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A novel multicompartimental system based on aminated poly(vinyl alcohol) microspheres/succinoylated pullulan microspheres for oral delivery of anionic drugs

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Abstract

Poly(vinyl alcohol) (PVA) microspheres were prepared by dispersion reticulation with glutaraldehyde and further aminated. These microspheres were firstly loaded with diclofenac (DF) and then entrapped in cellulose acetate butyrate (CAB) microcapsules by an o/w solvent evaporation technique for intestinal delivery of drug. The encapsulated PVA microspheres due to their low swelling degree in intestinal fluids, do not have enough force to produce the disruption of CAB shell, therefore different amounts of succinoylated pullulan microspheres (SP-Ms) (exchange capacity up to 5.2 meq/g) were co-encapsulated. The SP-Ms do not swell in acidic pH, but swell up to 20-times in intestinal fluids causing the rupture of CAB shell and facilitating the escape of loaded PVA microspheres.

Keywords: Multicompartimental systems; Ion exchange resin; Microencapsulation; Drug delivery systems

1. Introduction

Drug delivery systems enabled to precisely control the release rate or target drugs to a specific body site have an enormous impact on the healthcare. Among them, the administration of drugs to small intestine and colon by the oral route still remains one goal towards which numerous biotechnology companies are striving for (Gursoy and Benita, 2004; Kang et al., 2004; Xing et al., 2003; Krishnaiah et al., 2002). The main advantages presented by oral drug delivery are the ease of target accessibility, enhanced patient compliance owing to the non-invasive delivery method, and the possibility of local and systemic therapy.

Carrier technology offers an intelligent approach for oral drug delivery by coupling the drug to a carrier particle such as microspheres, nanoparticles, liposomes, etc., which modulates the release and the absorption characteristics of the drugs (Sánchez et al., 2003; Foss et al., 2004; Taira et al., 2004; Yamabe et al., 2003). Polymeric microspheres represent an important part of these particulate drug delivery systems due to their small size and efficient carrier characteristics. For instance, polymeric microspheres can be easily functionalized with anionic or cationic groups allowing high loading of drugs possessing opposite charges (Ouchi et al., 2002; Aral and Akbuga, 2003; Mocanu et al., 2004; Singh et al., 2004). In addition, the electrostatic interactions between drug and polymer increase the chemical stability or mask the unpleasant taste of the drugs (Borodkin and Sundberg, 1971).

However, the major drawback of this approach is the rapid release of drug within gastric fluids, before reaching the small and large intestine (Sriwongjanya, 1996).

In order to solve this problem different multicompartimental systems, obtained by polymer coating or microencapsulation, were proposed. The major part of authors used for coating or encapsulation of resins pH-sensitive polymers (Chourasia and Jain, 2004; Debunne et al., 2002; Rodriguez et al., 1998).

Previously, our research group have proposed microencapsulation of the loaded resins in CAB microcapsules. Consequently, after encapsulation the drug was not released in acidic buffer

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(resembling stomach pH), but neither at higher pH typical of intestine. Therefore, different strategies have been proposed to produce the release of drugs in intestine. In a first attempt we investigated the release of tetracycline from encapsulated sulfopropylated dextran microspheres and we found that the progressive swelling of the anionic resins in phosphate buffer at pH 7.4 producing the rupture of CAB shell and the escape of resins could allow the release of the drug (Fundueanu et al., 2005). In a second attempt, we encapsulated aminated poly(vinyl alcohol) (PVA) microspheres loaded with plasmid DNA for colonic delivery of the nucleic acids. Since aminated resins do not swell enough at intestine pH to produce the rupture of CAB shell, we incorporated in the network of microcapsules different amounts of enteric polymers (based on pH/thermo-sensitive polymers). The escape of loaded resins was possible through the halls created by their dissolution in intestinal fluids (Fundueanu et al., 2006).

In this paper, we proposed a new multiparticulate system based on cellulose acetate butyrate (CAB) microcapsules containing aminated PVA microspheres loaded with diclofenac (DF). Since the CAB is not an enterosoluble polymer and loaded PVA microspheres do not swell enough to produce the rupture of CAB shell neither in gastric nor in intestinal fluids, different amounts of succinoylated pullulan microspheres (SP-Ms) were together encapsulated. Therefore, the release of DF in intestinal fluids was possible due to the rupture of CAB shell under the pressure caused by the high swelling degree of SP-Ms. In acidic pH, these microspheres do not swell and therefore the drug is not released.

2. Materials and methods

2.1. Materials

PVA (M_w = 18,000 g/mol; hydrolysis mole = 98.4%) was purchased from Air Products and Chemicals Inc. (Utrecht, The Netherlands). Pullulan (Pul), M_w = 200,000 g/mol was purchased from Hayashibara Laboratories Ltd. (Okayama, Japan). Cellulose acetate butyrate, low viscosity, was purchased from Eastman Inc. (Kingsport, Tennessee, USA). Phospholipon 90G (phosphatidylcholine) (PC), was supplied by Rhône-Poulenc Rorer (Kôln, Germany). Glutaraldehyde (GA) (2.6 M aqueous solution) was supplied by Fluka AG (Seelze, Germany). *N*,*N*-Diethyl-*N*-(2-chloroethyl)amine hydrochloride (DCA HCl) was purchased from Fluka, AG (Buchs, Switzerland). Succinic anhydride (SA) (Fluka, AG) was purified by dissolving in chloroform and refluxing. 4-Dimethylaminopyridine (DMAP) was supplied by Fluka AG (Seelze, Germany). Sodium diclofenac (DF), used as model drug, was kindly supplied from Iassy Pharm (Iassy, Romania). All the other regents were purchased from Fluka at the highest purity grade available.

2.2. Preparation of PVA microspheres

PVA microspheres were synthesized by a suspension reticulation procedure with glutaraldehyde after a method previously reported (Fundueanu et al., 2006).

Briefly, 4 g of PVA was dissolved in 20 ml hot water. The solution was acidified with 1.6 ml 0.5 M H_2SO_4 solution, and then poured in 100 ml of disperding medium (1,2-dichloroethane) containing 1 g of CAB (as the dispersion agent).

This water/organic solvent emulsion was stirred for 30 min (stirring speed = 750 rpm), then 1–2 ml of GA was added, and the cross-linking reaction was carried out for 4 h at 50 °C. The cross-linked microspheres were recovered by filtration through a sintered glass filter, under vacuum. The removal of residuals was performed by washing the microspheres in the following order: 1,2-dichloroethane, acetone, hot water, cold water, methanol. Then, the microspheres were completely dried by overnight exposure to 60 °C, under vacuum.

2.3. Cationization of PVA microspheres

2.92 g (18 mmol) of PVA microspheres were swollen in 10.6 ml NaOH solution (50%, w/v). Thereafter, 11.42 g (66 mmol) DCA HCl were solubilized in 7 ml distilled water and added to the suspension. The mixture was left to react for 8 h under stirring at 80 °C. After cooling at room temperature, the microspheres were filtered and consecutively rinsed with water, 0.1 N HCl, water, methanol and dried from diethylether.

2.4. Determination of microsphere exchange capacity

The anion exchange capacity of cationized PVA microspheres was determined under dynamic conditions by standard methods (Helfferich, 1962).

Briefly, 50 mg of cationic microspheres were swollen in water into a glass column. A 0.1N NaOH solution (50 ml) was passed through the column and the microspheres in the –OH form, were washed with water until neutrality of the collected eluate. Thereafter, 50 ml 5% NaCl solution and 50 ml water were successively passed on the microsphere bed and the collected eluate was titrated with a 0.1N HCl solution. The content of quaternary ammonium groups was calculated using the following equation:

$$\mathrm{EC\ (meq/g)} = \left(\frac{n_{\mathrm{HCl}}}{a}\right) \times 0.1 \quad (1)$$

where EC is the exchange capacity, n_{HCl} the volume (ml) of HCl solution used for titration, and a is the weight of the microspheres. To determine the content of tertiary amino groups, 50 ml 0.1N HCl solution, 50 ml water, and 50 ml methanol were successively passed through the column, and the collected eluate was titrated with a 0.1N NaOH solution. The exchange capacity was calculated as follows:

$$\mathrm{EC\ (meq/g)} = \left[\frac{n_{\mathrm{HCl}} - n_{\mathrm{NaOH}}}{a}\right] \times 0.1 \quad (2)$$

2.5. Drug loading

Aminated PVA microspheres were loaded as follows: previously swollen, empty microspheres were placed in a chromatographic column and allowed to slowly settle and pack. A 0.1N NaOH aqueous solution was then passed through the packed column, and the microspheres in the –OH form were washed

with water. Thereafter, an excess of 0.1 M DF solution in warm water (3 mmol DF: 1 meq exchange capacity) was passed on the microspheres. Then, the loaded microspheres were extensively washed with water. The same protocol was used to load the microspheres in the –HCl form, with the exception that 0.1N NaOH solution was replaced with 0.1N HCl solution.

The amount of ionically linked drug was determined by difference, evaluating the amount of DF in the washing waters by spectrophotometrical analysis using a calibration curve.

2.6. Preparation of SP-Ms

Pullulan microspheres were prepared by suspension cross-linking procedure with epichlorohydrin with minor modification of the method previously reported by us (Fundueanu et al., 2003). Two grams of pullulan were dissolved in 10 ml NaOH solution (10%, w/v) under stirring, in the presence of 50 mg of $NaBH_4$. After a complete removal of the air bubbles under vacuum, the solution was poured in 50 ml of dispersion medium (1,2-dichloroethane) in which 1.2 g of CAB (as dispersion agent) were dissolved. The obtained w/o emulsion was stirred for 1 h, then 2 ml of ECH were added and the cross-linking reaction was carried out for 20 h at 50 °C. The cross-linked microspheres were recovered by filtration through a sintered glass filter, under vacuum. The removal of residuals was performed by washing the microspheres in the following order: 1,2-dichloroethane, acetone, water/acetic acid solution (30%, v/v), water, and methanol. Then, microspheres were completely dried after overnight exposure to 60 °C, under vacuum.

After preparation, the pullulan microspheres were carboxylated by succinoylation. The synthetic scheme for the preparation of SP-Ms is depicted in Fig. 1.

One gram (6.2 mmol) of pullulan microspheres (d = 60–120 μm) was swollen in 20 ml DMSO. Then, 2 g (20 mmol) of succinic anhydride and 0.2 g (1.85 mmol) DMAP were solubilized in 12 ml DMSO and added to the suspension. The mixture was left to react for 24 h under stirring at 50 °C. Finally, the microspheres were filtered and consecutively rinsed with DMSO, ethanol/water (1:1, v/v), methanol, and dried from diethylether.

The exchange capacity was determined by titrimetric analysis and gravimetrically from the increment of the weight of pullulan microspheres after succinoylation.

$$\mathrm{EC\ (meq/g)} = \frac{1000\mathrm{DS}}{162 + 99\mathrm{DS}} \tag{3}$$

$$\mathrm{DS} = \frac{162b}{100a - 99b} \tag{4}$$

where EC is the exchange capacity of the SP-Ms, DS the degree of substitution, a the weight of pullulan microspheres after succinoylation, b the increment of the weight of pullulan microspheres after succinoylation, 162 the molecular weight of pullulan and 100 is the molecular weight of succinic anhydride.

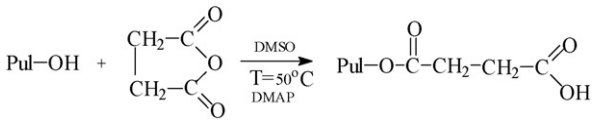

Fig. 1. Reaction of pullulan with succinic anhydride.

2.7. Preparation of CAB microcapsules

CAB microcapsules containing aminated PVA microspheres loaded with DF were obtained by an o/w solvent evaporation technique after a method developed by us with minor modifications (Fundueanu et al., 2005).

Two hundred milligrams of CAB plus 5 mg of phospholipon were dissolved in 1 ml chloroform, then 0.3 ml cyclohexane were added under stirring, as an inert solvent. Thereafter 200–150 mg of DF loaded PVA microspheres and 0–50 mg of SP-Ms were suspended for 2 min in the polymer solution. The obtained homogeneous suspension was dispersed, at a stirring speed of 450 rpm, into an external aqueous phase (50 ml, 1%, w/v PVA, 88% hydrolyzed) using an open cylindrical reactor (h = 120 mm, d = 60 mm), and a three blade turbine impeller. The encapsulation process started at 25 °C for 2 min, then the temperature was raised up to 50 °C, and the process continued for further 30 min. The obtained microcapsules were separated by filtration, washed with 100 ml water, and finally dried under vacuum at 50 °C.

2.8. Microcapsule drug loading

The amount of DF in CAB microcapsules was determined after dissolution of 25 mg of microcaspules in 2 ml chloroform followed by extraction with 100 ml phosphate buffer, pH 7.4. The amount of DF was determined by UV–vis spectrophotometric analysis using samples previously centrifuged for 10 min at 10,000 rpm, and expressed as weight of drug (mg)/weight of microcapsules (mg) × 100. The encapsulation efficiency of the drug was calculated as the ratio between the actual drug content and the theoretical drug content, and expressed as percentage.

2.9. Morphological and dimensional analysis

Microcapsule morphology was evaluated by optical and electron microscopy. Dried microcapsules were analyzed at 15–20 kV by scanning electron microscopy (SEM) (Cambridge S 360) after metallization by gold coating (Edwards Sputter coating S 150). Size and size distribution were evaluated by optical microscopy using an inverted microscope (Nikon Diaphot, Tokyo, Japan) equipped with a digital camera. Microcapsule size was determined by examining the microcapsule diameter on digital photomicrographs, considering at least 200 microspheres for each sample.

2.10. Swelling degree

The increase in volume of the PVA microspheres (free and loaded with DF) was determined at the equilibrium, placing the microspheres in an acid buffered solution at pH 1.2 (50 mM KCl + 64 mM HCl), phosphate buffered solution at pH 7.4 (20 mM NaH_2PO_4 + 80 mM Na_2HPO_4) or in demineralized water. The volume of the swollen microspheres (V_s) reported to

the dried volume (V_d) measured by placing the microspheres in a graduated cylinder ($d = 12$ mm), was defined as swelling factor ($q = V_s/V_d$).

2.11. In vitro drug release studies

In vitro drug release studies were determined by the bath method (Zografi et al., 1990), using different buffered solutions simulating the gastric juice (pH 1.2, KCl + HCl) or intestinal fluid (pH 7.4, $NaH_2PO_4 + Na_2HPO_4$).

Samples of the receiving buffer were withdrawn at different time intervals and the drug content was spectrophotometrically determined. The same volume of fresh receiving buffer was added to replace the volume of the withdrawn samples. During the entire release process, the morphological changes in the structure of CAB microcapsules were recorded by an optical microscope equipped with a digital camera.

Drug release kinetics were analyzed according to:

$$Y = Y_{max} \times (1 - e^{-kt}) \quad (5)$$

where Y is the time-dependent molar fraction of the released drug, Y_{max} the molar fraction of the finally released drug, and k ($=0.693/t_{1/2}$) is the first order rate constant for drug release.

3. Results and discussions

3.1. Preparation and characterization of aminated PVA microspheres

PVA microspheres were prepared by suspension cross-linking procedure with glutaraldehyde (Fig. 2, reaction 1).

The reaction was carried out in a water/organic solvent suspension using a 18.5% (w/v) acidified aqueous polymer solution. After preparation, cross-linked PVA microspheres were treated with *N*,*N*-diethyl-*N*-(2-chloroethyl)amine (Cl-A) (Fig. 2, reactions 2 and 3). During the amination, two successive reactions occur: the esterification of the hydroxyl groups of the PVA (reaction 2), and the quaternization of the newly introduced amino group (reaction 3). Therefore, the resulting derivatized microspheres possess three types of basic groups with different pK_b: two groups of moderate basicity and a strong quaternary ammonium basic group. The produced microspheres are characterized as evidenced by electron microscopy by a good spherical geometry and a smooth surface (data not shown), also the dimension of the major part of microspheres are situated between 50 and 160 μm. The main characteristics of the aminated microspheres are presented in Table 1.

3.2. Drug loading

In order to evaluate the ability of cationic microspheres to complex efficiently DF a series of binding experiments were performed (Table 1). Therefore, the cationic microspheres were prepared for loading either in –OH form and HCl form as follows:

–OH form of the strong quaternary ammonium group and free base of those with moderate basicity.
–HCl form of the amine with moderate basicity and Cl^- form of the strong quaternary ammonium group.

As shown in Table 1, the cationic microspheres bind more efficiently DF when the amine are in HCl form. Also, at lower cross-linking degrees the amount of loaded DF is slightly higher because of an increased accessibility of the sites of interaction in a more swellable matrix. It should be noticed firstly, the difference between swelling degree of the free and loaded PVA microspheres in water, this feature represents one of the key factors for the successful encapsulation of microspheres.

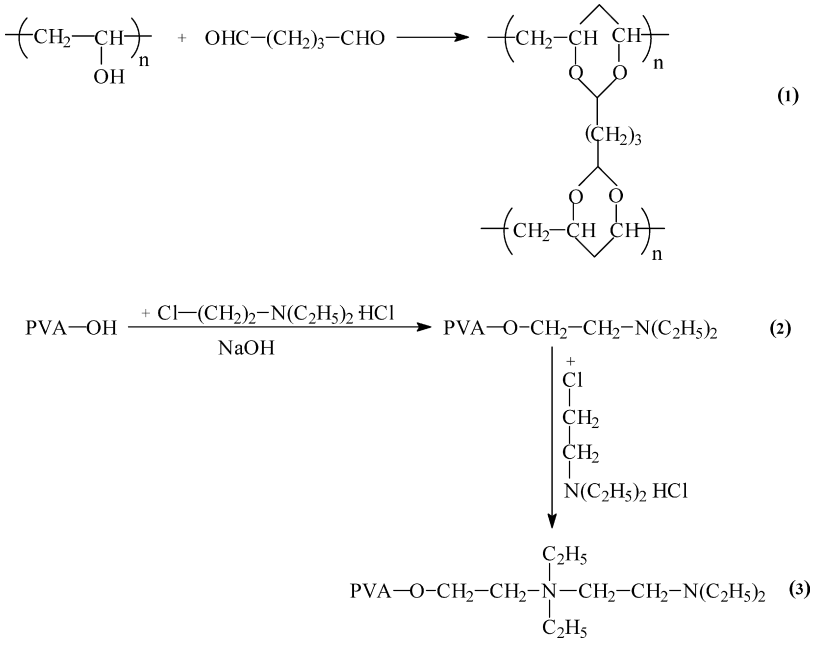

Fig. 2. Preparation of cross-linked and aminated PVA microspheres.

Table 1
Main characteristics of aminated PVA microspheres with and without bound DF

Sample code	Diameter (μm)	DC[a]	Exchange capacity (meq/g)		DF bound (%, w/w)		Efficiency (%)		Swelling degree ($q = V_s/V_d$)[b]		
			Moderate basicity	Strong basicity	–OH form	–HCl form	–OH form	–HCl form	H_2O	pH 1.2	pH 7.4
S_1	50–160	10	1.62 ± 0.12	1.33 ± 0.11					4.0 ± 0.2	3.6 ± 0.21	3.8 ± 0.18
S_1 + DF					20.2 ± 1.6	24.6 ± 2.2	42 ± 3.3	51.1 ± 4.5	2.2 ± 0.12		
S_2	50–160	20	1.48 ± 0.10	1.07 ± 0.08					1.8 ± 0.09	1.6 ± 0.11	1.7 ± 0.12
S_2 + DF					18.5 ± 1.1	20.9 ± 2	41.6 ± 2.6	47.1 ± 4.6	1.4 ± 0.10		

Data are the results of three independent experiments.
[a] DC, degree of cross-linking expressed as PVA/GA weight ratio.
[b] Swelling degree was determined in –HCl form of the microspheres.

Secondly, we intentionally used aminated microspheres with relatively high degree of cross-linking in reason of their low swelling degree even in gastric juice (protonated form) and impossibility to produce by their own swelling the rupture of CAB shell.

3.3. Preparation and characterization of succinoylated pullulan microspheres

Succinoylated pullulan microspheres (SP-Ms) were prepared taking into account their remarkable characteristics evidenced by high difference of swelling degrees in gastric (pH 1.2) with respect to intestinal fluids (pH 7.4).

SP-Ms with two different degree of acylation, was prepared by controlled reaction of pullulan with succinic anhydride (see Fig. 1) after a method previously reported in the literature (Bruneel and Schacht, 1994).

In this work, the succinoylation was performed in DMSO at 50 °C for 24 h using 4-dimethylaminopyridine as catalyst. The degree of esterification was controlled by the ratio of succinic anhydride to pullulan microspheres. The degree of succinoylation was determined by the titrimetric determination of the carboxylic groups as well as by gravimetric analysis. The characteristics of SP-Ms are given in Table 2. The remarkable difference between swelling degree in gastric (pH 1.2) and intestinal fluid (pH 7.4) was the most important factor for choosing these microspheres as additive for intestinal delivery of DF. This high difference is principally attributed to the higher exchange capacity of the pullulan microspheres. Practically, almost two –OH groups of the glucopyranoside unit were esterificated obtaining a high density of carboxylic groups. Therefore, in acidic medium these microspheres become unswellable because of the formed hydrogen bonds between carboxylic groups in their hydrogen form. In addition, the carbonyl group introduced by succinoylation adds a supplementary contribution to the forming hydrogen bonds increasing the hydrophobic character of pullulan microspheres. On the contrary, in intestinal fluid (pH 7.4) the hydrogen bonds are destroyed and the carboxylic groups are in ionic form, therefore these microspheres are characterized by a very high swelling degree.

3.4. Preparation of CAB microcapsules

CAB microcapsules containing aminated PVA microspheres loaded with DF were obtained by an o/w solvent evaporation procedure (Fundueanu et al., 2005). As it was stated the key factor for the successful encapsulation of PVA microspheres was the low swelling degree of loaded PVA microspheres (Table 1). Also, high percentage of DF loaded microspheres decrease the hydrophilic character of the PVA microspheres and therefore reduces the pulling out of the particles within the aqueous phase.

A first set of experiments was performed to determine the new optimal standard parameters such as type of solvents and polymer concentration, stirring speed, solvent evaporation temperature, amount of entrapped PVA microspheres. The optimal experimental parameters set up resulted in the formation of CAB microcapsules with properties suitable to pharmaceutical applications such as spherical shape, percentage of recovery, encapsulation efficiency, and drug release. Good results in terms of recovery, shape, size distribution, and encapsulation efficiency were obtained using chloroform as volatile solvent, cyclohexane as inert solvent, a CAB concentration of 20% (w/v), a stirring speed of 450 rpm, and a solvent evaporation temperature of 50 °C (Table 3). Also, the maximum amount of encapsulated PVA microspheres loaded with DF should not exceed 200 mg.

Table 2
Influence of succinic anhydride/pullulan weight ratio on the characteristics of the SP-Ms

Sample code	SA/Pul (w/w)	EC[a] (meq/g)	DS[b] determined		Swelling degree		
			Titrimetrically	Gravimetrically	H_2O	pH 1.2	pH 7.4
T #1	1	3.12 ± 0.2	0.71 ± 0.11	0.69 ± 0.12	1.6 ± 0.12	1.4 ± 0.13	9.2 ± 1
T #2	2	5.27 ± 0.2	1.78 ± 0.15	1.79 ± 0.18	1.4 ± 0.11	1.3 ± 0.10	21 ± 2

Data are the results of two independent experiments.
[a] EC, exchange capacity.
[b] DS, degree of substitution.

Table 3
Preformulatory studies: experimental parameters and microcapsule characteristics[a]

Sample code	$CHCl_3$ (ml)	CyH (ml)	Amount of CAB (mg)	Type of encapsulated Ms	Recovery (%)	DF content in Ms (%, w/w)	Encapsulated efficiency (%)	Diameter (μm)	Note
M #1	1	0.3	200	S_1 + DF (200 mg)	86.2 ± 7	12.8 ± 1.2	104 ± 9.7	365 ± 23	Sperical shape, large size distribution, no aggregation
M #2	1	0.3	200	S_2 + DF (200 mg)	92.5 ± 5	11.9 ± 0.7	116.4 ± 6.9	340 ± 28	Sperical shape, large size distribution, no aggregation

Data are the results of three independent experiments.
[a] The preparation of CAB microcapsules was performed at 50 °C and a stirring speed of 450 rpm.

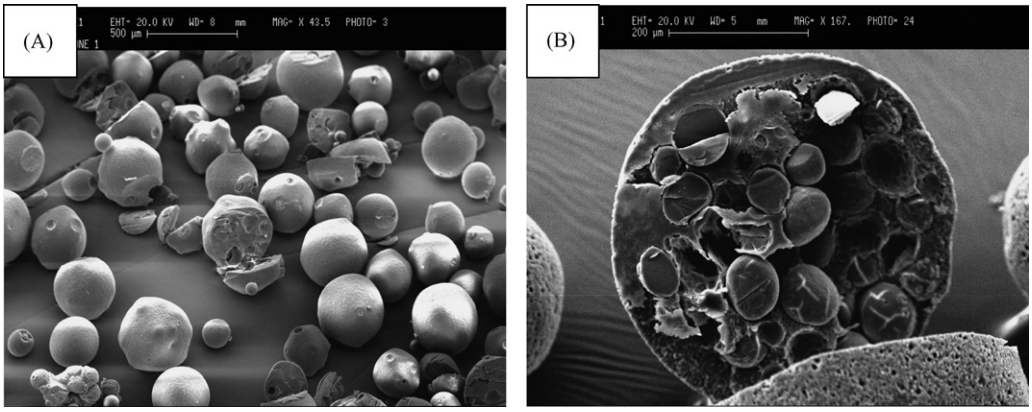

Fig. 3. Scanning electron micrographs of CAB microcapsules (sample P #9) containing both aminated PVA and SP-Ms: general view (A), and cross-section (B).

It should be noticed once again that a high viscosity of the organic phase reduces the partition of the loaded microspheres in aqueous phase during encapsulation. Also, the addition of 30% by weight of cyclohexane, miscible with chloroform but non-solvent for CAB, increase the volume of the organic phase with no significant decrease of the viscosity (Fundueanu et al., 2005), resulting in an increase of resin entrapment up to 200 mg. Moreover, the presence of the cyclohexane in the pores of the forming microcapsules after complete evaporation of chloroform (being bp of cyclohexane higher than that of chloroform) avoids the intimate contact between encapsulated resins and aqueous disperding medium, therefore the microspheres are encapsulated in the dried state (Fig. 3). However, after entrapment the loaded PVA microspheres do not swell enough to assure a pressure necessary to produce the rupture of CAB shell in intestine. Therefore, different amounts of SP-Ms with two different swelling degrees

Table 4
Influence of the percentage and exchange capacity of the co-encapsulated SP-Ms on the characteristics of CAB microcapsules containing loaded PVA microspheres with two different cross-linking degrees: PVA/GA weight ratio, respectively, 10 (section A) and 20 (section B)

Sample code	Amount of encapsulated S_1 + DF Ms (mg)	Amount of encapsulated SP-Ms (mg)	Recovery (%)	DF content in microcapsules (%, w/w)		Encapsulated efficiency (%)	Diameter (μm)
				Actual	Theoretical		
Section A							
P #1	190	10 (T #1)	84.0	11.20	11.68	95.9	341 ± 23
P #2	170	30 (T #1)	80.2	9.46	10.45	90.5	332 ± 33
P #3	150	50 (T #1)	78.8	8.49	9.22	92.0	350 ± 21
P #4	190	10 (T #2)	82.1	10.40	11.68	89.0	345 ± 24
P #5	170	30 (T #2)	80.0	9.20	10.45	88.0	360 ± 18
P #6	150	50 (T #2)	75.2	8.40	9.22	91.2	367 ± 17
Section B							
P #7	190	10 (T #1)	89.0	10.20	9.96	102.4	360 ± 35
P #8	170	30 (T #1)	86.2	8.60	8.91	96.5	362 ± 33
P #9	150	50 (T #1)	84.8	7.53	7.86	95.8	348 ± 28
P #10	190	10 (T #2)	88.1	9.6	9.96	96.3	345 ± 24
P #11	170	30 (T #2)	84.4	8.3	8.91	93.1	330 ± 32
P #12	150	50 (T #2)	82.3	7.20	7.86	91.6	370 ± 15

Data are the results of two independent experiments.

Table 5
Values of kinetic parameters for DF release[a]

Figure	Symbol	$t_{1/2}$ (h)	k (h^{-1})	Y_{max} (%)
Fig. 4	Filled squares	0.19	3.6	98.2
	Filled diamonds	0.62	1.1	97.3
	Filled triangles	2.8	0.24	46.0
	Asterisks	1.7	0.39	16.6
Fig. 5	Filled circles	1.54	0.45	75.7
	Filled triangles	1.7	0.40	51.1
	Filled squares	1.98	0.36	26.5
	Filled diamonds	1.75	0.40	16.6
	Open circles	0.38	1.84	3.8
Fig. 7	Filled circles	1.4	0.49	89.1
	Filled triangles	1.5	0.45	75.7
Fig. 8	Open circles	1.1	0.63	91.6
	Filled triangles	1.5	0.45	75.7

[a] For experimental details, see text.

(exchange capacity) were together encapsulated (Table 4A and B). An increased amount of encapsulated SP-Ms leads obviously to a decrease of drug content in CAB microcapsules because the total amount of encapsulated microspheres should not overpass 200 mg. On the other hand, the encapsulation efficiency slightly decreases since during co-encapsulation of SP-Ms, the carboxyl groups presented at the surface of microspheres could displace a small part of electrostatically bound DF. The higher the exchange capacity of the encapsulated SP-Ms, the higher the amount of dislocated DF and therefore the lower is the encapsulation efficiency. The cross-linking degree of aminated PVA microspheres slightly influences the microcapsule recovery. A higher cross-linking degree means a lower swelling degree (a decreased hydrophilicity) and therefore an easier entrapment of loaded PVA microspheres. During preparation, the pulling out of the microspheres situated at the peripheral site of microcapsules, within dispersion medium is lower for microspheres with higher cross-linking degree. However, these microspheres could alter the sphericity of the microcapsules (Fig. 3A).

3.5. Release studies

As previously stated (Fundueanu et al., 2005) the encapsulation of microspheres (loaded aminated PVA microspheres + SP-Ms) was possible due to the absence of an intimate contact between microspheres and the aqueous dispersion medium, realized by the presence of an inert solvent in the pores of the forming microcapsules. Also, after loading the aminated PVA microspheres become more hydrophobic with a lower swelling degree and therefore the partition of microspheres to the aqueous phase is more reduced. Moreover, SP-Ms in their protonated state are unswellable and less hydrophilic being easily encapsulated.

On the contrary, the release of drug should be favored by the access of the release fluid to the encapsulated microspheres followed by their extension.

The release profiles of DF from unencapsulated and encapsulated PVA microspheres in the absence of SP-Ms are depicted in Fig. 4. As can be easily seen, after 1 h the drug is released almost quantitatively even from microspheres with a higher

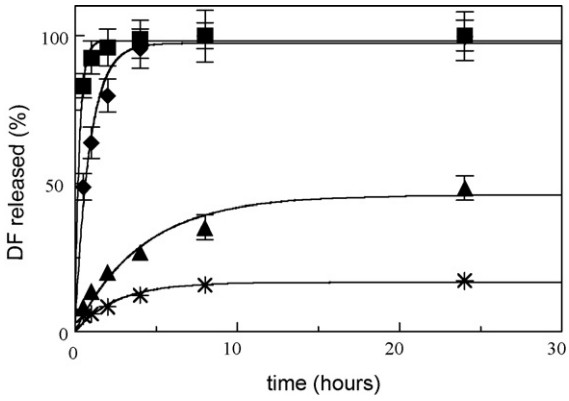

Fig. 4. Release profiles of DF in phosphate buffer at pH 7.4 from encapsulated PVA microspheres with 10 (▲), and 20 (✳) degree of cross-linking, in the absence of SP-Ms (samples M #1 and M #2, respectively). For comparison, the release profiles of DF from un-encapsulated PVA microspheres are depicted with 10 (■) and 20 (♦) degree of cross-linking (samples S_1 + DF and S_2 + DF, respectively). The continuous lines were calculated according to Eq. (5) with sets of parameters given in Table 5.

cross-linking degree. Oppositely, the amount of DF released from encapsulated microspheres in the absence of SP-Ms is low even after 24 h. The pressure created by their own swelling is not enough to produce the rupture of CAB shell and to facilitate the escaping of loaded PVA microspheres. However, a small amount of drug is released probably from the microspheres located at the periphery of microcapsules. In order to increase the amount of released drug in intestine, different amounts of SP-Ms were added keeping the total amount of encapsulated microspheres at 200 mg. As it was established, SP-Ms do not swell in acidic pH, but swell 10–20-fold in intestine causing the rupture of CAB microcapsules and faciliting the escape of loaded microspheres. Therefore, a higher amount of co-encapsulated SP-Ms caused a higher pressure against CAB shell and an easier escape of aminated PVA microspheres loaded with DF. In the gastric juice, the amount of released DF is very low (Fig. 5).

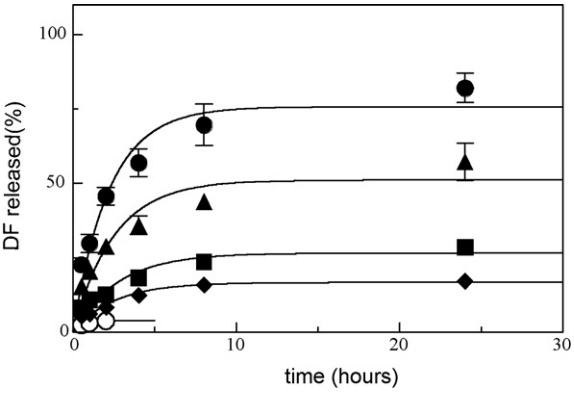

Fig. 5. Release profiles of DF in phosphate buffer at pH 7.4 from encapsulated PVA microspheres (S_2 + DF) in the presence of different percentage of SP-Ms (T #1): 0% (♦) (sample M #2), 5% (■) (sample P #7), 15% (▲) (sample P #8), and 25% (w/w) (●) (sample P #9). For comparison, the release profile of DF in gastric fluid is depicted, pH 1.2, from encapsulated PVA microspheres in the presence of 25% (w/w) SP-Ms (◯) (sample P #9). The continuous lines were calculated according to Eq. (5) with sets of parameters given in Table 5.

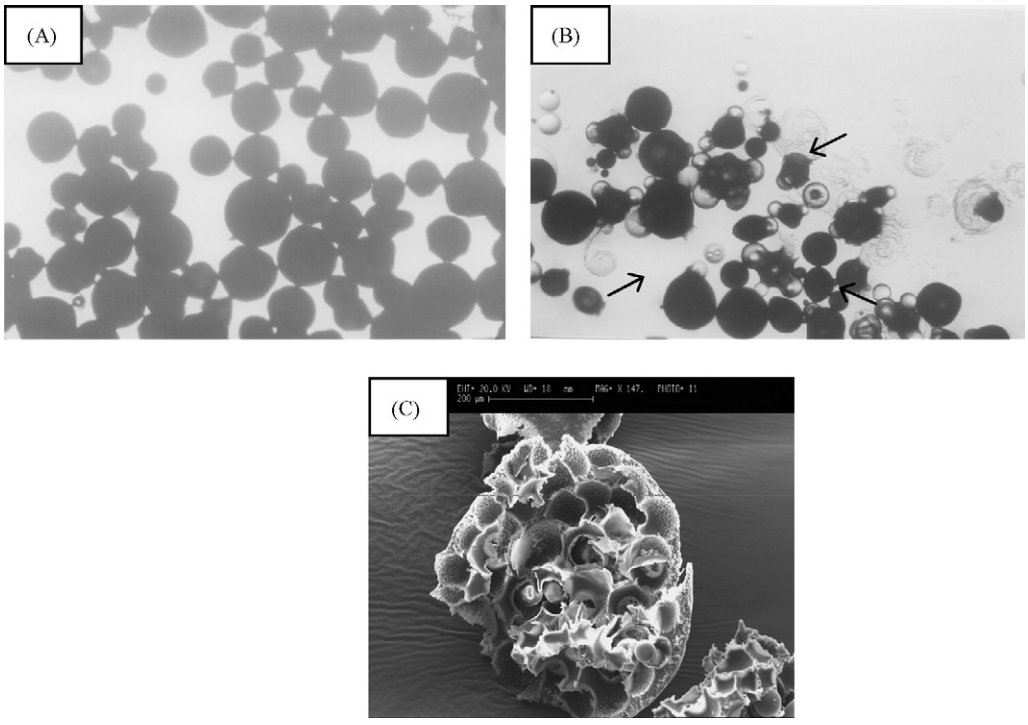

Fig. 6. Optical photomicrographs taken during release studies. Photomicrographs were taken at 5 min (A) and 1 h (B) after incubation in phosphate buffer, pH 7.4, $T = 25\,^{\circ}C$ (sample P #9). The arrows indicate the escape process of SP-Ms. Scanning electron microscopy of the microcapsule after release (C).

However, contrary to our expectation, the CAB microcapsules do not puff up within intestine under the pressure of swollen SP-Ms (even for those with higher swelling degree) because a part of these microspheres can "sneak" away through the halls smaller than their diameter in the maximum swollen state due to their hydrophylicity and flexibility (Fig. 6A and B). Therefore, even if the difference between swelling degrees of the two co-encapsulated SP-Ms is enough high, the release rate are very close (Fig. 7). After disruption of CAB microcapsules the escape of each loaded PVA microsphere (and therefore the release of drug) from their "eggshell" (Fig. 6C) occurs gradually by their own swelling. Therefore, after rupture of CAB microcapsules, two factors influence the escape of PVA microspheres: the thickness of the "eggshell", and the swelling degree of PVA microspheres. Obviously, since the microspheres are randomly distributed inside of CAB microcapsules the thickness of the shell is different and consequently the escape of microspheres will take place progressively. On the other hand, the higher the swelling degree of encapsulated PVA microspheres, the faster the release rate of the drug. In addition, DF was released almost quantitatively from encapsulated PVA microspheres with higher swelling degree (Fig. 8).

The time course for DF release reported in Figs. 4, 5, 7 and 8 conforms satisfactorily described by a single exponential process (see Eq. (5)). However, a very slow phase, not resolved within the experimental time scale, cannot be excluded. Val-

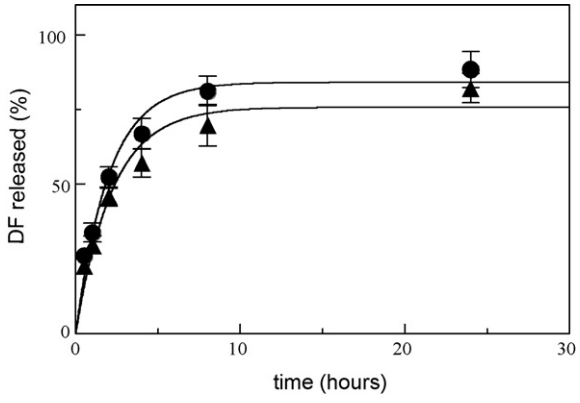

Fig. 7. Influence of exchange capacity of co-encapsulated SP-Ms on DF release profiles in phosphate buffer at pH 7.4: EC = 3.12 meq/g (▲) (sample P #9) and EC = 5.27 meq/g (●) (sample P #12). The continuous lines were calculated according to Eq. (5) with sets of parameters given in Table 5.

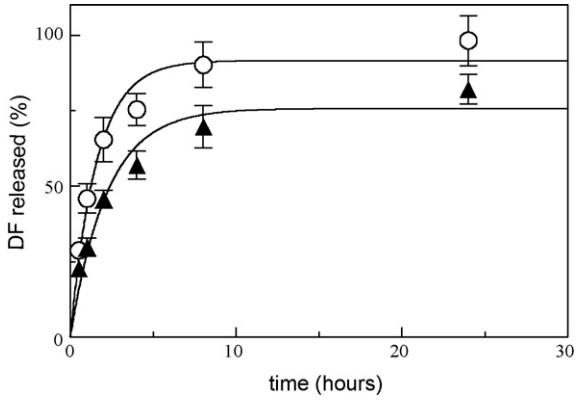

Fig. 8. Influence of cross-linking degree of encapsulated PVA microspheres on DF release profiles in phosphate buffer at pH 7.4: DC = 10 (◯) (sample P #3) and DC = 20 (▲) (sample P #9). The continuous lines were calculated according to Eq. (5) with sets of parameters given in Table 5.

ues of $t_{1/2}$, k, and Y_{max} for DF release obtained under different experimental conditions are given in Table 1.

4. Conclusions

A novel approach for enteric delivery of anionic drugs was proposed. The DF loaded PVA microspheres were co-encapsulated with free SP-Ms in CAB microcapsules by a o/w solvent evaporation technique. SP-Ms do not swell in acidic pH, but are able to puff up 10–20-fold in intestine causing the rupture of CAB microcapsules. After disruption of CAB microcapsules the dropping out of each loaded PVA microsphere (and therefore the release of drug) from their "eggshell" occurs gradually by their own swelling.

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