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Development and In Vitro Characterization of Chitosan-based Microspheres for Nasal Delivery of Promethazine

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ABSTRACT Conventional and composed promethazine-loaded microspheres were prepared by spray drying of chitosan solution systems and double water-in-oil-in-water (W/O/W) emulsion systems, respectively. Double emulsions were prepared in two different feed concentrations, with chitosan dissolved in both water phases, and ethylcellulose dissolved in oil phase. Swelling and bioadhesive properties of the microspheres depended on the chitosan content, type and the feed concentration of spray-dried system. Results obtained suggested that better ethylcellulose microcapsules with promethazine in the chitosan matrix were formed when less concentrated emulsion systems were spray-dried. Thus, in case of such a system, with ethylcellulose/chitosan weight ratio of 1:2, prolonged promethazine release was obtained.

KEYWORDS Promethazine, Chitosan, Ethylcellulose, Microspheres, Nasal delivery, Spray drying

INTRODUCTION

Promethazine belongs to the group of phenothiazines and has anticholinergic, sedative and antiemetic effects. It is predominantly used as an antiemetic or for the treatment of the motion sickness (Ruedas Rama et al., 2004). After administration it is cleared from the body by hepatic metabolism. Antiemetics are administrated orally or intravenously, despite the problems associated with acute emesis or invasiveness of parenteral administration. Nasal delivery of antiemetics could be considered as an alternative route to current oral and intravenous administration, as nasal formulations are not invasive and can be administrated to the patients with acute emesis. As nasally administrated drugs avoid first-pass hepatic metabolism, improved bioavailability can be expected (Gavini et al., 2005).

However, rapid mucociliary clearance reduces the residence time of nasal drug delivery system at the site of absorption. One of the ways to delay the mucociliary clearance and to prolong the contact between drug delivery system

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and mucosa is the use of bioadhesive polymers in nasal delivery system preparation (Vasir et al., 2003). Dry powder delivery systems such as microspheres are of special interest; the residence time in the nasal cavity is considerably increased in case of microspheres with swelling ability, as they hydrate in contact with the mucosa, forming the gel layer at the absorption site (Pereswetoff-Morath, 1998). Thus, bioadhesive microspheres are characterized by intimate and prolonged contact with the mucus layer, improved absorption of the drug, predictable and controlled drug release from polymeric device, leading to reduced frequency of drug administration (Illum, 2003; Vasir et al., 2003).

The aim of this study was to develop bioadhesive microspheres for nasal delivery of promethazine. Polymers used for the microsphere preparation were chitosan (CM) and ethylcellulose (EC). Dry powder delivery systems based on chitosan, such as chitosan microspheres, have proved to enhance the drug permeation across the nasal mucosa (Illum, 2003). Chitosan is natural biocompatible polycationic polymer, used in numerous pharmaceutical applications. Positively charged chitosan strongly interacts with negatively charged mucosal surface, providing prolonged contact between chitosan-based formulation and the absorption site (Singla & Chawla, 2001). Thus, determination of surface charge of the microspheres is of a great importance since it has a substantial influence on their bioadhesion properties (Berthold et al., 1996).

Except for chitosan, ethylcellulose was used for the preparation of promethazine-loaded microspheres. When a hydrophilic drug is entrapped into microspheres prepared with highly hydrophilic polymer such as chitosan, it is released readily with common initial burst due to fast polymer hydration and swelling. Modulated release rate of the drug from the swellable chitosan microspheres has been achieved with cross-linking agents like glutaraldehyde, citric acid, ascorbic acid or ascorbyl palmitate that reacted with chitosan forming covalent bonds with chitosan amino groups (Genta et al., 1998; Varshosaz et al., 2004). As a result, reduced bioadhesive properties of cross-linked microspheres have been observed. Therefore, the goal of adding lipophilic polymer such as EC to the formulation was to achieve a more sustained release by obtaining less hydrophilic system compared to conventional chitosan microspheres, leaving amino groups free for interaction with mucin.

Microspheres were produced by spray drying. The drug encapsulation efficiency, morphology, size distribution, zeta potential, swelling, and bioadhesive properties and in vitro drug release behavior were studied with respect to the type, polymeric composition and the drug content of spray-dried system.

MATERIALS AND METHODS

Reagents and Chemicals

The following materials were used as received: Chitosan of medium molecular weight, CM (M_r 400,000; deacetylation degree 83.5%, Fluka, Buchs, Switzerland), ethylcellulose, EC (Sigma, St. Louis, MO), promethazine hydrochloride (Sigma, St. Louis, MO). Simulated Nasal Fluid, SNF, was prepared as an aqueous solution containing 8.77 g NaCl, 2.98 g KCl and 0.59 g $CaCl_2$/L. These substances and all other chemicals or solvents used were of analytical grade and purchased from Kemika (Croatia).

Preparation of Microspheres

Drug free and drug-loaded chitosan-based microspheres were prepared by spray drying of the solutions and double W/O/W emulsions using Büchi 190 mini spray drier (Flawil, Switzerland) with a standard 0.5 mm nozzle. The liquid was fed to the nozzle with peristaltic pump, atomized by the force of the compressed air and blown together with a hot air to the chamber where the solvent in the droplets was evaporated. The dry product was then collected in a collection bottle. The drying conditions were as follows: spray flow rate of 0.25 L/hr, compressed air flow rate of 700 NL/hr, inlet air temperature of 135°C and outlet air temperature of 85°C.

For the solution system chitosan was solubilized in 0.5% acetic acid solution at 1% (w/v) concentration. Promethazine was dissolved in water at two different concentrations (1 and 0.75%, w/v). These solutions were than mixed with chitosan solution in a 1:6 (v/v) ratio and subjected to spray drying under process conditions described above. Thus, two different polymer/drug ratios (6:1 and 8:1, w/w) were obtained for the preparation of promethazine-loaded chitosan microspheres.

For the W/O/W (A) and W/O/W (B) emulsion systems, chitosan solution in 0.5% acetic acid represented inner water phase (1.5 and 1%, w/v, respectively) and the outer water phase (1 and 0.5%, w/v, respectively). The oil phase consisted of EC dissolved in ethyl ace-

tate (4 and 1%, w/v, respectively). Promethazine was dissolved in the inner water phase at the concentration of 10 and 2.5% (w/v), respectively. Primary W/O emulsions were prepared by simple ultrasonic homogenization (Cole-Parmer 4710 Series, Chicago, IL; 2 × 30 s, at 60 μW, with 30 s intervals) of the inner water phase and the oil phase, and were then added to the outer water phase under stirring conditions (900 rpm; magnetic stirrer). Weight ratio of EC dissolved in the oil phase and CM dissolved in the outer water phase (EC/CM) of the prepared W/O/W emulsions was 1:2 or 1:3.

Promethazine-free (empty) microspheres were prepared following the same procedure as for promethazine-loaded microspheres omitting promethazine.

The type and composition of spray-dried systems used for the preparation of promethazine-loaded microspheres is given in Table 1.

Scanning Electron Microscopy (SEM)

The shape and surface characteristics of the microspheres were observed by scanning electron microscopy. The microspheres were sputter-coated with Au/Pd using a vacuum evaporator (Edwards) and examined using a scanning electron microscope (Philips 500, Eindhoven) at 10 kV accelerating voltage.

Particle Size Analysis

A microscopical image analysis technique for determination of microsphere size distribution was applied. The morphology and particle size distributions (based on the number of particles) were determined in an Olympus BH-2 microscope equipped with a camera (CCD Camera ICD-42E; Ikegami Tsushinki Co., Japan) and computer-controlled image analysis system (Optomax V, Cambridge). The microspheres were dispersed on a microscope slide. A microscopical field was scanned by video camera. The images of the scanned fields were digitalized and analyzed by the software (Optomax V Software, Cambridge). In all measurements at least 3000 particles were examined.

Determination of the Drug Loading

Promethazine was extracted from microspheres with mixture of 0.1*M* HCl and 96% ethanol (3:2, v/v; 15 mL) under sonication in ultrasonic bath (Branson B1210E-DTH, Danbury, CT). The samples were filtered and the amount of promethazine was determined spectrophotometrically ($\lambda = 249$ nm; Ultrospec Plus, Pharmacia LKB, Cambridge, UK). Preliminary studies showed that the presence of dissolved polymers did not interfere with promethazine absorbance at 249 nm.

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) measurements were carried out on a scanning calorimeter (Mod. TA 4000, equipped with a measuring cell DSC 20 Mettler). The instrument was calibrated using indium as standard. Samples, containing 0.7–1.5 mg of active ingredient were placed in pierced aluminium pans and heated from 30–300°C at a rate of 10°C/min under air atmosphere.

TABLE 1 **The Type and Composition of Spray-Dried Systems in the Preparation of Promethazine-loaded Microspheres**

	Type of spray-dried system					
	Solution		W/O/W emulsion			
	PS1	PS2	PEA1	PEA2	PEB1	PEB2
Concentration CM (w/v, %)[a]	1	1	1	1	0.5	0.5
Concentration EC (w/v, %)[b]			4	4	1	1
Concentration CM (w/v, %)[c]			1.5	1.5	1	1
W/O/W (v/v/v)			0.2:1:8	0.2:1:12	0.2:1:4	0.2:1:6
EC/CM (w/w)[d]	0/1	0/1	1/2	1/3	1/2	1/3
Polymers[d]/promethazine (w/w)	6/1	8/1	6/1	8/1	6/1	8/1

[a]Refers to the solutions and outer water phase of W/O/W emulsions.
[b]Refers to the oil phase of W/O/W emulsions.
[c]Refers to the inner water phase of W/O/W emulsions.
[d]Refers to the polymers in oil and outer water phase of W/O/W emulsions.

Zeta-Potential of the Microspheres

Zeta-potential of the microspheres prepared was determined by photon correlation spectroscopy (Zetasizer 3000 HSA, Malvern Instruments, Malvern, UK) in 10 mM NaCl solution (pH 6.7) at 25°C.

Swelling Studies

The water absorbing capacity of each microsphere sample was determined by a volumetric method using a Franz diffusion cell apparatus. A water permeable polyamide membrane with 0.45 μm pore size was placed between the microsphere sample (5 mg) and receptor cell, which was filled with SNF. The whole system was thermostated at 37°C. The level of SNF in graduated part of Franz diffusion cell lowered due to liquid uptake of the microspheres. The amount of SNF, equal to the amount of SNF absorbed by the microspheres, was then added to the receptor cell (Cornaz et al., 1996). The liquid uptake of each sample was expressed as volume of SNF added per mg of the microspheres in 15 min swelling process (Garcia-Arieta et al., 2001).

Tensile Studies

Drug-loaded microspheres (60 mg) as well as drug-free microspheres were compressed into 5 mm diameter flat faced test disc, which was attached to a precise torsion balance. A piece of porcine nasal mucosa (2 cm^2) was mounted on the glass dish and placed on a mobile platform. The discs and the mucosal surfaces were brought in contact in simulated nasal fluid (SNF; an aqueous solution containing 8.77 g NaCl, 2.98 g KCl, and 0.59 g $CaCl_2$/L) pH 6.3 at 22°C. The value for the force of detachment was measured as a function of displacement, by lowering the mobile platform at the constant rate of 2 mm/min until total separation of the components was achieved. The work of fracture, equivalent to the total work of bioadhesion (TWA) was calculated as the area under the force/distance curve.

In Vitro Drug Release Studies

The drug release profiles of promethazine-loaded microspheres were evaluated using a Franz diffusion cell apparatus, since this model would allow the microspheres to hydrate slowly in a humid environment conditions designed to be similar to those encountered in the nasal cavity (Cheng et al., 2002). A polyamide membrane with 0.45 μm pore size was placed between the microsphere sample and receptor cell. The promethazine-loaded microspheres containing 1 mg of promethazine were sprinkled to the polyamide membrane. The receptor cell was filled with the SNF, thermostated at 37°C, and maintained in gentle agitation by means of a magnetic stirrer (600 rpm). At scheduled time intervals, the samples (0.5 mL) were withdrawn from receptor cell and replaced with fresh medium. The samples were assayed spectrophotometrically at 249 nm. All experiments were carried out in triplicate and average values were plotted.

Statistical Analysis

Statistical data analyses were performed using the Student's *t*-test with $p < 0.05$ as the minimal level of significance. Calculations were performed with the GraphPad Prism program (GraphPad Software, Inc., San Diego; www.graphpad.com).

RESULTS AND DISCUSSION

Characterisation of Microspheres

Six samples of promethazine-loaded and corresponding promethazine-free chitosan-based microspheres were prepared by spray drying of two different types of systems; solutions and double W/O/W emulsions. The main characteristics of the spray-dried systems are given in Table 1.

Spray drying of the solutions resulted in the conventional microspheres with promethazine dispersed in chitosan matrix (1:6 and 1:8, w/w).

Double W/O/W emulsions were prepared with no addition of external emulsifiers since chitosan was dissolved in both water phases of double emulsions. Chitosan, as amphiphilic polyelectrolyte, is known to stabilize emulsions combining electrosteric and viscosifying mechanisms. Since chitosan acts as a mixture of surfactants with different hydrophilic-lipophilic balance values due to different degrees of deacetylation, it has been shown to stabilize double W/O/W emulsions (Schulz et al., 1998; Rodriguez et al., 2002).

Formation of W/O/W emulsion was confirmed by light microscopy prior to spray drying. Double

emulsions differed in the concentration of the polymer solutions used for their preparation. Thus, W/O/W (A) emulsions were prepared with polymer solutions of higher concentrations than W/O/W (B) emulsions (Table 1).

Spray drying of W/O/W emulsions resulted in the composed microspheres, with EC (in oil phase) to CM (in outer water phase) weight ratios of 1:2 and 1:3 and, in case of promethazine-loaded microspheres, with drug to polymers weight ratios of 1:6 and 1:8, respectively (Table 1). Promethazine to EC weight ratio was kept constant (1:2, w/w) for all composed microspheres prepared.

The physical state of the encapsulated drug was determinated by DSC analysis of the pure drug, drug free and drug-loaded microspheres. The thermograms obtained indicated that promethazine was in the amorphous state in all microspheres prepared, regardless of the type and/or polymeric composition of spray-dried systems (data not shown).

The yields for all samples of microspheres were relatively high (31–54%), considering the preparation method used and the small amount of material (1 g) processed in each batch (Giunchedi et al., 2002).

As shown in Table 2, the yields of spray-dried microspheres were higher for conventional microspheres composed of chitosan only (samples PS1 and PS2), than for the microspheres with complex polymeric composition (samples PEA1, PEA2, PEB1 and PEB2). Similar decrease in yield in case of spray-dried loratadine-loaded microspheres composed of chitosan and ethylcellulose versus conventional microspheres composed of chitosan is known and was assigned to EC inducement to the spray-dried system (Martinac et al., 2005).

SEM analysis of the samples revealed that all microspheres prepared had spherical shape and similar surface morphology, regardless of the type and/or polymeric composition of spray-dried systems. Thus, SEM micrographs of the PEB1 promethazine-loaded and promethazine-free microspheres shown in Fig. 1 are representatives for all types of microspheres prepared. Smaller microspheres were characterized by surface indentations that could be attributed to the subsequent shrinking of the microspheres following the formation of the solid crust at the surface of the droplets due to the solvent evaporation (Wang & Wang, 2002). This effect is more evident for promethazine-free than for promethazine-loaded microspheres, since empty microspheres were more shrinked

TABLE 2 The Main Characteristics of Promethazine-loaded and Promethazine-Free Microspheres

Type of spray-dried system	Polymer/drug ratio (w/w)									
	6:1 (EC:CM = 1:2)[a]					8:1 (EC:CM = 1:3)[a]				
	Sample	Yield (%)[b]	Diameter (μm)	Drug loading (%)[c]	EE (%)[d]	Sample	Yield (%)[b]	Diameter (μm)	Drug loading (%)[c]	EE (%)[d]
Solution	PS1[e]	45.9 ± 0.9 (54.0 ± 0.5)	3.31 ± 1.47 (2.93 ± 0.99)	11.1 ± 0.4	77.8 ± 2.5	PS2[e]	48.3 ± 1.1 (54.0 ± 0.5)	3.23 ± 1.31 (2.93 ± 0.99)	9.1 ± 0.6	82.1 ± 5.4
W/O/W (A) emulsion	PEA1	36.9 ± 1.5 (34.2 ± 1.1)	3.77 ± 1.90 (3.43 ± 1.52)	13.4 ± 0.5	93.5 ± 3.7	PEA2	39.3 ± 1.1 (41.8 ± 1.0)	3.85 ± 1.93 (3.60 ± 1.67)	10.2 ± 0.4	95.7 ± 3.4
W/O/W (B) emulsion	PEB1	32.3 ± 1.2 (33.5 ± 0.5)	3.06 ± 1.39 (2.91 ± 1.2)	13.3 ± 0.6	98.2 ± 2.1	PEB2	35.9 ± 1.2 (36.9 ± 1.1)	3.05 ± 1.30 (2.95 ± 0.92)	10.4 ± 0.2	98,9 ± 1.0

Values in brackets refer to promethazine-free microspheres.
[a]EC : CM :weight ratio of EC dissolved in oil phase and chitosan dissolved in outer water phase of W/O/W emulsions.
[b]Product weight/total weight of starting components of the spay-dried system × 100.
[c]Actual drug content/examined quantity of microspheres × 100.
[d]Entrapment efficiency (EE): drug loading/theoretical drug loading × 100.
[e]samples with chitosan as the only polymer in composition.

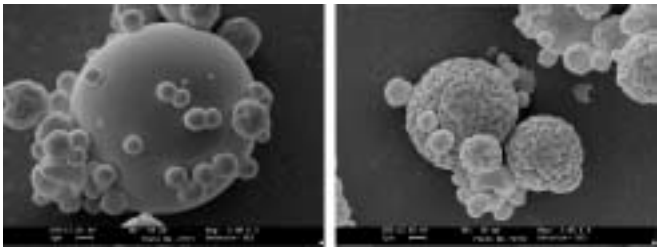

FIGURE 1 **SEM Micrographs of Promethazine-loaded (Left) and Promethazine-Free (Right) Microspheres (PEB1) Prepared by Spray Drying of W/O/W(B) Emulsion With EC/CM Weight Ratio of 1:2.**

than drug-loaded microspheres (Fig. 1). This observation was confirmed by the results obtained from the particle size analysis; the mean diameters of promethazine-loaded microspheres were larger than the mean diameters of promethazine-free microspheres for all types of spray-dried system (Table 2).

Particle size analysis also indicated that the feed concentration influenced the particle size distribution. Thus, spray-dried systems prepared with higher feed concentration (solutions and W/O/W (A) emulsions) had less than 5% of particles smaller than 2 μm, while spray-dried systems prepared with lower feed concentration (W/O/W (B) emulsions) had more than 20% of particles smaller than 2 μm, and consequently had the smallest mean diameters (3.06 ± 1.39 μm for the sample PEB1 and 3.05 ± 1.30 μm for the sample PEB2; Table 2). Influence of the feed concentration on the particle size distribution is well known (Pavanetto et al., 1994; Giunchedi et al., 2002).

Spray drying of solutions produced smaller microspheres than spray drying of double W/O/W (A) emulsions that were of comparable feed concentration (Table 2). Thus, mean diameters of the conventional microspheres (samples PS1 and PS2), were 3.31 ± 1.47 and 3.23 ± 1.31 μm, respectively, while mean diameters of the composed microspheres (samples PEA1 and PEA2), were 3.77 ± 1.90 and 3.85 ± 1.93 μm.

The zeta potential of the promethazine-loaded, promethazine-free microspheres, and EC microparticles that served as control was measured in 10 mM NaCl solution at 25°C (pH 6.7). The results obtained are shown in Fig. 2a. Zeta potential of EC microparticles was –(17.8 ± 1.6) mV. Solutions and W/O/W emulsions spray drying resulted in conventional and composed microspheres with no significant difference in the surface charge. All microspheres prepared were characterized by positive surface charge originating

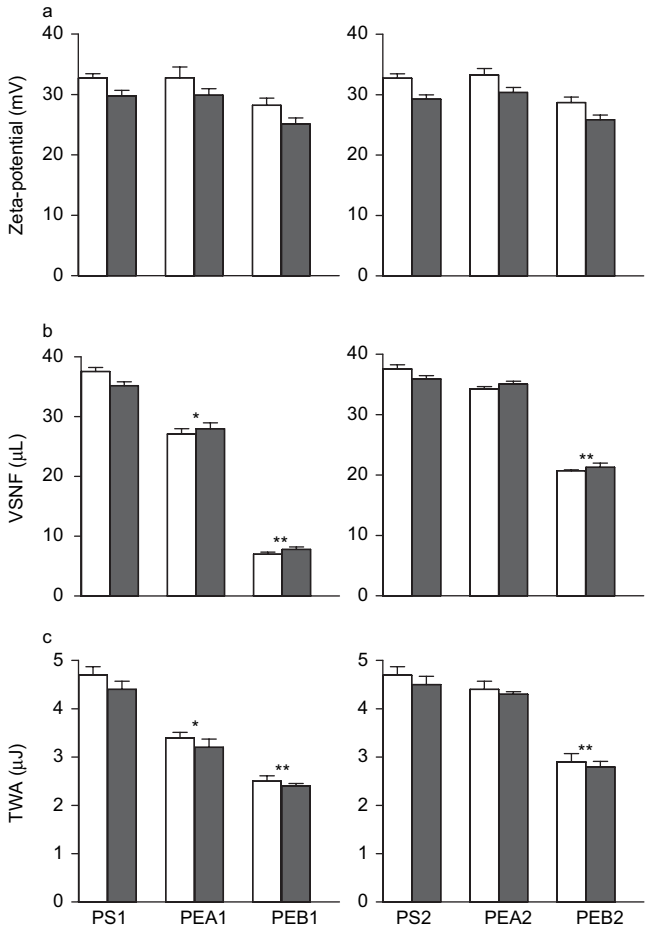

FIGURE 2 **Zeta Potential (a), Swelling (b) and Bioadhesive Properties (c) of Promethazine-Free (□) and Promethazine-loaded (■) Microspheres Prepared by Spray Drying of Solutions (PS), W/O/W(A) Emulsions (PEA) and W/O/W(B) Emulsions (PEB) With Polymer/drug Ratio of 6:1 (PS1, PEA1, PEB1) and 8:1 (PS2, PEA2, PEB2). Indicated Values are Means of at Least Three Experiments ± SD. *Differ from PS Microspheres ($P < 0.05$); **Differ from Other Microspheres ($P < 0.05$).**

from chitosan free amino groups. That observation is of great importance, since positively charged microspheres can interact with negatively charged mucus, exhibiting bioadhesive properties.

The entrapment efficiencies were high, ranging between 77.8 and 98.9% (Table 2). In the case of chitosan solution spray drying, the conventional microspheres prepared with theoretical polymer/drug ratio 8:1 showed higher promethazine entrapment (82.1%; sample PS2) than the conventional microspheres prepared with the theoretical polymer/drug ratio 6:1 (77.8%; sample PS1), indicating that less promethazine was entrapped as polymer/drug ratio decreased. In comparison to these systems composed of chitosan as the only polymer, spray drying of W/O/W emulsions, containing EC in the oil phase, resulted in increased

promethazine entrapment (92.8–98.9%; Table 2). Higher increase in promethazine entrapment was obtained when emulsions were prepared using less concentrated polymer solutions (samples PEB1 and PEB2).

Swelling Studies

Results of swelling studies with promethazine-loaded and promethazine-free microspheres are shown in Fig. 2b. There was no significant difference between swelling properties of promethazine-loaded and promethazine-free microspheres, due to hydrophilic nature of promethazine. Swelling properties of the microspheres prepared depended on the chitosan content in the formulation, since chitosan was the only component of the spray-dried systems with swelling abilities. Thus, promethazine-free conventional microspheres, made of chitosan only (promethazine-free samples PS1 and PS2) absorbed the highest volume of SNF (37.6 ± 1.1 μL/mg).

Swelling ability of the composed microspheres prepared by spray drying of double emulsions depended on the weight ratio of EC dissolved in oil phase and CM dissolved in outer water phase of W/O/W emulsions. Thus, microspheres with higher content of CM (EC/CM 1:3,w/w; samples PEA2 and PEB2) showed better swelling properties than the microspheres with lower content of CM (EC/CM 1:2,w/w; samples PEA1 and PEB1). At the same time, swelling properties of microspheres PEB were lower than swelling properties of microspheres PEA with the same EC/CM weight ratio (Table 2). Such results revealed that the characteristics of matrix formed depended not only on the EC/CM weight ratio, but also on the concentration of the polymeric solutions used for the emulsions preparation. More precisely, since EC/CM weight ratio of corresponding microspheres was kept constant (1:2 and 1:3), and since polymer solutions used for the preparation of emulsions A and B differed in the concentration, different oil to outer water phase volume ratios had to be used (1:8 and 1:12 for W/O/W (A) emulsions and 1:4 and 1:6 for W/O/W (B) emulsions, respectively; Table 1). Due to the relatively high solubility of ethyl acetate in water (8.7%, w/v) diffusion of ethyl acetate from oil phase into the outer aqueous phase occurred during the reemulsification process. The rate of ethyl acetate extraction depended on ethyl acetate to water volume ratio, as reported previously (Meng et al., 2003). It was shown that the volume of the outer water phase in the reemulsification step had a significant effect on the diffusion rate of ethyl acetate from the droplets into outer aqueous solution, and thereby on the characteristics of the resultant microparticles. With the volume increasing, the extraction or removal rate of ethyl acetate increased, resulting in rapid solidification of the microparticles. In this work, due to low ethyl acetate to water volume ratio in case of W/O/W (A) emulsions, rapid extraction of ethyl acetate from the outer perimeter of the dispersed droplets containing ethyl acetate produced a finite thickness barrier of ethylcellulose at the ethyl acetate/continuous phase interface, meaning that hardening of the ethylcellulose started prior to spray drying process which eventually caused EC precipitation. As such a system was kept under continuous stirring conditions, final product was obtained by spray drying of dispersion of EC precipitates within the chitosan solution. In case of W/O/W (B) emulsions ethyl acetate to water volume ratios were higher favoring the formation of EC microcapsules with entrapped promethazine. Such systems with EC microcapsules formed (samples PEB1 and PEB2) have proved to be less hydrophilic than the systems with EC precipitates dispersed in the chitosan matrix (PEA1 and PEA2), and consequently were characterized by moderate swelling properties. This observation could explain improved promethazine encapsulation in case of microspheres PEB compared to microspheres PEA (Table 2).

Bioadhesion Studies

Results of the tensile studies with promethazine-loaded and promethazine-free microspheres are shown in Fig. 2c. For all types of spray-dried systems, there was no significant difference in bioadhesion of promethazine-free and promethazine-loaded microspheres, owing to the hydrophilic nature of the drug. Similar observations in case of hyaluronic acid and chitosan gentamicin-loaded microspheres prepared by solvent evaporation method are known (Lim et al., 2000) where the degree of mucoadhesion was investigated by determining the mucociliary transport across an isolated frog palate. Results showed that the entrapment of hydrophilic gentamicin did not affect the mucoadhesive properties of the microspheres.

The highest total work of adhesion was measured for the conventional promethazine-free microspheres,

consisting of chitosan only (4.7 ± 0.3 μJ), followed by conventional promethazine-loaded microspheres (4.4 ± 0.3 and 4.5 ± 0.3 μJ for the samples PS1 and PS2, respectively). These results were in agreement with the highest swelling abilities of the conventional chitosan microspheres.

The addition of EC altered the bioadhesive properties of the microspheres, as shown also by zeta potential values. Results obtained suggested that EC was partially present at the surface of the composed microspheres, thus altering the surface properties originating from chitosan. However, all microspheres prepared were yet characterized by positive surface charge and sufficient bioadhesive properties (Fig. 2a,c).

Bioadhesive properties of the microspheres prepared by spray drying of double emulsions were also related to their swelling abilities. Thus, microspheres with higher content of CM (EC/CM 1:3, w/w; samples PEA2 and PEB2) were more bioadhesive than the microspheres with lower content of CM (EC/CM 1:2, w/w; samples PEA1 and PEB1), as they swelled better.

At the same time, microspheres PEA were more bioadhesive than corresponding microspheres PEB, regardless of the same EC/CM weight ratios (Fig. 2c). The correlation between swelling and bioadhesion of promethazine-loaded microspheres is shown in Fig. 3. It may be concluded that the bioadhesion properties depended not only on the chitosan content but on the type of spray-dried system as well, since increase in the total work of adhesion was not directly proportional to increase in swelling ability.

In Vitro Drug Release Study

The in vitro promethazine release was carried out using Franz diffusion cell, consisting of a donor and receptor cell that was filled with SNF. A polyamide membrane with 0.45 μm pore size was used to keep the microspheres on the donor side.

The release profiles of promethazine from the conventional chitosan microspheres (PS1 and PS2) revealed that as CM/promethazine ratio increased from 6:1 to 8:1, the release rate of the drug slightly decreased (Fig. 4). As the chitosan content increased, in the process of the hydration of the polymer, the thicker swollen gel layer was formed, and consequently drug diffusion through the thicker layer was slower (Lim et al., 2000).

Promethazine release profiles of the composed microspheres PEA (Fig. 5a) did no differ significantly from release profiles obtained for the conventional microspheres. Despite the EC inducement, the release of promethazine was not prolonged, and similar to conventional microspheres, about 80–90% of the drug was released in 2 h, while t_{50}% was about 30 min. According to the results of swelling studies, composed microspheres PEA were characterized by high swelling ability. Thus, promethazine release rate was determined only by the diffusion through the swollen chitosan matrix since it was not entrapped in EC microcapsules.

Promethazine release profiles of the composed microspheres PEB are shown in Fig. 5b. Release data for the microspheres PEB2 with EC/CM weight ratio of 1:3 were similar to release data obtained for the

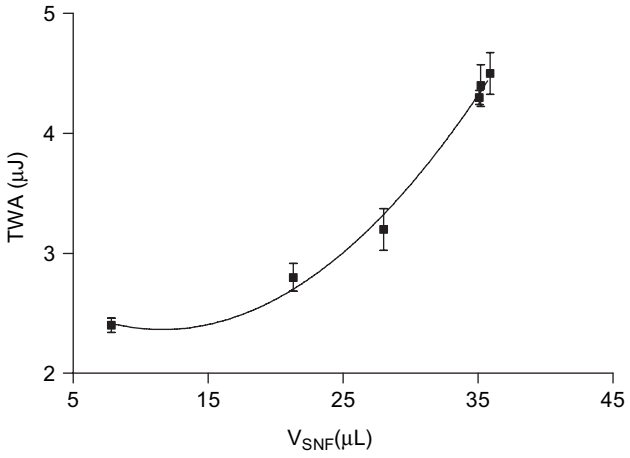

FIGURE 3 **Correlation Between Swelling and Bioadhesive Properties of Promethazine-loaded Chitosan-based Microspheres.**

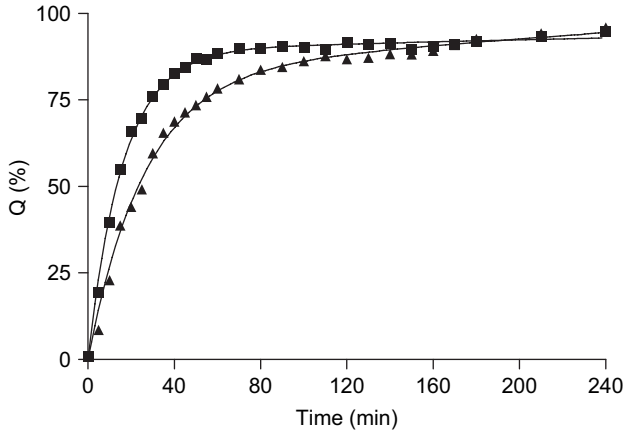

FIGURE 4 **The Release Profiles of Promethazine From the Microspheres Prepared by Spray Drying of Solutions With Chitosan to Promethazine Weight Ratio of 6:1 (■) and 8:1 (▲).**

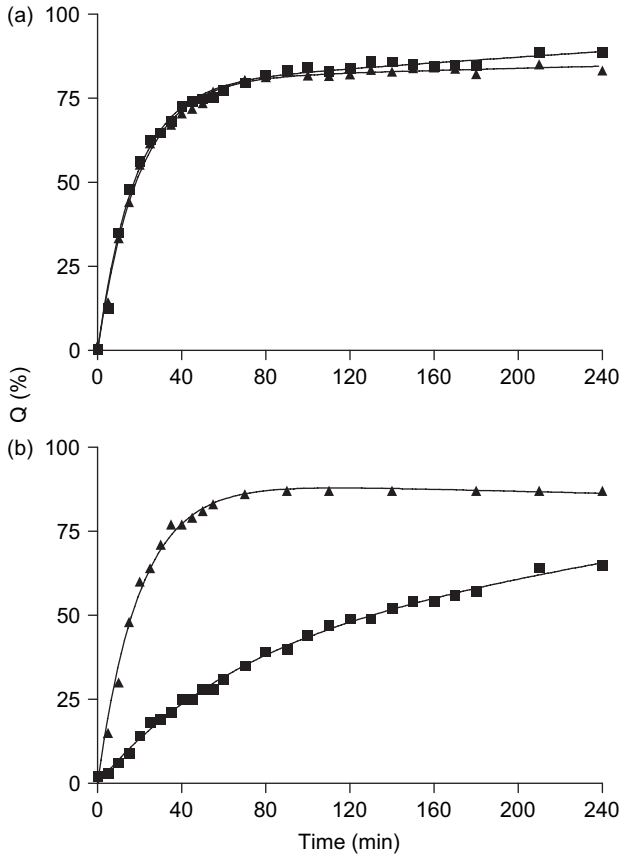

FIGURE 5 **The Release Profiles of Promethazine From the Microspheres Prepared by Spray Drying of W/O/W(A) Emulsions (a) and W/O/W(B) Emulsions (b) with EC Dissolved in the Oil Phase to CM Dissolved in the Outer Water Phase Weight Ratio of 1:2 (■) and 1:3 (▲).**

conventional and PEA composed microspheres. Sustained promethazine release is obtained only with PEB1 microspheres (EC/CM 1:2, w/w). In case of W/O/W (B) emulsions ethyl acetate to water volume ratios were higher (1:4 and 1:6, v/v, for PEB1 and PEB2, respectively), and less EC precipitates were formed than in case of W/O/W (A) emulsions. Thus, more EC microcapsules with entrapped promethazine were formed in the spray drying process, particularly in case of PEB1 microspheres with the highest ethyl acetate to water volume ratio (1:4). That could explain the slowest promethazine release rate from PEB1 microspheres. In addition, PEB1 microspheres with EC/CM ratio of 1:2, were characterized by moderate swelling ability which retarded promethazine release as well.

CONCLUSION

Considering these results, spray drying of W/O/W double emulsions have proved to be a good method to produce composed microspheres with moderate swelling, preserved bioadhesive properties and higher promethazine entrapment, compared to conventional chitosan microspheres. It seems like increased ethyl acetate to water volume ratio in less concentrated W/O/W(B) emulsion systems compared to W/O/W(A) emulsion systems resulted in the formation of EC microcapsules inside the chitosan matrix and improved promethazine encapsulation within the spray-dried product. Thus, in case of such a system, with EC/CM weight ratio of 1:2, prolonged promethazine release was obtained.

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