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Research paper

Freeze-dried chitosan/pectin nasal inserts for antipsychotic drug delivery

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ABSTRACT

The objective of this investigation was the development of chitosan/pectin based nasal inserts to improve bioavailability of antipsychotic drugs in the treatment of psychotic symptoms. In fact, the nasal route of administration ensures systemic availability avoiding the first-pass metabolism and obtaining more efficacious treatments. Chitosan/pectin polyelectrolyte complexes were prepared at pH 5.0 with different polycation/polyanion molar ratios and lyophilized in small inserts in the presence of chlorpromazine hydrochloride. The results show that higher amount of pectin in the complexes, with respect to higher amount of chitosan, produced a more evident porous structure of the nasal inserts, improving water uptake ability and mucoadhesion capacity. Finally, the presence of increasing amounts of pectin allowed the interaction with chlorpromazine hydrochloride inducing the formation of less hydratable inserts thus limiting drug release and permeation. This investigation verifies the formation of polyelectrolyte complexes between chitosan and pectin at pH values in the vicinity of the p*Ka* interval of the two polymers and confirms the potential of these complexes, capable of achieving antipsychotic drug delivery in the nasal cavity.

1. Introduction

Novel formulations and new routes of administration for psychotropic drugs can offer advantages over older formulations in terms of efficacy, tolerability and compliance. Short-acting and long-acting preparations are useful alternatives to the traditional formulations, which can provide more acceptable forms of medication for patients affected by schizophrenia or other psychotic disorders [1]. Actually, the antipsychotic drug chlorpromazine is available on the market in injectable, oral and rectal dosage forms. The intramuscular route is used primarily when rapid action is required to control acute severe symptomatology, while oral administration of sustained release capsules is used for the long-term treatment of psychiatric illness. In addition, conventional oral tablets, suppositories and syrups are available for the administration of the antipsychotic drug chlorpromazine. Recently, nasal delivery systems have been investigated with the aim of altering the pharmacokinetics of orally and parenterally administered drugs in a fashion that can enhance their pharmacologic profiles [2]. In fact, the large surface area, porous endothelial basement membrane and high total blood flow of the nasal mucosa ensure systemic availability of compounds under circumvention of the hepatic first-pass metabolism. Nasal administration of chlorpromazine hydrochloride can be useful for either acute treatment setting or long-term treatment of psychiatric illness. In the first case, pharmaceutical formulations able to provide immediate drug permeation across nasal mucosa and a rapid onset of action can be good candidate particularly for patients who may have difficulty swallowing tablets or for patients who refuse intramuscular therapy. In the second case, pharmaceutical formulations able to provide an extended drug permeation across nasal mucosa can be useful to reduce the number of required daily doses, thus improving compliance, and to eliminate peak-to-valley fluctuations, thus reducing the risk of adverse effects.

However, the mucociliary clearance mechanism that rapidly removes applied dosage forms from the absorption site can be a problem in nasal drug delivery. Generally, conventional nasal formulations such as liquid drops or sprays are rapidly cleared from the nose, and residence times in man of 12–15 min have been described [3]. Although the residence time of a liquid vehicle can be increased by increasing its viscosity [4], viscous solutions are difficult to administer as drops or sprays. Powder formulations have been shown to have longer nasal residence times [5] than solutions but require sophisticated delivery devices for deposition and accurate dosing. The purpose of this study was the preparation and characterization of lyophilized nasal inserts [6] able to deliver a unique dose of drug in the nasal cavity and achieve a controlled release of the active principle according to hydration/diffusion mechanisms. As mucoadhesive polymers can be used to prevent the rapid clearance of the drug formulation, chitosan/pectin polyelectrolyte complexes [7] were used in this study for the preparation of nasal inserts able to increase the residence time and control

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drug release due to the formation of a gelled system in which the drug can diffuse. 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 α(1–4) glycosidic bonds. Chitosan is a natural derivative of chitin consisting of glucosamine and N-acetylglucosamine. These polymers show interesting biological properties, including biocompatibility, biodegradability and mucoadhesivity. A suspension of chitosan/pectin complexes, with or without chlorpromazine hydrochloride, was lyophilized into small inserts. Morphological characteristics, water uptake, mucoadhesion, release and permeation studies were performed in order to investigate insert ability to deliver antipsychotic agents in the nasal cavity.

2. Materials and methods

2.1. Materials

Pectin from citrus peel (*M*r. 30,000–100,000; esterification degree 60%; p*K*a, 4.0), chitosan (*M*r. 150,000; deacetylation degree 97%; p*K*a, 6.3) and chlorpromazine hydrochloride used for this study were obtained commercially from Fluka (Milan, Italy). All other chemicals and solvents were of analytical grade and purchased from Carlo Erba (Milan, Italy). Water uptake, mucoadhesion, release and permeation studies were carried out in aqueous buffers with the following compositions (mM): 65.0 NaOH, 30.6 $C_6H_8O_7{\cdot}H_2O$, 68.8 HCl 37% for buffer solution pH 2.0; 4.2 $Na_2HPO_4{\cdot}10H_2O$, 100.0 KH_2PO_4, 45.5 NaCl for buffer solution pH 5.5; 8.4 $Na_2HPO_4{\cdot}10H_2O$, 7.4 KH_2PO_4, 94.0 NaCl for buffer solution pH 6.8; 6.7 $Na_2HPO_4{\cdot}10H_2O$, 1.4 KH_2PO_4, 136.9 NaCl for buffer solution pH 7.4.

2.2. Preparation of chitosan/pectin complex nasal inserts

Chitosan/pectin complexes were prepared as reported in a previous work [7]. Briefly, chitosan (0.50 mmoles of monomer) and pectin (0.50 mmol of monomer) were dissolved separately in 100 ml of acetate buffer pH 5.0 at the ionic strength of 50 mM. The chitosan solution was then added to pectin solution in various molar ratios (1:9, 3:7, 1:1, 7:3, 9:1; mol chitosan/mol pectin) and stirred at room temperature for 24 h. The precipitates were separated by ultracentrifugation at 10,000 rpm for 10 min (ALC 4239R centrifuge; Milan Italy), washed with deionised water, homogenized at 17,500 rev min^{-1} for 5 min (Ultra-Turrax, T 25 basic homogenizer; IKA, Dresden, Germany), suspended again in deionised water and finally freeze-dried (Christ Freeze Dryer ALPHA 1–2, Milan, Italy), obtaining five different chitosan/pectin complexes: $CH/PEC_{(1:9)}$, $CH/PEC_{(3:7)}$, $CH/PEC_{(1:1)}$, $CH/PEC_{(7:3)}$ and $CH/PEC_{(9:1)}$. As described in Bigucci et al., 2008, complex weight measurements, FT-IR spectra and TGA thermograms confirmed the formation of ionic bonds between chitosan and pectin. Loaded inserts (average diameter 5 mm, height 8 mm) were prepared by adding 100 μl of chlorpromazine hydrochloride (25 mg/ml, 50 mg/ml, 100 mg/ml or 200 mg/ml) aqueous solution (pH 5.5 phosphate buffer) to 10 mg of different complex/mannitol mixtures (9:1; w/w) obtaining four different complex/drug weight ratios (2:0.5, 2:1, 2:2 and 2:4). Mannitol was added, as a bulking agent in order to improve mechanical strength of lyophilized nasal inserts when handled [8]. The resultant suspensions were filled into polypropylene microcentrifuge tubes, allowed to settle to swell and remove air and finally lyophilized, obtaining cone-like shaped solid inserts. The inserts were stored in a desiccator until use. Unloaded inserts (10 mg) were prepared by the same procedure without the presence of chlorpromazine hydrochloride. Moreover, loaded inserts (10 mg) were produced only with mannitol and chlorpromazine hydrochloride as control formulations for release and permeation studies. Finally, in order to perform *in-vitro* water uptake studies, loaded and unloaded inserts (average diameter 7 mm, height 20 mm) were prepared starting from 100 mg of the different complex/mannitol mixtures (9:1; w/w).

2.3. Scanning electron microscopy (SEM) and porosity measurements

The morphology of nasal inserts was performed by SEM analysis. Inserts (10 mg) were cut with a razor blade to expose the inner structure, 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., England) using secondary electron imaging at 15 kV in order to examine the surface morphology and structure of the inserts.

Variation in porosity due to chitosan/pectin complex composition was determined using lyophilized samples, by means of a mercury porosimeter (Autopore IV 9500, Micromeritics Instrument).

2.4. Water uptake ability

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:

$$\%\ \text{Water Uptake}\ (\%\text{WU}) = (W_{Hip} - W_{Hp} - W_{Di}) \times 100/W_{Di}$$

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 influence of chlorpromazine hydrochloride on the water uptake behaviour of loaded inserts ($CH/PEC_{(1:9)}$ with different complex/drug weight ratios: 2:0.5, 2:1, 2:2 and 2:4) was also studied at pH 5.5, particular to human nasal secretions [9,10].

2.5. Insert mucoadhesion properties

The *in-vitro* mucoadhesion was measured in terms of the force needed to pull out a freshly excised sheep nasal mucosa (surface area 1 mm^2) from a tablet (100 mg) with an adapted tensiometer (Krüss 132869; Hamburg, Germany) as reported in a previous work [7]. For this study, tablets (weight of 100 mg) were prepared by direct compression of freeze-dried chitosan/pectin complex (compaction force: 18 kN) with a single-punch press (type Korsch, Korsch Maschinenfabrik No. 1.0038.86, Berlin, Germany). The nasal mucosa was fixed to a support with cyanoacrylate adhesive and then suspended from the tensiometer spring. The mucosa was lowered until it just contacted the surface of the tablet, previously immersed in phosphate buffers at pH 5.5 for 15 min. A 100-dyne force, measured by the torsion balance of the instrument as a negative force, was applied to the tablet for 30 s. Then, the nasal mucosa was raised until it was separated from the tablet. This point represents the adhesive bond strength between these elements and is expressed as a positive force in dyne.

2.6. In-vitro release studies

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 closed with Parafilm® film to avoid evaporation of release medium. The crucible was placed vertically into a release medium container

(filled with 10 ml of pH 5.5 phosphate buffer) and adjusted exactly to the height of the release medium surface so that the porous glass membrane was wetted but not submersed. 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. Chlorpromazine hydrochloride availability was determined as follows. The chromatographic system was composed of a Shimadzu (Milan, Italy) LC-10ATVP chromatographic pump and a Shimadzu SPD-10AVP UV–Vis detector set at 254 nm. Separation was obtained on a Phenomenex (Torrance, CA, USA) Sinergy Fusion-RP 80A (150 × 4.6 mm I.D., 5 μm) coupled to a Phenomenex (Torrance, CA, USA) SecurityGuard C18 guard cartridge (4 × 3.0 mm I.D., 5 μm). The mobile phase was composed of a mixture of acetonitrile – pH 3.0 solution of triethylamine (0.5%) 30:70 (v/v). The flow rate was 0.4 mL/min, and manual injections were made using a Rheodyne 7125 injector with a 20-μL sample loop. Data processing was handled by means of a CromatoPlus computerized integration system (Shimadzu Italia, Milan, Italy). A calibration curve was set up in the 10–500 μg ml^{-1} range; good linearity was found (r^2 = 0.9995). Repeatability assays were carried out on chlopromazine standard solutions, at concentrations corresponding to the lower and upper limit and the middle point of the calibration curve. Method precision was satisfactory: RSD% values of 3.2, 3.0 and 1.3 were obtained for chlopromazine concentrations of 10, 250 and 500 μg ml^{-1}, respectively.

2.7. In-vitro permeation studies across sheep nasal mucosa

Sheep nasal mucosa was obtained from local slaughterhouses. The turbinates were fully exposed by a longitudinal incision through the nose. The mucosa was carefully removed from the underlying bone by cutting with haemostatic forceps and pulling the mucosa off. To maintain the freshness of the specimen as far as possible, permeation studies were started immediately after the mucosa samples were excised. The permeation study was conducted in a Franz-type permeation cell with a diffusional area of 1.5 cm^2. At time zero, loaded inserts (10 mg) were placed in the donor compartment with their lateral surface in contact with the mucosa. The receiver phase (6.0 mL of a phosphate buffer solution, pH 5.5, maintained at 37 °C by means of a surrounding jacket) was stirred constantly, and at predetermined time intervals, samples of 100 μl were taken and replaced by fresh medium. The amount of chlorpromazine hydrochloride in the receiving phase was analysed by HPLC. The studies were carried on for 6 h. Flux data were plotted as the cumulative amount of drug that diffused from the mucosal to the serosal side of epithelium versus time. The permeability coefficient (P) was calculated using the following equation: $P = (dM/dt)/(M_0A)$, where dM/dt represents the permeability rate and M_0 stands for the initial concentration in the donor chamber, while A is the effective surface area of the mucosa.

2.8. Statistical analysis

ANOVA was used to determine statistical significance. Differences were considered to be significant for values of $P < 0.05$.

3. Results and discussion

3.1. Scanning electron microscopy (SEM) and porosity measurements

Figs. 1A and 1B show the morphology of the nasal inserts observed by scanning electron microscopy (SEM). The structure of the nasal inserts depends on the composition of chitosan/pectin complexes. For polyelectrolyte complexes, the interaction of polycation with polyanion leads to physically crosslinked hydrogels [11] that can retain great amount of water at the interior. As nasal inserts were obtained by freeze-drying, which consists of sublimation of the frozen water yielding to the formation of pores or channels in the polymer, all the samples were characterized by a sponge-like structure. In particular, the porous structure is more evident in CH/$PEC_{(1:9)}$, CH/$PEC_{(3:7)}$ and CH/$PEC_{(1:1)}$, than in CH/$PEC_{(7:3)}$ and CH/$PEC_{(9:1)}$ (Fig. 1A). In fact, the presence of higher amount of pectin in the complexes provided the formation of a three-dimensional interconnected network structure with larger and more homogeneous porosity with respect to complexes containing higher amounts of chitosan. CH/$PEC_{(1:9)}$, CH/$PEC_{(3:7)}$ and CH/$PEC_{(1:1)}$ seemed to have very suitable morphology as hydrogels for nasal administration due to their more evident sponge-like structure, which can provide great insert hydration and modulation of drug release in the nasal cavity. Finally, SEM study showed that the presence of chlorpromazine hydrochloride in the nasal inserts based on CH/$PEC_{(1:9)}$ produced a rough surface rather than smooth as unloaded samples; moreover, for CH/$PEC_{(1:9)complex/drug2:4(w/w)}$, the porous structure tends to disappear (Fig. 1B). This

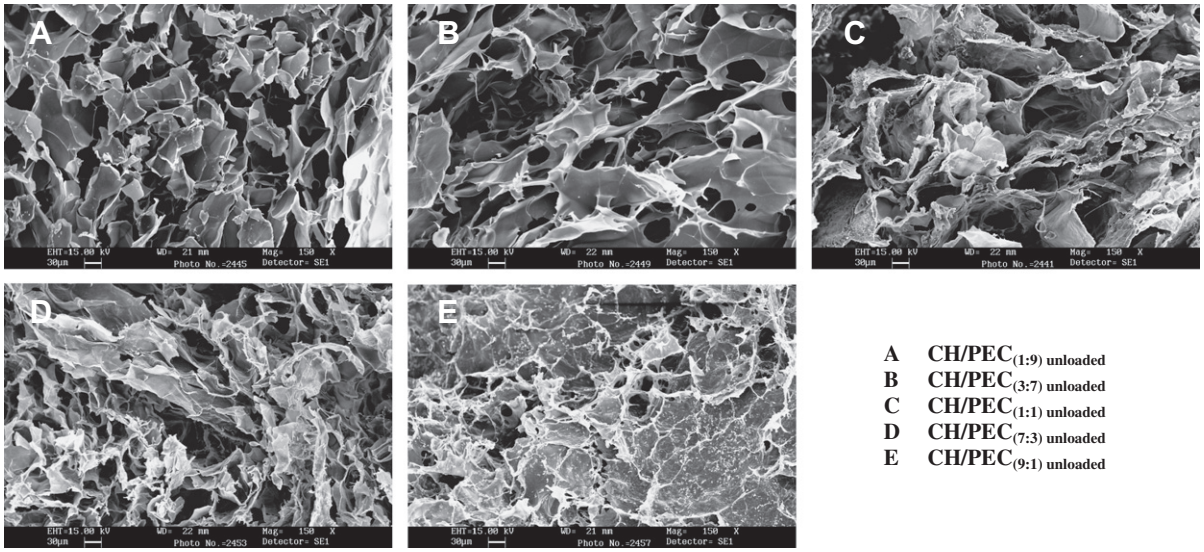

Fig. 1A. Scanning electron micrographs of the different chitosan/pectin complexes.

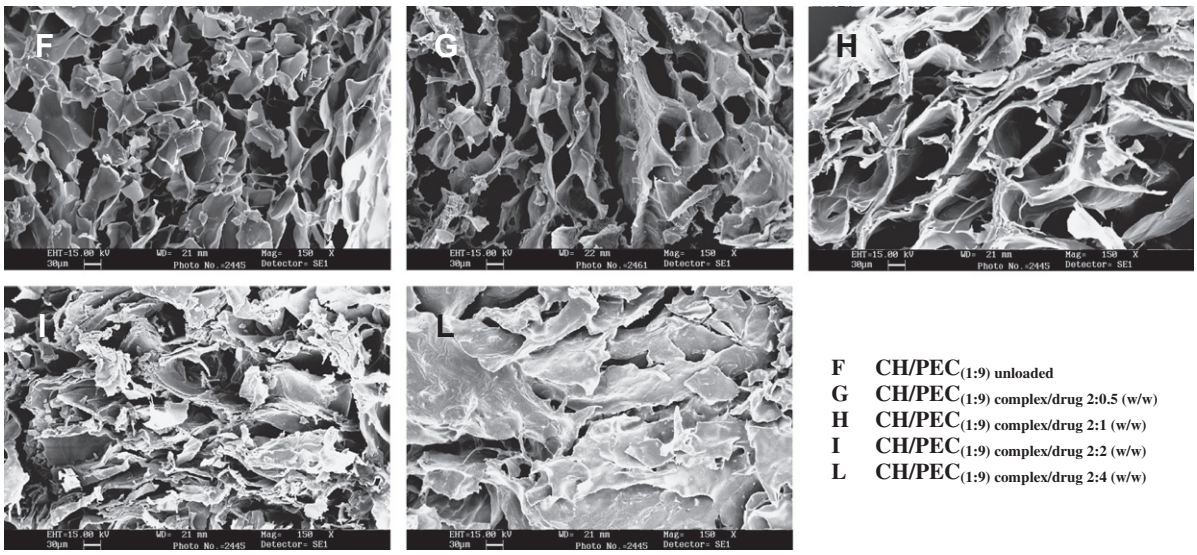

Fig. 1B. Scanning electron micrographs of the differently loaded CH/PEC$_{(1:9)}$.

behaviour can be correlated with possible complex/drug interactions during insert preparation procedure.

The porosity (%) of the different inserts determined by mercury intrusion analysis ranged from 72% to 93% (Table 1). In particular, the values obtained for unloaded CH/PEC$_{(1:9)}$, CH/PEC$_{(3:7)}$ and CH/PEC$_{(1:1)}$ and loaded CH/PEC$_{(1:9)\text{complex/drug2:0.5(w/w)}}$ and CH/PEC$_{(1:9)\text{complex/drug2:1(w/w)}}$ are consistent with highly porous networks (porosity > 90%). The values obtained for the other samples are reasonable for a less porous structure, being of the order of 75%.

3.2. Water uptake ability

Over the study period (6 h), all the nasal inserts hydrated without dissolution. Fig. 2 shows the results of water uptake studies of unloaded inserts under different pH conditions. Water uptake ability of chitosan/pectin complexes was strongly influenced by pH of the medium and by polycation/polyanion molar ratio. As can be seen, higher amount of pectin in the complexes provided greater water uptake. Moreover, water uptake ability was lower ($P < 0.05$) at pH 5.5 than at pH 7.4 for all the complexes analysed and lower ($P < 0.05$) than pH 2.0 for CH/PEC$_{(3:7)}$, CH/PEC$_{(1:1)}$, CH/PEC$_{(7:3)}$ and CH/PEC$_{(9:1)}$. In fact, when the complexes hydrated in the p*K*a interval of the two polysaccharides, the interactions between negative and positive charges in the polymeric network underwent only little or no modification, resulting in a lower water uptake. On the contrary, a large excess of free positive or negative charges appears inside the polymeric network at pH 2.0 and 7.4 allowing great water uptake. Fig. 2 shows that CH/PEC$_{(1:9)}$ provided the highest water uptake ability among all the complexes. As reported in the previous section, the presence of higher amount of pectin in the complexes provided the formation of a network structure with larger porosity with respect to complexes containing higher amounts of chitosan, thus favouring a greater entry of water.

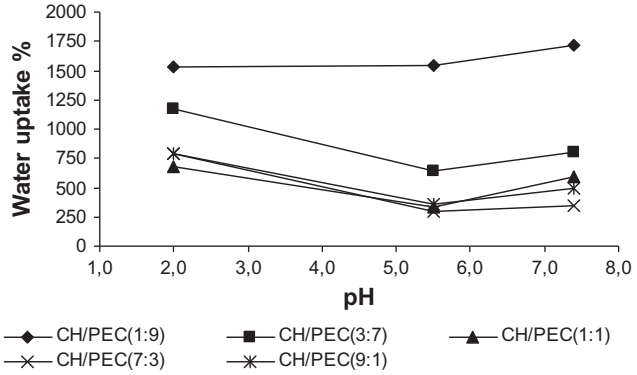

Fig. 2. Water uptake ability of nasal inserts in different pH conditions ($n = 5$, the SD did not exceed the 5%).

The influence of chlorpromazine hydrochloride on the water uptake ability of the insert based on CH/PEC$_{(1:9)}$ was also investigated at pH 5.5 (Fig. 3). The presence of chlorpromazine hydrochloride in the nasal insert gradually reduced water uptake. This behaviour can be explained due to the presence of the amino group (p*K*a 9.2) of chlorpromazine [12] able to interact with free negative charges (pectin carboxylate groups) in the complex network during the loading procedure, thus leading to the formation of less porous and hydratable inserts [13].

Table 1
Porosity and pore diameter of the different inserts determined by mercury intrusion analysis (mean ± SD, $n = 3$).

	Porosity (%)	Pore diameter (μm)
CH/PEC$_{(1:9)\text{unloaded}}$	93 ± 4	40 ± 6
CH/PEC$_{(3:7)\text{unloaded}}$	92 ± 5	50 ± 8
CH/PEC$_{(1:1)\text{unloaded}}$	93 ± 5	65 ± 10
CH/PEC$_{(7:3)\text{unloaded}}$	74 ± 3	35 ± 5
CH/PEC$_{(9:1)\text{unloaded}}$	72 ± 3	35 ± 6
CH/PEC$_{(1:9)\text{complex/drug2:0.5(w/w)}}$	92 ± 3	45 ± 7
CH/PEC$_{(1:9)\text{complex/drug2:1(w/w)}}$	93 ± 4	50 ± 8
CH/PEC$_{(1:9)\text{complex/drug2:2(w/w)}}$	77 ± 5	40 ± 9
CH/PEC$_{(1:9)\text{complex/drug2:4(w/w)}}$	69 ± 5	40 ± 8

3.3. Insert mucoadhesion properties

After administration into the nasal cavity and contact with the moist surface, freeze-dried insert hydration produces gelling networks able to interact with mucus as a result of physical entanglement and secondary bonding (i.e. H-bonding and Van der Waals attraction). In fact, polymers' water uptake ability, increasing the mobility of molecules, facilitates interpenetration and interaction with the mucus layer [14–16]. Fig. 4 shows the detachment force needed to pull out a sheep nasal mucosa from tablets based on

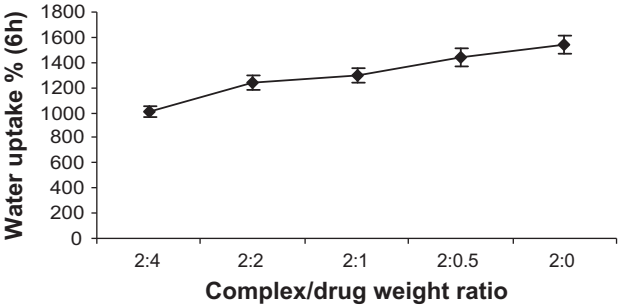

Fig. 3. Water uptake ability after 6 h of differently loaded (complex/drug weight ratios: 2:0.5, 2:1, 2:2 and 2:4) CH/PEC$_{(1:9)}$ at pH 5.5 (n = 5, the SD did not exceed the 5%).

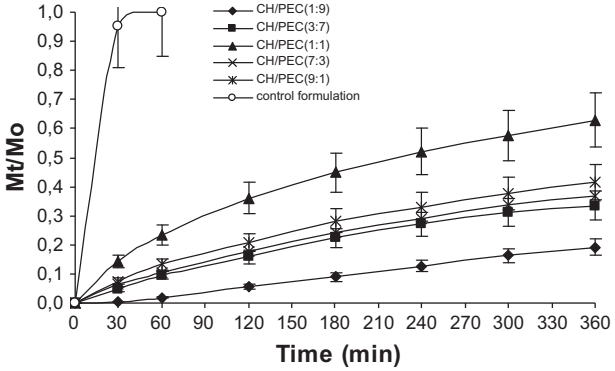

Fig. 5. Fractional amount of chlorpromazine hydrochloride released over time at pH 5.5 from control formulation and the different chitosan/pectin complexes. Each datum represents the average of three determinations ± SD.

chitosan, pectin and different chitosan/pectin complexes. As can be seen, pectin showed greater mucoadhesive capacity with respect to chitosan. At pH 5.5, mucus presents negative charges due to complete ionization of sialic acid (p*K*a 2.6) and sulphate residues in mucin glycoprotein [17]. Despite the presence of negative charges on pectin chains due to the ionization of the carboxyl groups (p*K*a of 4.0), pectin showed good mucoadhesive capacity. On the other hand, despite the presence of positive charges on chitosan chains due to the ionization of the amino groups (p*K*a of 6.3), chitosan showed lower mucoadhesive ability. This behaviour can be explained due to the different water uptake ability of pectin and chitosan, thus providing a more or less efficient chain mobility and physical entanglement with mucus, respectively. For the same reason, polyelectrolyte complexes containing high percentages of pectin (CH/PEC$_{(3:7)}$ and CH/PEC$_{(1:9)}$) showed the best *in-vitro* mucoadhesion ability among all the complexes.

3.4. In-vitro release studies

Release profiles from loaded (complex/drug weight ratio 2:2) inserts at pH 5.5 are shown in Fig. 5. As can be expected, control formulation provided an immediate release of chlorpromazine hydrochloride due to the fast dissolution of the mannitol insert. A decrease in drug release can be observed in the complexes containing high pectin amounts (CH/PEC$_{(1:9)}$ and CH/PEC$_{(3:7)}$) as a consequence of the high polymeric chain mobility (see mucoadhesion section) leading to a great entry of water in the nasal inserts thus forming a viscous network. Moreover, drug interactions with pectin contributed to the slower release observed for the insert based on CH/PEC$_{(1:9)}$. CH/PEC$_{(1:1)}$ showed the higher drug release due to the high degree of interaction between chitosan and pectin in the complex and the absence of free charges limiting water uptake and polymeric chain mobility. Finally, CH/PEC$_{(7:3)}$ and CH/PEC$_{(9:1)}$ provided a lower release of chlorpromazine hydrochloride than CH/PEC$_{(1:1)}$ as a consequence of the very low degree of interaction between the two polymers and the great amount of free positive charges, thus providing the formation of a more viscous network. Despite both CH/PEC$_{(1:9)}$ and CH/PEC$_{(9:1)}$ present an efficient polymeric chain mobility, a lower drug release was obtained with the complexes containing greater amounts of pectin. This behaviour can be correlated with drug diffusibility in differently viscous networks. Absolute viscosity measurements of solutions containing pectin (or chitosan) in presence of mannitol and chlorpromazine were performed, in order to simulate loaded insert environments. The viscosities of a pectin/mannitol/chlorpromazine and chitosan/mannitol/chlorpromazine solution (polymer/mannitol/drug, 3.6:0.4:4.0 mg/ml, in acetate buffer pH 5.0), obtained at 20 °C using an Ubbelohde capillary viscometer equipped with an electronic time-measuring unit ViscoClock (Schott, Mainz, Germany), were 8.58 cP and 3.02 cP respectively, thus confirming the presence of different viscous networks in the loaded inserts. Finally, the presence of increasing amount of chlorpromazine hydrochloride in na-

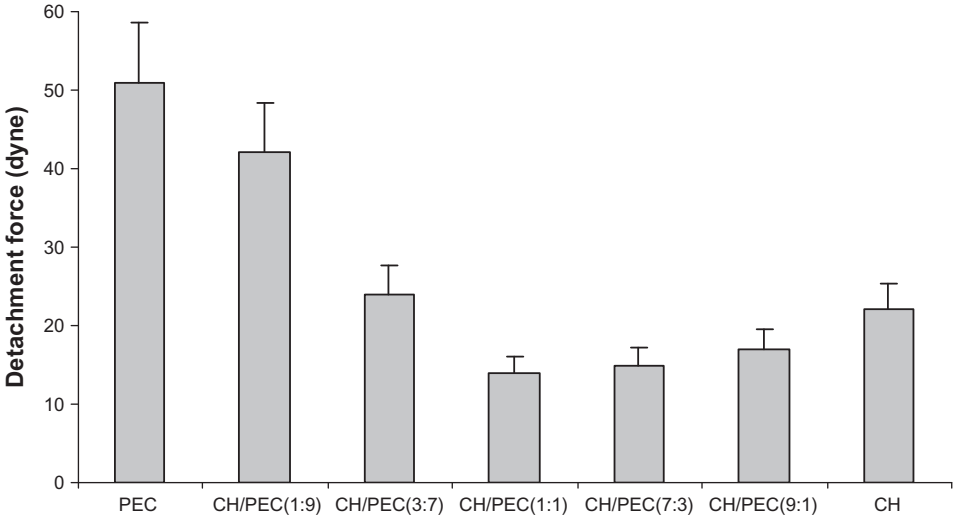

Fig. 4. Mucoadhesive capacity (expressed as detachment force, mean ± SD, n = 3) of chitosan hydrochloride, pectin and chitosan/pectin complexes at pH 5.5.

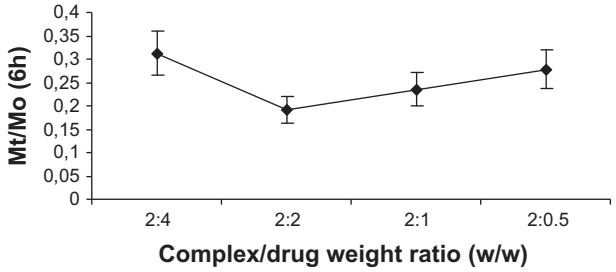

Fig. 6. Fractional amount of chlorpromazine hydrochloride released after 6 h from differently loaded (complex/drug weight ratios: 2:0.5, 2:1, 2:2 and 2:4) CH/$PEC_{(1:9)}$ at pH 5.5. Each datum represents the average of three determinations ± SD.

sal inserts gradually reduced drug release from the inserts based on complex CH/$PEC_{(1:9)}$ due to pectin/drug interactions providing a less porous and hydratable structure. In fact, the same trend in drug release profiles (Fig. 6) and water uptake data (Fig. 3) can be observed for the following complex/drug weight ratios: 2:0.5, 2:1 and 2:2. In the case of 2:4 weight ratio, the excess of chlorpromazine hydrochloride with respect to the complex made the insert unable to control its release anymore.

3.5. In-vitro permeation studies across sheep nasal mucosa

As reported earlier, CH/$PEC_{(1:9)}$, CH/$PEC_{(3:7)}$ and CH/$PEC_{(1:1)}$ seem to have very suitable morphology as hydrogels for nasal administration according to their sponge-like structure, which can provide great insert hydration and modulation of drug release in the nasal cavity. The permeation profiles and permeability coefficients (P) of chlorpromazine hydrochloride across sheep nasal epithelium from control formulation and CH/$PEC_{(1:9)}$, CH/$PEC_{(3:7)}$ and CH/$PEC_{(1:1)}$ are represented in Fig. 7. There were differences ($P \leqslant 0.01$) in permeation coefficients between control and complex formulations. This behaviour can be correlated with drug release profiles from nasal formulations influencing drug availability over time at the absorption site. Moreover, the presence of chlorpromazine hydrochloride in CH/$PEC_{(1:9)}$ did not provide significant differences ($P \geqslant 0.05$) in permeability coefficients (complex/drug 2:0.5, $P = 12 \times 10^{-4}$ (cm/min); complex/drug 2:1, $P = 11 \times 10^{-4}$ (cm/min); complex/drug 2:2, $P = 9 \times 10^{-4}$ (cm/min); complex/drug 2:4, $P = 12 \times 10^{-4}$ (cm/min)). For acute treatments of psychotic illness, usual dosage of chlorpromazine hydrochloride is 10–25 mg given by injection; this therapeutic dosage can be achieved by nasal administration of one nasal insert loaded with 10 or 20 mg of chlorpromazine hydrochloride. The insert prepared with CH/$PEC_{(1:1)}$ provided the highest drug amount permeated at each time with respect to the other inserts and a complete drug permeation in 6 h. However, a lag time of approximately 150 min suggests that this insert cannot be a good candidate for acute treatment setting, even if a direct transport of antipsychotic drugs to the brain via the olfactory region cannot be excluded, as reported from several authors [18]. For the long-term treatment of psychiatric illness, the insert prepared with CH/$PEC_{(1:1)}$ complex can be a good candidate as well as CH/$PEC_{(1:9)}$ and CH/$PEC_{(3:7)}$ complexes, as they can provide prolonged drug permeation up to and over 6 h, respectively. In particular, for chronic treatments, the average daily oral dose of chlorpromazine hydrochloride is of 25–75 mg for the mild cases and 75–150 mg for the more severe cases. Considering an oral bioavailability of approximately 30%, the anticipated transmucosal dose of drug should be 7.5–22.5 mg daily for the mild cases. For this reason, the daily therapeutic dosage can be achieved by nasal administration of one insert based on CH/$PEC_{(1:1)}$ complex loaded with 10 mg of chlorpromazine or by administration of one insert based on CH/$PEC_{(1:9)}$ or CH/$PEC_{(3:7)}$ complexes loaded with 20 mg of drug.

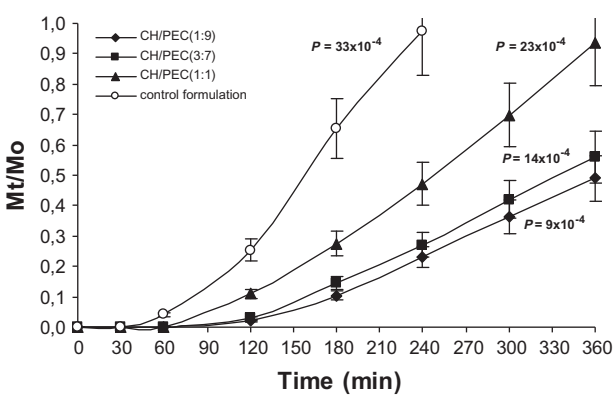

Fig. 7. Permeation profiles (mean ± SD, $n = 3$) and permeability coefficients P (cm/min) of chlorpromazine hydrochloride across the sheep nasal epithelium.

Naturally, control formulation and mucoadhesive formulations can be differently cleared from the nasal cavity by mucociliary mechanism thus providing different insert residence times and different drug bioavailability. McInnes et al. [19] reported the preparation of lyophilized inserts based on hydroxypropylmethylcellulose and found that the selection of adequate preparative conditions allows to extend their residence time in the nasal cavity up to 4–5 h. The different insert mucoadhesion ability of CH/PEC complexes suggests that nasal inserts based on CH/$PEC_{(1:9)}$ and CH/$PEC_{(3:7)}$, which show greater mucoadhesiveness than CH/$PEC_{(1:1)}$, could be more efficient for long-term treatments, even if *in vivo* studies must be performed in order to confirm this hypothesis. For this reason, the present work will be furthered by performing *in vivo* absorption studies in animal models.

4. Conclusions

The results in this study indicated that chitosan/pectin polyelectrolyte complexes can be employed for the formulation of mucoadhesive nasal inserts with different drug release properties. The selection of suitable chitosan/pectin molar ratio during complex preparation allowed the modulation of insert water uptake behaviour and chlorpromazine hydrochloride release and permeation at the administration site. This work has contributed to the understanding of chitosan/pectin polyelectrolyte complex formation and complexation with chlorpromazine hydrochloride and will be furthered by performing intranasal absorption studies in animal models.

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