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pH-sensitive polyelectrolyte films derived from submicron chitosan/Eudragit® L 100-55 complexes: Physicochemical characterization and *in vitro* drug release

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ABSTRACT: The objectives of this study were to prepare films from submicron chitosan/Eudragit® L100-55 polyelectrolyte complexes (CH/EL PEC) and to assess the influence of CH molecular weight and CH/EL mass ratio on their structure and drug-release properties. The films were obtained by a simple, environmentally friendly, casting/solvent evaporation method and the verapamil hydrochloride (VH) was used as model drug. Submicron size, narrow size distribution, and acceptable stability of CH/EL PECs were confirmed by DLS and laser Doppler microelectrophoresis. SEM analysis revealed nonporous inner structure and flat surface of the films. Interactions between comprising polymers and formation of CH/EL PEC were established by DSC and FT-IR spectroscopy. *In vitro* swelling and drug release studies revealed the pH sensitivity of the films, with burst drug release in acidic conditions (pH 1.2) and sustained release in phosphate buffers pH 5.8, 6.8, and 7.4. The slowest VH release was achieved from the films prepared from equal amounts of EL and CH of higher molecular weight, confirming the significance of the CH/EL ratio and CH molecular weight on their ability to sustain drug release. The obtained results suggested that presented, simple, and eco-friendly preparation procedure can be used to obtain pH-sensitive CH/EL PEC films with a promising potential as drug carriers. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 42583.

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INTRODUCTION

Ionic interactions between oppositely charged polymers in aqueous solutions lead to the formation of nonpermanent and stimuli-sensitive entities which are known as polyelectrolyte complexes (PECs).[1,2] Owing to their biodegradability and pH sensitivity, PECs are widely investigated as potential drug carriers in a various forms such as films, nanoparticles, microparticles, micelles, gels, and so on.[3–8]

Chitosans (CHs) are semisynthetic, linear copolymers of *N*-acetyl-D-glucosamine and D-glucosamine, derived from chitin by its partial deacetylation.[9] Due to their excellent biocompatibility, low toxicity, diversity, and unique cationic nature, CHs are nowadays among the most extensively investigated polymers in the field of drug delivery.[9,10] As cationic polymers, CHs are able to form PECs with polyanions of both natural (carboxymethylcellulose, xanthan, pectin, hyaluronic acid, and alginate) and synthetic origin (cross-linked poly(acrylic acid) polymers and polymethacrylate copolymers).[2] CH-based PEC films and particulate carriers exhibit mucoadhesivity, pH-dependent swelling, and drug release.[11,12] These properties depend not only on the type and characteristics of polyanion used but also on the functional characteristics of CH, such as molecular weight and degree of deacetylation.[13,14]

Eudragit® L100-55 (EL) is an anionic copolymer based on methacrylic acid and ethylacrylate, which is generally regarded as nontoxic and nonirritant excipient included in the FDA Inactive Ingredients Database.[15] It is soluble at pH above 5.5, and therefore, widely used as an enteric coating agent. The ability of

Table I. Composition of Submicron Dispersions and Characteristics of Corresponding CH/EL PECs (Mean ± S.D.; $n = 3$)

Formulation code	CH type	Volume of CH stock solution[a] (mL)	Volume of EL stock solution[a] (mL)	VH (mg)	Stirring time (min)	Z-average ± S.D. (nm)	PDI ± S.D.	ζ potential ± S.D. (mV)
LC9E1	LCH	180	20	100	30	470 ± 6	0.21 ± 0.02	29.1 ± 0.8
LC8E2	LCH	160	40	100	30	447 ± 4	0.21 ± 0.01	28 ± 1
LC7E3	LCH	140	60	100	30	398 ± 5	0.22 ± 0.03	30 ± 2
LC6E4	LCH	120	80	100	30	418 ± 8	0.23 ± 0.02	35.1 ± 0.8
LC5E5	LCH	100	100	100	30	408 ± 7	0.23 ± 0.03	34 ± 1
MC9E1	MCH	180	20	100	30	879 ± 2	0.29 ± 0.01	30 ± 2
MC8E2	MCH	160	40	100	30	800 ± 4	0.31 ± 0.01	29.5 ± 0.5
MC7E3	MCH	140	60	100	30	760 ± 20	0.32 ± 0.03	35.7 ± 0.4
MC6E4	MCH	120	80	100	30	672 ± 5	0.36 ± 0.03	35 ± 2
MC5E5	MCH	100	100	100	30	720 ± 20	0.40 ± 0.06	32 ± 2

[a] 0.1% w/w; pH 5.6.

EL to interact with CHs and form PECs under appropriate conditions was reported earlier.[11,16]

According to authors' knowledge, the possibility of film formation from CH/EL PEC submicron particles has not yet been investigated. To do so, a simple, organic-free, and environmentally friendly procedure was proposed and verapamil hydrochloride (VH), water-soluble calcium channel blocker, was used as a model drug. This drug has relatively short plasma half-life (2–5 h) and low bioavailability (20–30%) after oral administration due to its intensive first-pass metabolism.[17,18] These drawbacks can be overcome by the drug incorporation in extended-release tablets and films/patches intended for buccal and transdermal delivery.[18–20]

Therefore, the main objectives of this study were to prepare CH/EL PEC films from submicron CH/EL PEC dispersions and to investigate their basic physicochemical and biopharmaceutical properties, with special interest to assess their pH sensitivity and ability to sustain release of incorporated VH. Additionally, to investigate the influence of CH molecular weight on physicochemical properties of CH/EL PECs and drug release from CH/EL PEC films, two types of CH were used in this study, the one of low (50–190 kDa) and the other of medium (190–300 kDa) molecular weight.

EXPERIMENTAL

Materials

The following chemicals were used as supplied from their manufacturers. Low (LCH; 50–190 kDa, degree of deacetylation 75–85%) and medium (MCH; 190–300 kDa, degree of deacetylation 75–85%) molecular weight CHs were purchased from Sigma-Aldrich (Milwaukee, WI). EL was generously donated by Evonik Industries AG (Darmstadt, Germany). VH was kind gift from Hemofarm AD (Vršac, Serbia). All other chemicals and reagents were of the highest grade commercially available.

Methods

Preparation of Stock Polymer Solutions. A required amount of CHs was dispersed in deionized water to obtain a final concentration of 0.1% (w/w). Dissolution of CHs was achieved by addition of anhydrous acetic acid to a final concentration of 0.5% (w/w). EL solution (0.1% (w/w)) was prepared by dissolving a required amount of EL in deionized water containing 0.2% (w/w) sodium hydroxide. pH values of the CHs and EL stock solutions were adjusted to 5.6 by addition of 0.1 M sodium hydroxide and anhydrous acetic acid, respectively. Prior to use, these stock solutions were filtered through 0.22 μm membrane filters (Millipore, Bedford, MA).

Preparation of Submicron CH/EL PEC Dispersions. Ten different formulations of CH/EL PEC dispersions were prepared by dropping the EL stock solution in the CH stock solution under vigorous magnetic stirring to prevent formation of large coacervates. The two solutions were mixed in different proportions as presented in Table I and stirred magnetically for additional 30 min at room temperature. The final volume of the resulting dispersion was 200 mL for each formulation. Prior to addition of the EL solution, 100 mg of VH was dissolved in CH solution.

To investigate the structure of submicron particles, the dispersion was ultracentrifuged at 15,000 rpm for 30 min by Eppendorf Centrifuge 5417R (Eppendorf, Hamburg Germany) at 25°C. Obtained precipitate was washed with deionized water, dried at 50°C to a constant mass, and subjected to the thermal and FT-IR analysis as described below.

Preparation of PEC Films. The PEC films were obtained by a simple casting/solvent evaporation method. Briefly, 200 mL of the freshly prepared PEC dispersion was cast into a Petri dish (9 cm inner diameter) and dried at 50°C to a constant weight. The dried films were rehydrated with bidistilled water, and gently cut into square pieces (20 × 20 mm) using a razor blade. Cut films were washed with bidistilled water, dried at 50°C to a constant weight, and stored in well-closed glass containers at room temperature for further investigation.

Particle Size Measurements

Measurements of the PECs size and size distribution (polydispersity index (PDI)) were performed on freshly prepared

dispersions placed in glass cuvettes by dynamic light scattering using a Zetasizer NanoZS90 instrument (Malvern Instruments, Malvern, UK). Each measurement was performed in a triplicate under a fixed angle of 90° at 25°C and the average values were calculated.

ζ-Potential Measurements. The ζ-potentials of the investigated dispersions were measured in folded capillary cell by laser Doppler microelectrophoresis using a Zetasizer NanoZS90 instrument (Malvern Instruments, Malvern, UK). Prior to the measurements, the operating conditions were confirmed and adjusted using a calibrated latex dispersion supplied by the instrument manufacturer. The ζ-potential was calculated from the electrophoretic mobility using Smoluchowski's equation. Each measurement was repeated three times at 25°C and the average values were calculated.

Film Structure Analysis. The surface and inner structure film analysis was performed using a scanning electron microscope DSM 940 A (Zeiss, Oberkochen, Germany). Prior to observation, the dried films were transferred onto double-sided tape and sputter coated with gold in a BioRad E 5100 coater (Bio-RAD Microscience Division Cambridge, MA).

Differential Scanning Calorimetry. The DSC measurements were carried out using a DSC 1 instrument (Mettler Toledo, Schwerzenbach, CH). The samples were crimped in a standard 40 μL aluminum pan and heated from 25 to 320°C at a heating rate of 10°C/min under a constant nitrogen flow rate of 50 mL/min. The empty sealed pan was used as a reference.

Fourier Transform Infrared (FT-IR) Spectroscopy. The attenuated total reflectance FT-IR spectra were recorded using a Nicolet iS10 FT-IR Spectrometer (Thermo Fisher Scientific, Cambridge, UK) in the wavelength range between 4000 and 650 cm^{-1} with a resolution of 4 cm^{-1}.

***In Vitro* Drug Release Studies.** To determine *in vitro* release of VH, drug-loaded films were placed in a glass bottles containing 50 mL of dissolution medium. The bottles were placed in an orbital shaking water bath and gently shaken for 8 h with a shaking speed of 50 rpm at 37°C. The following media were used for release studies: 0.1 M HCl (pH 1.2), USP phosphate buffer solutions of pH 5.8, 6.8, and 7.4. After 15, 30, 60, 90, 120, 180, 240, 300, 360, 420, and 480 min, 4 mL of samples were withdrawn, filtered through a 0.45 μm Millipore filter (Millipore, Bedford, MA), and replaced with equal amount of fresh medium. The drug concentration was determined spectrophotometrically using an Evolution 300 spectrophotometer (Thermo Fisher Scientific, Cambridge, UK) at 278 nm. The drug release experiments were repeated three times for each formulation in each dissolution media separately. The average value was taken as the value of the percentage cumulative drug released and plotted *versus* time.

Swelling Studies. Swelling ability of the films was determined under the same conditions as those described for drug-release studies. After 15, 30, 60, 120, 180, 240, 300, 360, 420, and 480 min, the films were removed from the glass bottles, blotted on filter paper to remove excess water from the surface, and weighed immediately on a Sartorius AE240 single pan balance (Göttingen, Germany). The swelling ratio (SR %) of the particles was calculated according to the following equation:

$$SR\% = 100 \times \frac{W_t - W_o}{W_o} \quad (1)$$

Where W_t is the weight of the swollen film at time t and W_0 is the initial weight of the film. For each formulation, swelling experiments were repeated three times in each medium separately and the average value was taken as the value of the percentage swelling ratio.

RESULTS AND DISCUSSION

PECs Size Measurements

The mean values of hydrodynamic radius (Z-average) and size distribution (PDI) of CH/EL PECs are shown in Table I. Submicron size and unimodal size distribution were confirmed for all investigated CH/EL PECs, with the Z-average in the range between 398 ± 5 and 879 ± 2 nm. It is clear that both investigated variables (CH molecular weight and CH/EL ratio) affect the size and size distribution of the PECs. The larger PECs were formed when CH of higher molecular weight was used. This could be ascribed to the influence of chain length of the polycation since the shorter polymer chains could form denser and hence smaller particles. The initial size reduction was observed with increasing amount of added EL, most likely due to more intensive polycation/polyanion interaction and consequently tighter packing of the polymer chains. A preliminary study showed that most of the prepared PEC formulations have the smallest size (the lowest Z-average) and the narrowest size distribution (the lowest PDI) at pH 5.6 with exception of MC7E3, MC6E4, MC5E5, and LC5E5 PECs whose size slightly decreased with increasing pH to 7.4 (the results not shown). However, at the same time, PDI values of these formulations significantly increased (from 0.3 to 0.6, depending on the formulation). For that reason, all the formulations used for preparation of PEC films have been prepared at pH 5.6.

ζ-Potential Measurements

The average ζ-potential values of submicron PECs were in the range from 28 ± 1 to 35.7 ± 0.4 mV (Table I). Colloidal dispersions are considered to be stable when the absolute value of the ζ-potential is higher than 25 mV.[21] Therefore, all investigated PECs dispersions can be considered as relatively stable. The positive values of ζ-potential can be ascribed to excess of protonated —NH_2 groups of CH in comparison to deprotonated —COOH groups of EL under reaction conditions (pH 5.6). Surprisingly, ζ-potential was not significantly altered by changing CH/EL mass ratio. This was reported earlier in literature for CH-based PEC nanoparticles and could be ascribed to more significant influence of CH positive charge density in comparison to that of polyanion.[22,23] Since the starting pH value of polyanion solution was 5.6, it is expected that charge density of polyanion was much lower than that of CH.

Film Structure Analysis

Figure 1 shows the SEM images of the LCH/EL and MCH/EL films. As can be seen in Figure 1(c), the surface of the MCH/EL film was flat and nonporous. On the other hand, the surface of LCH/EL film was rough and slightly porous [Figure 1(a)].

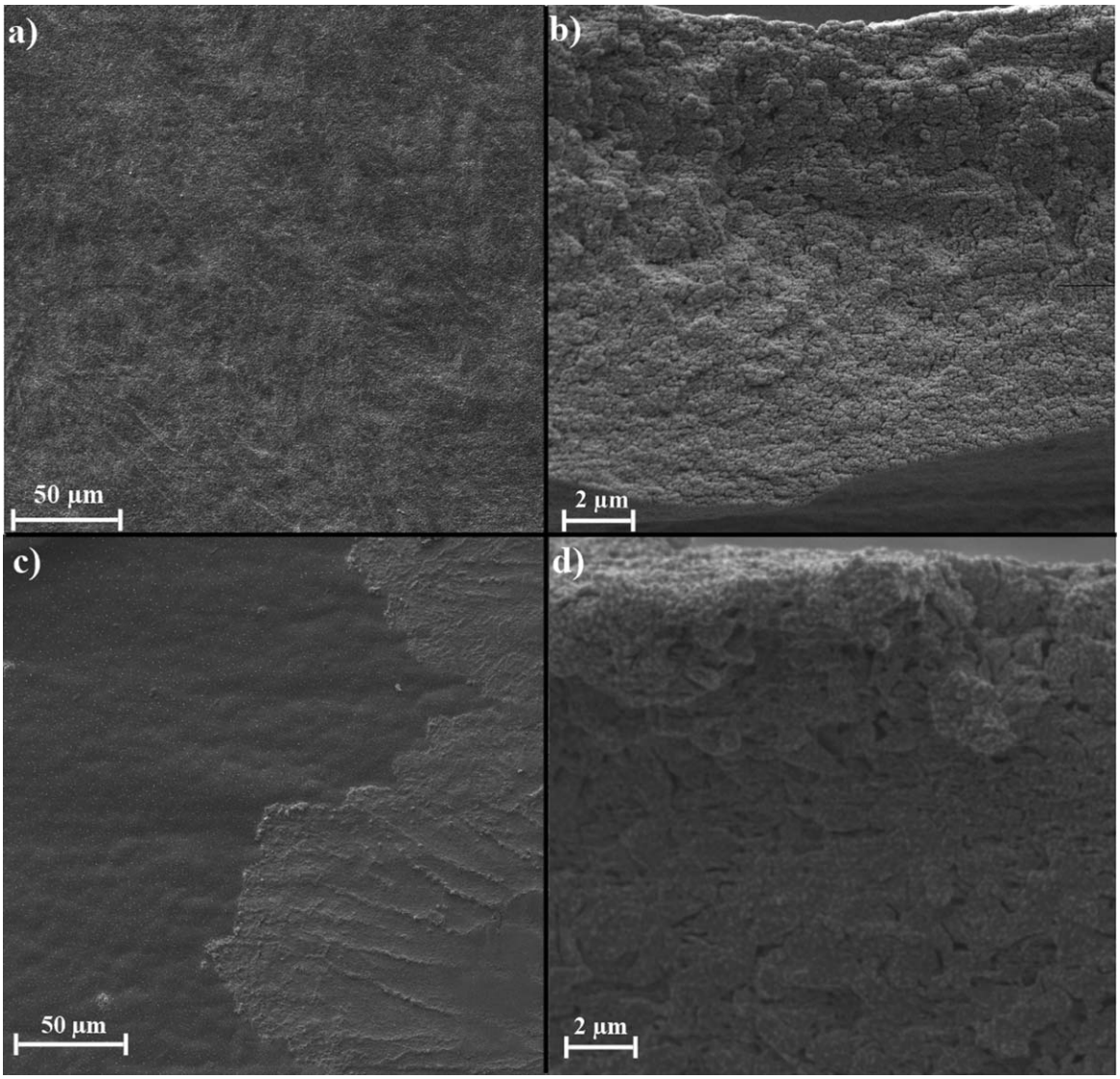

Figure 1. SEM micrographs of (a) LCH/EL films surface, (b) LCH/EL films inner structure, (c) MCH/EL films surface, and (d) MCH/EL films inner structure.

Closer look to the cross-section of both the films showed the differences in the inner structure of the two films. Namely, the SEM images of cross-sections taken at magnification of 5000× [Figure 1(b,d)] reveal that both the films were consisted of sub-micron irregular shaped particles and that the MCH/EL film had a firmer structure compared to that of LCH/EL film. Such compact structure explains the slower VH release from MCH/EL films in comparison to that of LCH/EL films, and indicates that formation of MCH/EL PEC is more enhanced. It should be emphasized that significantly smaller particles were observed in the structure of LCH/EL film in comparison with those of MCH/EL film, which is in line with the results of PECs size measurements presented in Table I.

Differential Scanning Calorimetry (DSC)

DSC was used to investigate the state of embedded drug and the possible drug/polymer and polymer/polymer interactions. Figure 2 presents the DSC thermograms of VH, LCH, MCH, EL, L(M)CH-EL PEC submicron particles, and VH-loaded L(M)CH/EL films. As expected, the thermal profiles of LCH and MCH were similar showing the initial endothermic bands related to the water evaporation with the peaks at 105 and 93°C, respectively. The broad exothermic events with peaks at 308°C for LCH and 307°C for MCH correspond to the polymer decomposition.[24] The DSC curve of EL exhibited two broad endothermic events, the one in the range between 40 and 100°C as a consequence of water evaporation, and the other with the peak at 191°C attributable to the melting of the EL crystalline portion.[25] Additionally, the glass transition of the amorphous fraction of EL was observed at 128°C.[26]

The DSC curves of the PECs showed endotherms with peaks at 122°C (LCH/EL PEC) and 108°C (MCH/EL PEC) which could be ascribed to the water evaporation. The absence of peaks related to the degradation of individual polymers and the appearance of new broad exothermic events on these thermograms could be explained by ionic interaction between

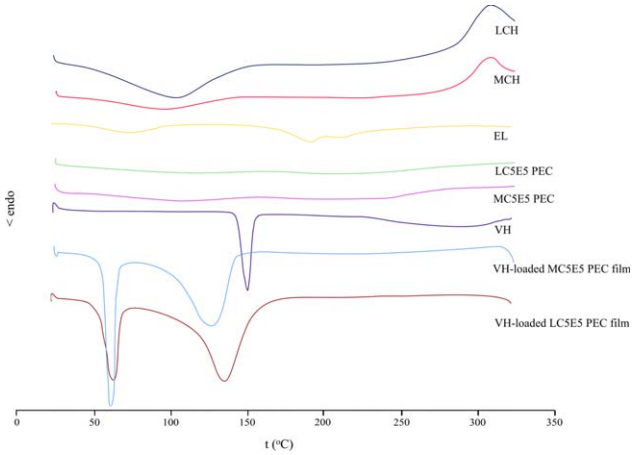

Figure 2. DSC heating curves of VH, LCH, MCH, EL, L(M)C5E5 submicron PEC particles and VH-loaded L(M)C5E5 films. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

the two polymers and subsequent formation of the CH/EL PEC.

In the DSC curve of VH, sharp endothermic peak was observed at 149°C which undoubtedly corresponds to its melting point.[27] On the thermogram of the VH-loaded films, this endothermic event was broadened and shifted toward lower temperatures suggesting the presence of the drug and decrease of its crystallinity.[28] The decrease of the crystallinity could be ascribed to the difficulty of drug crystals formation in highly viscous surrounding during film formation.[29] This can be further confirmed by comparing thermograms of VH-loaded LCH/EL and MCH/EL films. Namely, it is known that viscosity of CH solution increases by increasing the molecular weight of CH.[13] Therefore, the drug crystallization was more difficult when the MCH was used and this peak was shifted toward lower temperatures in the case of VH-loaded MCH/EL films. DSC curves of VH-loaded LCH/EL film and MCH/EL film showed endothermic peaks at 63 and 60°C, respectively. Similar observations have been reported earlier for PEC films consisted of chitosan and carboxylate polyanions, which could be ascribed to the release of water bonded to the films by the several hydrophilic groups in their structure (—OH, $—NH_2$, $—NHCOCH_3$, —COOH, and $—COO^-—^+NH_3—$).[24,30] These endothermic events occurred at different temperatures with different intensities of enthalpy changes on thermograms of PEC films in comparison to those of the individual polymers suggesting that both preparation procedure and polyelectrolyte complexation had impact on the amount and the state of embedded water.[30] The higher content of water embedded in the films compared to the amount of water bonded to the hydrophilic groups of the individual polymers is most probably related to the three-dimensional porous structure of PEC network.

Fourier Transform Infrared (FT-IR) Spectroscopy

FT-IR spectroscopy was used to confirm the PEC formation, presence of the drug in the films, and to investigate the nature of chemical bonding between the two polymers. The FT-IR spectra of LCH, MCH, EL and L(M)CH-EL PEC submicron particles are shown in Figure 3(a). The FT-IR spectra of the both CHs exhibited a weak absorption band at 2872 cm^{-1} due to the C—H stretching, one band at 1654 cm^{-1} due to the carbonyl group stretching of the secondary amide, three bands at 1586, 1417, and 1318 cm^{-1} related to the N—H bending vibration (amine I band), N—H stretching of the amide and ether bonds, and the amide III band, respectively, and three additional bands peaks at 1149, 1061, 1026, and 892 cm^{-1} related to the C—O—C stretching vibration.[24] The spectrum of EL showed a broad band in the range between 3500 and 2500 cm^{-1} associated to O—H vibrations, bands of the C—H vibrations are at 2982, 2934, 1477, 1446, and 1383 cm^{-1}, two characteristic intensive bands of the carbonyl group vibration, one of the carboxylic acid group other of esterified carboxylic groups at 1698 and 1724 cm^{-1}, respectively, and two bands associated to C—O stretching vibrations of ester groups at 1254 and 1156 cm^{-1}.[16] These bands of the individual polymers could be observed in the spectra of LCH/EL and MCH/EL PECs, confirming the presence of the LCH (or MCH) and EL in the structure of the PEC. More importantly, a new absorption

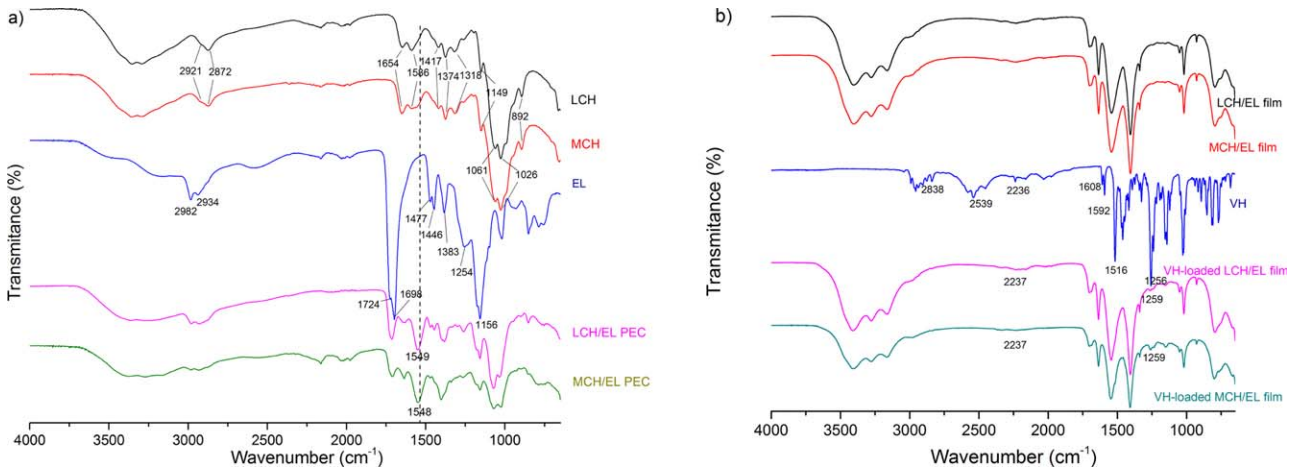

Figure 3. The FT-IR spectra of (a) LCH, MCH, EL, L(M)CH-EL PEC submicron particles and (b) VH, placebo, and VH-loaded L(M)CH/EL films. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

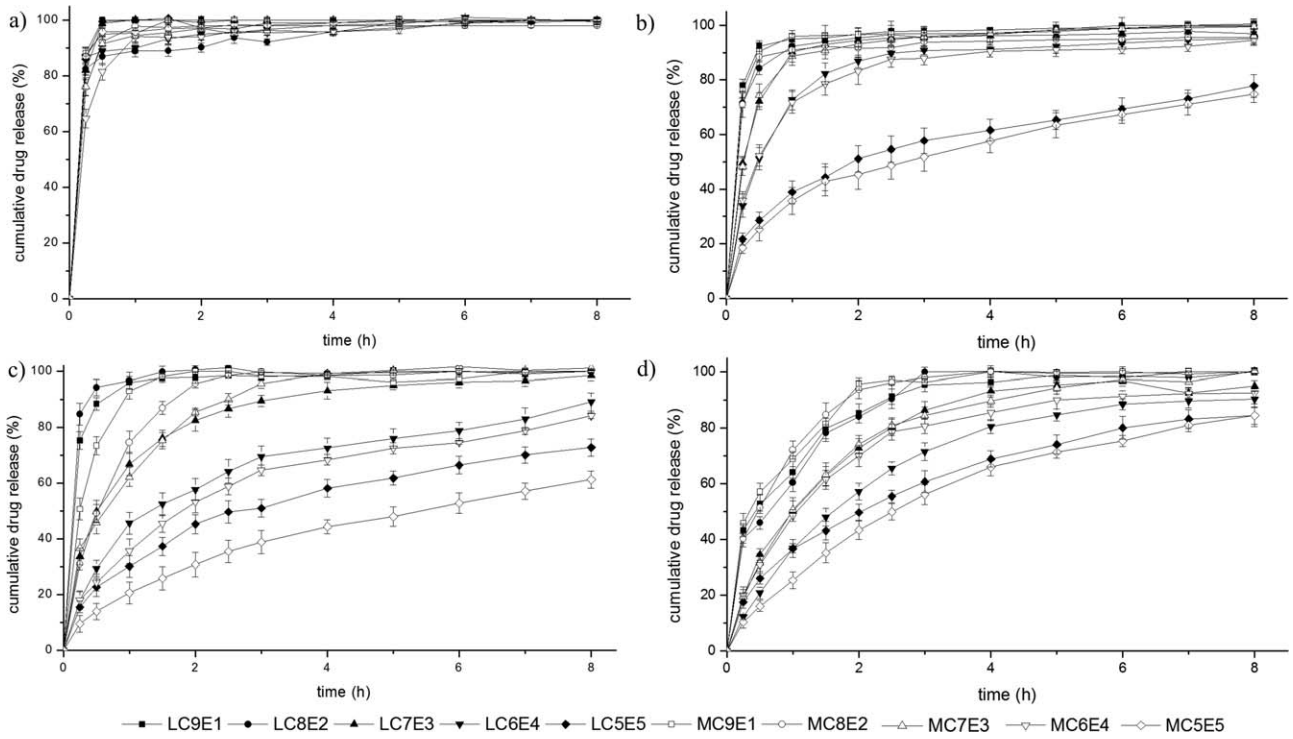

Figure 4. VH release profiles from films in (a) 0.1 M HCl, (b) phosphate buffer pH 5.8, (c) phosphate buffer pH 6.8, and (d) phosphate buffer pH 7.4. Experiments were performed in triplicate ($n = 3$) for each formulation. Error bars represent standard deviation.

bands with peaks at 1549 and 1548 cm^{-1} were observed on the spectrum of LCH/EL and MCH/EL PECs, respectively. The appearance of these bands is the consequence of the interaction between L(M)CH and EL, and formation of the PEC. These findings were consistent with previously published reports related to films comprised of CH and different carboxylate polyanions.[24]

The spectra of VH, placebo, and VH-loaded L(M)CH/EL films are presented in Figure 3(b). The VH spectrum shows the following absorption bands attributable to its functional groups: weak bands of C—H stretching of the methoxy group at 2838 cm^{-1}, broad band between 2800 and 2300 cm^{-1} due to N—H stretching vibrations of the protonated amine, C—N stretching vibrations at 2236 cm^{-1}, skeletal stretching vibrations of the benzene ring at 1608, 1592, and 1516 cm^{-1}, and C—O stretching vibrations of the aromatic ethers at 1256 cm^{-1}.[31] As it is depicted in Figure 3(b), some of these bands were presented on the spectra of the VH-loaded LCH/EL and MCH/EL films, confirming the presence of the drug in the films, while the other VH-related bands were overlapped by the more intensive bands of the consisting polymers. Furthermore, comparison of FT-IR spectra of VH, placebo, and VH-loaded films did not reveal any sign of interaction between VH and the polymers. No significant difference was observed between films derived from CHs of different molecular weights.

In Vitro Drug Release Studies and Drug Release Kinetics

The drug release profiles from VH-loaded LCH/EL and MCH/EL films in 0.1 M HCl, phosphate buffers at pH 5.8, 6.8, and 7.4 are shown in Figure 4(a–d), respectively. It is evident that all prepared films demonstrated pH-sensitive drug release behavior. This was expected since the ionic interaction between consisting polymers and consequent stability of the films are strongly dependent on the degree of ionization of CH and EL. Since the consisting polymers are the weak base and the weak acid, respectively, their ionization degrees depend on the pH value of the surrounding medium. The release of VH was the slowest in phosphate buffer at pH 6.8, suggesting that the ionic interactions between the two polymers are more intensive at pH 6.8, in comparison to the acidic and the weakly alkaline medium. As can be seen in Figure 4(a), the release of the model drug in 0.1 M HCl was rapid, reaching the cumulative value of more than 80% of entrapped VH for less than 1 h of experiment for all the investigated formulations. This was due to the intensive protonation of the free $—NH_2$ groups of CHs and the $—COO^-$ groups of EL, which caused the dramatic weakening of the intermolecular interactions between the two polymers. The fully protonated and unbounded CHs molecules dissolved and diffused in surrounding medium leaving the unprotected drug to dissolve as well. On contrary, under slightly alkaline conditions, the larger number of carboxylate groups of EL was deprotonated and available in its anionic form for interactions with cationic moieties of CHs. Nevertheless, under such conditions, significantly lower percentage of the free $—NH_2$ groups of CH is protonated, which again leads to the weakening of the CH/EL ionic forces, and thus to the destabilization of the PECs films. Therefore, the release of the drug was more sustained when the films were incubated in phosphate buffer at pH 6.8 [Figure 4(c)] than in slightly acidic (pH 5.8, Figure 4b) or alkaline medium (pH 7.4, Figure 4d).

Table II. Values of Fitted Parameters by Functional form Suggested by Peppas and Korsmeyer for VH-Loaded LC5E5 and MC5E5 Films in Phosphate Buffers at pH 5.8, 6.8, and 7.4

		Phosphate buffer 5.8		Phosphate buffer 6.8		Phosphate buffer 7.4	
Drug release model	Fitted parameters	LC5E5	MC5E5	LC5E5	MC5E5	LC5E5	MC5E5
Korsmeyer–Peppas	r^2	0.992	0.993	0.991	0.997	0.994	0.989
	n	0.360	0.392	0.445	0.539	0.456	0.622
	k_k	8.608	6.805	4.461	2.265	5.465	2.036

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As Figure 4(b–d) shows, the drug release rate significantly decreased when the content of EL increased. This could be attributed to the more intensive polyelectrolyte complexation in the presence of higher amount of polyanion. Therefore, the slowest drug release was achieved from the films obtained from equal amounts of CH and EL (CH/EL mass ratio = 1/1), with cumulative drug release in phosphate buffer at pH 6.8 less than 73% and 62% for LCH/EL and MCH/EL films, respectively, after 8 h of the experiment. It should be emphasized that further increase of EL content during preparation of PEC dispersions led to the formation of large snow-like PEC coacervates which precipitated immediately. Therefore, formulation obtained from EL in excess was not suitable for preparation of PEC films.

The influence of CH molecular weight on the drug release was also evident in the phosphate buffers [Figure 4(b–d)]. Namely, the drug release was obviously slower from MCH/EL films, which can be related to the lower solubility of MCH in dissolution medium in comparison to the LCH. Besides, the CSs of higher molecular weight in aqueous media can form gels of higher viscosity.[32] Once the hydration of the films in dissolution medium starts, MCH forms more viscous hydrogel layer at the films surface than LCH, making in that way drug diffusion in surrounding medium more difficult.

The slowest release of entrapped VH was achieved from LC5E5 and MC5E5 films. Since several mechanisms were likely to control the rate of release over time, release data from these films was fitted the empirical Korsmeyer–Peppas power law form:[33]

$$f_t = kt_n \quad (2)$$

where f_t is the fraction of dissolved drug at time t, k is the constant incorporating structural and geometric characteristics of the drug dosage form, and n is the release exponent that depends on the release mechanism and the shape of the formulation tested.

The obtained results are given in Table II. The fitted values of n reflect "sub-Fickian" ($n < 0.5$), near-Fickian ($n \sim 0.5$), and anomalous ($n > 0.5$) diffusion, depending on the formulation.[33]

Swelling Studies

As can be seen in Figure 5, water uptake capacity of the CH/EL PEC films was undoubtedly pH dependent. In acidic medium, all the films exhibited rapid and intensive swelling, reaching the values of SR % in the range between 65.84 and 570.04% for the first 15 min of experiment. This rapid swelling was followed by complete erosion of the films. These observations are in complete agreement with the results of drug release obtained in the same medium. As described above, intensive protonation of the —COO^- groups of EL resulted in overall PEC destabilization and was followed by intensive protonation of CH. Protonated CH rapidly formed transparent hydrogel which dissolved completely in surrounding medium. Based on this finding, it can be concluded that swelling process controls the drug release from films in acidic surrounding. Slightly slower swelling of MCH/EL films in comparison with LCH/EL films was a consequence of firmer gel formation when CH of higher molecular weight was used for preparation of the films. However, this difference is not significant for potential application of the films, because both the LCH/EL and MCH/EL films were completely dissolved for less than 1 h. Completely different swelling behavior of the films was observed in phosphate buffers at pH 5.8, 6.8, and 7.4 [Figure 5(b–d)]. Namely, slow weight loss was observed during 8 h of experiment in these three media, suggesting better stability of the films at pH 5.8, 6.8, and 7.4. At pH 5.8 [Figure 5(b)], films with higher EL content showed initial weight loss (negative SR % values), most likely due to the dissolving of unbound EL which is soluble at pH > 5.6. This can be confirmed by comparing SR % values of films having different amount of EL. Namely, by increasing the amount of EL in films, the initial weight loss was more evident. Further weight loss was mostly the consequence of the slow release of incorporated model drug, which is in accordance with the results of drug release experiments. By increasing pH value of the surrounding medium to 6.8, the observed difference between the films with high and low EL content was less evident [Figure 5(c)]. The remarkable decreases in SR % values by the time were observed at this pH value. This can be explained by continuous stabilization of PEC at this pH value which is in line with the results of *in vitro* drug release studies. That is, the release of VH was much slower at pH 6.8 in comparison to the release at lower pH values, as a consequence of more intensive interaction between the two polymers.

CONCLUSIONS

CH/EL PEC films were successfully obtained from submicron aqueous CH/EL PEC dispersions by a simple casting and solvent evaporation procedure. Prior to the casting, stability, unimodal size distribution, and submicron size of the CH/EL PECs were confirmed by dynamic light scattering and laser Doppler microelectrophoresis. SEM analysis confirmed that MCH/EL films were flat-surfaced and consisted of tightly packed submicron particles. Interaction between CH and EL, and consequent

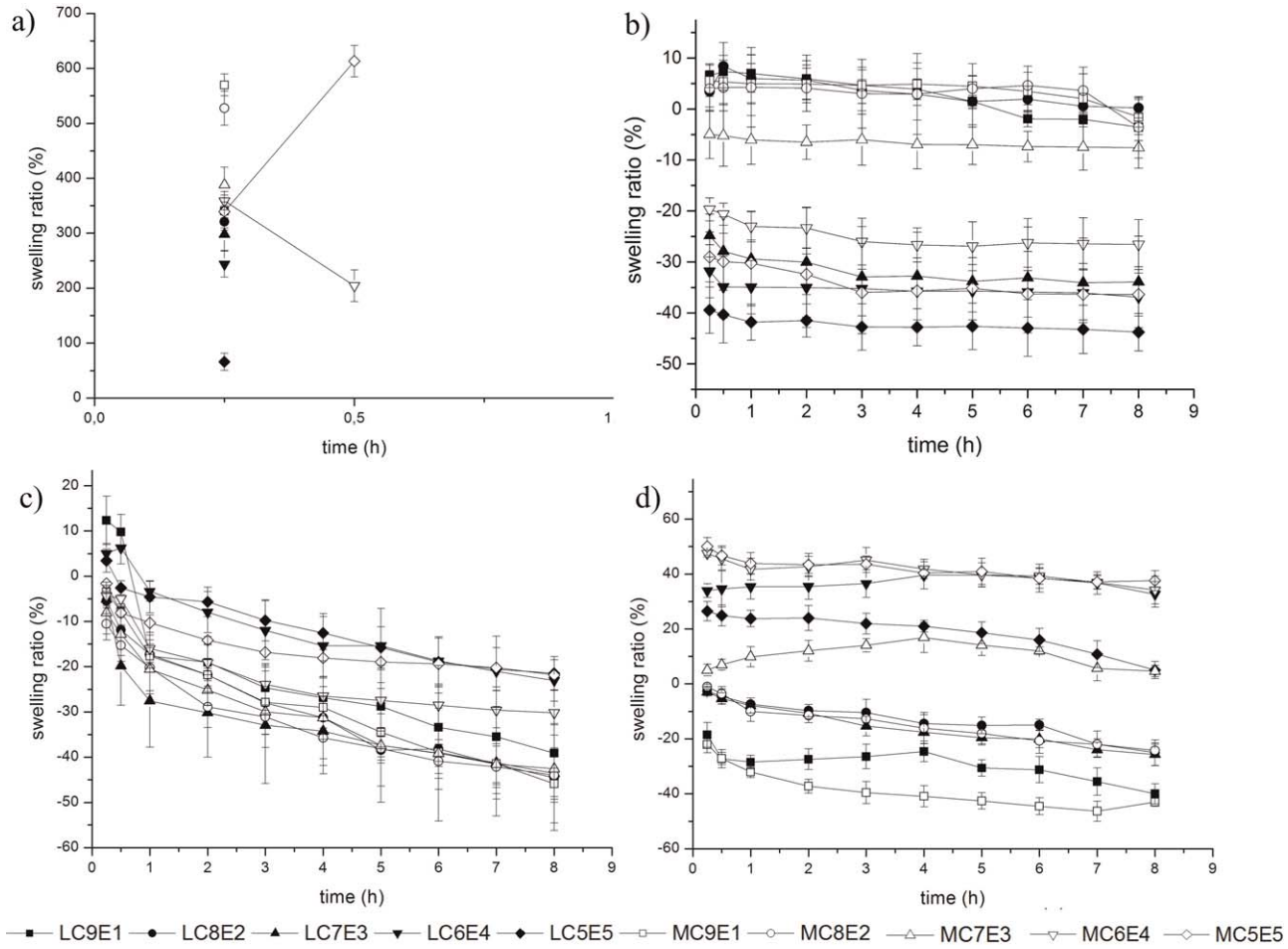

Figure 5. Swelling ratios (%) of films in (a) 0.1 M HCl, (b) phosphate buffer pH 5.8, (c) phosphate buffer pH 6.8, and (d) phosphate buffer pH 7.4. Experiments were performed in triplicate ($n = 3$) for each formulation. Error bars represent standard deviation.

formation of CH/EL PECs were confirmed by DSC and FT-IR spectroscopy. DSC analysis pointed that crystallization of the drug during film formation was obstructed, especially when MCH was used, most likely by the high viscous surrounding. *In vitro* swelling and drug release experiments revealed the pH sensitivity of the films. All the films exhibited sustained release at pH 5.8, 6.8, and 7.4, and rapid and intensive swelling accompanied by the burst drug release in acidic medium. The slowest release was achieved in phosphate buffers at pH 5.8 and 6.8 from the films derived from equal amounts of MCH and EL, confirming the significance of the CH molecular weight and CH/EL ratio on pH sensitivity of the films. The ability of MCH/EL films to provide sustained release of VH is comparable to the recently reported results of the *in vitro* VH release from buccoadhesive tablets consisted of various polysaccharides, implying that sustained release could be achieved from buccal films that could be considered more convenient for the patients.[20] Obtained results suggest that proposed, simple, organic solvent-free preparation procedure can be effectively used to obtain PEC films from CH/EL submicron particles with a promising potential as pH-sensitive drug carriers. Further studies will be focused on improvement of mechanical and mucoadhesive properties of CH/EL films intended for buccal delivery of VH.

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REFERENCES

1. Moustafine, R. I.; Margulis, E. B.; Sibgatullina, L. F.; Kemenova, V. A.; Van den Mooter, G. *Eur. J. Pharm. Biopharm.* **2008**, *70*, 215.
2. Hamman, J. H. *Mar. Drugs* **2010**, *8*, 1305.
3. Birch, N. P.; Schiffman, J. D. *Langmuir* **2014**, *30*, 3441.
4. Agüero, L.; Garcia, J.; Valdés, O.; Fuentes, G.; Zaldivar, D.; Blanco, M. D.; Katime, I. *J. Appl. Polym. Sci.* **2013**, *128*, 3548.
5. Čalija, B.; Milić, J.; Cekić, N.; Krajišnik, D.; Daniels, R.; Savić, S. *Drug Dev. Ind. Pharm.* **2013**, *39*, 77.

6. Li, G.; Song, S.; Zhang, T.; Qi, M.; Liu, J. *Int. J. Biol. Macromol.* **2013**, *62*, 203.
7. Park, M. R.; Chun, C.; Ahn, S. W.; Ki, M. H.; Cho, C. S.; Song, S. C. *J. Control. Release* **2010**, *147*, 359.
8. Türkoğlu, T; Taşcıoğlu, S. *J. Appl. Polym. Sci.* **2014**, 131.
9. Dash, M.; Chiellini, F.; Ottenbrite, R. M.; Chiellini, E. *Prog. Polym. Sci.* **2011**, *36*, 981.
10. Anitha, A.; Sowmya, S.; Kumar, P. T.; Deepthi, S.; Chennazhi, K. P.; Ehrlich, H.; Tsurkan, M.; Jayakumar, R. *Prog. Polym. Sci.* **2014**, *39*, 1644.
11. Čalija, B.; Cekić, N.; Savić, S.; Daniels, R.; Marković, B.; Milić, J. *Colloid. Surf. B* **2013**, *110*, 395.
12. Bigucci, F.; Luppi, B; Cerchiara, T; Sorrenti, M.; Bettinetti, G; Rodriguez, L.; and Zecchi, V. *Eur. J. Pharm. Sci.* **2008**, *35*, 435.
13. Čalija, B.; Cekić, N.; Savić, S.; Krajišnik, D.; Daniels, R.; Milić, J. *Arch. Pharm. Res.* **2011**, *34*, 919.
14. Gåserød, O.; Smidsrød, O.; Skjåk-Bræk, G. *Biomaterials* **1998**, *19*, 1815.
15. Chang, R. K.; Peng, Y.; Trivedi, N.; Shukla, A. J. In Handbook of Pharmaceutical Excipients, 6th ed.; Rowe, R. C.; Sheskey, P. J.; Quinn, M. E., Eds.; Pharmaceutical Press: London, **2009**; p 525.
16. Jelvehgari, M.; Zakeri-Milani, P.; Siahi-Shadbad, M. R.; Loveymi, B. D.; Nokhodchi, A.; Azari, Z.; Valizadeh, H. *AAPS PharmSciTech* **2010**, *11*, 1237.
17. Waller, J. R; Waller, D. G. *Medicine* **2014**, *42*, 538543.
18. Kusum Devi, V.; Saisivam, S.; Maria, G. R.; Deepti, P. U. *Drug Dev. Ind. Pharm.* **2003**, *29*, 495.
19. Emami, J; Varshosaz, J; Saljoughian, N. *DARU J. Pharm. Sci.* **2008**, *16*, 60.
20. Aboutaleb, A. E; Abdel-Rahman, A. A.; Samy, E. M.; El-Naggar, M. G. *Unique J. Pharm. Biol. Sci.* **2013**, *1*, 48.
21. Roland, I.; Piel, G.; Delattre, L.; Evrard, B. *Int. J. Pharm.* **2003**, *263*, 85.
22. Alonso-Sande, M.; Cuña, M.; Remuñán-López, C.; Teijeiro-Osorio, D.; Alonso-Lebrero, J. L.; Alonso, M. J. *Macromolecules* **2006**, *39*, 4152.
23. Du, J.; Sun, R.; Zhang, S.; Govender, T.; Zhang, L. F.; Xiong, C. D.; Peng, Y. X. *Macromol. Rapid Comm.* **2004**, *25*, 954.
24. Silva, C. L.; Pereira, J. C.; Ramalho, A.; Pais, A. A.; Sousa, J. J. *J. Membrane Sci.* **2008**, *320*, 268.
25. Ceballos, A.; Cirri, M.; Maestrelli, F.; Corti, G.; Mura, P. *Farmaco.* **2005**, *60*, 913.
26. Miller, D. A.; DiNunzio, J. C.; Yang, W; McGinity, J. W.; Williams, R. O. *Pharm. Res.* **2008**, *25*: 1450.
27. Yoshida, M. I.; Gomes, E. C. L.; Soares, C. D. V.; Cunha, A. F.; Oliveira, M. A. *Molecules* **2010**, *15*, 2439.
28. Pang, J.; Luan, Y.; Li, F.; Cai, X.; Du, J.; Li, Z. *Int. J. Nanomed.* **2011**, *6*, 659.
29. Puttipipatkhachorn, S.; Nunthanid, J.; Yamamoto, K.; Peck, G. E. *J. Control. Release* **2001**, *75*, 143.
30. Ostrowska-Czubenko, J.; Gierszewska-Drużyńska, M. *Carbohydr. Polym.* **2009**, *77*, 590.
31. Li, Y.; Wong, H. L.; Shuhendler, A. J.; Rauth, A. M.; Wu, X. Y. *J. Control. Release* **2008**, *128*, 60.
32. Agnihotri, S. A.; Mallikarjuna, N. N.; Aminabhavi, T. M. *J. Control. Release* **2004**, *100*, 5.
33. Costa, P.; Lobo, J. M. S. *Eur. J. Pharm. Sci.* **2001**, *13*, 123.