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Chitosan salts as nasal sustained delivery systems for peptidic drugs

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Abstract

The aim of this study was to describe a sustained drug release system based on chitosan salts for vancomycin hydrochloride delivery. Chitosan lactate, chitosan aspartate, chitosan glutamate and chitosan hydrochloride were prepared by spray-drying technique. Vancomycin hydrochloride was used as a model peptidic drug, the nasal sustained release of which should avoid first-pass metabolism in the liver. This in-vitro study evaluated the influence of chitosan salts on the release behaviour of vancomycin hydrochloride from the physical mixtures at pH 5.5 and 7.4. In-vitro release of vancomycin was retarded by chitosan salts and, in particular, chitosan hydrochloride provided the lowest release of vancomycin.

Introduction

The nasal cavity is one of the most attractive routes for peptide and protein delivery because of its greater permeability than other administration routes and avoidance of first-pass metabolism in the liver (Natsume et al 1999). Various bioadhesive materials, including chitosan, have been proposed for nasal delivery of drugs and vaccines (Illum et al 1987, 1994; Calvo et al 1997; McDermott et al 1998; Bacon et al 2000). Chitosan is a polysaccharide comprising copolymers of glucosamine and *N*-acetylglucosamine and can be derived by the partial deacetylation of chitin. It is insoluble at alkaline and neutral pH values, but forms salts with inorganic and organic acids such as hydrochloric acid, lactic acid, acetic acid and glutamic acid (Paul & Sharma 2000). Chitosan salts can bind strongly to negatively charged materials such as cell surfaces and mucus (Illum et al 2001). Mucus contains mucins that have different chemical constitutions but some contain significant proportions of sialic acid. At physiological pH, sialic acid carries a net negative charge and, as a consequence, mucin and chitosan can demonstrate strong electrostatic interaction when in solution. Unlike other absorption promoters, chitosan appears to be non-toxic and well tolerated by man. Chitosan delivery systems have the ability to increase the residence time of drug formulations in the nasal cavity, thereby providing the potential for improved systemic medication. Consequently, the combination of bioadhesion and sustained-release effects has made chitosan a candidate for the delivery of drugs via the nasal cavity (Soane et al 2001).

Materials and Methods

Materials

High-molecular-weight chitosan (MW 600 000, viscosity 400 mPas (1% solution in 1% acetic acid), degree of deacetylation 80%) was purchased from Fluka (Buchs, Switzerland). Aspartic acid, glutamic acid, hydrochloric acid, lactic acid and mucin (Type II: crude, from porcine stomach) were all purchased from Fluka-Sigma-Aldrich. Vancomycin hydrochloride was the generous gift of Eli Lilly (Italy).

Preparation of chitosan salts

The preparation of chitosan salts was described in a previous article (Orienti et al 2002). Briefly, 0.25 g of chitosan (1.55 mmol glucosamine) was dissolved in 50 mL water containing

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different acids (aspartic, glutamic, lactic and hydrochloric) in 1:1 molar ratios (1:1 mol monomer:mol acid) at room temperature. The solutions were spray-dried (Buchi Mini Spray Dryed, B-191, Switzerland) with an inlet temperature of 105 °C and the products obtained were collected.

Chitosan salt characterization by Fourier transform infrared spectrometry

Infrared (IR) spectra were recorded with a Jasco FT-IR-410 spectrophotometer. The samples were prepared by processing compressed KBr disks.

Viscosity tests

The viscosity of all the chitosan salt solutions were tested before spray-drying at 37 °C, by a Visco Star-R (Fungilab-Spain) viscosimeter.

Preparation of the drug–polymer physical mixture

1.55 mmol of chitosan salts (0.46 g of 1:1 chitosan monomer–aspartate, 0.50 g of 1:1 chitosan monomer–glutamate, 0.39 g of 1:1 chitosan monomer–lactate, 0.43 g of 1:1 chitosan monomer–hydrochloride) and 1.55 mmol vancomycin hydrochloride (2.30 g) were weighed and mixed in a mortar until homogeneity.

Swelling of the drug-polymer mixtures

To quantify the swelling of the drug–polymer mixtures in acidic and alkaline environments, disks of approximately 20 mg in weight were prepared by a punch press working at 7 ton cm^{-2}. The disks were immersed in 10 cm^3 volume pH 5.5 or pH 7.4 aqueous buffers at 37 °C and weighed after each hour for 5 h. The swelling ratio was determined as the ratio between the weight of the hydrated disks (Wh) at each time and the initial weight of the dry disks (Wd).

Muco-adhesion properties

The muco-adhesion behaviour was evaluated by mixing 1 mL of a mucin suspension (0.05% w/v) with 1 mL of chitosan salts solution for 24 h at pH 5.5 and pH 7.4 at 37 °C under continuous stirring. Mucin–chitosan interaction was evaluated by an instrument equipped with a 50 mW He-Ne laser (532 nm) and thermostated at 37 °C (90 Plus Particle Sizer Analyzer, Brookhaven). Measurements were carried out by fixing the scattering angle at 90°. Results were the combination of three 5-min runs for a total correlation function (ACF) accumulation time of 15 min. The diffusion coefficient was evaluated from the time autocorrelation function, $g^2(\tau)$, using the forced single-exponential fit (Equation 1) (Chu 1974; Berne & Pecora 1976).

$$g^2(\tau) = Ae^{-2\Gamma\tau} + B \quad (1)$$

$$\Gamma = Dq^2 \quad (2)$$

$$q = (4\pi n/\lambda_0)\sin(\theta/2) \quad (3)$$

where τ is the delay time, both A and B are constant, D is the translational diffusion coefficient, q is the scattering vector, n is the refractive index of pure solvent, λ_0 is the wavelength of incident light in-vacuo and θ is the scattering angle. The hydrodynamic radius, R_H, was calculated using Stokes-Einstein equation:

$$R_H = k_B T/6\pi\eta D_0 \quad (4)$$

where k_B, T and η are the Boltzmann constant, the absolute temperature and the solvent viscosity, respectively.

In-vitro release studies

To detect the amount of free drug available from the drug–polymer mixtures, the solid mixtures (50 mg) were introduced into a donor cell containing 3 mL of pH 5.5 and pH 7.4, respectively (Behl et al 1998; Tengamnuay et al 2000; Washington et al 2000), separated by a dialysis membrane (MW cut off = 14 000) from a receiving compartment containing 10 mL of the same aqueous buffer, which was replaced after time intervals suitable to guarantee sink conditions throughout the runs. The system was thermostated at 37 °C and the drug was spectrophotometrically detected in the receiving phase.

Statistical analysis

All the data are the arithmetic means of results from three experiments ± s.d. Statistical data were analysed using Student's *t*-test, with $P \leq 0.05$ as minimum level of significance.

Results and Discussion

Fourier transform infrared spectrometry

As reported in our previous article (Orienti et al 2002), the FT-IR spectra of chitosan depict characteristic absorption bands at 3436, 2916 and 2850 cm^{-1}, which represent the presence of the OH group, CH_2 and CH_3 groups (aliphatic groups). The amino group has a characteristic absorption band in the region of 3400–3500 cm^{-1}, which must have been masked by the absorption band due to OH group (Shanmugasundaram et al 2001). Chitosan showed the characteristic band of the amino group (-NH_2) at 1669 cm^{-1} (Figure 1). In the spectrum of spray-dried chitosan hydrochloride, the characteristic absorption band at about 1669 cm^{-1} disappeared, giving rise to two new bands located at 1631 and 1522 cm^{-1} (Table 1). This behaviour reflects the interaction between the amino groups and the HC1. Moreover, the spectrum of spray-dried chitosan lactate, chitosan aspartate and chitosan glutamate showed a large peak (-NH_2) at 1582, 1623 and 1631 cm^{-1}, respectively. The large shift of these vibrations to higher wavenumbers compared with the usual wavenumbers of the amino groups proves the formation of a carboxylate between the -COO^- groups of the acids

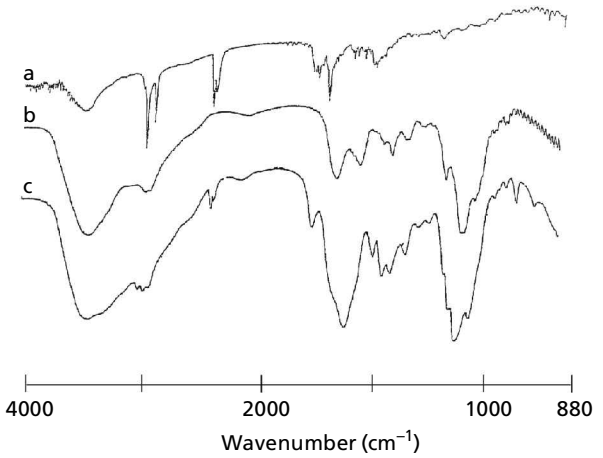

Figure 1 IR spectra of chitosan (a), chitosan hydrochloride (b) and chitosan lactate (c).

and the -NH_3^+ groups of chitosan (Lorenzo-Lamosa et al 1998). Consequently, it seems reasonable to conclude that chitosan was ionically interacted with the acids.

Viscosity tests

The viscosity of all the chitosan salt solutions before spray-drying at 37 °C decreased as follows: chitosan hydrochloride (350 cPs) > chitosan glutamate (80 cPs) > chitosan aspartate (50 cPs) > chitosan lactate (40 cPs). This can be attributed to the ability of different acids to form a salt with the amino groups of chitosan. The viscosity of the different salts can influence the swelling and consequently the release behaviour – the higher the viscosity, the lower the swelling and the lower the release (Figure 4).

Swelling of the drug–polymer mixtures

The presence of hydrophilic groups, like the carboxyl group in the molecule of acid and amine groups in the molecule of chitosan, affected the water uptake of the drug–polymer mixtures. In particular, at pH 5.5 all forms of both the amino and carboxylic acid groups of amino

Table 1 Wavenumbers of peaks in FT-IR spectra for characteristic regions.

	NH_2, NH_3^+	OH, CH_2 and CH_3
Chitosan	1669 cm^{-1}	3436, 2916 and 2850 cm^{-1}
Chitosan hydrochloride	1631–1522 cm^{-1}	//
Chitosan lactate	1582 cm^{-1}	//
Chitosan aspartate	1623 cm^{-1}	//
Chitosan glutamate	1631 cm^{-1}	//

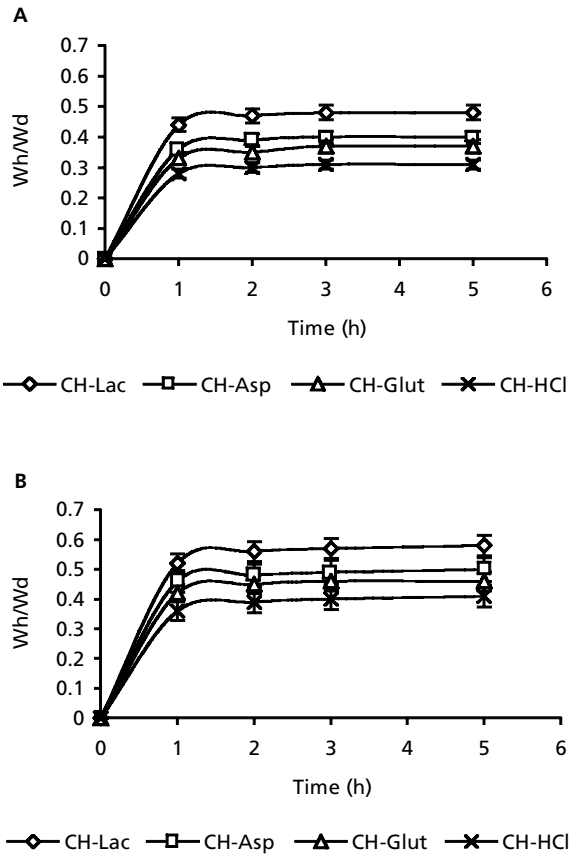

Figure 2 Swelling (± s.d.; n = 3) of the chitosan salts at pH 5.5 (A) and pH 7.4 (B). Wh/Wd, swelling ratio (weight of hydrated disks/initial weight of dry disks); CH-Lac, chitosan lactate; CH-Asp, chitosan aspartate; CH-Glut, chitosan glutamate; CH-HCl, chitosan hydrochloride.

acids and chitosan will exist (i.e. NH_3^+, COO^-, NH_2 and COOH (Figure 2A)). Within the amino acids there also exists the possibility of intra-molecular H-bonding between the COOH groups and NH_2 groups of amino acids or NH_2 groups of chitosan and OH or $COOCH_3$ groups elsewhere within the network (Macleod et al 1999). This H-bonding may result in a tightening of the amino-acid network leading to a reduced swelling capacity (Yao et al 1997). Similarly the interaction between the chitosan and amino acids would cause a tightening of the network resulting in less swelling.

The swelling of chitosan lactate can be explained by the ionisation of the NH_2 groups at pH 5.5. Therefore, the network will be looser as a result of suboptimal NH_3^+–COO^- ionic interaction and decreased H-bonding possibilities caused by the charged NH_3^+ species.

At pH 7.4, COOH groups of lactic acid will be ionised, but there also exists the unionised species of chitosan. This condition means that swelling will increase because the network is not tightly bound. Similarly for chitosan aspartate and chitosan glutamate, there will be an excess of COOH groups (Figure 2B). However, there will be a relatively higher amount of the ionised species and consequently the swelling is higher than at pH 5.5.

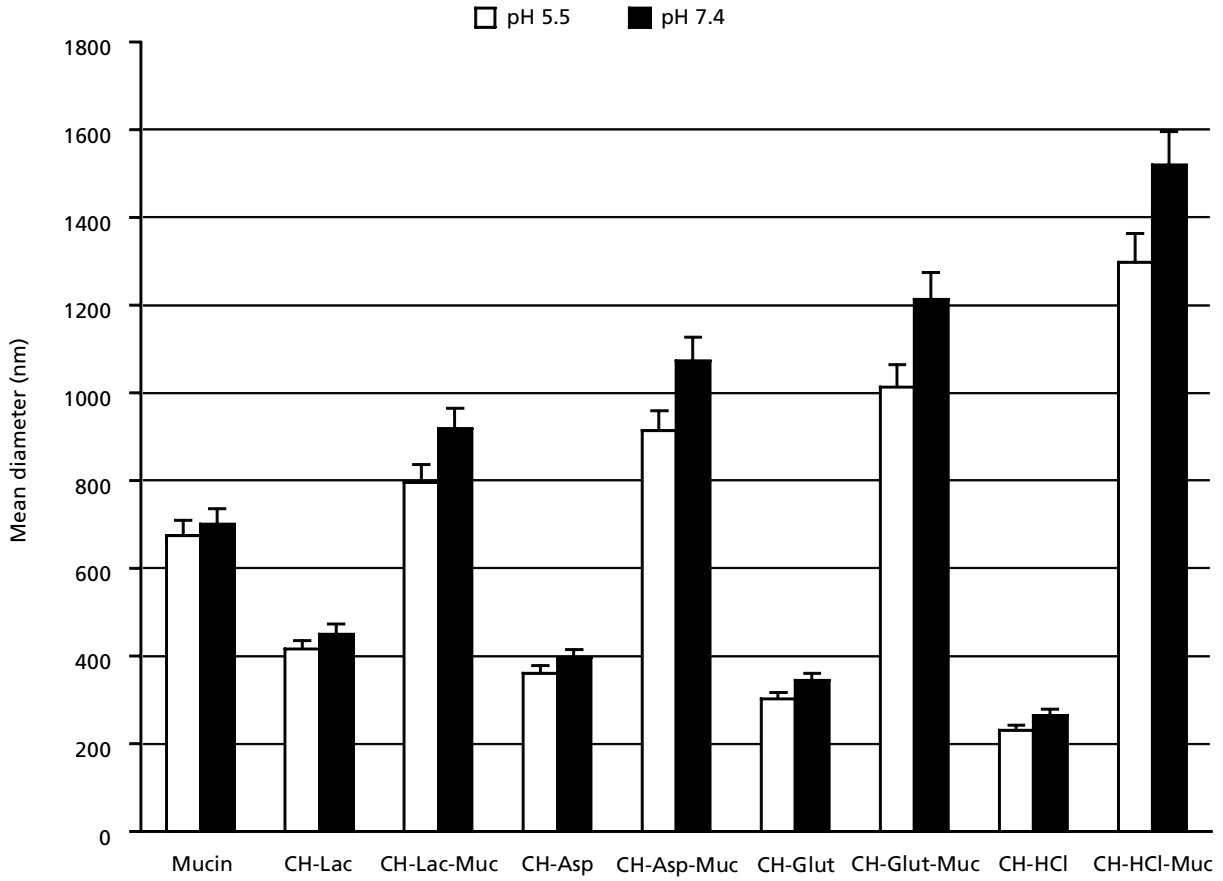

Figure 3 Mean size of chitosan salts in pH 5.5 and pH 7.4 aqueous buffer at 37 °C. The data were obtained by dynamic light scattering measurements and each value represents the average of three determinations ± s.d. CH-Lac, chitosan lactate; CH-Asp, chitosan aspartate; CH-Glut, chitosan glutamate; CH-HCl, chitosan hydrochloride; Muc, mucin.

Muco-adhesion properties

Chitosan mucoadhesive properties are presumably due to the formation, depending on environmental pH, of either secondary chemical bonds such as hydrogen bonds or ionic interactions between the positively charged amino groups of chitosan and the negatively charged sialic acid residues of mucus glycoproteins or mucins (Lehr et al 1992; Rossi et al 2001). The interaction of chitosan salts with mucin dispersion showed a higher in-vitro muco-adhesion at pH 7.4 than at pH 5.5. In fact, a light increase in the mean size of chitosan salts with mucin was observed (Figure 3).

Chitosan salts and mucin macromolecules are characterized by a more extended conformation at pH 7.4 than pH5.5, where a contraction of the polymeric coil occurs. This could prevent a deep inter-penetration between polymer and mucin chains and therefore affect the chitosan interaction properties.

In-vitro release studies

Free-drug availability, expressed as fractional release over time, was lower from the physical mixtures than the pure drug at each pH analysed (Figure 4A, B). This may be attributed to the establishment of interactions between the drug and the swelled polymer in solution, decreasing the free-drug concentration in the releasing aqueous phase. In particular, the release of vancomycin from the physical mixtures was in the order: chitosan lactate > chitosan aspartate > chitosan glutamate > chitosan hydrochloride. This behaviour was in accordance with the swelling of the drug–polymer mixtures (Figure 2) and the viscosity test. In particular, a direct correlation was observed between the viscosity of the salts and the antibiotic release – among the different salts, chitosan hydrochloride provided the lowest release of the drug. Moreover, the free-drug availability was higher for all the physical mixtures at pH 7.4 than at pH 5.5. This may be attributed to the physico-chemical characteristics of these mixtures characterized by enhanced swelling ability at alkaline pH.

Conclusions

Chitosan salts could serve as potent candidates for antibiotic delivery in nasal systems. The presence of the chitosan salts slows down the release of vancomycin hydrochloride at pH 5.5 and pH 7.4, guaranteeing a sus-

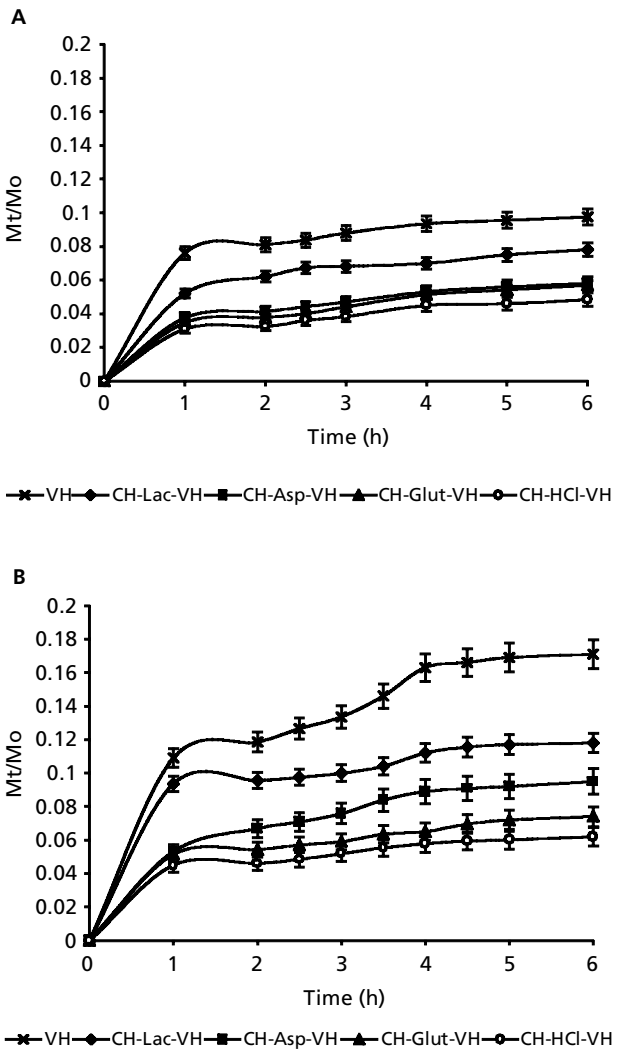

Figure 4 Fractional release of vancomycin hydrochloride from the mixtures with chitosan salts at pH 5.5 (A) and pH 7.4 (B). Each value represents the average of three determinations ± s.d. Mt, cumulative amount (mg) released at each time; Mo, total amount (mg) present in donor cell; CH-Lac, chitosan lactate; CH-Asp, chitosan aspartate; CH-Glut, chitosan glutamate; CH-HCl, chitosan hydrochloride, VH, vancomycin hydrochloride.

tained release at acidic pH and alkaline pH of drug in the nasal cavity. Among the different salts, chitosan hydrochloride provided the lowest release of vancomycin.

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