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Research paper

Spray-dried microspheres based on methylpyrrolidinone chitosan as new carrier for nasal administration of metoclopramide

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Abstract

The work purpose was to study the application of 5-methylpyrrolidinone chitosan (MPC) for preparing mucoadhesive microparticles for the nasal administration of drugs.

Microspheres were produced by the spray-drying technique using MPC; metoclopramide hydrochloride (MC) was chosen as model drug. Chitosan microparticles were prepared as a comparison.

The microparticles obtained were characterised (encapsulation efficiency, morphology, size and drug release behaviour). *In-vitro* mucoadhesive tests, swelling tests and *ex-vivo* studies using sheep nasal mucosa were performed. The hydrogel formation from microspheres was studied in different media and at different pHs.

Microspheres are able to control the *in-vitro* MC release. MPC microparticles show good *in-vitro* mucoadhesive properties and *ex-vivo* controlled permeation profiles. The hydrogel formation is dependent mainly on the medium used: ionically crosslinked hydrogel was hypothesized.

These *in-vitro* and *ex-vivo* preliminary results show that spray-dried microspheres based on MPC could be a suitable nasal delivery system for the administration of metoclopramide.

Keywords: Microspheres; Nasal delivery; Spray-drying; Mucoadhesion; Nasal sheep mucosa

1. Introduction

In recent years, chitosan derivatives have been studied to improve polymer solubility at different pH values and to promote the permeability of anionic drugs thereby avoiding the precipitation of drug–polymer complexes [1–3].

5-Methylpyrrolidinone chitosan (MPC) is a chitosan derivative in which the aminogroups of glucosamine units of the polysaccharide backbone are partially substituted by methylpyrrolidinone (MP) in position 5. It belongs to the class of the gel-forming reabsorbable biopolymeric substituted chitosans possessing documented biological significance. This chitosan derivative combines the biocompatibility of chitosan and the hydrophilic characteristics of the pyrrolidinone moiety, being particularly susceptible to the hydrolytic action of lysozyme [4].

Previous studies demonstrated the efficacy of MPC in the biomedical field: in particular it has been used in the human dental surgery and in accelerating wound and ulcer healings [5,6]. As well as chitosan, MPC shows an antimicrobial activity against a broad spectrum of bacteria [4]; more recently Muzzarelli et al. reported that methylpyrrolidinone chitosan and other modified chitosans exert effective fungistatic action [7]. Moreover, the mucoadhesive

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and penetration enhancement properties in both buccal and vaginal environments of methylpyrrolidinone chitosan has been assessed [8]. Taking into account these considerations, aim of this work was the study of chitosan derivatized with MP as nasal carrier with penetration enhancement properties as well as chitosan [9,10].

Nasal drug delivery has generated interest as an alternative route for the administration of drugs and biomolecules that are susceptible to enzymatic or acidic degradation and first-pass hepatic metabolism. Possible pathways for a drug to permeate across the nasal mucosa are passive transportation, carrier mediated, transcytosis and transport through intercellular tight junctions [11]. However nasal delivery has limitations which have restricted its use to the delivery of a few drug molecules. The permeability of nasal mucosa is normally low for polar molecules: for small polar drugs the bioavailability is generally in the region of 10% and for peptides such as calcitonin and insulin normally not above 1% [9].

Another factor of importance for low membrane transport is the general rapid clearance of the administered formulation from the nasal cavity due to the mucociliary clearance mechanism. It has been shown that for both liquid and powder formulations that are not mucoadhesive, the half life of clearance are in the order of 15–20 min.

Different delivery systems based on mucoadhesive polymers have been developed which are able to increase the residential time of the formulation at the absorption site of the drugs [12–14]. In particular chitosan has been employed in the preparation of micro- and nanoparticles as nasal delivery systems [15,10,16–18]. It is able to efficiently deliver polar drugs (including peptides) to the systemic circulation and therapeutically relevant bioavailabilities. The mechanism of action of chitosan in improving the transport of polar drugs across the epithelial membrane is believed to be a combination of bioadhesion and transient opening of the tight junctions in the cell membrane to enable the passage of polar drugs [19]. The use of mucoadhesive systems as microspheres, provide a drug protection from enzymatic degradation, increase the contact time with the nasal mucosa and permits the control of the drug release from formulation [18] The aim of this work was the possible application of MCP for the preparation of mucoadhesive microparticles for the nasal administration of drugs. A non-derivatized chitosan (CH) has been used as a comparison; metoclopramide hydrochloride has been chosen as model drug.

2. Materials and methods

2.1. Materials

Metoclopramide hydrochloride (MC) was kindly given by AMSA (Milano, Italy); chitosan food grade 90 (CH) was provided by Istituto di Biochimica, Università Politecnica delle Marche (Ancona, Italy); 5-methylpyrrolidinone chitosan (MPC) was synthesized by Prof. Muzzarelli (Istituto di Biochimica, Università Politecnica delle Marche, Ancona, Italy). Silicon oil (Tegiloxan 3®) was kindly given by Goldschmidth (Essen, Germany); mucin, M-2378, Type II, Crude, from porcine stomach, bound sialic acid: 1%, desiccate was provided from Sigma–Aldrich (St.Louis, MO, USA); ultra pure-water was prepared with the MilliQ R4 system Millipore (Milano, Italy); acetonitrile Chromasolv®, sulphuric acid 96% and $HClO_4$ 70% for analysis were obtained from Riedel-de Haen (Milano, Italy); membrane filters (45 mm, 0.45 μm pore size) and nylon and PTFE syringe filters 13 mm, 0.2 and 0.45 μm porosity were supplied by Alltech Italia Srl (Sedriano, Milano, Italy).

All other solvents and chemicals were of analytical grade.

2.2. Synthesis of 5-methylpyrrolidinone chitosan

Crustacean chitosan powder was suspended in water. Levulinic acid was then poured into the reaction vessel at 20 °C; pH ranged 3.7–4.5. Sodium borohydrided solution was delivered over a time period of at least 3 h, to reach pH 5.6. The resulting solution was dialysed [20,21].

2.3. Preparation of microspheres

The microspheres were produced by the spray-drying method. Aqueous solutions containing different concentration of polymer (50 and 66% w/w, Table 1) were prepared dissolving MPC and MC in ultra-pure water, under stirring and a room temperature. The total concentration of solid in solution was 1% w/v.

CH microparticles were prepared as reference dissolving chitosan and drug in 0.5% acetic acid solution, under stirring and a room temperature.

As a comparison, blank microspheres were prepared from a 1% w/v CH or MPC solution, in the conditions described above.

The microspheres were obtained by spraying the solutions through the nozzle (0.7 mm diameter) of a spray dryer (co-current flow type) model Mini Spray Dryer Büchi B-191 (Büchi Labortechnik AG, Flawil, Switzerland). The conditions of the spray-drying process were: inlet air temperature 120 °C; outlet air temperature 60 °C; pump ratio 13%; aspirator ratio 73%; flow control 400/600 l/h and spray rate of feed about 8 ml/min.

Table 1
Composition (% w/w) of spray-dried microspheres

Formulation	MC	MPC	CH
MPC 1	50.0	50.0	–
MPC 2	33.3	66.7	–
MPC	–	100.0	–
CH 1	50.0	–	50.0
CH 2	33.3	–	66.7
CH	–	–	100.0

The solid microparticles were then harvested from the apparatus collector and kept under vacuum for 24 h, at room temperature.

2.4. Microspheres characterisation

Drug content and encapsulation efficiency were evaluated. Ten milligrams of microspheres were dissolved/dispersed in ultra-pure water (250 ml) and stirred for 30 min. Metoclopramide concentration was determined by means of HPLC analysis. Samples were filtrated before analysis by PTFE syringe filters 13 mm (0.45 μm porosity). Encapsulation efficiency was calculated from the ratio between the real drug content and the theoretical amount of drug in microspheres and expressed in per cent. Results are means of triplicate experiments (standard deviation, SD within 0.1).

The particle size of the microparticles was measured using Coulter Laser Diffraction (Coulter LS 100 Q Laser Sizer, Beckman Coulter, Miami, FLA, USA). Microspheres were suspended in silicon oil (Tegiloxan 3®) and stirred in vortex for analysis. The average particle size was expressed as the volume-surface diameter, d_{vs} (μm) [22].

The particle size distribution was expressed in terms of SPAN Index determined from the following equation:

$$\text{SPAN} = (d_{90} - d_{10})/d_{50} \quad (1)$$

where d_{10}, d_{50} and d_{90} are the diameter sizes and the given percentage value is the percentage of particles smaller than that size. A high SPAN value indicates a wide size distribution [23].

The morphological attributes of the microparticles were studied by Scanning Electron Microscopy (SEM). A small amount of powder was spread on an aluminium stub, which was placed after gold sputtering in an SEM chamber (Zeiss DMS 962, Zeiss, Germany). Photographs were taken at an acceleration voltage of 20 kV and under argon atmosphere.

2.5. In-vitro swelling studies

In-vitro swelling properties of the spray-dried microspheres were evaluated by a Coulter laser diffraction. The variations of particle size versus time were evaluated using the Coulter laser diffraction apparatus above described. The samples were prepared suspending the microspheres in phosphate buffer (pH 7.0) and particle size distributions and the mean diameters (d_{vs}) were measured, under magnetic stirring, after 5 min. The results are express as Swelling Index (SI) calculated as follows:

$$\text{Swelling Index} = (d2 - d1)/d1 \quad (2)$$

with $d1$ is the d_{vs} measured in silicon oil and $d2$ is the d_{vs} after 5 min on contact with the buffer solution.

2.6. In-vitro mucoadhesion studies

The *in-vitro* mucoadhesion evaluation was performed on drug-loaded microspheres by a method described previously [10]. Briefly, a filter paper ($d = 2.28$ cm; $A = 4.08$ cm^2) was saturated with 2% w/v mucin solution for 10 min in a chamber with controlled humidity (90–100%) and room temperature. Ten milligrams of microparticles were spread out onto the disk, putted in "cruet stand", which was subject to an air flow (flux = 6.37 m/s) for 15 s. Microparticles sticking on the disk surface were recovered by washing the filter paper with water: the volume then was adjusted to 50 ml and the amount of the drug was determined by an UV spectrophotometric analysis, at 309 nm. Mucoadhesion behaviour of the microspheres was expressed as the percentage of microspheres remaining on the disk after the air stream (M_a), calculated applying the following equation:

$$M_a = [(M \times MC_a)/MC] \times 100 \quad (3)$$

where M is the quantity of microparticles spread out onto the disk, MC_a and MC are the amount of the drug detected in the adhered microspheres and contained in M, respectively.

Each determination was carried out in triplicate (SD less than 2).

Statistical differences were determined using Kruskal–Wallis test and the post hoc Dunn's multiple comparisons test (GraphPad Prism, version 2.01; GraphPad Software Incorporated). Differences between groups were considered to be significant at $P < 0.05$.

2.7. In-vitro release tests

In-vitro release tests were performed in 400 ml phosphate buffer (pH 7.0) using the USP Apparatus n.1, at 37 °C and 50 rpm (Erweka DT 70, Erweka GmbH, Heusenstamm, Germany. At fixed time intervals (5, 10, 15, 20, 25, 30, 45, 60, 120 and 180 min), 1 ml samples were withdrawn and replaced with the same volume of dissolution medium. Metoclopramide content in the dissolution samples was measured by UV spectrophotometric analysis at 309 nm. The dissolved amount of drug at each time was expressed as a percentage of the dose.

The dissolution rate of the metoclopramide as raw material was performed using the same conditions reported above.

Each experiment was performed in triplicate (SD less than 5).

2.8. Ex-vivo drug permeation studies

2.8.1. Tissue preparation

For these studies, ovine tissue was used. Nasal mucosa was dissected immediately after slaughter of Sardinian sheep. Turbinates were obtained by cutting out the frontal part of the nasal conch. The excised tissue was stored directly on ice during the transportation to the laboratory.

2.8.2. Permeation test

The method of permeation test has been previously described in detail [10]. CH 2 and MPC 2 batches and metoclopramide as pure drug were tested. An amount of 7.5 mg of pure drug or encapsulated into microspheres was employed. Working conditions were: 400 ml of phosphate buffer pH 7.0, 37 °C and 50 rpm. MC permeated across the mucosa was determined by the HPLC [10]. The results are expressed as cumulative drug penetration vs time. Each experiment was performed in triplicate (SD less than 9).

The permeation results were compared for statistical significance using Student's *t*-test at 5% significance level.

The effective permeability coefficient, P_{eff}, under steady state conditions across excised mucosa has been mathematically expressed, as follow:

$$P_{eff} = (\mathrm{d}c/\mathrm{d}t)_{ss} V/(A\mathrm{CD}) \quad (4)$$

where $(\mathrm{d}c/\mathrm{d}t)_{ss}$ is the time-dependent change of concentration in the steady state, A is permeation area, V is the volume of the receiver compartment and CD is the initial concentration in donor compartment [24].

The lag time (t_{lag}) was obtained by extrapolating the linear portion of the curve to the abscissa.

2.9. Test for the study of hydrogel formation from microspheres

The hydrogel formation from microspheres was studied in water milliQ and in buffer solutions at different pH values. In particular the following media were used:

HCl/KCl buffer (pH 5.5)
Acetate buffer (pH 5.5)
Phosphate buffer (pHs 6.6, 7.0, 7.4)

To verify the possible influence of the drug in hydrogel formation both loaded and drug-free microspheres were tested.

The tests were carried out using a six-wells plate containing 1.5 ml of the suitable medium; a cellulose acetate membrane was put in contact with the fluid. An amount of 10.0 mg of microspheres were spread out the membrane after sieving and incubated at 37 °C.

After 3 h, the hydrogel formation was observed.

Hydrogels were dried in an air circulating oven and then treated with distilled water until dissolution or dissolved in different media for possible understanding the nature of the hydrogels obtained at diverse operative conditions.

3. Results and discussion

3.1. Microspheres preparation and characterisation

All the microspheres were produced by the spray-drying method with yields of production of 30–50%. Drug content determinations show incorporations of the model drug, MC, close to the theoretical values and in particular within the range 95–100%. Formulations CH 1 and CH 2 show the lowest drug content values being the encapsulation efficiencies of 94.5 and 95.5%, respectively.

Particle size and particle size distribution of the microparticles were measured using the Coulter laser diffraction method and the results are expressed as d_{vs} [22] and as SPAN Index [23], calculated by Eq. (1), respectively. Particle size analyses indicate that microparticles have d_{vs} values of about 6–9 μm. As reported in Table 2, the drug-free formulations CH and MPC and the microspheres characterized by the highest drug to polymer ratio of 1:1 (w/w), CH 1 and MPC 1, do not present any remarkable differences in terms of size: in fact they show d_{vs} range of 8.2–8.5 μm. On the contrary CH 2 and MPC 2, whose drug to polymer ratio is 1:2 (w/w), have smaller size then other formulations: in particular d_{vs} values are 5.92 and 7.38 μm, respectively.

Moreover the preparations are characterised by narrow size distributions with regard to polymeric composition and drug loading as highlighted by the SPAN Index values. In fact as already observed from d_{vs} data, also SPAN Indexes of CH 2 and MPC 2 are highest compared to the values of the microspheres based on the corresponding polymer (CH or MPC), indicating, thus, a wider size distribution. On the other hand, MPC 1 shows the lowest value compared to MPC and MPC 2. The same behaviour is noticed in case of CH 1.

The morphological analyses of the microparticles, studied by Scanning Electron Microscopy (SEM), show differences among microspheres based on the same polymer but with different drug to polymer ratio as well as drug-free microparticles CH and MPC.

Microspheres CH 1, CH 2 and CH containing chitosan, have spherical shape and rough surface; some invaginations appear on the CH surface. Different morphology of particles based on methylpyrrolidinone chitosan is observed: MPC, the drug-free formulation, shows some invagination. The loading of the drug modifies the morphological characteristics of the microparticles with regard to the amount of drug encapsulated: when the drug to polymer ratio is 1:2 (w/w), as in case of MPC 2, spherical particles with smooth surface are obtained (Fig. 1). When the drug content is increased (MPC 1) microparticles loose their spherical shape and show irregular surface.

Table 2
Dimensional characterization of microspheres prepared

Formulation	$d_{\text{vs}} \pm$ SD (μm)	SPAN Index
MPC 1	8.50 ± 1.0	1.42
MPC 2	7.39 ± 0.4	1.60
MPC	8.07 ± 0.2	1.44
CH 1	8.15 ± 0.1	1.53
CH 2	5.92 ± 0.6	1.90
CH	8.21 ± 1.0	1.74

Data are presented as means ± SD ($n = 3$).

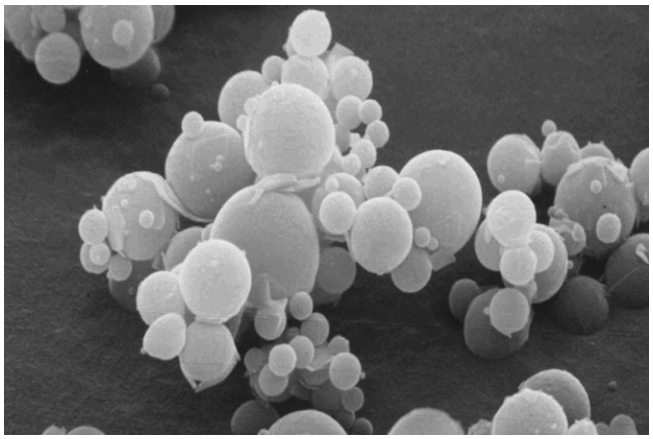

Fig. 1. SEM pictures of MPC 2 microparticles.

3.2. In-vitro swelling studies

In-vitro swelling properties of the spray-dried microspheres were evaluated by Coulter Laser Diffraction and expressed as Swelling Index (SI) estimated by use of equation Eq. (2). Fig. 2 reports SI values of the microspheres tested. Drug-free microspheres based on chitosan, CH, show SI value (0.42) remarkable lower then the corresponding microspheres containing derivatized chitosan, MPC (2.35) indicating that, in the case of the microspheres constituted only by polymer, the methylpyrrolidinone chitosan swells much more then chitosan. However, in case of drug-loaded microspheres based on chitosan, the drug encapsulation increases the swelling properties of the particles: in fact both CH 1 and CH 2, drug-loaded microspheres, present a higher swelling capability of drug-free particles CH (0.91 and 1.89 compared to 0.42, respectively). On the contrary the loading of MPC microparticles determines a decrease of the SI, which is dependent on the amount of drug encapsulated: the swelling decreases when the drug loading increases as shown by the SI values of MPC 2 and MPC 1 (1.65 and 0.55, respectively).

Thus, drug-loaded microparticles based on chitosan show always higher SI values then the corresponding drug-loaded microspheres based on methylpyrrolidinone chitosan.

The obtained results highlight the better swelling properties of the chitosan derivative compared with the chitosan; this could be ascribed to the methylpyrrolidinone moiety, MP, which characterized the derivatized chitosan. On the other hand, when microspheres are loaded with the drug, the swelling effect of MPC microspheres decreases with regard to the amount of the drug loaded. It could be hypothesized that the MP moiety is involved in slightly charge interaction with metoclopramide hydrochloride. The drug might interfere between the polymer chains, taking them close each other, avoiding the moving of the chains away and reducing the swelling process. The swelling properties of MPC microspheres are supported by studies of water uptake (data not reported) which showed that microspheres rapidly and easily swell and gel after absorption of small amounts of water.

3.3. In-vitro mucoadhesion studies

The results of *in-vitro* mucoadhesion tests, expressed as percent of microspheres attached calculated using the equation Eq. (3), are reported in Fig. 3. All the microspheres prepared show good mucoadhesion properties as the percentages found in the range of 80–97%. Microspheres based on chitosan present significative mucoadhesion capability differences ($P < 0.05$); in particular CH 2

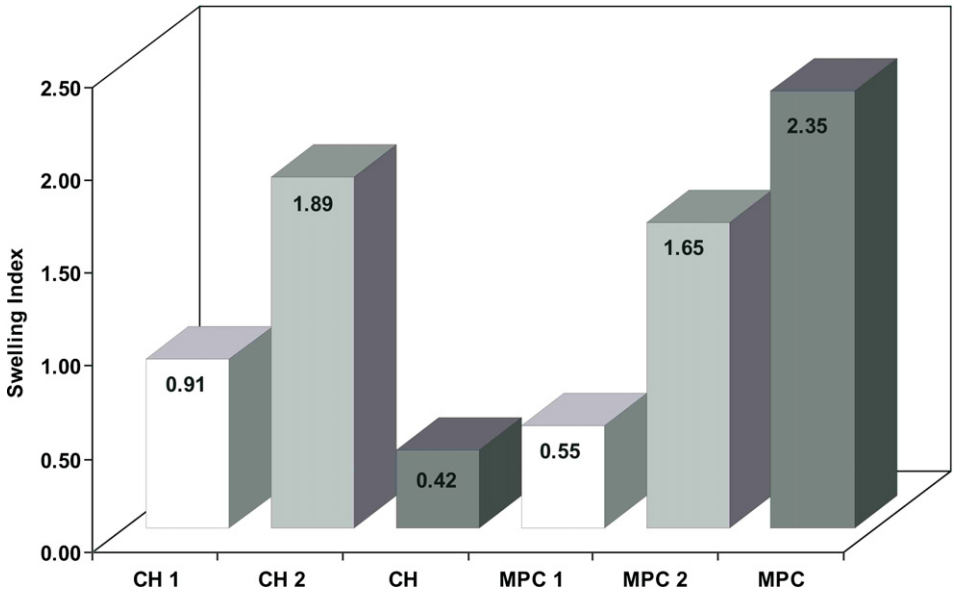

Fig. 2. Swelling properties of spray-dried microspheres expressed as Swelling Index.

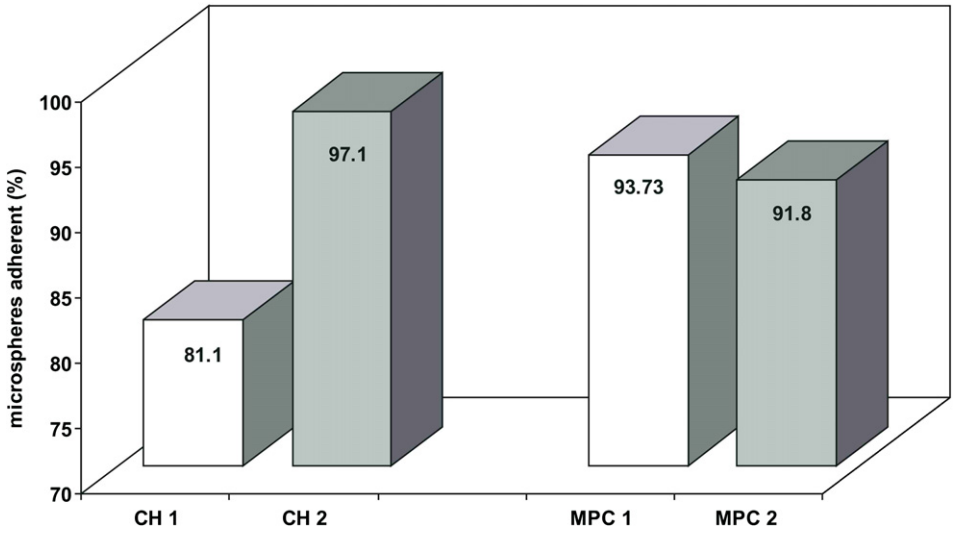

Fig. 3. *In vitro* mucoadhesion evaluation of spray-dried microspheres.

microspheres have the highest values while CH 1 shows the lowest values: the percentage of adhesion of CH 1 microspheres is always statistically different from the other formulations ($P < 0.05$).

Microspheres based on MPC present a mucoadhesion capability independent from the amount of drug encapsulated ($P > 0.05$). The comparison of microspheres based on different polymer but containing the same amount of the drug, CH 1 vs MPC 1 and CH 2 vs MPC 2, shows that CH 1 is significantly less mucoadhesive than MPC 1 (81.1 vs 93.7%) ($P < 0.05$); on the contrary CH 2 shows no statistically differences in mucoadhesive properties of the corresponding derivatized chitosan formulation MPC 2 (97.1 vs 91.8%) ($P < 0.05$). This indicates that the bioadhesion is influenced by the amount of the drug loaded with regard to the polymer.

3.4. In-vitro release tests

In-vitro drug release tests of microspheres were carried out in phosphate buffer (pH 7.0) within 180 min. The dissolution rate of metoclopramide (raw material) was performed as a comparison. The results, reported in Fig. 4, show that the drug dissolves quickly and total drug is recovered after 5 min in the dissolution medium. Spray-dried microspheres are able to control the *in-vitro* MC release with regard to the amount of drug loaded: total drug is released from CH 1 and MPC 1, characterized by the highest drug content, after about 10 min while microparticles CH 2 and MPC 2, containing more polymer, show a slower release rate and 100% of MC is recovered in phosphate buffer after about 1 h. No relevant differences are found between the two chitosans.

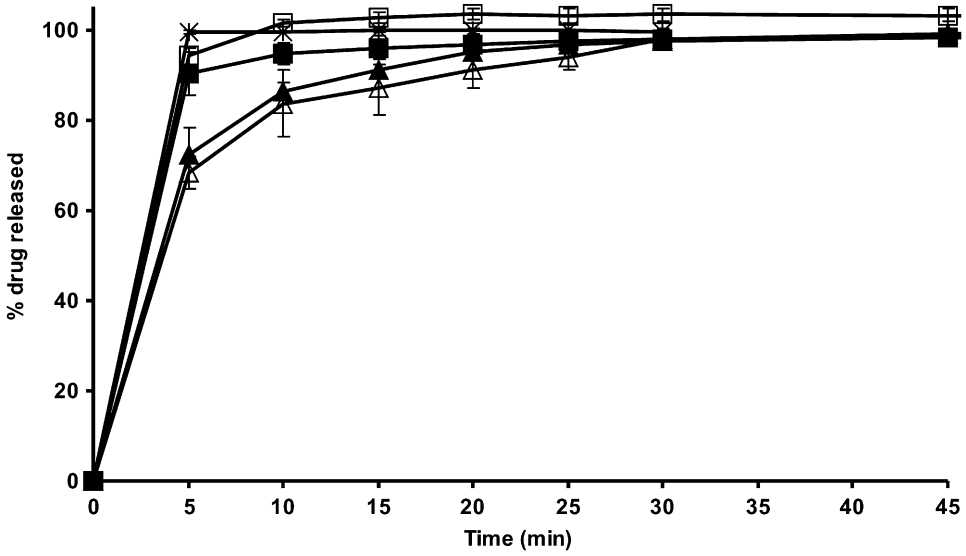

Fig. 4. *In-vitro* drug release study of spray-dried microspheres: (□) CH 1; (■) MPC 1; (△) CH 2; (▲) MPC 2 compared to the dissolution rate of the pure drug (∗). Data are presented as means ± SD ($n = 3$).

3.5. Ex-vivo drug permeation studies

CH 2 and MPC 2 batches, chosen on the bases of the *in-vitro* results, were tested and compared to the pure drug. The permeation profiles of metoclopramide from the two formulations, over a 3 h time period, are shown in Fig. 5.

The permeation of the drug (raw material) is rapid: after 1 h the % amount of drug penetrated is about 4-fold higher ($P < 0.05$) than from both drug-loaded formulations CH 2 and MPC 2 and it stays, after 3 h, higher (about 2-fold) in case of MPC 2 ($P < 0.05$) and 1-fold with respect to CH 2 ($P > 0.05$), indicating the capability of the polymers to control drug permeation rate through the sheep mucosa; in particular MPC 2 shows a linear permeation profile ($R^2 = 0.9969$) along the time considered for the *ex-vivo* experiments. The loaded microspheres have also similar P_{eff} values, calculated by Eq. (4), such as 0.0139 and 0.0104 mg/cm^2 min (CH 2 and MPC 2, respectively). However, statistical differences ($P < 0.05$) are revealed between two formulations based on chitosan (CH) or derivatized chitosan (MPC) after 90 min and a slightly different behaviour concerning the lag time is also found: in fact it corresponds to 16 min in case of CH 2 while MPC 2 shows a virtual zero lag time.

3.6. Study of hydrogel formation from microspheres

During the *ex-vivo* experiments gel formation was observed. The study was carried out in different media (water milliQ and in buffer solutions) and at different pHs (5.5, 6.8, 7.0, and 7.4). Results show that microspheres dissolve in water but when they are put in contact with buffer solutions, the process of gel formation is dependent on the medium used. When HCl/KCl or acetate buffer (pH 5.5) is employed, a gelling process of microspheres occurs; the resulted gel dissolves after dilution in water. However, when phosphate buffers at pHs 6.6, 7.0, 7.4 are used, a hydrogel forms, which does not dissolve in water regardless the amount used but it dissolves in acidic medium such as HCl 0.01 N (pH 2.02) or HCl 0.1 N (pH 1.2). Drug-free microspheres present the same behaviour of loaded microparticles indicating that the hydrogel formation is independent from the presence of the drug but it depends on the medium used. These preliminary results show that MPC gels in the range of pH considered (5.5–7.4) when buffer solutions are employed. In particular phosphate ions seem to be essential for the hydrogel formation: in fact as MPC is charged at neutral and slightly basic pHs, it is able of ionic interactions with phosphate anions; the anions work as crosslinkers able to interconnect the polymeric chains leading to the formation of an ionically crosslinked hydrogels. These hydrogels do not dissolve by adding distilled water, as confirmation of the ionic nature of the link in the hydrogel formed. The acidic medium determines a decrease of the pH value which decreases the charge density on the MPC chains and thus the chance of crosslinking with phosphate anions. It is known in literature that the cationic charge of the ammonic groups of chitosan form ionic hydrogel with anions or anionic molecules containing phosphates [25]. In addition, results show that when other ions such as Cl^- or acetate are employed, a gel formation is observed but it rapidly dissolves after water dilution. This could be due to either the lower pH values (5.5) (and consequently a lower charge density of MPC), and the kind of anions involved.

The influence of the medium on the ionically crosslinked hydrogel is confirmed of the MPC behaviour in water, where, even if the pH is neutral and thus the chitosan is charged, gel formation is not observed but microspheres dissolve.

The importance of the hydrogel formation from microspheres containing MPC is that the MPC is charged at the

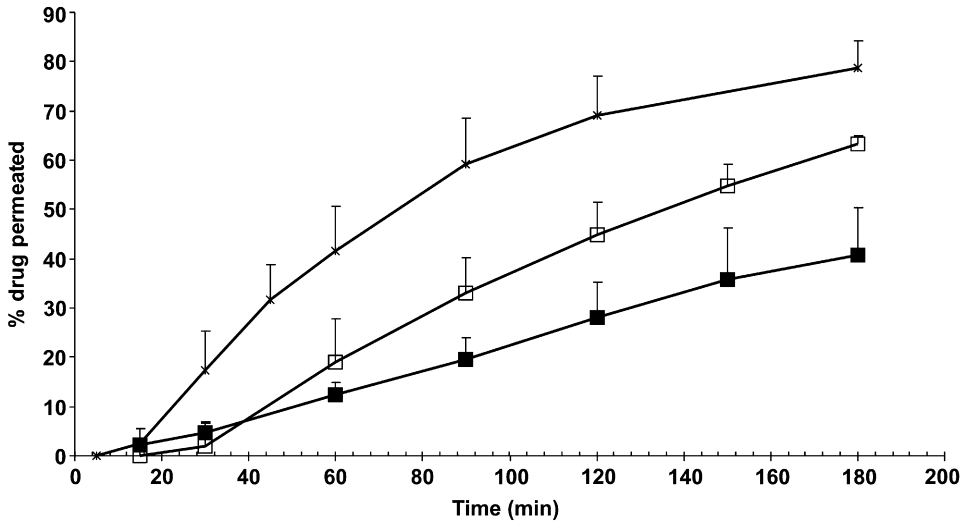

Fig. 5. *Ex-vivo* permeation of MC through sheep nasal mucosa as pure drug (∗) or loaded into CH 2 (□) and MPC 2 (■). Data are presented as means ± SD ($n = 3$).

physiological pH and thus it could be able to form ionic interactions with anions or anionic molecules of the mucous or of the epithelial cells of the mucosa, creating a network responsible of the swelling and/or the drug release and penetration control from microspheres as shown from the *ex-vivo* permeation results. Obviously, the properties of this network will depend on crosslinking density which is dependent on crosslinker dimension, total charge density of the polymer and crosslinker.

4. Conclusion

Microspheres containing metoclopramide based on chitosan derivatized as 5-methylpyrrolidinone chitosan can be easily produced by a spray-drying technique. They show similar properties of microparticles made by chitosan chosen as reference with respect to size and *in-vitro* release behaviour. However microparticles based on MPC are characterized by better mucoadhesiveness, less swelling capability and more prolonged *ex-vivo* permeation profile than particles containing chitosan; moreover they are able to provide a gel (when they were put in contact with aqueous solutions) which shows different properties dependent on the medium used. These properties make microspheres based on derivatized chitosan suitable for the nasal administration: in fact the mucoadhesiveness might prolong the residential time of the formulation inside the nasal cavity while a moderate swelling could avoid potential mucosal damages or inconveniences to the possible users.

On the basis of these preliminary results, MPC microspheres may be considered a promising nasal delivery system based on chitosan derivative able to control the drug penetration through the nasal sheep mucosa.

Acknowledgement

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References

[1] J.H. Hamman, M. Stander, H.E. Junginger, A.F. Kotze, Enhancement of paracellular drug transport across mucosal epithelia by *N*-trimethyl chitosan chloride, S.T.P. Pharm. Sci. 10 (2000) 35–38.

[2] M. Thanou, M.T. Nihot, M. Jansen, J. Coos Verhoef, H.E. Junginger, Mono-*N*-carboxymethyl chitosan (MCC), a polyampholytic chitosan derivative enhances the intestinal absorption of low molecular weight heparin across intestinal epithelia in vitro and in vivo, J. Pharm. Sci. 90 (2001) 38–46.

[3] Y. Chung, C. Kuo, C. Chen, Preparation and important functional properties of water-soluble chitosan produced through Maillard reaction, Bioresour. Technol. 96 (2005) 1473–1482.

[4] R. Muzzarelli, Depolymerization of metyl pyrrolidinone chitosan by lysozyme, Carbohydr. Polym. 19 (1992) 29–34.

[5] P. Giunchedi, I. Genta, B. Conti, R.A.A. Muzzarelli, U. Conte, Preparation and characterization of ampicillin loaded methylpyrrolidinone chitosan and chitosan microspheres, Biomaterials 19 (1998) 157–161.

[6] P.C. Berscht, B. Nies, A. LiebGndiirfer, J. Kreutert, Incorporation of basic fibroblast growth factor into methylpyrrolidinone chitosan fleeces and determination of the in vitro release characteristics, Biomaterials 15 (1994) 593–600.

[7] R.A.A. Muzzarelli, C. Muzzarelli, R. Tarsi, M. Miliani, F. Gabbanelli, M. Cartolari, Fungistatic activity of modified chitosans against *Saprolegnia parasitica*, Biomacromolecules 2 (2001) 165–169.

[8] G. Sandri, S. Rossi, F. Ferrari, M.C. Bonferoni, C. Muzzarelli, C. Caramella, Assessment of chitosan derivatives as buccal and vaginal penetration enhancers, Eur. J. Pharm. Biopharm. 21 (2004) 351–359.

[9] L. Illum, Nasal drug delivery-possibilities, problems and solutions, J. Control. Release 87 (2003) 187–198.

[10] E. Gavini, G. Rassu, V. Sanna, M. Cossu, P. Giunchedi, Mucoadhesive microspheres for nasal administration of an antiemetic drug, metoclopramide: in-vitro/ex-vivo studies, J. Pharm. Pharmacol. 57 (2005) 287–294.

[11] P. Arora, S. Sharma, S. Garg, Permeability issues in nasal drug delivery, Drug Discov. Today 7 (2002) 967–975.

[12] H.O. Alpar, S. Somavarapu, K.N. Atuah, V.W. Bramwell, Biodegradable mucoadhesive particulates for nasal and pulmonary antigen and DNA delivery, Adv. Drug. Del. Rev. 57 (2005) 411–430.

[13] M. Tafaghodi, S. Abolghasem Sajadi Tabassi, M.R. Jaafari, S.R. Zakavi, M. Momen-Nejad, Evaluation of the clearance characteristics of various microspheres in the human nose by gamma-scintigraphy, Int. J. Pharm. 280 (2004) 125–135.

[14] C. Callens, J.P. Remon, Evaluation of starch–maltodextrin–Carbopol® 974P mixtures for the nasal delivery of insulin in rabbits, J. Control. Release 66 (2000) 215–220.

[15] E. Gavini, A.B. Hegge, G. Rassu, V. Sanna, C. Testa, J. Karlsen, P. Giunchedi, Chitosan microspheres for the nasal administration of Carbamazepine: in vitro and in vivo studies, Int. J. Pharm. 307 (2006) 9–15.

[16] G. Fundueanu, M. Constantin, A. Dalpiaz, F. Bortolotti, R. Cortesi, P. Ascenzic, E. Menegatti, Preparation and characterization of starch/cyclodextrin bioadhesive microspheres as platform for nasal administration of Gabexate Mesylate (Foys) in allergic rhinitis treatment, Biomaterials 25 (2004) 159–170.

[17] A.M. Dyer, M. Hinchcliffe, P. Watts, J. Castile, I. Jabbal-Gill, R. Nankervis, A. Smith, L. Illum, Nasal delivery of insulin using novel chitosan based formulations: a comparative study in two animal models between simple chitosan formulations and chitosan nanoparticles, Pharm Res. 19 (2002) 998–1008.

[18] M.D. Abd El-Hameed, I.W. Kellaway, Penetration and in vitro characterisation of mucoadhesive polymeric microspheres as intranasal delivery systems, Eur. J. Pharm. Biopharm. 44 (1997) 53–60.

[19] L. Illum, Nasal drug delivery: new developments and strategies, Drug Discov. Today 7 (23) (2002) 1184–1189.

[20] R.A.A. Muzzarelli, P. Ilari, M. Tomasetti, Preparation and characteristic properties of 5-methyl pyrrolidinone chitosan, Carbohydr. Polym. 20 (1993) 99–106.

[21] R.A.A. Muzzarelli, U.S. Patent 5,378,472, 1995.

[22] I.C. Edmundson, Advances in Pharmaceutical Sciences 2, in: H.S. Bean, J.E. Carless, A.H. Beckett (Eds.), Academic Press, London, 1967, p. 950.

[23] R.R. Dubey, R.H. Parikh, Studies of PLGA microspheres, Pharm. Tech. Eur. 16 (2004) 23–34.

[24] S. Lang, P. Langguth, R. Oschmann, B. Traving, H.P. Merkle, Transport and metabolic pathway of thymocartin (TP4) in excised bovine nasal mucosa, J. Pharm. Pharmacol. 48 (1996) 1190–1196.

[25] J. Berger, M. Reist, J.M. Mayer, O. Felt, R. Gurny, Structure and interactions in chitosan hydrogels formed by complexation or aggregation for biomedical applications, Eur. J. Pharm. Biopharm. 57 (2004) 35–52.