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Research Paper

Novel mucoadhesive nasal inserts based on chitosan/hyaluronate polyelectrolyte complexes for peptide and protein delivery

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Abstract

Objectives The purpose of this study was the preparation and characterisation of mucoadhesive nasal inserts based on chitosan/hyaluronate polyelectrolyte complexes prepared at various pHs and at different molar ratios.

Methods A suspension of chitosan/hyaluronate complexes with or without the model drugs (vancomycin or insulin) was lyophilised into small inserts. Complexation yield, FT-IR spectra and thermogravimetric analysis were used to study the degree of interactive strength between polyions. In-vitro swelling, mucoadhesion and release tests were performed in order to investigate delivery of vancomycin and insulin in the nasal cavity.

Key findings The results indicated that the selection of complex preparative conditions allows modulation of insert swelling and mucoadhesion ability. Nasal inserts containing vancomycin or insulin had showed completely different drug release behaviour.

Conclusions Chitosan/hyaluronate polyelectrolyte complexes can be used for the formulation of mucoadhesive nasal inserts for the delivery of peptide and protein drugs.

Keywords insulin; mucoadhesion; nasal administration; polyelectrolyte complexes; vancomycin

Introduction

Over the past decades, the nasal route of administration has gained interest among mucosal sites as a non-invasive alternative for systemic delivery of drugs with poor oral bioavailability, including peptide and protein drugs.[1] This is because the large surface area, porous endothelial basement membrane and high total blood flow of the nasal mucosa ensure a rapid absorption of compounds, whilst circumventing hepatic first-pass metabolism.[2] Moreover, the accessibility of the nasal route provides a quick and easy route for self-medication compared with other routes, thus improving patient compliance.[3]

A major problem of nasal drug delivery is the mucociliary clearance mechanism, which rapidly removes non-mucoadhesive dosage forms from the absorption site.[4] Mucoadhesive polymers can be used to prevent rapid clearance of the drug formulation, increasing the nasal residence time and thereby allowing longer absorption times. Moreover, a more intimate contact with the nasal mucosa provides a higher concentration gradient and thus increases absorption.[5] Another factor that limits the bioavailability of nasally administered peptides and proteins is their poor ability to cross mucosal membranes. To enhance nasal absorption of large-molecular-weight and polar molecules, absorption enhancers such as surfactants, bile salts, phospholipids and fatty acids have been widely employed. The absorption of such polar molecules across the nasal mucosa can also be greatly increased by the use of polymers that are able to transiently open the tight junctions between epithelial cells.[6] Among different polymers, chitosan, the N-deacetylated product of the polysaccharide chitin, is gaining importance in medical and pharmaceutical applications because of its good mucoadesion and absorption-enhancing ability.[7–9] Moreover, chitosan has the ability to form hydrogels that are able to control the rate of drug release from the delivery system as well as protecting the drug from chemical and enzymatic degradation at the administration

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site. In particular, when chitosan is cross-linked or complexed with an oppositely charged polyelectrolyte, a three-dimensional network is formed in which the drug can be incorporated to control its release.[10]

In this study, chitosan and hyaluronic acid were selected to produce polyelectrolyte complexes, with the aim of investigating their possible application for peptide/protein delivery. The physicochemical properties of chitosan/hyaluronate polyelectrolyte complexes have been widely characterised, but few works present their application in the field of drug delivery. In this work, the release behaviour of two peptide/protein drugs with different ionic natures and molecular weights (i.e. vancomycin and insulin) from mucoadhesive nasal inserts based on chitosan/hyaluronate polyelectrolyte complexes was evaluated. Nasal inserts prepared with chitosan/hyaluronate complexes obtained in different preparative conditions were characterised with respect to mucoadhesion potential, swelling ability and drug release behaviour.

Materials and Methods

Materials

Sodium hyaluronate (molecular weight (relative MW) 1 650 000), low-viscosity chitosan (relative MW 150 000; viscosity ≤ 200 mPa·s at 1% in 1% acetic acid and 20°C; deacetylation degree 97%), high-viscosity chitosan (relative MW 600 000; viscosity ≥ 400 mPa·s at 1% in 1% acetic acid and 20°C; deacetylation degree 90%) and vancomycin hydrochloride (MW 1485.71) were purchased from Fluka (Buchs, Switzerland). Bovine insulin (MW 5733.49) was purchased from Sigma-Aldrich (St Louis, MO, US). Mannitol was obtained from Carlo Erba (Milan, Italy). All chemicals were analytical grade. Venlafaxine (1-[2-(dimethylamino)-1-(4-methoxyphenyl)ethyl]cyclohexanol) used as the internal standard (IS) was kindly provided by Wyeth (Madison, NJ, US). Methanol and acetonitrile (both HPLC grade) were purchased from Sigma-Aldrich; 37% (w/w) hydrochloric acid (HCl), 85% (w/w) phosphoric acid and monobasic potassium phosphate pure for analysis (> 99%) were purchased from Carlo Erba. Ultrapure water (18.2 MΩ cm) was obtained by means of a MilliQ apparatus by Millipore (Milford, US).

Preparation of chitosan/hyaluronate complex

The interactions between polyelectrolytes of opposite charge depend on the degree of ionisation, the strength of the alkaline or acidic sites they bear and their charge density. Chitosan is a weak base with an intrinsic pKa near 6.3 and a low charge density, with a maximum of only one charge per monomer in the fully deacetylated chitosan. Hyaluronic acid is a weak polyacid (pKa 2.9) with a very low charge density, since only one charge can be present every two monomers. In solution, strong electrostatic interactions take place between the $-NH_3^+$and $-COO^-$ groups of chitosan and hyaluronic acid, respectively, which allow complex precipitation.[10,11] In the case of chitosan/hyaluronate polyelectrolyte complexes, it has been demonstrated that when the molar ratio between $-NH_3^+$ groups and $-COO^-$ groups in solution is 1, all the anionic sites of hyaluronic acid are involved in a polyelectrolyte complex of 1/1 type with the cationic sites of chitosan.[12] In fact, the formation of chitosan/hyaluronate polyelectrolyte complexes obeys the simple stoichiometric reaction represented by the equation: α[PA] = β[PC], where α and β are the degrees of ionisation of the ionisable sites of the polyanionic and polycationic chains, respectively (independently of their nature) and [PA] and [PC] represent the total concentrations of ionisable sites brought about by the polyanionic and polycationic chains, respectively, at the maximum complexation.[13] The mechanism of formation and the composition of chitosan/hyaluronate polyelectrolyte complexes can be influenced by the nature of the polymer in excess and the molar mass of the polyions.

In the current study, chitosan/hyaluronate polyelectrolyte complexes were prepared at various pH values and at different molar ratios. Polymer concentration (5.0 mM) and pH range (2.0–5.0) were selected on the basis of the solubility and viscosity characteristics and pKa values of chitosan and hyaluronic acid (Table 1).

Sodium hyaluronate and low/high-viscosity chitosan (0.75 mmol monomer in 150 ml) were separately dissolved in HCl (pH 2.0) or acetate buffers (pH 3.0, 3.5, 4.0, 5.0) at the same ionic strength (50 mM). High-viscosity chitosan/hyaluronate polyelectrolyte complexes were not prepared at pH 5.0 because of the poor solubility of high-viscosity chitosan in this medium. Then, 5 ml, 15 ml, 25 ml, 35 ml or 45 ml of a chitosan solution was added to 45 ml, 35 ml, 25 ml, 15 ml or 5 ml of sodium hyaluronate solution, respectively, (total volume 50 ml) and stirred at room temperature for 24 h, thus obtaining different chitosan/hyaluronic acid molar ratios (1 : 9, 3 : 7, 1 : 1, 7 : 3, 9 : 1). The precipitate was separated by ultracentrifugation at 10 000 rev/min for 10 min (ALC 4239R centrifuge; ALC srl, Milan, Italy), washed with deionised water and finally dried under vacuum to constant weight.

Table 1 Theoretical concentrations of $-NH_3^+$ and $-COO^-$ (mM) obtained for a complexation reaction between chitosan and sodium hyaluronate (both 5 mM), as a function of pK_a values of the two polysaccharides, chitosan/hyaluronate molar ratio and complexation pH

	Chitosan/hyaluronate molar ratio									
	9 : 1		7 : 3		1 : 1		3 : 7		1 : 9	
Complexation pH	$-NH_3^+$	$-COO^-$	$-NH_3^+$	$-COO^-$	$-NH_3^+$	$-COO^-$	$-NH_3^+$	$-COO^-$	$-NH_3^+$	$-COO^-$
5.0	4.27	0.49	3.32	1.48	2.37	2.47	1.42	3.46	0.47	4.45
4.0	4.50	0.46	3.50	1.39	2.50	2.32	1.50	3.25	0.50	4.18
3.5	4.50	0.40	3.50	1.20	2.50	2.00	1.50	2.80	0.50	3.60
3.0	4.50	0.28	3.50	0.84	2.50	1.40	1.50	1.96	0.50	2.52
2.0	4.50	0.05	3.50	0.16	2.50	0.27	1.50	0.38	0.50	0.49

Thermogravimetric analysis (TGA)

Mass losses were recorded with a Mettler TA 4000 apparatus equipped with a TG 50 cell on 8–10 mg samples in open alumina crucibles (β = 10 K/min, static air atmosphere, 30–300°C temperature range). Measurements were carried out at least in triplicate.

Fourier-transform infrared (FT-IR) spectroscopy

Mid-IR (650–4000 cm^{-1}) spectra were recorded on powder samples using a Spectrum One Perkin-Elmer FT-IR spectrophotometer (resolution 4 cm^{-1}; Perkin Elmer, Wellesley, MA, US) equipped with a MIRacle ATR device (Pike Technologies, Madison, WI, US).

Manufacture of nasal inserts

The precipitated chitosan/hyaluronate polyelectrolyte complexes prepared as described above were separated by ultracentrifugation at 10 000 rpm for 10 min, homogenised at 17 500 rpm for 5 min (Ultra-Turrax, T 25 basic homogeniser; IKA, Dresden, Germany), washed with deionised water, resuspended in deionised water and finally freeze-dried (Christ Freeze Dryer ALPHA 1-2, Milan, Italy). Loaded inserts were prepared by adding 100 μl vancomycin (0.9 mg/ml) or insulin (3.5 mg/ml) aqueous solution to 10 mg of different freeze-dried complex/mannitol mixtures (9 : 1 w/w), thus obtaining inserts with the same molar amount of vancomycin or insulin (6×10^{-2} mmoles). In order to avoid complex destabilisation due to the presence of media with different pH and ionic strength, the aqueous solutions used for the loading process were the same as those used for the complex formation procedure (pH range 2.0–5.0). Mannitol was added as a bulking agent to improve the mechanical strength of the lyophilised nasal inserts during handling.[14] The resultant suspensions were filled into polypropylene microcentrifuge tubes, allowed to settle to swell and remove air and finally lyophilised. The inserts were stored in a desiccator until use. Loaded inserts (10 mg) with only mannitol and drugs were produced as standard formulations for insulin and vancomycin release studies.

Unloaded inserts (10 mg) were prepared by the same procedure without the presence of drugs for in-vitro mucoadhesion studies. All inserts had a cone-like shape (average diameter 5 mm, height 8 mm) and weight in the range of 10–10.35 mg. Unloaded and loaded inserts (100 mg) were prepared by the same procedure for water uptake studies.

Water uptake

In order to quantify the water uptake ability of the different chitosan/hyaluronate polyelecrolyte complexes, accurately weighed unloaded inserts (100 mg) were placed on filter papers (d = 40 mm) soaked in different media (pH 2.0, pH 5.5 and pH 7.4 phosphate buffers) and positioned on top of a sponge (5 cm × 5 cm × 2 cm) previously soaked in the hydration medium and placed in a Petri dish filled with the same buffer to a height of 0.5 cm.

Water uptake was determined as weight increase of the insert after 6 h, according to the following equation: % water uptake (%WU) = $((W_{Hip} - W_{Hp} - W_{Di}) \times 100)/W_{Di}$, where W_{Hip} is the weight of hydrated insert and wet filter paper, W_{Hp} is the weight of wet filter paper and W_{Di} is the initial weight of the dry insert. The same procedure was used for loaded inserts in order to study the influence of insulin and vancomycin on the swelling behaviour in physiological conditions (pH 7.4).

Insert mucoadhesion properties

In-vitro mucoahesion studies were performed by adapting a method of Bertram and Bodmeier.[15] Twenty grams of a hot agar/mucin solution (1 and 2% w/w, respectively, in pH 7.4 phosphate buffer) was cast on a Petri dish (10 cm diameter) and left to gel at 4–8°C for 3 h. The inserts (100 mg) 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 7.4 buffer at 25°C. The adhesion potential was directly related to the residence time of the inserts on the plate.

In-vitro release studies

The diffusion apparatus used for in-vitro release studies is shown in Figure 1. Loaded inserts (10 mg) were placed on the sintered-glass filter plate (pore size 90–150 μm) of a Borosil glass filter crucible (inner diameter 2.0 cm, capacity 15 ml) and the whole system was sealed with Parafilm to avoid evaporation of release medium. The crucible was placed vertically into a release medium container (filled with 10 ml pH 7.4 phosphate buffer) and adjusted exactly to the height of the release medium surface so that the porous glass membrane was wetted but not submerged. The experiments were performed at 37°C under magnetic stirring. Samples of 300 μl were taken at predetermined time points and replaced by fresh medium.

Vancomycin hydrochloride availability was evaluated by HPLC analysis, as reported previously.[16] Insulin availability was determined as follows. The chromatographic system comprised a Jasco (Tokyo, Japan) PU-2089 chromatographic pump and a Jasco FP-2020 spectrofluorimetric detector. Separation was performed on an Agilent (Waldbronn, Germany) Eclipse XDB-C_8 reverse-phase column (150 × 4.6 mm i.d., 5 μm) coupled to a Phenomenex (Torrance, CA, US) SecurityGuard C_8 guard cartridge (4 × 3.0 mm i.d., 5 μm). The mobile phase was a mixture of acetonitrile (24% v/v) and 100 mM sodium sulphate adjusted to pH 3.0 with phosphoric acid (76%, v/v). The mobile phase was filtered through a Phenomenex membrane filter (47 mm membrane, 0.2 μm, nylon) and degassed by an ultrasonic apparatus. The flow rate was 1.0 ml/min and injections were via a 20 μl loop. Venlafaxine was used as the IS. The excitation wavelength

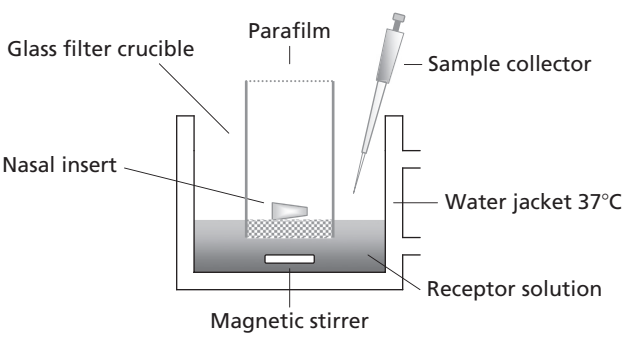

Figure 1 Scheme of the release apparatus.

was 276 nm and fluorescence emission was monitored at 306 nm. Simulated release solutions obtained from the different kinds of nasal inserts were spiked with 10 μl IS solution; the mixture was then diluted suitably with the mobile phase and injected into the HPLC apparatus. Full method validation was performed with good results in terms of linearity ($y = 1.721x + 0.012$, $r^2 = 0.9996$) over the 0.10–30.0 μg/ml insulin concentration range, limit of detection (0.03 μg/ml) and limit of quantification (0.10 μg/ml), precision (relative standard deviation < 3.6%) and accuracy (recovery percentage > 90.0%).[17]

Statistical analysis

All the experiments were done in triplicate. Results are expressed as mean ± SD. Statistical analysis of the effect of formulation type and drug loading on water uptake, effect of chitosan concentration and pH on complex weight and effect of formulation type and pH on water uptake were tested using two-way analysis of variance (ANOVA). The effect of formulation type on drug release data was tested using one-way ANOVA. Individual differences between means were tested using Tukey's test. The criterion for statistical significance was $P < 0.05$.

Results

Chitosan/hyaluronate polyelectrolyte complex yield

Figure 2 shows the effect of chitosan/hyaluronate molar ratio on complex formation at various pHs. For both chitosan types (low and high viscosity), the molar ratio for maximum insoluble complex formation was 1 : 1–3 : 7 at pH 5.0 and pH 4.0, 3 : 7 at pH 3.5 and pH 3.0, and 1 : 9 at pH 2.0.

In order to investigate the effect of pH preparative conditions and chitosan molecular weight on the delivery of vancomycin and insulin from the insert in the nasal cavity, low-viscosity chitosan/hyaluronate complexes prepared at 3 : 7 molar ratio in pH 2.0, 3.5 and 5.0 (l-CH/$HY_{pH2.0}$, l-CH/$HY_{pH3.5}$, l-CH/$HY_{pH5.0}$) and high-viscosity chitosan/hyaluronate complexes prepared at 3 : 7 molar ratio in pH 2.0 and 3.5 (h-CH/$HY_{pH2.0}$, h-CH/$HY_{pH3.5}$) were selected for the following studies.

Thermogravimetric analysis

Figure 3 shows the thermograms of low-viscosity chitosan, sodium hyaluronate, l-CH/$HY_{pH2.0}$, l-CH/$HY_{pH3.5}$ and l-CH/$HY_{pH5.0}$. Low-viscosity chitosan and sodium hyaluronate degraded at about 300°C and 250°C (inflection point temperatures), respectively. The thermal degradation of polyelectrolyte complex occurred at lower temperatures than the polysaccharides alone. In particular, l-CH/$HY_{pH2.0}$ degraded at 200°C, l-CH/$HY_{pH5.0}$ at 220°C and l-CH/$HY_{pH3.5}$ at 225°C. High-viscosity chitosan degraded at 310°C, h-CH/$HY_{pH2.0}$ at 225°C and h-CH/$HY_{pH3.5}$ at 215°C (thermograms not shown).

FT-IR spectroscopy

Figure 4 shows the FT-IR spectra of sodium hyaluronate, low-viscosity chitosan and chitosan/hyaluronate complexes formed at different pHs. Sodium hyaluronate showed the typical $\nu_{C=O}$ band of carboxylate at 1607 cm^{-1}. Chitosan showed the characteristic $\nu_{C=O}$ band of amide at 1648 cm^{-1} and δ_{N-H} band of amine at 1582 cm^{-1}. All the complexes showed the δ_{N-H} band characteristic of protonate amine at 1547 cm^{-1}.

Water uptake

Figure 5 shows the results of water uptake studies of unloaded inserts under different pH conditions. CH/$HY_{pH2.0}$ showed

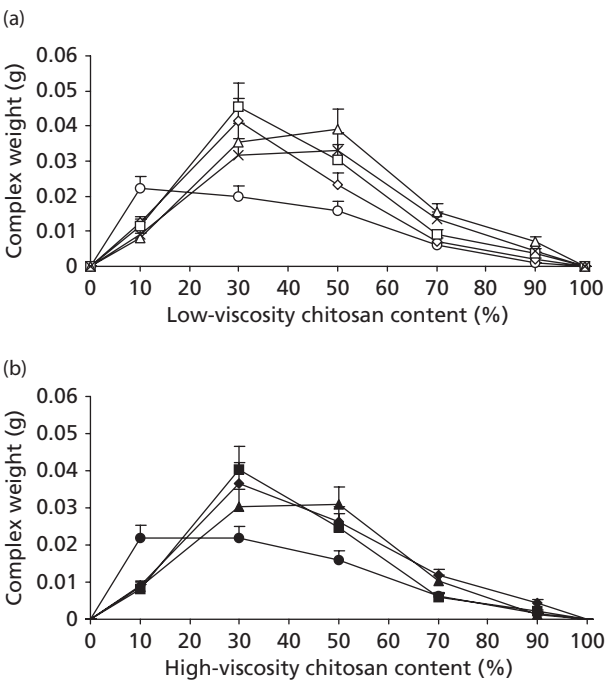

Figure 2 Solid complex weights as a function of chitosan/hyaluronate molar ratio and pH conditions. (a) where pH is 2.0 (-○-), 3.0 (-◇-), 3.5 (-□-), 4.0 (-△-) and 5.0 (∗); (b) where pH is 2.0 (-●-), 3.0 (-◆-). Values are mean ± SD (n = 3 experiments).

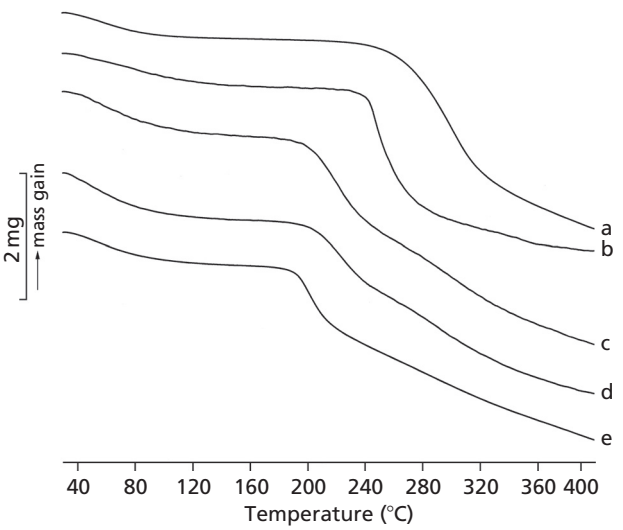

Figure 3 Thermogravimetric analysis of (a) low-viscosity chitosan, (b) sodium hyaluronate, and low-viscosity chitosan–hyaluronate complexes at (c) pH 5.0 (l-CH/$HY_{pH5.0}$), (d) pH 3.5 (l-CH/$HY_{pH3.5}$) and (e) pH 2.0 (l-CH/$HY_{pH2.0}$). l-CH, low viscosity chitosan; HY, hyaluronate.

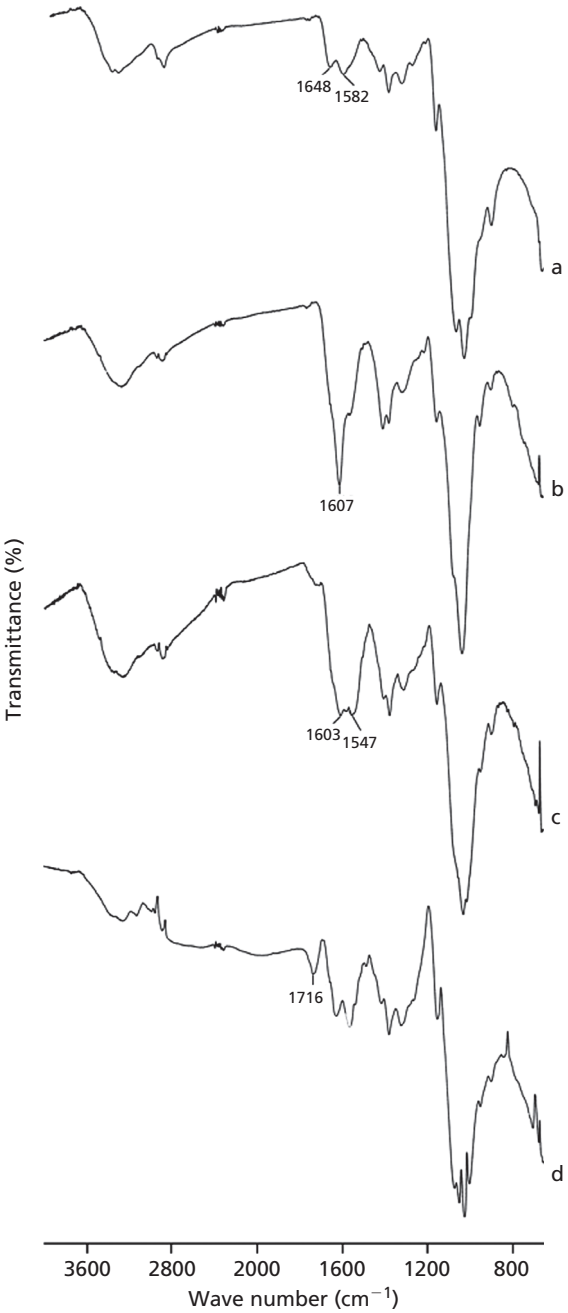

Figure 4 FT-IR spectra of (a) chitosan, (b) sodium hyaluronate, and low-viscosity chitosan–hyaluronate complexes at (c) pH 3.5 (l-CH/$HY_{pH3.5}$) and (d) pH 2.0 (l-CH/$HY_{pH2.0}$). l-CH, low viscosity chitosan; HY, hyaluronate.

greater swelling ability than CH/$HY_{pH3.5}$ and CH/$HY_{pH5.0}$ at all pHs studied. CH/$HY_{pH5.0}$ showed higher water uptake than CH/$HY_{pH2.0}$ only at pH 7.4. Moreover, the swelling ability of all the complexes was lower at pH 5.5 than pH 2.0 or 7.4. The influence of insulin and vancomycin on water uptake was also investigated at pH 7.4 (Table 2). The presence of insulin in nasal inserts enhanced water uptake, whereas the presence of vancomycin reduced water uptake.

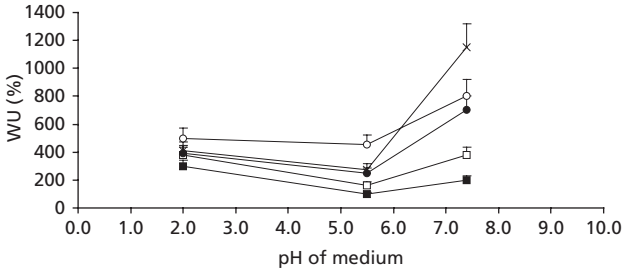

Figure 5 Effect of pH on water uptake (%WU) after 6 h of nasal inserts. Values are mean ± SD (n = 3 experiments). Where (-○-) 1-CH/$HY_{pH2.0}$, (-×-) 1-CH/$HY_{pH5.0}$, (-■-) h-CH/$HY_{pH3.5}$, (-□-) 1-CH/$HY_{pH3.5}$ and (-●-) h-CH/$HY_{pH2.0}$. h-CH, high-viscosity chitosan; l-CH, low-viscosity chitosan; HY, hyaluronate.

Table 2 Influence of insulin and vancomycin on insert water uptake (%WU after 6 h) at pH 7.4

	Unloaded inserts	Vancomycin-loaded inserts	Insulin-loaded inserts
l-CH/$HY_{pH2.0}$	800 ± 18	760 ± 17	830 ± 16
l-CH/$HY_{pH3.5}$	380 ± 15	366 ± 10	459 ± 15
l-CH/$HY_{pH5.0}$	1149 ± 28	1113 ± 18	1257 ± 24
h-CH/$HY_{pH2.0}$	700 ± 22	659 ± 18	733 ± 12
h-CH/$HY_{pH3.5}$	200 ± 16	191 ± 11	277 ± 15

h-CH, high-viscosity chitosan; l-CH, low-viscosity chitosan; HY, hyaluronate.

Mucoadhesion properties of inserts

The residence time of inserts on the agar/mucin plate can be used to evaluate mucoadhesion ability: the longer the residence time, the higher the mucoadhesion potential. The results were as follows: l-CH/$HY_{pH2.0}$, 105 ± 8 min; l-CH/$HY_{pH3.5}$, 10 ± 1 min; l-CH/$HY_{pH5.0}$, 50 ± 5 min; h-CH/$HY_{pH2.0}$, 53 ± 5 min; h-CH/$HY_{pH3.5}$, 3 ± 1 min. l-CH/$HY_{pH2.0}$ showed the highest mucoadhesion tendency, in line with its higher swelling ability.

In-vitro release studies

Release profiles from loaded (insulin and vancomycin) inserts at pH 7.4 are shown in Figure 6. As expected, standard formulations provided fast and complete drug release because of immediate solubilisation of nasal inserts. A good correlation between drug release profiles and water uptake data was observed for all the complexes analysed.

Discussion

Chitosan/hyaluronate complexes can be obtained in a pH range of 2.0–5.0 and at different molar ratios. Since the pKa value of chitosan was 6.3, in the 2.0–5.0 pH range the theoretical percentage ionisation of chitosan (-NH_3^+) was about 100%. In contrast, the percentage ionisation of hyaluronic acid (pKa 2.9) decreased with decreasing pH of the media. The theoretical percentage ionisation of hyaluronic acid (-COO^-) at pH 2.0, 3.0, 3.5, 4.0 and 5.0 was 11%, 56%, 80%, 93% and 99%, respectively. This suggests that much larger amounts of hyaluronic acid

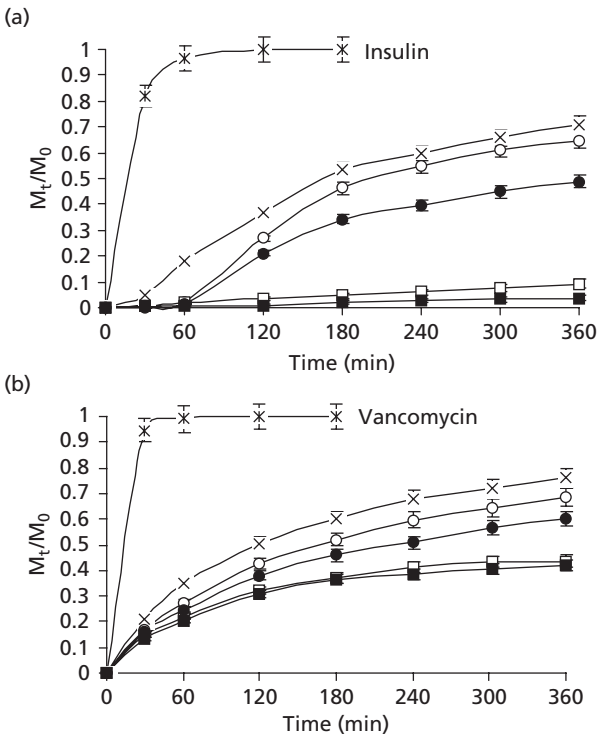

Figure 6 Fractional amount of insulin and vancomycin hydrochloride released from nasal inserts at pH 7.4. Values are mean ± SD (n = 3 experiments). Where (-⁎-) Mannitol, (-×-) 1-CH/$HY_{pH5.0}$, (-○-) 1-CH/$HY_{pH2.0}$, (-□-) 1-CH/$HY_{pH3.5}$, (-●-) h-CH/$HY_{pH2.0}$, (-■-) h-CH/$HY_{pH3.5}$. h-CH, high-viscosity chitosan; l-CH, low-viscosity chitosan; HY, hyaluronate; M_t/M_0 is the fractional amount of drug released over time.

molecules were required to interact with chitosan molecules at low pH values.[18] Moreover, at pH 2.0, the limited ionisation of hyaluronic acid provided low amounts of solid complex at any ratio (Table 1).

The thermal stability of chitosan/hyaluronate complexes was investigated by thermogravimetric analysis. The thermal degradation of chitosan and sodium hyaluronate occurred at higher temperatures than that of the different polyelectrolyte complexes. The difference was more important in the case of l-CH/$HY_{pH2.0}$ (200°C) than l-CH/$HY_{pH5.0}$ (220°C) and l-CH/$HY_{pH3.5}$ (225°C). This destabilisation can be related to the loss of organisation of the complexes in the solid state[12] and was in accordance with the complex yield results (see Figure 2). Similar behaviour was obtained for the complexes based on high-viscosity chitosan.

Complexes were analysed by FT-IR spectroscopy to confirm the chitosan/hyaluronic acid interaction. The shift in amine band to 1547 cm^{-1} in the spectrum of complexes indicated a change in environment of amine group through its interaction with hyaluronic acid.[19,20] Moreover, an increased intensity of undissociated carboxyl group band ($\nu_{C=O}$ at 1716 cm^{-1}) can be observed in the spectrum of chitosan/hyaluronate complex prepared at pH 2.0.

Chitosan/hyaluronate complexes showed ionic interactions between positively charged chitosan and negatively charged hyaluronic acid. The swelling ability of the different complexes can be modulated by selection of appropriate preparative pH condition. The low degree of interaction between chitosan and hyaluronic acid and the great amount of free positive charges (Table 1) in CH/$HY_{pH2.0}$ enhanced complex swelling at all pH values considered (2.0, 5.5 and 7.4). Despite the presence of free negative charges, CH/$HY_{pH3.5}$ and CH/$HY_{pH5.0}$ provided lower water uptake because of the high degree of interaction between the two polyions. Only for CH/$HY_{pH5.0}$ did the large excess of free negative charges increase water uptake at pH 7.4 compared with CH/$HY_{pH2.0}$.

The swelling ability of all the complexes analysed was lower at pH 5.5 than pH 2.0 or 7.4. When the complexes were hydrated in pH 5.5 (i.e. in the pKa interval of the two polysaccharides), the interactions between negative and positive charges were not modified. Table 2 shows that the presence of insulin or vancomycin in nasal inserts enhanced and reduced water uptake, respectively. Vancomycin is a glycopeptide with low molecular weight and an isoelectric point (pI) of 7.2; the presence of protonated amino groups in the pH 2.0–5.0 range suggested the possibility of ionic interactions with free negative charges in the complex network (particularly for CH/$HY_{pH3.5}$ and CH/$HY_{pH5.0}$) during the loading procedure and the formation of less rehydratable inserts.[15] On the other hand, insulin is a protein with higher molecular weight than vancomycin and a pI of 5.4. Despite the presence of free positive charges on the insulin surface (pH 2.0–5.0), suggesting the possibility of ionic interactions with polyelectrolyte complexes,[21] during the loading procedure the steric hindrance of the protein induced a loss of organisation in the polymeric chain and the formation of more rehydratable inserts. At pH 7.4 a different distribution of free charges in the polymer network and drug molecules can occur. In fact, an excess of free negative charges can appear in all polyelectrolyte complexes, which can be further increased by the presence of insulin (pI 5.4) but not by the presence of vancomycin (pI 7.2). The large excess of negative charges contributed to the increased water uptake of insulin-loaded inserts. Finally, the same behaviour can be observed for the complexes based on low-viscosity chitosan and high-viscosity chitosan.

After administration into the nasal cavity and contact with the moist surface, hydration of the lyophilisate insert produces gelling networks that are able to interact with mucus as a result of physical entanglement and secondary bonding, mainly H-bonding and van der Waals attractions. In fact, a polymer's swelling ability, increasing the mobility of molecules, facilitates interpenetration and interaction with the mucous layer.[22–24] l-CH/$HY_{pH3.5}$ showed the lowest mucoadhesive ability due to its poor hydration in aqueous media. On the other hand, l-CH/$HY_{pH2.0}$ and l-CH/$HY_{pH5.0}$ adhered tightly to mucin as a consequence of their greater swelling. However, l-CH/$HY_{pH5.0}$ showed lower mucoadhesion than l-CH/$HY_{pH2.0}$ as a result of electrostatic repulsion[25] between the negatively charged mucin (pKa of sialic acid 2.6)[26] and negatively charged complex at pH 7.4 (see Table 1). The same behaviour can be observed for high-viscosity chitosan complexes, in accordance with water uptake data.

As reported in the section on water uptake, the presence of insulin in nasal inserts enhanced water uptake whereas vancomycin reduced water uptake. This behaviour did not significantly influence drug release, however. In fact, from the release profiles, the key factor affecting insulin and vancomycin availability seemed to be the molecular weight of the

drug.[27] Although vancomycin induced the formation of less rehydratable inserts whereas insulin induced the formation of more rehydratable inserts, their release seemed to be driven by drug diffusibility in the gelled formulation. This behaviour was particularly evident for the inserts based on the lowest hydratable complexes (l-CH/$HY_{pH3.5}$ and h-CH/$HY_{pH3.5}$) where the limited water uptake enhanced differences in drug diffusibility. As regards the more hydratable inserts (based on l-CH/$HY_{pH2.0}$, l-CH/$HY_{pH5.0}$ and h-CH/$HY_{pH2.0}$), a balance between the effect of drug on complex swelling ability and drug diffusibility made only minimal differences to the cumulative amount of insulin or vancomycin released. Moreover, the ionisation of vancomycin and insulin molecules, according to their isoelectric points, did not affect the release behaviour. Although at pH 7.4 repulsive forces between negative charges of the polymeric network and insulin molecules (pI 5.4) can exist, insulin availability was not enhanced compared with vancomycin, which is near neutrality (pI 7.2) at this pH value. This further confirmed that the different release behaviour obtained with insulin- and vancomycin-loaded inserts relates to the different molecular weights of the drugs.

Conclusions

Our results indicate that chitosan/hyaluronate polyelectrolyte complexes can be used for the formulation of mucoadhesive nasal inserts with different drug release properties. Selection of suitable conditions for preparation of the complexes allowed modulation of insert swelling behaviour and insulin or vancomycin release at the administration site. This work has contributed to the understanding of chitosan/hyaluronate polyelectrolyte complex formation and complexation with peptide and protein drugs and will be furthered by performing intranasal absorption studies in animal models.

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Conflict of interest

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

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