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Research paper

Chitosan and poly(methyl vinyl ether-co-maleic anhydride) microparticles as nasal sustained delivery systems

T. Cerchiara[a], B. Luppi[b,*], G. Chidichimo[a], F. Bigucci[b], V. Zecchi[b]

[a]Department of Chemistry, Calabria University, Arcavacata di Rende (CS), Italy
[b]Department of Pharmaceutical Sciences, Bologna University, Bologna, Italy

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Abstract

An original dosage form for nasal delivery based on the encapsulation of hydrophilic drug in chitosan-poly(methyl vinyl ether-co-maleic anhydride) (CH-PVM/MA) microparticles prepared by spray-drying technique was developed. Microparticles were characterized in terms of morphology, size, swelling properties, encapsulation efficiency and drug release. The physical state of the drug and the polymer was determined by scanning electron microscopy (SEM) and infrared spectroscopy (IR). Propranolol hydrochloride (PH) was a β-blocker, used for the treatment of hypertension and was chosen as a model of hydrophilic drug. SEM studies showed spherical particles with smooth surfaces for chitosan hydrochloride (CH-HCl), whereas rather gross surface defects resulted from the incorporation of poly(methyl vinyl ether-co-maleic anhydride) (PVM/MA). In vitro release studies revealed a sustained release of propranolol HCl from microparticles and in particular chitosan hydrochloride provided the lowest release of drug.

Keywords: Chitosan; Poly(methyl vinyl ether-co-maleic anhydride); Spray-drying; Propranolol hydrochloride; Mucoadhesion; Franz cells

1. Introduction

Nasal drug delivery has generated widespread interest among the scientific comunity as an alternative route for the administration of drugs and biomolecules that are susceptibile to enzymatic or acidic degradation and first-pass hepatic metabolism [1].

Antihypertensives (propranolol, nifedipine, nitroglycerine, hydralazine and so on) have been shown to produce considerable systemic effects when administered via the nasal cavity. However, like other routes, nasal delivery also has its limitations, which have restricted its use to the delivery of only a few drug molecules [2]. Recently, microsphere technology has been applied in designing formulations for nasal drug delivery. Drugs which are not absorbed from such dosage forms, stand better chance of absorption when formulated in 'gelling' microspheres made by using biocompatible materials e.g. starch, gelatin, albumin, chitosan and dextran [3]. In particular, chitosan (CH) is a positively charged linear polysaccharide that is bioadhesive and able to interact strongly with the nasal epithelial cells and the overlaying mucus layer thereby providing a longer contact time for drug [4]. Bioadhesive systems can be in the form of powders as well as liquids or liquid gelling systems. The powders can be administered as freeze-dried or spray-dried particles or microspheres.

Poly(methyl vinyl ether-co-maleic anhydride) (PVM/MA) is widely used for pharmaceutical purposes as a thickening and suspending agent, denture adhesive and adjuvant for transdermal patches [5]. The oral toxicity of all these polymers is quite low (LD_{50} in guinea pigs is 8–9 g/kg per os from data supplied by ISP Corp.). It is a biodegradable polyanhydride and could be an appropiate copolymer for the preparation of particulate dosage forms with bioadhesive or mucoadhesive properties [6]. In fact, when polyanhydrides hydrolytically degrade, the product of each cleaved anhydride bond contains two carboxylic acid groups. In accordance with the adsorption theory of adhesion, carboxylic groups would enhance the ability of

* Corresponding author. Department of Pharmaceutical Sciences, Bologna University, Via S. Donato 19/2, 40127 Bologna, Italy. Tel./fax: +39 051 253664.
E-mail address: bluppi@biocfarm.unibo.it (B. Luppi).

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polymers to form hydrogen bonds with components from the mucosa [7].

Propranolol hydrochloride (PH) is an effective non-selective β-adrenergic blocking agent that has a widespread use for angina pectoris and hypertension. It also has a short biological half-life (2–3 h) [8]. After oral administration it is completely absorbed, however, the systemic availability is relatively low again due to metabolism by the liver during its first passage through the portal circulation [9].

The aim of this work was firstly to entrap propranolol hydrochloride within CH-PVM/MA microparticles prepared by spray-drying technique and secondly to characterize the formulation in terms of morphology, size, swelling properties, drug loading and release.

2. Materials and methods

2.1. Materials

High-molecular-weight chitosan (MW 600,000, viscosity 400 mPa s (1% solution in 1% acetic acid) degree of deacetylation 80%) was purchased from Fluka (Buchs, Switzerland). Poly(methyl vinyl ether-co-maleic anhydride) (Gantrez AN 119, MW 216,000) was purchased from ISP (Milan, Italy). Mucin (Type II: crude, from porcine stomach) was purchased from Fluka-Sigma-Aldrich. Propranolol hydrochloride was purchased from Sigma-Aldrich.

2.2. Preparation of CH-PVM/MA microparticles

Chitosan (0.25 g; 1.55 mmoles of glucosammine) was dissolved in 0.1 N HCl (50 ml) to which was added, dropwise, a PVM/MA solution (0.24 g; 50 ml; 1.55 mmoles of dimer) at room temperature. The solution was spray-dried (Büchi Mini Spray Dryed, B-191; Switzerland). The drying conditions were as follows: inlet temperature, 110 °C; outlet temperature, 49 °C; air flow rate, 600 N l/h. As a comparison, chitosan (0.25 g; 1.55 mmoles of glucosammine) dissolved in 0.1 N HCl (50 ml) was spray-dried in the same conditions (CH-HCl).

The drug-loaded microparticles were prepared by dissolving 0.46 g (1.55 mmoles) of PH in 100 ml of CH-PVM/MA solution or CH-HCl solution prepared by description above. The solution was spray-dried and a fine white powder was obtained.

2.3. Determination of drug content

The loading efficiency of PH in the microparticles was determined spectrophotometrically by measuring at the maximum wavelength of 290 nm, the amount of encapsulated drugs after extraction from the microparticles. An equivalent of 10 mg of powered microparticles was extracted with 10 ml of 0.1 N HCl by stirring for 5 h. The resulting acidic solution containing the extracted drug was assayed using UV spectroscopy [9].

2.4. Fourier transform infrared spectrometry

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

2.5. Scanning electron microscopic (SEM) studies

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

2.6. Swelling of the CH-HCl and CH-PVM/MA polymers

In order to quantify the swelling of CH-HCl and CH-PVM/MA in acidic and alkaline environments, disks approximately 20 mg in weight were prepared by a punch press working at 7 ton/cm^2. The disks were immersed in 10 cm^3 volume pH 5.5 or pH 7.4 aqueous buffers at 37 °C and weighed after 1 hour (time corresponding at the higher swelling degree). The swelling ratio was determined as the ratio between the weight of the hydrated disks (Wh) at $t = 1$ h and the initial weight of the dry disks (Wd) according to the following equation:

$$(Wh - Wd)/Wd$$

where Wh is the weight of the hydrated disks and Wd is the initial weight of the dry disks.

2.7. Muco-adhesion properties

The muco-adhesion behaviour was performed as described elsewhere [10]. Briefly, 1 ml of a mucin suspension (0.05%w/v) was mixed with 1 ml of spray-dried CH-HCl and CH-PVM/MA microparticles solution for 24 h at pH 5.5 and pH 7.4 at 37 °C under continuous stirring. Mucin–microparticles interaction was evaluated by photon correlation spectroscopy (PCS) using an instrument (Brookhaven 90-PLUS) with an He–Ne laser beam at a wavelength of 532 nm (scattering angle of 90°) [11,12].

In addition the residence time was determined. In particular, a freshly cut 5 cm piece of bovine nasal mucosa was obtained and cleaned by washing with isotonic saline solution. One hundred milligram of microparticles were placed on mucosal suface, which was fixed over a polyethylene support. The bovine nasal mucosa was throughly washed with phosphate buffer solution (pH 7.4) at the rate of 5 ml/min using a peristaltic pump. Sixty

minutes after administration of microparticles, the concentration of the drug in the collected perfusate was spectrophotometrically determined. The microparticles amount corresponding to the drug amount in perfusate was calculated. The adhered microparticles amount was estimated from the difference between the applied microparticles amount and the flowed microparticles amount. The ratio of the adhered microparticles was computed as per cent mucoadhesion.

2.8. In vitro release studies

Franz diffusion cells were used in the in vitro release studies [13]. Fifty milligram of microparticles were applied across a dialysis membrane (Mw cut off = 14.000) mounted in a Franz diffusion cell. The donor compartment contained 3 ml of pH 5.5 and pH 7.4, respectively [3,14,15], and the receiver compartment was filled with 10 ml of the same aqueous buffer, which was stirred with a magnetic stirring bar. The cells were thermostated at 37 °C. At set time intervals, 0.5 ml samples were withdrawn from the receiver compartment and the drug was spectrophotometrically detected. The aqueous buffer contained in the receiving compartment was replaced with an equal quantity to maintain a constant volume.

2.9. Statistical analysis

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

3. Results and discussion

3.1. FTIR

Fig. 1 shows the FT-IR spectra of CH, spray-dried CH-PVM/MA and PVM/MA. Amine bands including minor amide I and amide II bands of chitosan itself are located at 1750 and 1669 cm^{-1}, respectively. The carbonyl absorption bands of PVM/MA itself appeared at 1779 and 1730 cm^{-1}. For spray-dried CH-PVM/MA, absorption bands at 1717 and 1617 cm^{-1}, which can be assigned to the free carbonyl groups of PVM/MA and to carboxyl groups of PVM/MA complexed with amino groups of chitosan respectively, were observed.

3.2. Entrapment efficiency (EE) within microparticles

The EE was about 92% for CH-PH microparticles and 89% for CH-PVM/MA-PH microparticles as expected from spray-drying preparation technique.

3.3. Structure of microparticles (SEM)

Analysis by scanning electron microscopy revealed a regular spherical shape of the spray-dried CH-HCl microparticles (Fig. 2A) with a smooth surface. However,

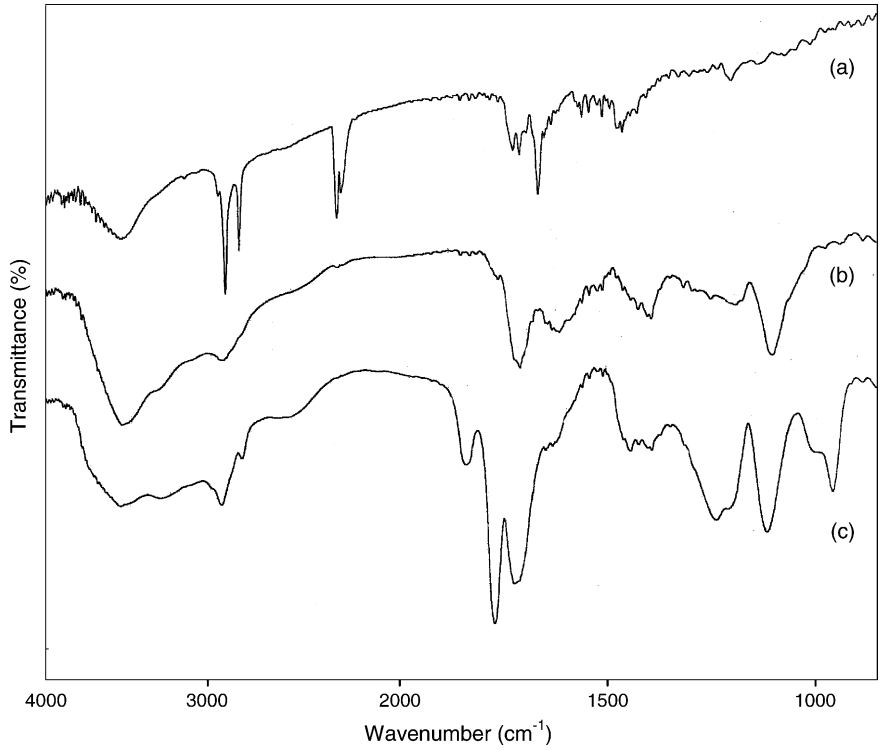

Fig. 1. IR spectra of: (a) CH; (b) CH-PVM/MA; (c) PVM/MA.

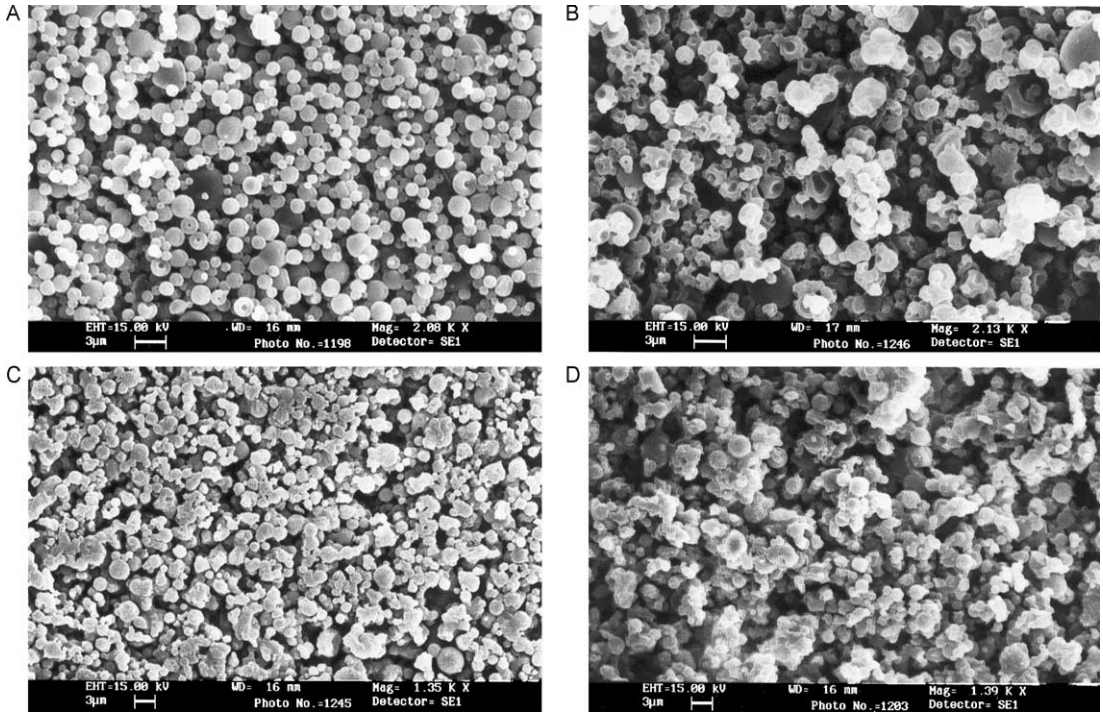

Fig. 2. SEM: (A) spray-dried CH-HCl; (B) spray-dried CH-PVM/MA; (C) spray-dried CH-HCl-PH; (D) spray-dried CH-PVM/MA-PH.

incorporation of PVM/MA significantly influenced the surface morphology resulting in the production of blow-holes and irregular microparticles (Fig. 2B). This could be due to the different precipitation ability of CH-HCl and PVM/MA during the course of solvent removal, thus forming a skin layer able to prevent the solvent inside the nascent microparticles from removal and resulting in the deformation of microparticles. These findings suggest that physical properties of polymer solution played an important role in the morphology formation of microparticles.

Moreover, the images demonstrated that the incorporation of drug significantly alters the surface morphology of the CH-HCl and CH-PVM/MA microparticles. The spray-dried CH-HCl-PH and (CH-PVM/MA)-PH microparticles (Fig. 2C and D) have a quite regular shape and a rough surface.

3.4. Influence of pH on the swelling of CH-HCl and CH-PVM/MA polymers

Table 1 showed the result of the swelling studies of the CH-PVM/MA and CH-HCl polymers under different pH values. Swelling degree of CH-PVM/MA increased with the increasing the pH value from 5.5 to 7.4. This behaviour can be explained by the following. CH-PVM/MA polymer presented ionic interaction between positively charged chitosan and negatively charged PVM/MA. At different pHs, the charge balance inside the gel and therefore the degree of interaction between the two polymers were different [15]. When the pH value was 5.5, the polyacid was neutralized and due to the free ammonium groups of chitosan, free positives charges appeared inside the gel. Their mutual repulsion and the entry of water together with counterions to neutralise these charges caused swelling. When the pH value was 7.4, CH and PVM/MA were oppositely charged and ammonium groups of CH were totally engaged by ionic interaction with carboxyl groups of PVM/MA. In this case the swelling can be explained due to the excess of carboxyl groups in the network (CH:PVM/MA molar ratio is 1:2) and appearing of free negatives charges in the gel. As concerns CH-HCl polymer, the swelling degree at different pHs was similar due to the presence of the positively charged ammonium groups both at pH 5.5 and pH 7.4.

3.5. Muco-adhesion properties

Fig. 3 showed CH-HCl and CH-PVM/MA mucoadhesive properties. CH-HCl and CH-PVM/MA mean size increased in presence of mucin dispersion due to the secondary chemical bonds such as hydrogen bonds or ionic interactions between the positively charged amino groups of

Table 1
Swelling ratio (Wh − Wd/Wd; weight of hydrated disks-initial weight of dry disks/initial weight of dry disks) of CH-HCl and CH-PVM/MA polymers at pH 5.5 and pH 7.4 values

pH Values	(Wh − Wd)/Wd	
	CH-PVM/MA	CH-HCl
5.5	0.53 ± 0.05	0.28 ± 0.04
7.4	0.69 ± 0.05	0.36 ± 0.03

Each data represents the average of three determinations ± SD.

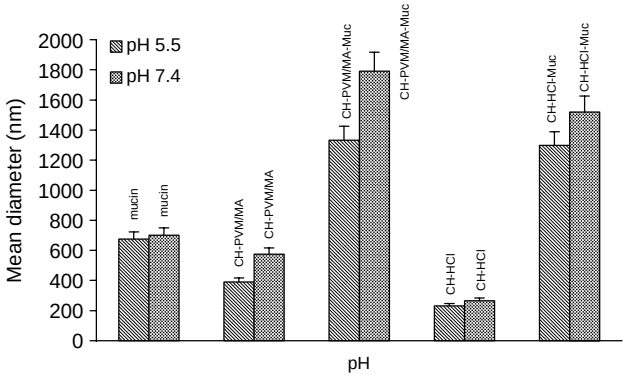

Fig. 3. Mean size of CH-Cl and CH-PVM/MA with and without mucin at pH 5.5 and pH 7.4 aqueous buffer at 37 °C. The data were obtained by DLS measurements and each datum represents the average of three determinations ± SD.

chitosan and the negatively charged sialic acid residues of mucus glycoproteins or mucins [16–18]. Moreover, the interaction of CH-HCl and CH-PVM/MA with mucin dispersion showed a higher in vitro muco-adhesion at pH 7.4 than at pH 5.5. Infact at pH 7.4, sialic acid carries a net negative charge and providing strong electrostatic interactions between mucin and chitosan.

In addition, the microparticles adequately adhere on nasal mucosa. Infact, 60 min after microparticles administration the percentages of mucoadhesion were 78% for CH-PVM/MA and 67% for CH-HCl. These results may be explained on the bases of swelling rates: CH-PVM/MA microparticles swelled more rapidly to provide a more highly mucoadhesive system.

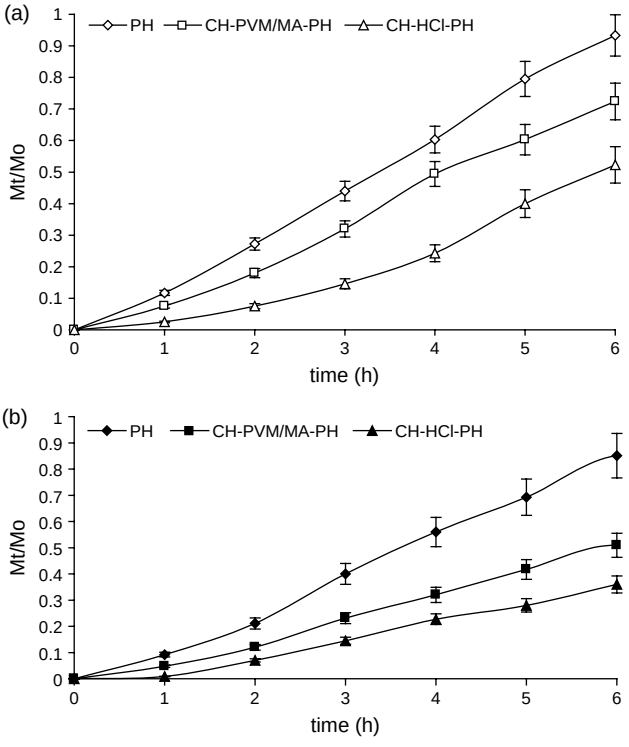

Fig. 4. Fractional release of propranolol hydrochloride from the microparticles at pH 5.5 (a) and pH 7.4 (b). Each data represents the average of three determinations ± SD.

3.6. In vitro release studies

Fig. 4(a and b) showed the release profiles of propranolol HCl from CH-HCl and CH-PVM/MA microparticles at pH 5.5 and 7.4 phosphate buffers. Free drug availability, expressed as fractional release over time was lower from microparticles than the pure drug at each pH analyzed. This may be attributed to the gelling ability of the polymers hindering drug diffusion thus decreasing the free-drug concentration in the releasing aqueous phase. In particular, the release of propranolol HCl from the microparticles was higher for CH-PVM/MA than CH-HCl and for all microparticles at pH 7.4 than pH 5.5. This behaviour was in accordance with the higher swelling ability of CH-PVM/MA than CH-HCl and the higher swelling ability of all microparticles at alkaline pH with respect to acidic pH (see Table 1).

4. Conclusions

CH-PVM/MA and CH-HCl microparticles slowed down the release of propranolol hydrochloride guaranteeing a sustained release at acidic pH and alkaline pH in the nasal cavity. In particular chitosan hydrochloride provided the lowest release of drug.

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