous surface. In fact, the ionization of carboxyl groups of pectin (pK_a 3.5) enhanced polymer swelling and thus physical entanglement with mucus. For the same reason, hydrogels containing the highest percentage of pectin showed the best in-vitro mucoadhesion.[15]

Determination of drug loading

The amount of vancomycin incorporated in the microspheres was in the range of 26.7–31.7% and 16.1–19.0% for 2 : 1 and 4 : 1 polymer/drug weight ratio, respectively. These data can be attributed to the nature of the spray-drying mechanism, which provides high drug loading, particularly in the case of drying of solutions or well-stabilized suspensions. Different pectin/chitosan and polymer/drug weight ratios did not significantly affect drug loading.

In-vitro release studies

The release data of microspheres prepared at different chitosan/pectin and polymer/drug ratios are shown in Table 1. The lowest drug release was obtained with Pec_{10}-Ch_1. In
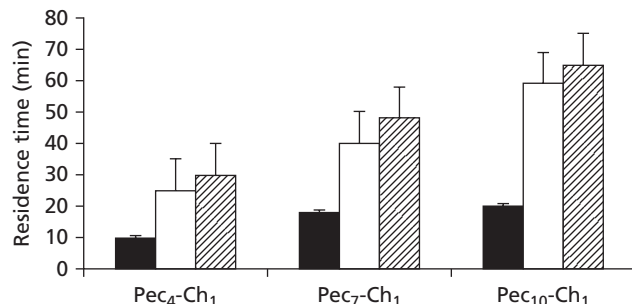
Figure 4 Mucoadhesive ability, expressed as residence time on agar/mucin plate, of pectin/chitosan hydrogels at pH 2.0 (black columns), pH 5.5 (white columns) and pH 7.4 (hatched columns). Data represent the mean of three determinations ± SD.

fact, the presence of increasing amounts of pectin from Pec_4-Ch_1 to Pec_{10}-Ch_1 produced a more hydrated and viscous network in the gelled microspheres thus limiting drug diffusion. Moreover, for all the hydrogels analysed, the medium pH (2.0, 5.5 or 7.4) influenced vancomycin availability according to swelling behaviour. Finally, the drug availability decreased by raising the polymer/drug weight ratio from 2 : 1 to 4 : 1. The release data of microspheres (2 : 1 polymer/drug weight ratio chosen as an example) in the presence of enzymes are shown in Figure 5. The presence of pectinolytic enzymes provided an abrupt release due to polymer degradation.[16–18] The ability of the enzyme to degrade the different polymeric networks was highly dependent on the hydration of pectin/chitosan microspheres (Pec_{10}-Ch_1 > Pec_7-Ch_1 > Pec_4-Ch_1). Finally, the fractional amount of vancomycin released from Pec_{10}-Ch_1 (chosen as an example) in a pH-gradient (2.0–7.4) incubation medium (Figure 6) showed that at pH 2.0 only a slight drug release occurred, while at higher pH values the cumulative amount of drug released increased due to the presence of pectinase.

Discussion

In this study pectin/chitosan hydrogels for colon-specific delivery of vancomycin were prepared by ionic crosslinking technique. Pectin/chitosan hydrogels were stabilized by electrostatic interaction between positively charged chitosan (NH_3^+) and negatively charged pectin (COO^-) as confirmed by FT-IR analysis. At the pH value of preparation (5.0), a pK_a of 3.5 for pectin and a pK_a of 6.5 for chitosan would mean that they had an extensive degree of ionization and so the electrostatic interaction between the polymers was favoured. Moreover, the possibility exists of intramolecular H-bonding between the COOH groups of pectin or NH_2 groups of chitosan and OH, OCH_3 or $COOCH_3$ groups elsewhere within the hydrogel.

After contact with pH 2.0, 5.5 or 7.4 aqueous buffers, pectin/chitosan microspheres swelled. Different swelling behaviour of pectin/chitosan hydrogels can be explained by considering the charge state of the polymers as well as the pectin/chitosan ratio.[6,19] As swelling medium pH changes (2.0, 5.5 and 7.4), the charge balance inside the gelling network, and therefore the degree of interaction between chitosan and pectin, is modified and swelling occurs because of the dissociation of the complex. In acidic medium, pectin
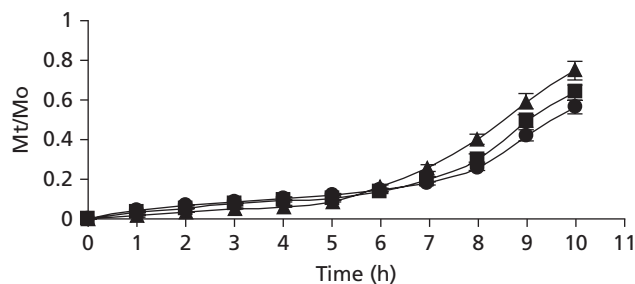
Table 1 Fractional amount of vancomycin hydrochloride released after 6 h from Pec_4-Ch_1, Pec_7-Ch_1 and Pec_{10}-Ch_1 microspheres (2 : 1, 4 : 1; polymer/drug weight ratio) at pH 2.0, 5.5 and 7.4

Microsphere type	Polymer/drug weight ratio	pH of aqueous buffers		
		2.0	5.5	7.4
Pec_4-Ch_1	2:1	0.089 ± 0.003	0.146 ± 0.007	0.233 ± 0.010
	4:1	0.070 ± 0.002	0.096 ± 0.004	0.173 ± 0.005
Pec_7-Ch_1	2:1	0.075 ± 0.005	0.112 ± 0.006	0.169 ± 0.005
	4:1	0.065 ± 0.003	0.084 ± 0.001	0.121 ± 0.007
Pec_{10}-Ch_1	2:1	0.060 ± 0.003	0.083 ± 0.001	0.114 ± 0.004
	4:1	0.053 ± 0.001	0.078 ± 0.003	0.098 ± 0.002

Data represent the mean of three determinations ± SD.

Figure 5 Vancomycin hydrochloride release profiles from Pec_4-Ch_1 (●), Pec_7-Ch_1 (■) and Pec_{10}-Ch_1 (▲) microspheres (2 : 1; polymer/drug weight ratio) in the presence of pectinolytic enzymes at pH 5.5. Mt/Mo corresponds to vancomycin fractional amount released at each time. Data represent the mean of three determinations ± SD.
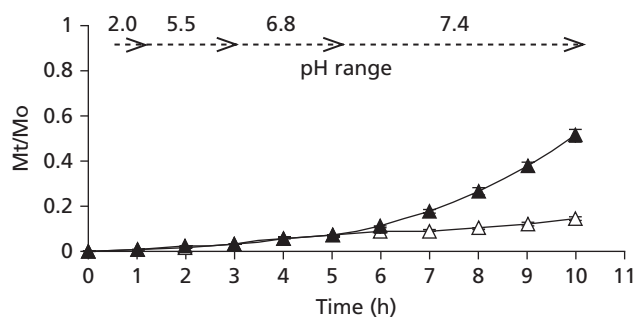

Figure 6 Fractional amount of vancomycin hydrochloride released from Pec_{10}-Ch_1 (2 : 1; polymer/drug weight ratio) with (▲) or without (△) pectinase in a pH-gradient incubation medium. Mt/Mo corresponds to vancomycin fractional amount released at each time. Data represent the mean of three determinations ± SD.

is neutralized and free positive charges (NH_3^+) appear inside the gel; in basic medium, chitosan is neutralized and free negative charges (COO^-) appear inside the gel. The mutual repulsion between positive or negative charges and the entry of water, together with counter-ions to neutralize these charges, cause swelling. All the pectin/chitosan hydrogels present an excess of pectin, which allows a higher swelling degree at pH 5.5 and 7.4 with respect to pH 2.0. Moreover, the presence of a high amount of pectin in the hydrogel (Pec_{10}-Ch_1) provided a greater amount of free negative charges, thus greater water uptake, with respect to low weight ratio pectin/chitosan hydrogel (Pec_4-Ch_1).

Pectin and chitosan were used for many colon-specific drug delivery systems as they are known to provide an intimate contact with the intestinal mucosa, allowing longer absorption times. Mucoadhesion is becoming an important strategy to improve drug efficacy in local diseases of the colon. The interaction between mucus and hydrophilic polymers is a result of physical entanglement and secondary bonding, mainly H-bonding and van der Waals attraction. Moreover, polymers' swelling ability, increasing the mobility of molecules, facilitates interpenetration and interaction with the mucus layer.[20–22] Pectin is known for good mucoadhesion related to a balance between available hydrogen bonding sites and an open expanded conformation.[23] Chitosan has

hydroxyl, amide and amine groups able to provide hydrogen bonds and its linear molecule expresses a good chain flexibility and thus a good physical entanglement. The cationic nature of chitosan can provide strong electrostatic interactions with mucus or a negatively charged mucosal surface.[10] Clearly the mucoadhesive capacity of chitosan and pectin depends on environmental pH, which can influence the ionization degree of the two polysaccharides and mucin. At pH values higher than 2.6, mucus presents negative charges due to complete ionization of sialic acid (pK_a 2.6) and sulfate residues in mucin glycoprotein.[24] Mucoadhesion studies performed on pectin/chitosan microspheres revealed that the key factor influencing hydrogel adhesion to the mucosal surface is their swelling ability and not electrostatic interactions. In fact a good correlation between swelling and mucoadhesion data can be observed, suggesting that suitable swelling of the hydrogels can improve the physical entanglement with the mucus.

Swelling of microspheres is an important factor affecting the diffusion of incorporated drug. It has been found that drug diffusion in highly hydrated hydrogels is faster than that in less-hydrated hydrogels. This behaviour can be observed only for hydrogels in which the water uptake does not provide highly viscous networks. In the case of pectin/chitosan hydrogels, microspheres with a great amount of pectin showed high swelling and low drug availability, thus confirming the formation of highly viscous hydrogels. Moreover, the presence of colonic bacteria can influence drug availability from biodegradable delivery systems such as pectin/chitosan microspheres. Drug-release profiles confirmed that vancomycin availability in the presence of pectinase was nearly four times as much as drug availability without pectinolytic enzyme.

Conclusions

This investigation describes a new colonic drug delivery system, consisting of pectin/chitosan microspheres containing vancomycin. The microspheres, easily prepared from aqueous solution by spray-drying method, could be capable of achieving a colon-specific delivery of drug due to their pH-dependent swelling ability, mucoadhesion characteristics and enzyme-dependent degradation. In fact in the presence of pectinase, the glycoside bonds of pectin were degraded and a considerable amount of vancomycin was released in a short time.

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

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