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Preparation and characterization of controlled release matrices based on novel seaweed interpolyelectrolyte complexes

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ABSTRACT

Novel interpolyelectrolyte complexes (IPECs) between naturally sulfated polysaccharides of the seaweed *Polysiphonia nigrescens* (PN) and cationized agaroses (CAG) and Eudragit E (EE) were prepared using an organic solvent free process, characterized, and explored for controlled drug release. Tablets containing model drug ibuprofen and IPECs were prepared by direct compression. Drug release in acid medium was low owing to the low solubility of ibuprofen in that condition and to the matrix action. Zero order drug release was determined in the buffer stage (pH = 6.8), with Fickian diffusion predominating over relaxation during the initial phases. Relaxation appears to increase along the release process and even overcomes diffusion for some systems. Drug release profiles could be controlled by varying the content of IPECs in the tablets. Also, the change in molecular weight and the degree of substitution of the components allowed altering the release profiles.

1. Introduction

Interpolyelectrolyte complexes (IPECs) are a class of polymer complexes, which are formed readily between most polyanions and polycations by ionic association of repeating units of the polymer chains (Lowman, 2000). Many IPECs form hydrogels (Satish et al., 2006; Hennink and van Nostrum, 2002). In general, hydrogels have been extensively employed for controlled delivery of drugs. However, potential applications of IPECs as hydrogels for controlled drug release are still limited.

Sometimes hydrogels are crosslinked to prevent matrix erosion. The degree of crosslinking affects the solubility of hydrogels. Most covalent crosslinking reagents are very toxic, i.e. glutaraldehyde, epichlorohydrin (Hennink and van Nostrum, 2002; Dulong et al., 2004), even in trace quantities, and further purification is required after preparation. The crosslinking agent can also affect the integrity of the drug to be trapped (if the drug is added to the preparation before the crosslinking step). In this sense, one advantage of IPECs is that those problems are avoided because the crosslinking occurs by means of ionic linkages (Hennink and van Nostrum, 2002; Peppas et al., 2000).

Among the research works concerned with the use of IPECs in monolithical matrix systems for controlled release of drugs, the following systems can be highlighted: Eudragit E–Eudragit L (Moustafine et al., 2005a, 2006), Eudragit E–alginate (Moustafine et al., 2005b, 2009), chitosan–Eudragit L (Moustafine et al., 2008), chitosan–alginate and chitosan–carrageenan (Tapia et al., 2002, 2004), chitosan–pectin and chitosan–arabic gum (Meshali and Gabr, 1993), chitosan–polyacrylic acid (de la Torre et al., 2003) and chitosan–carbopol (Park et al., 2008).

In the present work we have investigated novel IPEC systems, with special emphasis in their potential use as controlled drug release matrices. The investigated systems include the IPEC between Eudragit E (EE) and the polysaccharides extracted from the seaweed *Polysiphonia nigrescens* (PN) (Prado et al., 2008a), and the IPECs between cationized agaroses (CAG) obtained by chemical synthesis (Prado et al., 2011) and the polysaccharides of *P. nigrescens*. This seaweed is an agarophyte distributed worldwide, and constitutes an important biomass in the coasts of Mar del Plata (Province of Buenos Aires, Argentina). Polysaccharides from this seaweed have a rather regular structure and a high degree of sulfation (Prado et al., 2008a). To the best of our knowledge, IPECs CAG–PN are the first IPECs reported completely based on

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seaweed polysaccharides, both in the cationic and the anionic components.

2. Materials and methods

2.1. Materials

Basic butylated methacrylate copolymer, commercialized with the trademark Eudragit® E PO (EE) was kindly provided by Etilfarma S.A./Evonik Degussa Argentina S. A. Basic butylated methacrylate copolymer (EE) is a copolymer of 2-dimethylaminoethyl methacrylate, methyl methacrylate and *n*-butyl methacrylate, having a mean relative molecular mass of about 150,000. The ratio of dimethylaminoethyl methacrylate groups to butyl methacrylate and methyl methacrylate groups is about 2:1:1. The content of dimethylaminoethyl groups is between 20.8% and 25.5% (calculated from the dried substance).

Cationized agaroses were obtained by chemical synthesis as earlier depicted (Prado et al., 2011). It should be mentioned that the nomenclature used in the present study for cationized agaroses differs from that employed previously; herein, the acronym CAG is followed by the degree of substitution of the product determined by elemental analysis (DS_{EA}), i.e. CAG19 denotes cationized agarose with DS_{EA} of 0.19. For the preparation of IPECs, CAG19 (DS_{EA} = 0.19, M_n = 134.5 kDa, M_w = 242.0 kDa) and CAG77 (DS_{EA} = 0.77, M_n = 196.6 kDa, M_w = 354.0 kDa) were used.

In this research work, the two major extracts obtained from *P. nigrescens* were employed (Prado et al., 2008a): the first aqueous extract at room temperature, PNRT1 (M_n = 7.0 kDa), and the second extract at 70 °C, PN702 (M_n = 25.1 kDa). Fig. 1a–c shows the structures of the different polymers used for IPECs preparation. All the reagents employed were of analytical grade. Ibuprofen (IBF), complying with USP requirements, was purchased from Droguería Todofarma S.A. (Buenos Aires, Argentina).

2.2. Repeating unit calculation

The polymer solution concentration was calculated according to the repeating units of each polyelectrolyte. As a result, a value of 278 Da was employed for EE (Fig. 1a). Values of 956 and 350 Da were calculated for CAG19 and CAG77, respectively (Fig. 1b) (Prado et al., 2011). For the polysaccharides of *P. nigrescens* (PN) (Fig. 1c), repeating units of 395 and 336 Da were estimated for PNRT1 and PN702, respectively. Values were obtained applying the following equation:

$$\mathrm{RU} = \frac{(G \times 162) + (\mathrm{AG} \times 144) + (\mathrm{SO_3Na} \times 103) - \mathrm{SO_3Na}}{\mathrm{SO_3Na}} \quad (1)$$

In Eq. (1), G is the relative composition of galactose (G = 1, as the results are expressed with regard to agarose) (Prado et al., 2008a) and 162 is the molecular weight of the anhydrous galactose unit. Likewise, AG is the relative composition of anhydrogalactose and 144 is the molecular weight of the anhydrous anhydrogalactose unit. In turn, SO_3Na is the relative sulfate composition and 103 is the molecular weight of SO_3Na group.

2.3. Preparation of IPEC complexes

According to the manufacturer, EE is soluble in acid media up to pH = 6.0. Hence, IPECs EE–PN were prepared dissolving separately EE (1.390 g), PNRT1 (1.975 g) and PN702 (1.680 g) in 1 L of pH = 5.0 0.05 M acetic acid/sodium acetate buffer. In turn, IPECs CAG–PN were prepared by separately dissolving CAG19 (4.780 g), CAG77 (1.750 g), PNRT1 (1.975 g) and PN702 (1.680 g) in 1 L of distilled water.

Solutions (5 mM) of the corresponding cationic and anionic polymers were poured simultaneously in a vessel fitted with magnetic stirring. The agitation was maintained for 1 h and the system was allowed to stand for another hour. After isolation by centrifugation for 10 min (13,000 × *g*), the precipitate was washed with distilled water. The centrifugation and washing process was repeated twice. IPEC suspensions were lyophilized. The dried products were sieved and the particle size fraction less than 250 μm (60 mesh U.S. sieve) was used.

2.4. Turbidity, elemental analysis, and FT-IR spectra

For turbidity studies EE (13.9 mg), PNRT1 (19.8 mg) and PN702 (16.8 mg) were separately dissolved in 100 mL of pH = 5.0 0.05 M acetic acid/sodium acetate buffer. IPECs CAG–PN were prepared by separately dissolving CAG19 (47.8 mg), CAG77 (17.5 mg), PNRT1 (19.8 mg) and PN702 (16.8 mg) in 100 mL of distilled water.

For all IPECs, turbidity measurements were carried out as follows. Different volumes of the cationic polymer solution (0.5 mM of repeating units; see Section 2.2) were added to volumes of the anionic polymer solution (0.5 mM), for different molar mixing ratios in the range of 1:9 (0.11)–9:1 (9.00), with a constant final volume. The molar mixing ratios are molar ratios of repeating units of the cationic polymer to the ones of the anionic polymer. The procedure was also repeated for all mixtures but reversing the order of addition of the polymers. The system was allowed to stand for 1 h and then shaken vigorously. The relative turbidity was calculated as the ratio of the absorbance of a certain mixture at 600 nm to the maximum absorbance of each curve at 600 nm, multiplied by 100.

The composition of IPECs and raw materials was investigated by elemental analysis, using a Carlo Erba EA 1108 CHNS elemental analyzer (Carlo Erba, Milan, Italy).

FT-IR spectra of the IPECs, physical mixtures and the raw materials were determined with a 510P Nicolet FTIR spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA) employing the KBr disk method, in the range 4000–500 cm^{-1}. 32–64 scans were taken with a resolution of 2–4 cm^{-1}.

2.5. Microscopy results and specific surface areas

SEM images of the IPEC particles, the physical mixtures and the individual polymers, were obtained. A Zeiss DSM 982 Gemini electronic microscope (Carl Zeiss, Oberkochen, Germany), equipped with a field emission gun (FEG) and an in-lens secondary electron detector, was employed. Acceleration voltages were 2–4 kV. The magnifications used were in the range 500×–50,000×.

Particle size determination was performed using a Primo Star microscope (Carl Zeiss, Oberkochen, Germany) equipped with a graduated ocular, previously calibrated with a Zeiss graduated object micrometer. The accuracy of this system is ±1 μm. For each sample, 625 particles at random were chosen and their Feret diameters (Lieberman and Lachman, 1980) were measured. Preliminary observations of particle shape were also performed.

From the Feret diameter, the mean volume surface diameter (d_{vs}) was calculated. d_{vs} can be defined by the following equation:

$$d_{vs} = \frac{\sum n_i X_i^3}{\sum n_i X_i^2} \quad (2)$$

where n_i is the number of particles in each size interval (sizes between 0 and 250 μm were taken, with intervals of 10 μm) and X_i, the arithmetical mean of each size interval employed.

N_2 adsorption isotherms (77 K) were determined for IPECs by the volumetric technique. Samples were previously degassed overnight at 310 K with a final pressure of 1.33×10^{-4} Pa (10^{-6} mm Hg). The conventional Brunauer, Emmet and Teller (BET) procedure

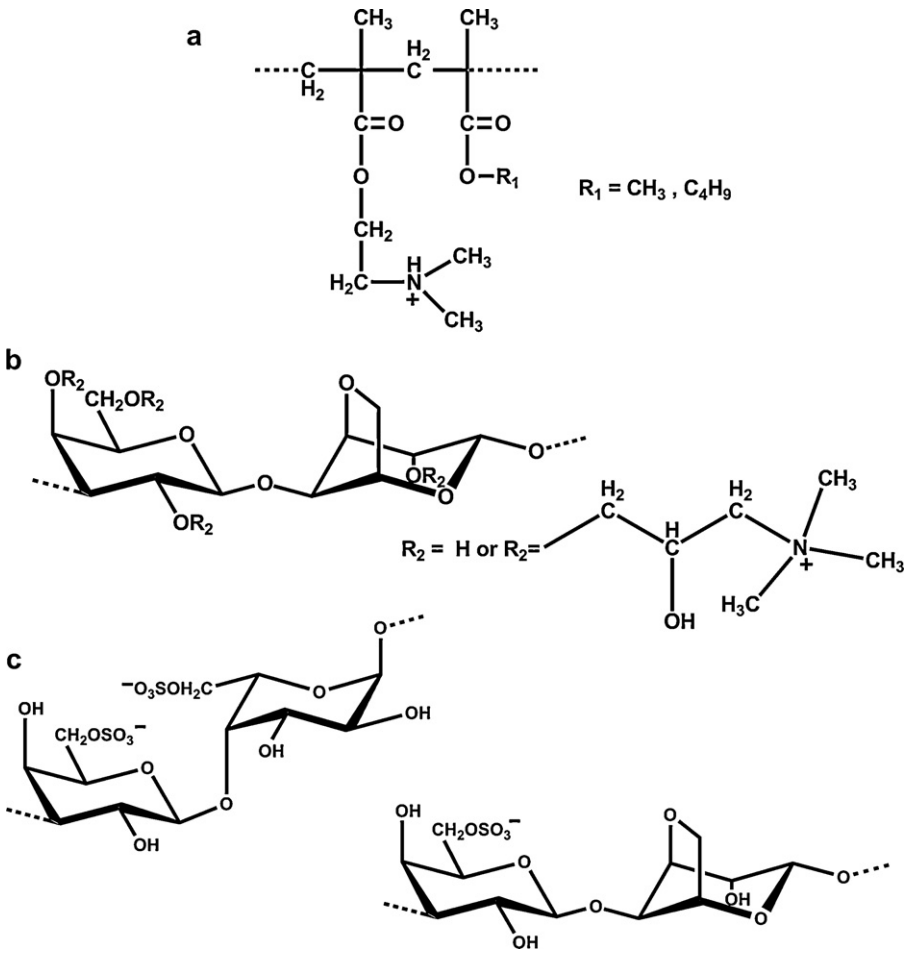

Fig. 1. Repeating units of basic butylated methacrylate copolymer (Eudragit E, EE) (a), cationized agaroses (CAGs) (b) and diads present in the polysaccharides of *P. nigrescens* (PN) (c).

was applied to evaluate the specific surface areas of the samples. A Gemini 2360 sorption instrument (Micromeritics Instrument Corp., Norcross, GA, USA) was used.

2.6. Static angles of repose, preparation of tablets and compactibility profiles

The flow properties of the IPECs were evaluated semi quantitatively by measuring the static angle of repose (Lieberman and Lachman, 1980; USP 30/NF 25, 2007). A funnel filled with the IPEC was maintained 2 cm above a graduated surface, the funnel was emptied and the angle of repose was calculated measuring the diameter of the cone formed.

Tablets were prepared in order to examine basic aspects, including swelling and erosion, compactibility, and drug release. For the swelling and erosion tests, round flat tablets were prepared (total weight 100 ± 1 mg, 7.0 ± 0.1 mm of diameter) by compressing the solid IPEC or the physical mixture. A hydraulic press (W.A. Whitney, Rockford, IL, USA) was employed. A compression force of 500 kp was applied to the tablets.

To determine the compactibility profile, tablets of the same characteristics were prepared, but applying compression forces in the range 300–1000 kp and its hardness was measured with an automatic durometer (Vanderkamp VK200, VanKel Technologies, Inc., NJ, USA). The mean of three determinations is informed.

For drug release tests, IPEC and IBF was manually mixed and tablets were prepared (100 ± 1 mg or 150 ± 1 mg) by compressing 50 mg IPEC and 50 mg IBF or 100 mg IPEC and 50 mg IBF, respectively. For comparative purposes tablets containing 100 mg of the physical mixture and 50 mg IBF were also prepared. The compression force was 500 kp.

2.7. Degree of swelling, erosion and ibuprofen release profiles

The degree of swelling of tablets of the IPECs and the physical mixtures was investigated, simulating the physiological conditions of the gastrointestinal tract (Moustafine et al., 2005a, 2006). For this purpose, tablets were placed in a tared basket of the dissolution tester and immersed for 2 h in HCl solution (pH = 1) and afterward in Na_3PO_4 buffer (pH = 6.8). Then, the test was continued for another 22 h. The temperature of the medium was $37.0 \pm 0.5\,^{\circ}C$. Measurements consisted in removing the basket from the medium, drying by filter paper and weighting in an analytical balance. Weight differences were measured at 15 min and from 0.5 h to 8 h every 30 min; an equilibrium value of swelling was determined after 24 h, as evaluated by further determinations.

The degree of swelling ($S\%$) at each time was calculated by means of the equation:

$$S\% = \frac{m_2 - m_1}{m_1} \times 100 \quad (3)$$

where m_1 is the weight of the initial tablet (dry) and m_2 is the weight of the swollen tablet at different times. The informed results are the mean of three determinations.

The degree of erosion ($E\%$) of the IPECs and the corresponding physical mixtures was determined by lyophilizing the tablets after

reaching the equilibrium swelling values (24 h) and was calculated by the equation:

$$E\% = \frac{m_1 - m_3}{m_1} \times 100 \quad (4)$$

where m_1 is the weight of the initial tablet (dry) and m_3 is the weight of the lyophilized tablet after erosion. The informed results are the mean of three determinations.

The release profile of IBF was determined employing a dissolution equipment (Alycar Instruments, Argentina) that meets USP requirements for Apparatus 1 (basket) A rotating speed of 100 rpm was used and the temperature of the medium was 37.0 ± 0.5 °C. The dissolution medium employed was 750 mL of 0.1 M HCl (pH = 1) for the first 2 h, then 250 mL of 0.20 M Na_3PO_4 solution were added to reach a pH of 6.8 ± 0.05 (if necessary, pH was rapidly adjusted with 2 M HCl or 2 M NaOH); the test was allowed to continue for six more hours (total release time evaluated: 8 h) (Moustafine et al., 2005a, 2006; USP 30/NF 25, 2007). Tablets containing 50 mg IPEC and 50 mg IBF, 100 mg IPEC and 50 mg IBF, and 100 mg of physical mixtures and 50 mg IBF were tested.

Aliquots of 3 mL were taken every 30 min, and were filtered immediately through a 0.45 μm PVDF membrane. The reduction of volume was taken into account for the calculations. The amount of IBF released was determined spectrophotometrically at 221 nm (Cary 1E, Varian Inc., Palo Alto, CA, USA). The results informed for each tablet formulation are the mean of three determinations. Previous studies indicated that IPECs or physical mixtures did not interfere with the determination of the model drug.

3. Results and discussion

3.1. Formation and characterization of the different IPECs

Fig. 1 shows the structures of the cationic and anionic components employed for IPEC formation. EE (Fig. 1a) is a synthetic polymer, whereas CAG (Fig. 1b) is a natural polysaccharide modified with 2-hydroxy-3-(N,N,N-trimethylammonium)propyl groups. EE presents tertiary amino groups whose protonation depends on the pH. Unlike the former, CAG possesses quaternary ammonium groups that remain cationic in the whole range of pH. In this work two cationized agaroses were employed CAG19 and CAG77 that differ mainly on their degree of substitution (0.19 and 0.77, respectively) (Prado et al., 2011).

In Fig. 1c, the structures of the main diads present in the agarans of *P. nigrescens* (PN) are shown. The negative charge of the sulfate groups in PN is also independent of pH. The polysaccharides of *P. nigrescens* employed presented different molecular weight (PNRT1, M_n = 7.0 kDa and PN702, M_n = 25.1 kDa) (Prado et al., 2008a).

The IPECs are labeled according to the following nomenclature: the use of the word IPEC, followed by the name of the cationic and the anionic components; both components are separated by a hyphen.

3.2. Turbidity, elemental composition, and FT-IR spectra

IPECs formation was initially examined by turbidity measurements. Turbidity is caused by the formation of insoluble complexes that reduce transmitted light by scattering. Maximum turbidity of the IPECs was achieved for a molar mixing ratio of 1:1 (ratio of repeating units), known as stoichiometric complexes (Thünemann et al., 2004). For molar mixing ratios different from 1:1, overcharging of the complexes could lead to non-stoichiometric complexes with increased solubility and, consequently, to higher light transmission. Similar curves were obtained when the order of addition of polymer solutions was inverted, indicating that IPEC composition is independent of the mixing order.

In order to confirm the stoichiometry of each component in the different solid IPECs, elemental analysis was performed. Table 1 shows experimental values and those calculated considering a 1:1 interaction of the repeating units. The experimental N:S molar ratio is also presented. A good correlation between experimental and calculated values was found. A slight increase in the N:S molar ratio above the value expected (equal to 1) was detected for all the IPECs, this is due to the presence of small quantities of seaweed proteins as impurities in the PN extracts. In the elemental analysis of PNRT1 and PN702, N values of 0.89% and 1.35% were obtained, respectively. No elemental S was found in EE and CAG. Accordingly, all the sulfur in the IPECs arises from the anionic polysaccharides of *P. nigrescens* (PNRT1 or PN702).

In the FT-IR spectra of IPEC EE–PNRT1 and IPEC EE–PN702 the disappearance of the absorption bands at 2770 and 2824 cm^{-1} corresponding to the non-ionized dimethylamino groups in EE spectrum was detected, indicating the formation of an ammonium salt. Those signals were present in raw materials and physical mixtures (obtained by dry mixing the powders of the raw materials) spectra. A wide and weak band at 2550 cm^{-1} was also detected in the IPECs spectra, and has been assigned to the interaction of the quaternary ammonium with the polyelectrolyte of opposite charge (Moustafine et al., 2005a, 2005b, 2006). On the other hand, the band at 822 cm^{-1} of PNRT1 and PN702 assigned to the sulfate group in the primary hydroxyl (C-6) of the galactose, was shifted to 809 and 811 cm^{-1}, respectively, in the IPECs. There were also differences in the fingerprint region between the spectra of the IPECs EE–PN and the corresponding components and physical mixtures.

The spectra of the four IPECs CAG–PN presented similar trends to the aforementioned IPECs. The band at 822 cm^{-1} (sulfate in C-6) of PNRT1 and PN702 was not observed in IPEC CAG19–PNRT1 and IPEC CAG19–PN702, whereas a weak band shifted to 812 cm^{-1} was found in IPEC CAG77–PNRT1 and IPEC CAG77–PN702. The band at 1482 cm^{-1} (C—H bending of the methyl groups of the quaternary ammonium) of the cationic substituent of CAG, shifted to 1469–1473 cm^{-1} in the IPECs. For the physical mixtures, the bands at 822 cm^{-1} and 1482 cm^{-1} remained in the same position as in the raw materials. The IPECs also showed differences in the fingerprint regions with regard to the respective physical mixtures.

3.3. Microscopy results and specific surface areas

SEM microscopy (Fig. 2) was used for the characterization of raw materials, physical mixtures and IPEC particles in solid state, providing information of shape, particle size, surface and presence of pores. EE appeared as particles of irregular shape (Fig. 2a), whereas PN presented fibrillar structures, of larger size. PNRT1 samples (Fig. 2d) were very similar to those found in PN702. In turn, CAG19 particles (Fig. 2b) showed a branched reticular structure that became more evident at high magnifications, whereas CAG77 particles (Fig. 2c) were amorphous and smooth. In physical mixtures, the raw materials particles maintained their original individual characteristics and could be easily distinguished.

In contrast, all the studied IPECs presented very different characteristics when compared with the raw materials; these characteristics were inherent to each IPEC in particular. IPEC EE–PNRT1 and IPEC EE–PN702, were in turn similar to each other, presenting irregular shapes with a reticular three-dimensional structure (Fig. 2e and f) and pores in the range 250–600 nm.

The particles of the four IPECs CAG–PN presented irregular characteristic shapes with smooth surfaces (Fig. 2g and h). No differences were identified among the different IPECs CAG–PN. From the images, it can be inferred that IPECs based on EE (IPECs EE–PN) presented porous structures, whereas IPECs CAG–PN were non-porous. We had previously reported porous structures in IPECs based on EE and kappa carrageenan (Prado et al., 2008b) and

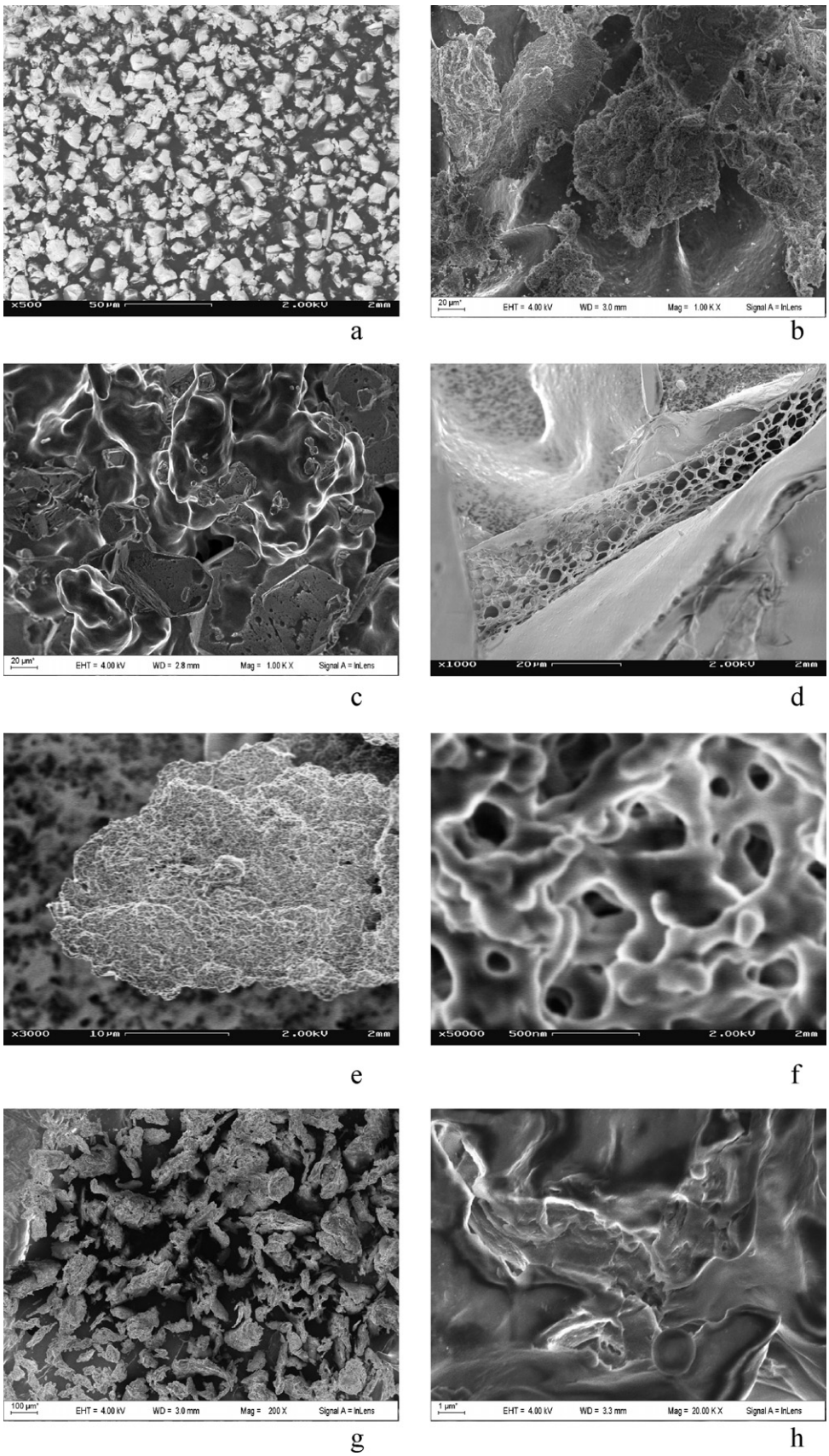

Fig. 2. Scanning electron micrographs of Eudragit E 500× (a); CAG19 100× (b); CAG77 1000× (c); PNRT1 100× (d); IPEC EE–PN702 3000× (e); 50,000× (f); IPEC CAG19–PNRT1 200× (g); ×20,000 (h).

Table 1
Elemental analysis of IPECs.

IPEC	Experimental value, %				Calculated value, %				Experimental N:S molar ratio
	C	H	N	S	C	H	N	S	
EE–PNRT1	47.69	6.88	2.33	4.88	47.25	6.76	2.15	4.92	1.16
EE–PN702	47.46	6.85	2.63	5.45	47.63	6.80	2.36	5.41	1.10
CAG19–PNRT1	44.99	6.15	1.19	2.46	45.28	6.08	1.08	2.48	1.11
CAG19–PN702	45.40	6.18	1.26	2.64	45.28	6.07	1.13	2.60	1.09
CAG77–PNRT1	43.06	6.26	2.27	4.64	43.56	6.22	2.04	4.66	1.12
CAG77–PN702	43.18	6.21	2.52	5.14	43.57	6.21	2.23	5.10	1.12

Table 2
Mean volume surface diameter (d_{vs}), BET area and theoretical area for the IPECs.

IPEC	d_{vs} (μm)	BET area ($m^2\ g^{-1}$)	Theoretical area ($m^2\ g^{-1}$)
EE–PNRT1	86	0.985	0.070
EE–PN702	89	0.990	0.067
CAG19–PNRT1	189	0.050	0.032
CAG19–PN702	190	0.045	0.032
CAG77–PNRT1	195	0.054	0.031
CAG77–PN702	178	0.048	0.034

non-porous structure for IPECs based on cationized starch (with the same group as in this work) and kappa carrageenan (Prado et al., 2009). Unfortunately, no SEM results have been reported for other IPEC systems in order to perform comparisons.

The Feret diameter of particles and the mean volume surface diameter (d_{vs}) of each IPEC were calculated from observations by optical microscopy (Lieberman and Lachman, 1980). The results are presented in Table 2. Optical microscopy was also useful to perform preliminary observations on the shape of the particles. The observations were in agreement with those by SEM.

In Table 2, the results of BET areas of the different IPECs are presented. The theoretical areas of non-porous monodispersed spheres, of the same diameter to that determined for each sample (considering a density of 1 g cm^{-3}) were included in the last column of the table, for comparative purposes (Lieberman and Lachman, 1980). The BET area of IPEC EE–PNRT1 and IPEC EE–PN702 (0.985 and 0.990 $m^2\ g^{-1}$) was one order of magnitude higher than the theoretical one for a sample with similar volume surface diameter (d_{vs}, 86 and 89 μm). This points to a porous structure in agreement with SEM observations. The four IPECs CAG–PN presented BET areas consistent with theoretical values and with SEM observations, where they appeared as non-porous materials.

3.4. Static angles of repose and compactibility profiles

The static angles of repose for each IPEC are shown in Table 3. A description of the flow properties (USP 30/NF 25, 2007) is included in the same table. IPECs EE–PN presented better flowability than IPECs CAG–PN. There was almost no difference between the two IPECs EE–PN or among the four IPECs CAG–PN. Flowability could be improved even more by applying spray drying (Singh and Naini, 2007).

In Fig. 3, the compactibility profiles of IPECs are shown. The mean of three determinations is informed with a confidence interval of 95%. In general, all the IPECs presented good compactibility properties, achieving appropriate hardness values for low compression forces.

Table 3
Static angles of repose for IPECs.

IPEC	Static angle of repose (°)	Flow properties
EE–PNRT1	31	Good
EE–PN702	32	Good
CAG19–PNRT1	37	Fair – aid not needed
CAG19–PN702	37	Fair – aid not needed
CAG77–PNRT1	38	Fair – aid not needed
CAG77–PN702	37	Fair – aid not needed

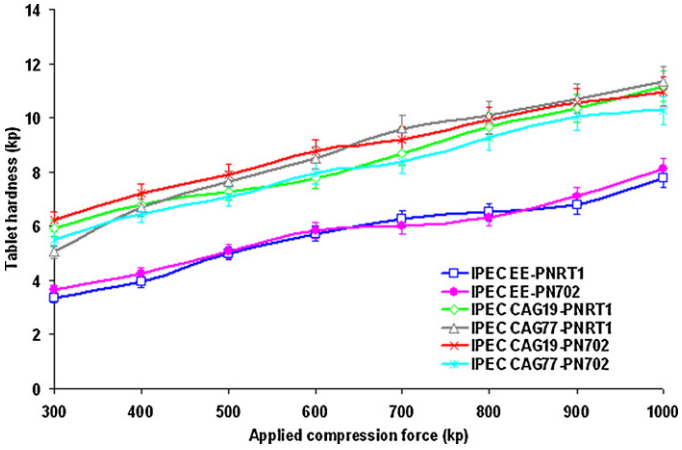

Fig. 3. Compactibility profile of tablets containing 100 mg of IPECs.

3.5. Degree of swelling, erosion and ibuprofen release profiles

Swelling profiles for IPEC EE–PNRT1 and IPEC EE–PN702 reached values of 126% and 158%, respectively, at the end of the acid stage (2 h) (Fig. 4). For the buffer stage (pH = 6.8), swelling values sharply rose to 204% and 261%, respectively. Equilibrium values of 150% and 188% were determined after 24 h, at the end of the buffer stage. This behavior could be due to the gradual neutralization of the protonated dimethylamino groups, causing reduction in the interaction between both polymers and subsequent relaxation of the matrix. Moreover, these free dimethylamino groups could intervene as

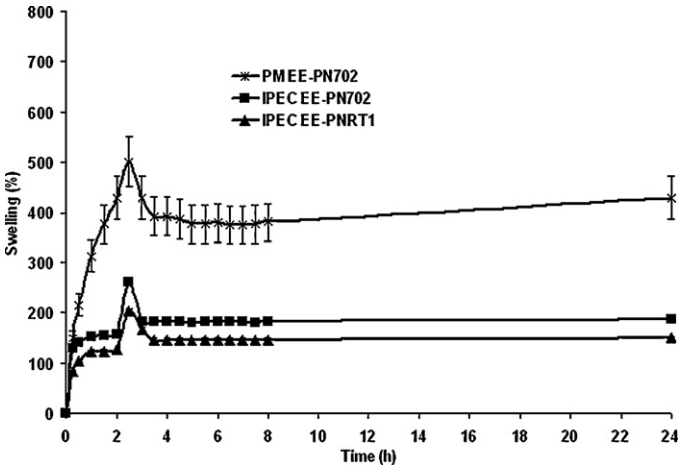

Fig. 4. Swelling profile of tablets containing 100 mg of IPECs EE–PN or 100 mg of physical mixtures.

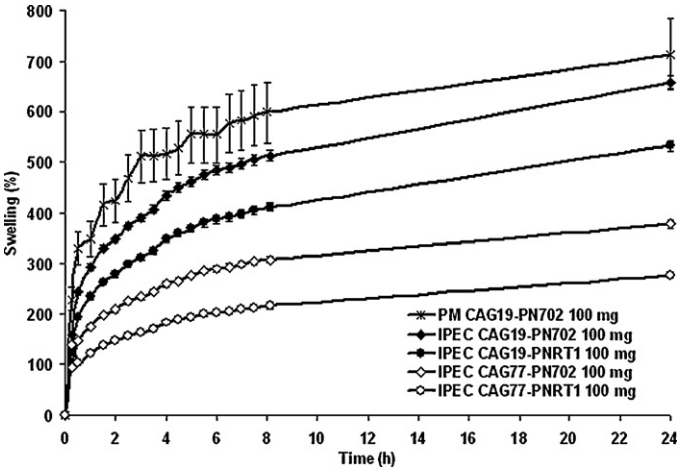

Fig. 5. Swelling profile of tablets containing 100 mg of IPECs CAG–PN or 100 mg of the physical mixture.

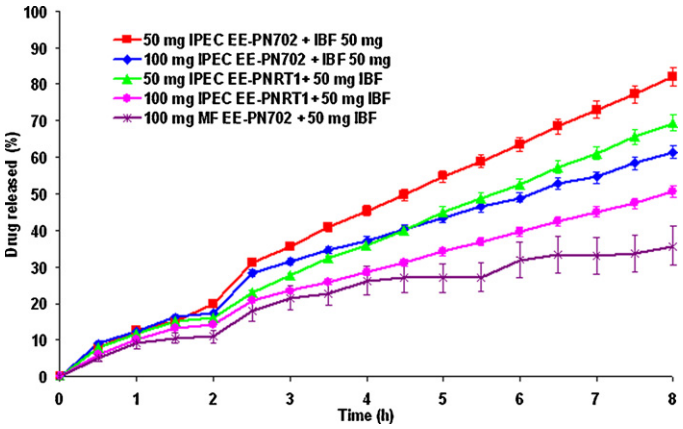

Fig. 6. Release of IBF from tablets containing 50 or 100 mg of IPECs EE–PN (plus 50 mg IBF), or 100 mg of the physical mixture (plus 50 mg IBF).

acceptors in hydrogen bonding with the hydroxyls groups of PN, and the rearrangement would lead to the equilibrium swelling values. An increase in the molecular weight of the anionic component was related to a higher experimental swelling value. The sharp increase in swelling followed by a reduction, after an increase of the pH, seems to be a typical behavior of IPECs that have EE as the cationic component; this is the case of IPEC Eudragit E100–Eudragit L100 (Moustafine et al., 2005a, 2006), IPEC Eudragit E–alginate (Moustafine et al., 2009) and the IPEC EE–kappa carrageenan (Prado et al., 2008b).

The physical mixtures PM EE–PN exhibited higher values of swelling than its IPEC, likely due to negligible interactions between both polymers that act independently in the physical mixture. Erosion after 24 h for IPEC EE–PN702, IPEC EE–PNRT1 and the physical mixture was 2.7%, 3.4% and 6.7%, respectively.

IPECs CAG–PN presented a more gradual increase in swelling after pH change, probably because the ionization of their ionic groups was independent of the pH (Fig. 5). As seen in Fig. 5, swelling values followed the order IPEC CAG19–PN702 > IPEC CAG19–PNRT1 > IPEC CAG77–PN702 > IPEC CAG77–PNRT1. An increase in swelling was related to an increase in the molecular weight of the anionic component, and to a decrease in the degree of substitution of the cationic component. For IPEC chitosan–Eudragit L100 and IPEC chitosan–Eudragit L100–55, a higher swelling was reported when chitosan of higher molecular weight was employed (Moustafine et al., 2008).

The physical mixture PM CAG19–PN702 showed a gradual increase in swelling, with a value of 423% at the end of the acid stage and of 713% after 24 h (Fig. 5). The values of erosion after 24 h for the different IPECs were low (2.2–3.5%) and slightly higher (6.7%) for the physical mixtures. From erosion values of all the IPECs, it can be concluded that this phenomenon is less important than swelling, at least, for the experimental conditions employed.

Ibuprofen (IBF) release profiles from tablets containing 50 or 100 mg of the different IPECs and 50 mg of IBF are presented in Figs. 6 and 7. For comparative purposes, drug release profiles of tablets containing the physical mixtures and IBF are also shown in the same figures. The profiles in the figures show two distinguished regions: acid stage and buffer stage. The comparison of the release values during the first 30 min (first experimental point) with those corresponding to equivalent successive intervals, allowed to detect the presence or the lack of a "burst release" effect (Huang and Brazel, 2001) for each IPEC system; this effect is difficult to predict a priori. IPECs EE–PN presented a small and consistent degree of burst release; this was also found in IPEC EE–kappa carrageenan, another EE based IPEC (Prado et al., 2008b). On the contrary, IPECs CAG–PN presented a release in the first interval similar to that in the next one, not exhibiting that effect. Burst release in monolithic hydrophilic matrices formed by the IPECs could be due to some drug trapped on the surface of the matrix during compression, especially in the case of high drug loads, leading to immediate drug release. Burst release would then occur in the IPECs that are less efficient in quickly generating a layer of hydrogel, capable of controlling the release of the drug on the surface. Nevertheless, the existence of heterogeneity in the IPEC matrix that gives rise to different microdomains should not be neglected (Huang and Brazel, 2001).

During the acid stage, release values were low for IPECs EE–PN (11.0–20.0%) and for IPECs CAG–PN (11.0–14.7%). This could be attributed mainly to the action of the matrix and to the low solubility of IBF in the acid medium. The sharp change in pH from the acid to the buffer stage generated a relative increase in the amount of drug released for most IPEC systems. The pH change affected release not only because of the variation of the matrix swelling (in the case of IPECs EE–PN) but also because the carboxylic group of ibuprofen ionized in the buffer stage, turning the drug more soluble in that condition. The solubility of IBF at pH = 1.0 is 0.027 mg mL^{-1} and at pH = 7.0 it increases to 10.1 mg mL^{-1}, namely 374 times higher (Higgins et al., 2001). Hence, 20.25 mg of IBF would be the theoretical maximum amount that can be dissolved in the 750 mL of the acid stage medium (45.5% of the total IBF content in the tablets).

The IPEC systems prepared in this study led to a variety of release profiles with near zero order kinetics, in the buffer stage. For IPECs

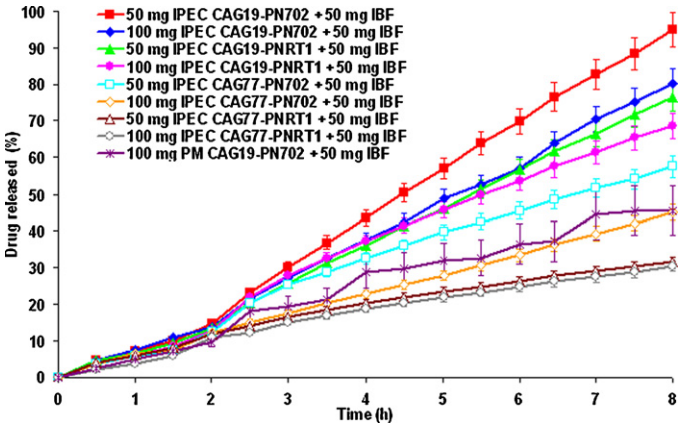

Fig. 7. Release of IBF from tablets containing 50 or 100 mg of IPECs CAG–PN (plus 50 mg IBF), or 100 mg of the physical mixture (plus 50 mg IBF).

EE–PN, the molecular weight of the anionic component (25.1 kDa for PN702 and 7.0 kDa for PNRT1) and the amount of the IPEC in the tablets formulation were varied. 100 mg IPEC EE–PNRT1 and 50 mg IPEC EE–PNRT1 formulations (plus 50 mg IBF in each case) achieved releases of 50.7% and 69.4%, respectively, after 8 h of test (Fig. 6). Instead, 100 mg EE–PN702 and 50 mg EE–PN702 formulations achieved values of 61.4% and 82.0%, respectively, for the same time (Fig. 7). It follows that decreasing the molecular weight of the anionic component decreased drug release. For IPECs CAG–PN, in addition to the variation in the degree of substitution of the cationic component (CAG) and the amount of IPEC in the tablets, the molecular weight of the anionic component was also modified. In this way, final release values from 30.5% for 100 mg IPEC CAG77–PNRT1 formulation to 95.0% for 50 mg IPEC CAG19–PN702 formulation were achieved. With regard to the variation in the molecular weight of PN, similar conclusions as for the IPECs EE–PN are attained. On the other hand, the increase in the degree of substitution of CAG (from 0.19 in CAG19 to 0.77 in CAG77) decreased release, keeping other conditions constant. For the two cationized agaroses, the molecular weight (M_n) was varied from 134.5 kDa in CAG19 to 196.6 kDa in CAG77. Accordingly, in this case, the influence of the lower degree of substitution of CAG19 would have been more important than its lower molecular weight, as the cationic component of lower molecular weight allowed for a quicker release. It should be taken into account that unlike PNs which differ mainly in their molecular weigh, CAG19, of lower molecular weight is actually composed of longer chains of polysaccharide than CAG77, which was further degraded by the more drastic conditions of its substitution reaction, as determined by light scattering measurements. The higher molecular weight of CAG77 is due to the higher number of substituting groups in the polysaccharide backbone. The higher release of IPEC Eudragit L100–chitosan and IPEC Eudragit L100-55–chitosan with the increase in the molecular weight of chitosan has been reported (Moustafine et al., 2008).

Comparison of the matrix swelling and drug release profiles for the same IPEC system indicates that the evaluated systems showing higher swelling were the ones that released the model drug faster. For IPECs EE–PN, the IPEC EE–PN702 presented higher swelling and drug release than IPEC EE–PNRT1. The swelling order for IPECs CAG–PN: IPEC CAG19–PN702 > IPEC CAG19–PNRT1 > IPEC CAG77–PN702 > IPEC CAG77–PNRT1, agreed with that found for the drug release.

The release from tablets composed of the physical mixtures PM EE–PN or PM CAG–PN plus IBF was slower than that observed in the respective IPECs, despite its higher swelling. Physical mixtures of pectin–chitosan and pectin–arabic gum released chlorpromazine slower than the corresponding IPECs (Meshali and Gabr, 1993), and we found the same behavior in the release of IBF from physical mixtures EE–kappa carrageenan (Prado et al., 2008b); however, in the case of physical mixtures of cationized starch–kappa carrageenan, we found a faster release than in the IPEC, possibly because the starch in the physical mixture acted as a tablet disintegrant exposing IBF to rapid dissolution (Prado et al., 2009). In all cases the physical mixtures presented widely dispersed results among replicates, as reflected in the amplitude of the error bars corresponding to a confidence level of 95% in the figures of the release profiles (Figs. 6 and 7).

Because sink conditions were not attained in the acid stage, only experimental release data from the buffer stage were fitted to a mathematical model (Model I) based in previous proposals (Peppas, 1985; Siepmann and Peppas, 2001). Accordingly, experimental data corresponding to fractions of drug released ≤60% were employed. Model I equation is as follows:

$$\frac{M_t}{M_\infty} = k(t - t')^n + \alpha \quad (5)$$

where M_t/M_∞ is the fraction of drug released at time t, k, the apparent release constant that takes into account the geometrical and structural characteristics of the drug release system, t, the time elapsed since the beginning of the release test, t', the duration of the acid stage (t' = 2 h), n, the release exponent and α, a term that considers the amount of drug released in the acid stage (0–2 h).

The model characteristic parameters, evaluated by non-linear regression analysis of the results corresponding to the buffer stage, are shown in Table 4. As can be inferred from R^2 values (≥0.991), the model appropriately describes the experimental data. For cylindrical geometry, as is the case of the tablets prepared in this article, a release exponent (n) of 0.45 points to Fickian diffusion transport, whereas values of n between 0.45 and 0.89 suggest non-Fickian transport. In turn, values of n close to 0.89 indicate that the system releases the drug with a zero order kinetics (Case II transport) independently of the real drug release mechanism (Peppas, 1985; Siepmann and Peppas, 2001).

As can be seen in Table 4, n values were close to 0.89 for all the IPEC formulations (range $0.81 < n < 1.02$). For all the IPEC systems evaluated, the k parameter for the formulation prepared with 50 mg of a given IPEC, was higher than the one for the formulation that contained 100 mg of the same IPEC. In general, this indicates that the k value could be modified by changing the proportion of the IPEC in the tablets. However, the extent of the variation depends on the specific IPEC system considered. Thus, the 50 mg IPEC CAG77–PNRT1 formulation presented a k value of 5.60, barely higher than 5.30 for the 100 mg IPEC CAG77–PNRT1 formulation. Modeling experimental dissolution data for the physical mixtures, led to low R^2 values (0.969–0.979) (Table 4).

To further study the mechanisms involved in drug release, experimental data from the buffer stage were fitted to a second model (Model II) based on previous proposals (Peppas, 1985; Siepmann and Peppas, 2001). The equation representing the model used is:

$$\frac{M_t}{M_\infty} = k_1(t - t')^m + k_2(t - t')^{2m} + \alpha' \quad (6)$$

The meaning of terms M_t/M_∞, t and t', is the same as for Model I. The model characteristic parameters k_1, k_2, and m, evaluated by non-linear regression analysis, are presented in Table 4.

It is worth mentioning that in this case, the term α' added is the respective experimental release value at time = 2 h (end of the acid stage) and was included in order to reduce the number of model parameters to be adjusted. As shown in Table 4, R^2 values were greater than or equal to 0.992. Accordingly, Model II seems to adequately describe the experimental release data from IPECs matrices. On the contrary, dissolution data for the physical mixtures did not show a good fit to Model II.

The fact that the release of drug from the physical mixtures could not be estimated from the swelling tests and did not show a good fit to Models I and II, provide additional support to the observations on the important differences between the behavior of physical mixtures and their corresponding IPECs.

The first term on the right side of Model II equation represents Fickian diffusional contribution, F, whereas the second term represents the Case II matrix relaxation, R. The ratio of both contributions can be calculated as follows:

$$\frac{R}{F} = \frac{k_2 t^m}{k_1} \quad (7)$$

The model characteristic parameters estimated in Table 4 and the experimental data from the IPEC systems were used to build Figs. 8 and 9, namely to represent the R/F ratio versus the drug release percentage.

The results shown in Figs. 8 and 9 indicate that in general, the contribution of Fickian diffusion dominated all formulations, when

Table 4
Model characteristic parameters of Model I and Model II.

IPECs	IPEC:IBF (mg:mg)[a]	Model I				Model II			
		k	n	α	R^2	k^1	k^2	m	R^2
EE–PNRT1	50:50	9.15	0.96	18.40	0.999	8.83	2.76	0.63	0.999
	100:50	5.19	1.02	18.14	1.000	6.56	2.10	0.59	0.993
EE–PN702	50:50	10.36	0.93	25.57	0.999	11.31	4.68	0.51	0.998
	100:50	5.96	1.00	25.43	0.999	9.94	3.58	0.47	0.992
CAG19–PN702	50:50	13.64	1.00	16.29	1.000	9.98	5.20	0.65	1.000
	100:50	12.93	0.87	14.27	0.998	8.36	5.33	0.56	0.997
CAG19–PNRT1	50:50	13.11	0.89	12.50	0.994	9.44	3.53	0.63	0.999
	100:50	12.80	0.81	14.20	0.998	9.50	5.68	0.50	0.999
CAG77–PN702	50:50	9.50	0.85	14.19	0.999	11.01	1.50	0.60	0.999
	100:50	5.51	0.95	12.94	0.998	4.98	0.70	0.80	0.997
CAG77–PNRT1	50:50	5.60	0.81	9.78	0.991	0.70	0.40	0.57	1.000
	100:50	5.30	0.86	8.29	0.991	6.00	0.60	0.58	0.998
Physical mixtures	PM:IBF (mg:mg)[a]	Model I				Model II			
		k	n	α	R^2	k^1	k^2	m	R^2
EE–PN702	100:50	8.18	0.55	12.81	0.979	8.18	0.57	0.50	0.978
CAG19–PN702	100:50	6.79	0.88	13.46	0.969	7.49	2.76	0.52	0.970

[a] Weight ratio between IPEC (or physical mixture) and ibuprofen in the tablet.

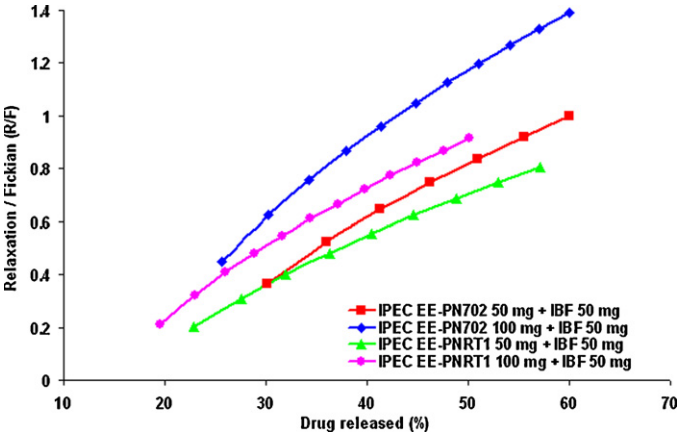

Fig. 8. Ratio between matrix relaxation and Fickian diffusion contributions (R/F), for 50 or 100 mg IPECs EE–PN (plus 50 mg IBF) formulations.

the percentage of drug released was low (short times). In turn, for a particular drug release percentage, the contribution of matrix relaxation was relatively more important for formulations containing greater amounts of IPEC. In addition, for each formulation, the relaxation of the matrix also became more important when the percentage of drug released increased, even exceeding diffusion in the cases of tablets of 50 mg IPEC EE–PN702 plus 50 mg IBF, 50 mg IPEC CAG19–PN702 plus 50 mg IBF, 100 mg IPEC CAG19–PN702 plus 50 mg IBF and 50 mg IPEC CAG19–PNRT1 plus 50 mg IBF formulations.

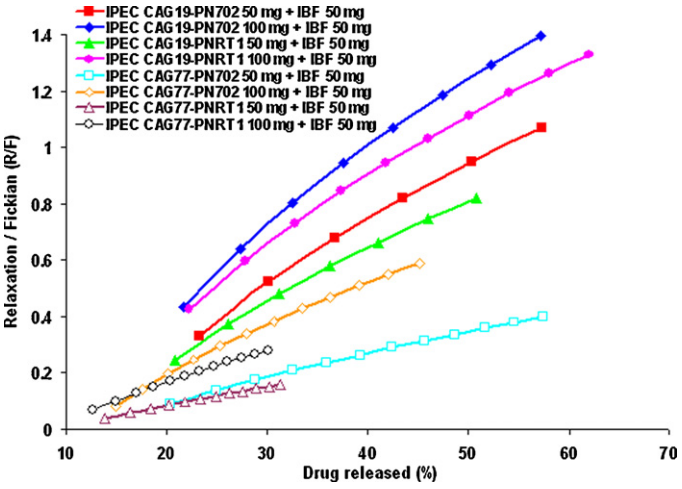

Fig. 9. Ratio between matrix relaxation and Fickian diffusion contributions (R/F), for 50 or 100 mg IPECs EE–PN (plus 50 mg IBF) formulations.

4. Conclusions

Novel interpolyelectrolyte complexes were prepared and characterized to evaluate their potential as controlled release matrices. Turbidity results indicated that IPEC composition was not affected by the order of addition of components. The elemental analysis confirmed the formation of stoichiometric complexes (1:1 interaction of the repeating units). FT-IR spectra of IPECs was different from the starting materials and their physical mixtures as a result of the ionic interactions between the opposite charged polyelectrolyte chains. Solid IPEC particles showed characteristic shapes and IPECs EE–PNs presented porous structures in line with their BET areas. All the IPECs tested exhibited good flowability and compactibility properties allowing their use for full-scale manufacture.

Tablets were prepared by direct compression, which is an advantageous method from both operational and financial viewpoints. No organic solvents were used during the preparation of formulations, nor in the extraction of polysaccharides from *P. nigrescens* nor in the synthesis of CAGs. Swelling behavior of IPEC tablets was very different from the ones determined for physical mixtures tablets. These monolithic matrix systems released the drug with near zero-order kinetics during the buffer stage. According to a model that allows an adequate description of experimental dissolution data in the buffer stage, Fickian diffusion appears to predominate initially over relaxation, with the latter increasing along the release process, to even surpass the first in some systems. The results indicated that release profiles could be controlled by altering the amount of IPEC in the tablets. Also, the change in molecular weight and the degree of substitution of the components allowed varying release profiles. These results could be particularly useful to companies interested in controlled release technologies, but limited to the direct compression technique.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijpharm.2012.03.016.

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