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ORIGINAL ARTICLE

Carrageenans from *Sarcothalia crispata* and *Gigartina skottsbergii*: Structural Analysis and Interpolyelectrolyte Complex Formation for Drug Controlled Release

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Abstract

The aims of the present study were to characterize for the first time the carrageenan extracted from cystocarpic stage of *S. crispata* collected in the Patagonian coast of Argentina, and to prepare interpolyelectrolytic complexes (IPECs) between the polysaccharide extracted from cystocarpic stage of *Sarcothalia crispata* and *Gigartina skottsbergii* thalli, and basic butylated methacrylate copolymer (Eudragit E), in order to test their potential for the controlled release of ibuprofen as model drug. The structural determination revealed that the polysaccharides extracted from *S. crispata* and *G. skottsbergii* were mainly constituted by κ-carrageenan, particularly in the case of *G. skottsbergii*; however, significant amounts of ι- and ν-carrageenan were also detected in both polygalactans. The differences in diad composition and possibly in their distribution along the polysaccharide chain of both carrageenans would favor a different arrangement in the resulting IPEC structure. The smaller pores observed by scanning electron microscopy in the IPEC of *S. crispata* suggest that the kinks in the polysaccharide backbone are evenly distributed, resulting in a slower ibuprofen release compared to the IPEC of *G. skottsbergii*.

Keywords *Sarcothalia crispata* · *Gigartina skottsbergii* · Carrageenan · IPECs · Controlled drug release

Introduction

Carrageenans are non-fibrillar sulfated galactans extracted from red algae. These polysaccharides consist of linear chains of alternating 3-linked β-D-galactopyranosyl and 4-linked α-D-galactopyranosyl units, the 4-linked unit often occurring as 3,6-anhydropyranosyl moiety. Additionally, hydroxyl groups in these repeating units may be substituted by sulfate groups. The sulfation pattern of the 3-linked unit and the presence/absence of anhydrogalactose in the 4-linked unit define different properties of these polygalactans, which have been traditionally classified into six basic forms ι-, κ-, λ-, μ-, ν-, and θ-

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carrageenans (Campo et al. 2009; Stortz and Cerezo 2000; Usov 2011). As gelling, stabilizing, and viscosity-binding agents, carrageenans have been extensively used in food, cosmetic, and pharmaceutical industries (McHugh 2003; Rinaudo 2008; Li et al. 2014).

In recent years, there has been an increasing interest in obtaining chemical modifications of these galactans (Prado and Matulewicz 2014; Barahona et al. 2015) or interactions with other natural or synthetic polymers (Prado et al. 2008a, 2009; Khan et al. 2017). This aims to improve their properties or achieve new ones, especially in the release of pharmaceuticals and agrochemicals, among others. Undoubtedly, obtaining novel products through such modifications implies adding value to these natural products and turning them into commercially attractive commodities. Non-covalent crosslinked gels have the benefit of avoiding the use of crosslinking agents. These agents may affect the integrity of the substances to be entrapped and are often toxic; thus, they have to be removed before the hydrogels can be applied (Hennink and van Nostrum 2012). 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). IPECs may provide a valuable tool to design drug delivery systems (Dautzenberg et al. 2000; Moustafine et al. 2009; Dalmoro et al. 2017; Moustafine et al. 2017).

Several species of the order Gigartinales, included in the Gigartinaceae, Phyllophoraceae, Caulacantaceae, Cystocloniaceae, Hypnaceae, and Soleriaceae families, produce κ-carrageenans, characterized for their gelling properties (Usov 2011). Both Gigartinaceae species studied here, *Sarcothalia crispata* (Bory) Leister and *Gigartina skottsbergii* Setchell & N.L.Gardner, grow in the subtidal on the Patagonian coast of Argentina. *G. skottsbergii* has been irregularly exploited, mainly by harvesting detached thalli cast ashore (Piriz 1996), while there are no records of exploitation of *S. crispata*. The systems of polysaccharides of both tetrasporic and cystocarpic stages of *G. skottsbergii* have been thoroughly analyzed in our laboratory (Matulewicz et al. 1989; 1990; Ciancia et al. 1993), while the carrageenans of *S. crispata* have received less attention (Jouanneau et al. 2011).

In view of the above mentioned features, the aims of this study were to characterize for the first time the carrageenans extracted from cystocarpic stage of *S. crispata* collected in the Patagonian coast of Argentina, and to obtain the IPECs for the carrageenans from both cystocarpic species, *S. crispata* and *G. skottsbergii*, comparing their properties and behavior in the controlled release of ibuprofen as model drug. Their performance as a matrix for controlled release was confronted with our previous results reported for a benchmark standard constituted by a similar stoichiometric IPEC of Eudragit E and commercial κ-carrageenan (Prado et al. 2008a).

Materials and Methods

Algal Collection and Polysaccharide Extraction

Sarcothalia crispata and *Gigartina skottsbergii* were collected from the subtidal in Cabo Raso (65° 15′ 22″ W, 44° 18′ 42″ S), Argentina, by scuba diving during 2014 and 2015. Specimens were found at a depth of 4–7 m below mean tide level. Sometimes, *S. crispata* was found in tide pools.

Batches of specimens were pooled by duplicate, air-dried, and milled. The dried sample of seaweed (50 g) was extracted with water (2 L) with mechanical stirring for 16 h at room temperature. The residue was removed by centrifugation and the supernatant was dialyzed (Spectra/Por molecular weight cutoff of 3500 Da), concentrated, and freeze-dried. The residue was extracted two times more with water and the crude products of each extraction were pooled. Yield of carrageenan from *S. crispata* (SC), 20.5 g; yield of carrageenan from *G. skottsbergii* (GS), 15.0 g.

General Methods

Carbohydrate content was determined by the phenol–sulfuric acid method (Dubois et al. 1956) without previous hydrolysis of the polysaccharide, using galactose as standard; a factor of 0.9 was used for converting monosaccharide to polysaccharide residue (expressed in Table 1 as carbohydrate, % anhydro). Protein content was estimated by the method of Lowry et al. (1951), using bovine serum albumin as standard. Sulfate content was measured using the turbidimetric method of Dodgson and Price (1962) after hydrolysis of the samples with 1 M HCl for 4–5 h at 105–110 °C. Sulfate content was also measured using ion chromatography with conductimetric detection after hydrolysis of the samples with 2 M CF_3CO_2H at 121 °C for 2 h employing the following technique (Prado et al. 2008b): after evaporation to dryness under nitrogen atmosphere and redissolution in high purity water from a Milli-Q system, the hydrolyzates were analyzed using a DIONEX DX-100 ion chromatography system fitted with an AS4A column (4 × 250 mm), an AMMS-II micromembrane suppressor, and a conductivity detector (eluent 1.8 mM Na_2CO_3/1.7 mM $NaHCO_3$, flow rate 2 mL min^{-1}).

In order to determine the 3,6-anhydrogalactose content in the polysaccharide from *S. crispata* after alkaline treatment, the one-pot method described by Navarro and Stortz (2003) was carried out. The polysaccharide (2 mg) was dissolved in water (0.4 mL) with $NaBH_4$ (1 mg). After 1 h, 5 M NaOH (1.7 mL) was added and the solution was heated at 80 °C in a water bath. The cyclization reaction was stopped after 5 h by neutralization with 4 M CF_3CO_2H. The solvent was evaporated-off and the residue derivatized to the acetylated alditols and analyzed by gas-liquid chromatography (GLC), according to Stevenson and Furneaux (1991).

Table 1 Yields and analyses of the extracts obtained from and *S. crispata* and *G. skottsbergii* by extraction with water at room temperature

Extract	Yield[a] (%)	Carbohydr (% anhydro)	Sulfate (% $NaSO_3$)	Protein (%)	Monosaccharide composition[b] (mol %)		Gal:AnGal:sulfate (molar ratio)
					D-Gal	D-AnGal	
SC	41 ± 0.3[c]	72 ± 0.1	37 ± 1.2	2.4 ± 0.7	74 ± 5	26 ± 5	1.00:0.39:1.12
GS	30±7[c]	58 ± 7	30 ± 1.5	3.9 ± 0.3	59 ± 4	41 ± 3	1.00:0.60:1.20

[a] Yields are given for 100 g of dry matter

[b] GS contains 5% of glucose

[c] Data correspond to two replicates and are expressed as mean ± SD

Monosaccharide Composition

Monosaccharide composition was determined by GLC of the hydrolyzate after reductive hydrolysis and acetylation according to Stevenson and Furneaux (1991). The absolute configuration of galactose was determined by the method of Cases et al. (1995). The absolute configuration of 3,6-anhydrogalactose was determined by the procedure of Navarro and Stortz (2003).

Methylation Analysis

SC (20 mg) was converted to the triethylammonium salt for methylation according to Ciucanu and Kerek (1984) (powdered NaOH in dimethyl sulfoxide-iodomethane). The methylated product (yield 15 mg), after reductive hydrolysis, was derivatized to the acetylated alditols and monosaccharide composition was analyzed by GLC (or GLC-MS).

Gas-Liquid Chromatography

GLC was carried out on a Hewlett–Packard 5890A gas chromatograph equipped with a flame-ionization detector and fitted with a capillary column (0.25 mm i.d. × 30 m) WCOT coated with a 0.20 μm film of SP 2330. Chromatography was carried out (a) isothermally at 220 °C for alditol acetates and (b) from 160 to 210 °C at 2 °C min^{-1}, then from 210 to 240 °C at 5 °C min^{-1} for partially methylated alditol acetates. Nitrogen was used as the carrier gas, with a head pressure of 15 psi and a split ratio of 100:1. The injector and detector temperature was 240 °C.

A native and a methylated derivatized κ-carrageenan were used as standards for GLC peak assignations. When necessary, assignations were corroborated by GLC–MS analyses carried out on a Shimadzu QP 5050A apparatus. Chromatography was performed on the SP-2330 capillary column using similar conditions to those described above, but using helium as a carrier gas at a flow rate of 0.7 mL min^{-1} and a split ratio of 11:1. Mass spectra were recorded over a mass range of 30–600 Da, using an ionization potential of 70 eV.

Spectroscopic Methods

Infrared Spectroscopy

Infrared spectra of the samples were recorded with a Thermo Scientific Nicolet IS50 FTIR spectrophotometer, equipped with an ATR accessory, at 4000–500 cm^{-1}; 100 scans were performed with a resolution of 4 cm^{-1}. Signals were assigned according to Matsuhiro (1996) and Pereira et al. (2011).

NMR Spectroscopy

Proton NMR spectra were obtained on a Bruker Avance II 500 spectrometer provided with a 5-mm probe, at room temperature, using ca. 20 mg polysaccharide in 0.4 mL of D_2O. The parameters were an acquisition time of 4.4 s, a pulse angle of 30°, a pulse delay of 1 s, a spectral width of 7.50 kHz, and 16–50 scans. The ^{1}H-^{13}C HSQC technique was supplied by the spectrometer manufacturer; spectra were recorded at room temperature and were obtained at a base frequency of 500 MHz for ^{1}H and 125 MHz for ^{13}C. Acetone was added as internal standard (referred to Me_4Si by calibrating acetone methyl group to 31.3 ppm in ^{13}C, 2.21 ppm in ^{1}H). Resonances were assigned according to Ciancia et al. (1993) and Pérez Recalde et al. (2016).

Scanning Electron Microscopy (SEM)

Native carrageenans and IPECs were metalized by sputtering with a thin layer of gold in argon atmosphere. The observations were performed in a LEO EVO 40XVP microscope (CCT-CONICET, Bahía Blanca), equipped with a thermoionic emission gun and secondary electrons detector (SE). A 7-kV accelerating voltage was used. Digimizer (2018) version 5.3.3. was used to estímate the relative areas of the pores of the IPECs.

Preparation and Characterization of IPECs, and Drug Delivery Testing

Preparation of IPECs The percentages of the diads present in the polysaccharides samples were determined from the areas of the α-anomeric signals in the ^{1}H NMR spectra. These percentages together with the molecular weight values of the repeating units of each ideal diad (κ-carrageenan 408, ι-carrageenan 510, μ-carrageenan 528, ν-carrageenan 630) (Fig. 1) led to the determination of the corresponding equivalent repeating unit (ERU) according to the following equation:

$$ERU = \frac{\kappa_{diad\%} \times 408 + \iota_{diad\%} \times 510 + \mu_{diad\%} \times 528 + \nu_{diad\%} \times 630}{\kappa_{diad\%} \times 1 + \iota_{diad\%} \times 2 + \mu_{diad\%} \times 2 + \nu_{diad\%} \times 3}$$

In this equation, the numerator indicates the mass corresponding to 100 repeating units of the polysaccharide, whereas the denominator indicates the number of charges (sulfates) present in the same number of repeating units. The Eudragit E equivalent repeating unit was calculated taking into account that it is based on dimethylaminoethyl methacrylate, butyl methacrylate, and methyl methacrylate with a ratio of 2:1:1; the monomers are randomly distributed along the copolymer chain (Evonik Industries, Technical Information INFO 7.1/E, July 2015). Thus, the concentrations of the polysaccharide and Eudragit E PO (EE) solutions were calculated according to the equivalent repeating unit of each polyelectrolyte: 288 and 308 Da for SC and GS, respectively, and 278 Da for EE.

Solutions of SC (985 mg), GS (1042 mg), and EE (945 mg) in 500 mL of 0.05 M acetic acid/sodium acetate buffer (pH 5.0) were separately prepared. Then, solutions of SC and EE, and GS and EE were simultaneously poured in a vessel and agitated with a magnetic stirrer during an hour at room temperature. The mixtures were allowed to stand without agitation during an hour, and centrifuged at 9000 rpm. Precipitates were washed twice with distilled water. Finally, IPECs suspensions were freeze-dried.

Elemental Analyses Elemental analyses were performed by duplicate on an EAI Exeter Analytical, Inc. CE-440 apparatus (USA).

Preparation of Tablets For IPEC swelling and erosion tests, tablets containing only IPECs were prepared (100 ± 1 mg total weight). For ibuprofen release test, IPECs and ibuprofen were manually mixed and tablets containing 100 mg IPECs plus 50 mg ibuprofen were prepared (150 ± 1 mg total weight). Resulting tablets were round, flat-faced and had a diameter of 7.0 ± 0.1 mm. Compression was performed in a hydraulic press, employing an ad hoc die and punch set.

Swelling and Erosion of Tablets The degree of swelling, carried out in 0.05 M phosphate buffer of pH 7.2 ± 0.05 and at a temperature of 37 °C, after 8 h ($S\%_{8h}$) was calculated by means of the equation:

$$S\%_{8h} = \frac{m_2 - m_1}{m_1} \times 100$$

where m_1 is the weight of the initial dry tablet and m_2 the weight of the swollen tablet after 8 h of the drug release test. The informed results are the mean of three determinations.

The degree of erosion of the IPECs after 8 h ($E\%_{8h}$) was determined by the equation:

$$E\%_{8h} = \frac{m_1 - m_3}{m_1} \times 100$$

where m_1 is the weight of the initial dry tablet and m_3 the weight of the tablet subjected to 8 h of the drug release test and then lyophilized. The informed results are the mean of three determinations.

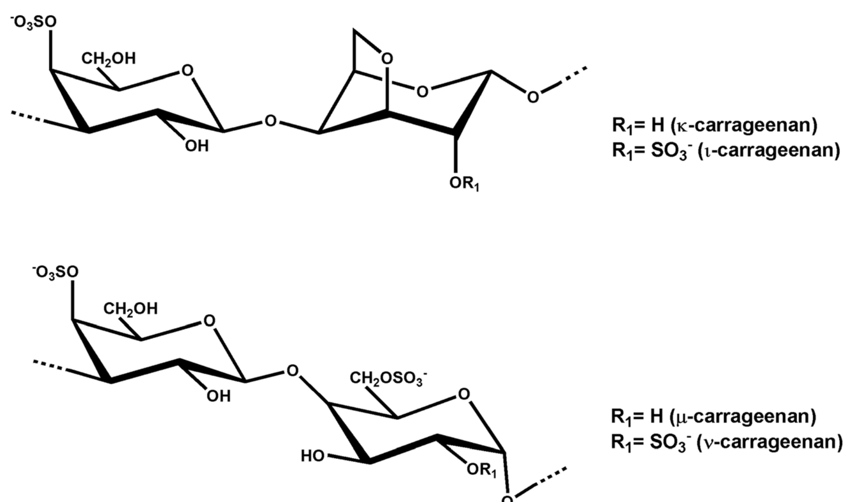

Fig. 1 Repeating units of κ-, ι-, μ-, and ν-carrageenans

Ibuprofen Release from Matrix Tablets The release of ibuprofen from matrix tablets was determined using a standard dissolution tester that complies with USP requirements for apparatus I (basket) (Avic Industrial, Buenos Aires, Argentina). A rotating speed of 100 rpm and temperature of 37.0 ± 0.5 °C were used in all the experiments. The dissolution medium consisted of 1000 mL 0.05 M phosphate buffer of pH 7.2 ± 0.05 (total release time of 8 h). Aliquots of 3 mL were taken from the solution every 30 min without any volume replacement. To calculate concentrations, corrections in the total volume were taken into account. The amount of ibuprofen released was determined spectrophotometrically at 221 nm (Cary 1E, Varian Inc., Palo Alto, CA, USA). The results informed for each kind of tablet are the mean of three determinations. Previous assays carried out in our laboratory indicate that these polymers do not interfere with the determination of the model drug.

Statistical Methods Analytical determinations were carried out by duplicate and the corresponding data are expressed as mean ± standard deviation. Drug delivery assays were carried out by triplicate and tested by non-linear regression, model fitting accepted for $R^2 \geq 0.998$.

Results and Discussion

Characterization of Carrageenans from *S. crispata* and *G. skottsbergii*

Extraction from autumn thalli of the cystocarpic stage of *S. crispata* rendered SC with a yield ca. 41% and its composition is depicted in Table 1. The D-configuration of 3,6-anhydrogalactosyl and galactosyl residues is in agreement with carrageenan as a major product. Alkaline treatment led to an increase in 3,6-anhydrogalactose content, giving a galactose:3,6-anhydrogalactose molar ratio of 1:1. This indicated the presence of sulfate on C-6 of the 4-linked unit; these units (precursor units) render 3,6-anhydrogalactose or its 2-sulfated derivative not only by alkaline treatment, but also by the biosynthetic pathway in the alga. These units are also known as kinks or helix breaking residues, since their $^4C_1$ conformation precludes helix formation (Rees et al. 1982).

Structural analysis of SC was carried out by methylation analysis and NMR spectroscopy.

Bearing in mind the backbone feature of carrageenans, based on equal amounts of 3-linked and 4-linked residues, methylation analysis of SC showed (Table 2) a structure consistent with a galactan backbone of the κ-family: major sulfation on the 4-position of the 3-linked galactose (2,6-di-*O*-methylgalactose, 51%), and 4-linked 3,6-anhydrogalactose (3,6-anhydro-2-*O*-methylgalactose, 27%) and 3,6-anhydrogalactose 2-sulfate (3,6-anhydrogalactose, 9%). Considering the composition of the native polysaccharide (galactose:3,6-anhydrogalactose ratio 1.00:0.39, Table 1), some cyclization of the 4-linked 6-sulfate units occurred during methylation, i.e., galactose 6-sulfate and galactose 2,6-disulfate to 3,6-anhydrogalactose and 3,6-anhydrogalactose 2-sulfate residues (galactose:3,6-anhydrogalactose ratio 1.00:0.56, Table 2). The presence of 3-/4-*O*-methylgalactose (7%) can be mainly assigned as derived from 4-linked galactose 2,6-disulfate residues, according to the signals found in the ^{1}H-NMR spectrum of the native polysaccharide (see below).

Table 2 Composition (mol %) of monosaccharides produced by permethylation and hydrolysis of the carrageenan extracted from *S. crispata*

Monosaccharide	Structural unit[a]	Mol %[b]
2,4,6-Me_3 Gal	G	3 ± 1[b]
2,3,6-Me_3 Gal	D	1 ± 0.1
2-Me AnGal	DA	27 ± 2
D-AnGal	DA2S	9 ± 0.5
2,6-Me_2 D-Gal	G4S	51 ± 3
2-Me Gal	G4S,6S or D3S,6S	2 ± 0.3
3-Me Gal	D2S,6S	7 ± 2

[a] Nomenclature according to Knutsen et al. (1994)

[b] Data correspond to two replicates and are expressed as data ± SD

Table 3 gives the assignments (Ciancia et al. 1993) and areas of the anomeric protons of the α-units linked to β-D-galactose 4-sulfate obtained from the ^{1}H-NMR spectrum of SC. The major area corresponded to the resonance of the anomeric proton of 3,6-anhydrogalactose indicating the prevalence of the κ-diad (G4S → DA, 56%); minor amounts of ι-diad (G4S → DA2S, 20%) and ν-diad (G4S → D2S,6S, 24%) were also detected (Fig. 1).

The two-dimensional heteronuclear single quantum coherence (HSQC) represents one of the primary 2D techniques in structural organic chemistry (Claridge 1999). This technique provides richer and less unambiguous information. For

Table 3 ^{1}H NMR assignment (ppm) of the anomeric signals of α-D-galactose units linked to β-D-galactose 4-sulfate for the carrageenans from *S. crispata* and *G. skottsbergii*

Carrageenan	Signal	α-unit	Area%
S. crispata	5.50	D2S,6S	12
	5.27	DA2S	10
	5.09	DA	28
G. skottsbergii	5.50	D2S,6S	9
	5.27	DA2S	6
	5.09	DA	35

Nomenclature according to Knutsen et al. (1994)

example, overlapping protons directly attached to carbons with different shifts may be separated in the carbon dimension, whereas overlapping carbons may be separated by their direct attachment to protons in the proton dimension (Wen et al. 2013). Figure 2 shows the HSQC spectrum of SC in which all resonances for the above diads were assigned (Table 4). The diad percentage determined for SC is similar to that reported by Jouanneau et al. (2011) for the water extracted carrageenan WE of *S. crispata* harvested in Chile. In the latter, κ-carrabiose was the main unit (51%) as in our case, but ι-carrabiose (27%) and ν-carrabiose (17%) moieties were found in slightly higher and lower amounts, respectively, and minor percentages of μ-carrabiose moieties were detected (5%). Falshaw et al. (2001) also reported the diad composition for the carrageenan from *S. crispata*, collected in Chile, but extracted with lime, 0.1 M $Ca(OH)_2$, or with 0.02 M NaOH (both at 95 °C). Even though these conditions, which produce total or partial cyclization of the precursor units, were different from the one used in our manuscript (water at room temperature), the sum of the percentages of κ-carrabiose moieties and its precursor (μ-carrabiose) was 62% and the sum of the percentages of ι-carrabiose moieties and its precursor (ν-carrabiose) was 32%. For SC (Table 3), the κ-carrabiose moieties represented 56% and the sum of ι/ν-carrabiose moieties was 44%, values also slightly different to those reported by Falshaw et al. (2001).

The FTIR spectrum of SC (Fig. S1) showed important bands at 1240 cm^{-1}, 926 cm^{-1} (3,6-anhydrogalactose), and 844 cm^{-1} (sulfate on C-4 of 3-linked galactose), and small shoulder at 805 cm^{-1} (sulfate on C-2 of 3,6-anhydrogalactose). The broad peak at 844 cm^{-1} overlapped signals at 830 cm^{-1} (sulfate on C-2 of 4-linked galactose) and 820 cm^{-1} (primary sulfate on the 4-linked unit) (Matsuhiro 1996; Pereira et al. 2011).

Carrageenan GS from the cystocarpic stage of *G. skottsbergii* was obtained with a yield of ca. 30% (Table 1). The ^{1}H NMR (Table 3) and the HSQC spectra (Table 5; Fig. 3) indicated that GS was composed of a mixture of κ-ι/ν-carrageenans with prevalence of the κ-diad (70%) and lower but significant amounts of ι- and ν-diads (12 and 18%, respectively). The assignments and areas of the anomeric protons of the α-units linked to β-D-galactose 4-sulfate in the ^{1}H-NMR spectrum of GS were also determined according to Ciancia et al. (1993) (Table 3).

The FTIR spectrum of GS (Fig. S1) was, as a whole, similar to the spectrum of SC: bands at 1240 cm^{-1} (sulfate), 927 cm^{-1} (3,6-anhydrogalactose), 845 cm^{-1} (sulfate on C-4 of 3-linked galactose), and a small one at 803 cm^{-1} (sulfate on

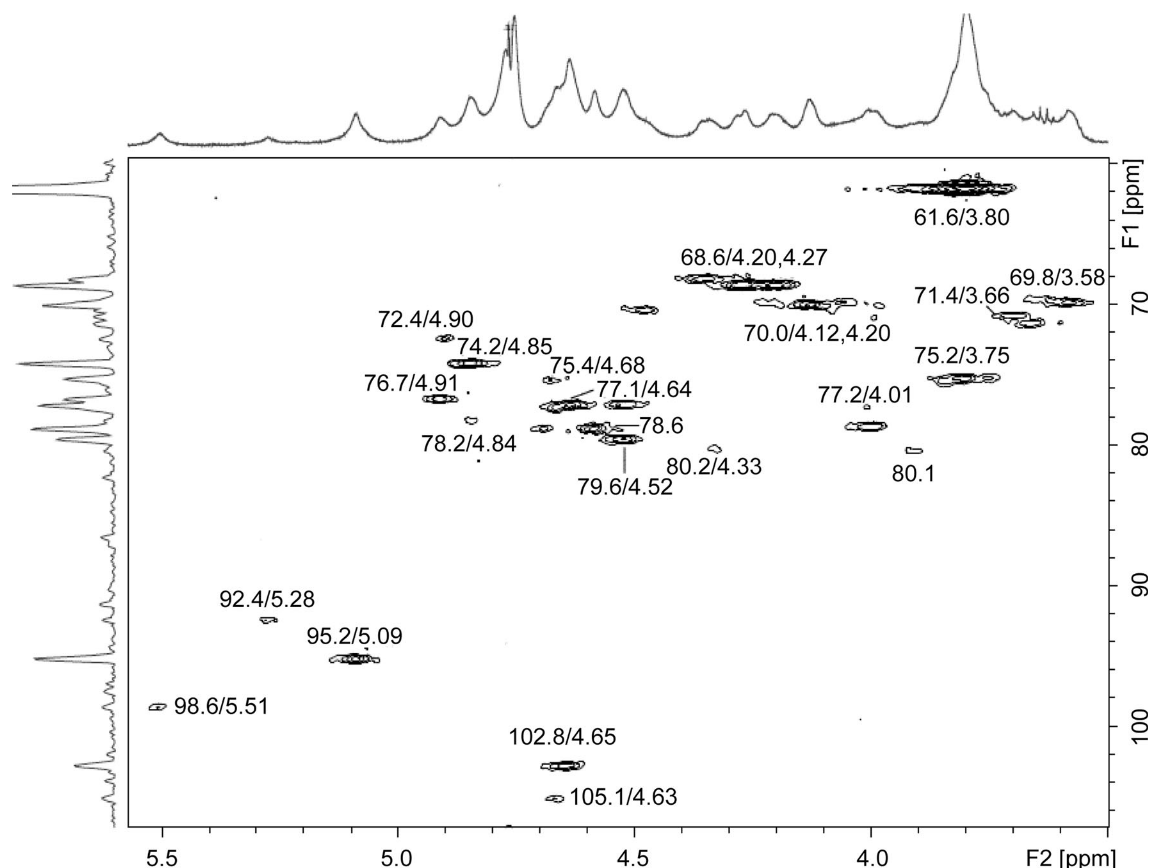

Fig. 2 HSQC spectrum of the carrageenan from *S. crispata*

Table 4 HSQC signal assignments (ppm) of carrageenans from *S. crispata*

Structural unit[a]	C-1/H-1	C-2/H-2	C-3/H-3	C-4/H-4	C-5/H-5	C-6/H6,6′
κ-diad						
G4S	102.8/4.65	69.8/3.58	79.6	74.2/4.85	75.2/3.75	61.6/3.80
DA	95.2/5.09	70.0/4.13	79.6/4.52	78.6/4.59	77.1/4.64	70.0/4.13, 4.20
ι-diad						
G4S	102.8/4.65	69.8/3.58	77.2/4.01	72.4/4.90	75.2/3.75	61.6/3.80
DA2S	92.4/5.28	75.4/4.68	78.2/4.84	78.6	77.1/4.61	70.0/4.13, 4.20
ν-diad						
G4S	105.1/4.63	71.4/3.66	80.1	71.4/3.66	75.2/3.75	61.6/3.80
D2S,6S	98.6/5.51	76.7/4.91	68.6/4.27	80.3/4.33	68.6/4.20	68.6/4.20, 4.27

[a] Nomenclature according to Knutsen et al. (1994)

C-2 of 3,6-anhydrogalactose). Signals at 830 cm^{-1} (sulfate on C-2 of 4-linked) and 820 cm^{-1} (sulfate on C-6 of 3-linked galactose) were overlapped by the broad peak at 845 cm^{-1} (Matsuhiro 1996; Pereira et al. 2011).

The characteristics of GS were slightly different from that studied previously in our laboratory (Matulewicz et al. 1989) which was reported to be composed of similar amounts of gelling and soluble carrageenans of the κ-/ι- and μ-/ν-type, respectively. It should be kept in mind that μ- and ν-carrageenans are biochemical precursors of κ- and ι-carrageenans (Usov 2011). It has been also proposed for other carrageenophytes that the physical properties of carrageenan and chemical composition of the thallus may vary with different environmental conditions (Durako and Dawes 1980; Reis et al. 2008), though there is still no agreement among different authors on the incidence of abiotic factors on carrageenan composition (Garbary et al. 2011 and literature therein). The population studied by Matulewicz et al. (1989) was located in Bahía Camarones, a protected environment where the algae are less exposed to wave action than in Cabo Raso (the collection location of specimens for this manuscript), and where supposedly a higher proportion of gelling carrageenan would confer advantages for the algal population. Yet to assess, the incidence of water motion on polygalactan composition requires sampling throughout several years in both locations (Garbary et al. 2011), in order to have a clear picture of physiological acclimation of the individuals to given environmental conditions.

The comparison of SC and GS shows similar diad contents, but SC presents a lower proportion of κ-structure and a higher content of ι- and ν-structures than the latter. From the chemical and spectroscopic characterization of SC and GS, there is no evidence of polymer branching.

Scanning Electron Microscopy

SEM micrographs of the native carrageenan from *S. crispata* exhibited a laminar structure with a smooth surface and numerous circular perforations of different sizes (Fig. 4a), while the native carrageenan from *G. skottsbergii* showed fibers grouped into bundles (Fig. 4b).

IPEC particles of EE-SC and EE-GS presented irregular shape. At low magnification, the surface of the IPEC EE-SC had a homogeneous particulate surface, while the IPEC EE-GS showed a heterogeneous particulate appearance. At higher magnification, both IPECs showed a sponge-like structure, with regular pores in the IPEC EE-SC (0.12 ± 0.01 μm of diameter) with a uniform distribution (Fig. 4c), and with irregular cavities (0.41 ± 0.04 μm of

Table 5 HSQC signal assignments (ppm) of carrageenans from *G. skottsbergii*

Structural unit[a]	C-1/H-1	C-2/H-2	C-3/H-3	C-4/H-4	C-5/H-5	C-6/H6,6′
κ-diad						
G4S	102.7/4.64	69.7 /3.56	79.6	74.2/4.86	75.1/3.80	61.6/3.80
DA	95.1/5.10	69.8/4.13	79.6/4.52	78.6/4.59	77.2/4.64	69.5/4.20, 4.27
ι-diad						
G4S	102.7/4.64	69.7/3.56	77.0/4.02	72.2/4.91	75.2/3.80	61.6/3.80
DA2S	92.3/5.28	75.2/4.68	78.1/4.84	78.6/4.69	77.2/4.64	68.8/4.13, 4.20
ν-diad						
G4S	105.1/4.65	71.1/3.66	80.2	71.1/3.66	75.1/3.80	61.6/3.80
D2S,6S	98.2 /5.50	76.7/4.91	68.4/4.27	80.2/4.36	68.4/4.27	68.0/4.35

[a] Nomenclature according to Knutsen et al. (1994)

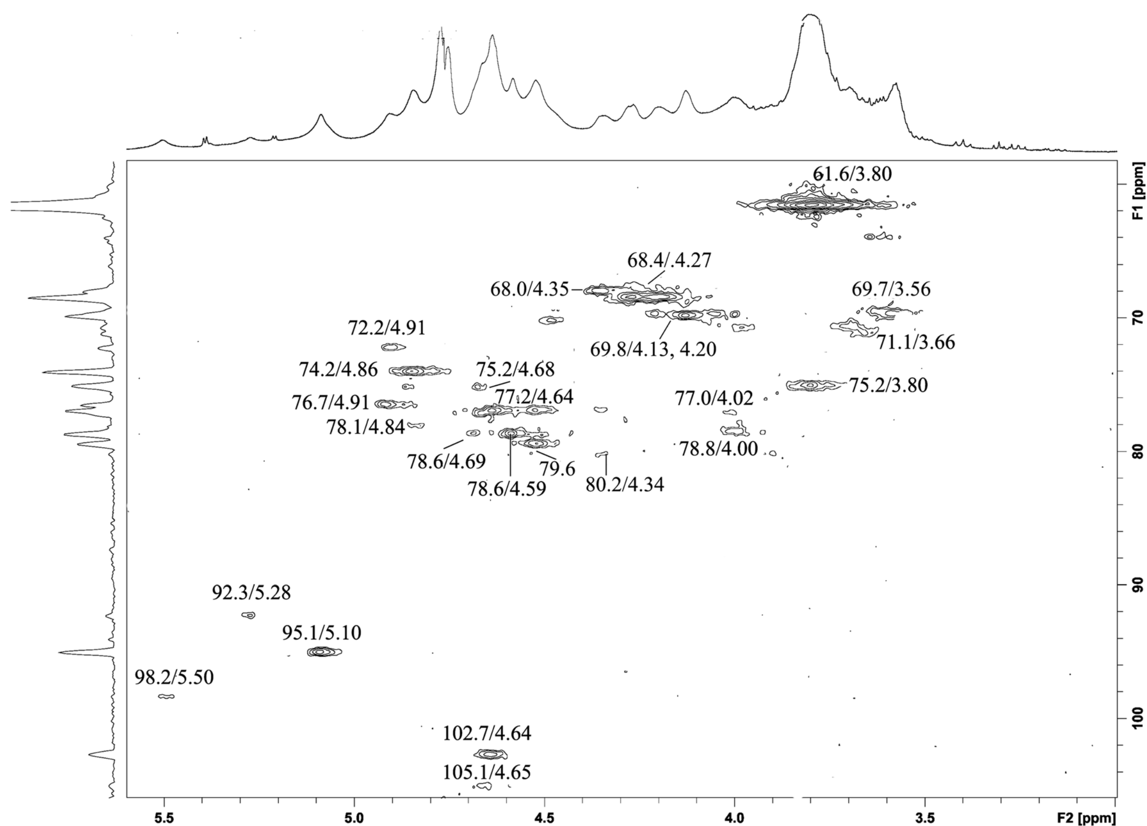

Fig. 3 HSQC spectrum of the carrageenan from *G. skottsbergii*

length × 0.19 ± 0.02 μm of width) in the IPEC EE-GS (Fig. 4d). The IPEC EE-SC presented 9.06 ± 0.2 pores/μm^2, and the IPEC EE-GS had 5 ± 0.4 pores/μm^2. In the IPEC EE-SC, 6.4 ± 0.3% of the total area was covered with pores, while in the IPEC EE-GS, pores represented 11.7 ± 0.6% of the total area of the IPEC.

Composition of the IPECs

According to the manufacturer, EE is soluble in acidic medium up to pH 6.0 due to hydration of protonated dimethylamine groups. GS and SC have ionized sulfate groups in the whole range of pH and are stable at pH 5.0–7.0. Thus, an acetic acid/

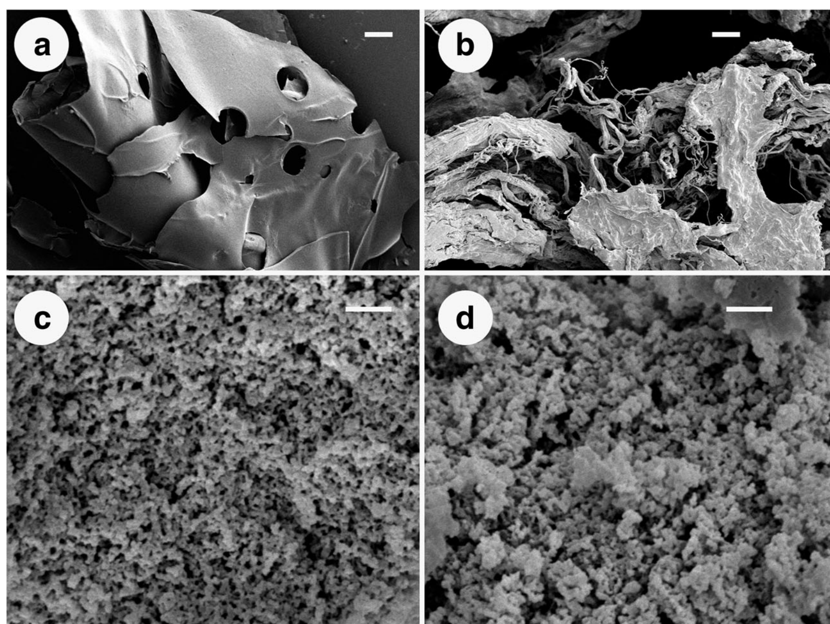

Fig. 4 SEM micrographs of **a** native carrageenan of *S. crispata*, **b** native carrageenan of *G. skottsbergii*, **c** IPEC EE-SC, and **d** IPEC EE-GS. Scale bars: **a** = 100 μm; **b** = 40 μm; **c**, **d** = 1 μm

sodium acetate buffer of pH 5.0 was used in order to obtain solutions of both reactants.

In order to confirm the interaction or binding ratio of each component in the solid IPEC, elemental analysis was carried out: N:S molar 1.00:0.98 for SC and 1.00:0.96 for GS, indicating the formation of stoichiometric IPECs in both cases.

Comparison of the FTIR spectra of EE, SC, GS, and the corresponding IPECs showed that the two bands at 2768 and 2819 cm^{-1} present in the spectrum of EE and absent in those of the polysaccharides were negligible in the IPECs, thus indicating the formation of the ammonium salt (Fig. 1S) (Moustafine et al. 2006; Prado et al. 2008a).

Swelling and Erosion of Tablets

Final swelling and erosion values were determined for the tablets. The ones containing the IPEC EE-SC initially presented a swelling value of 223% and an erosion of 23% after 8 h, whereas the IPEC EE-GS exhibited a swelling value of 240% and an erosion of 28%. Higher swelling and erosion of the IPEC EE-GS tablets are consistent with its higher drug release values. Given the swelling and erosion values obtained for both IPEC tablets, both mechanisms would contribute to drug release.

Ibuprofen Release from Matrix Tablets

Ibuprofen release profiles from matrix tablets containing stoichiometric IPECs of Eudragit E and the polysaccharides from *S. crispata* or *G. skottsbergii* (IPEC EE-SC and IPEC EE-GS) are presented in Fig. 5.

Release values are higher for the IPEC EE-GS than for the IPEC EE-SC, with values after 8 h of 35% for the former and of 20% for the latter. As we employed the same cationic polymer in both IPECs (EE) and the anionic polysaccharides (SC and GS) present a similar (but not equal) diad composition, we postulate that differences in the diads composition and in the sequence in which the aforementioned diads are distributed along the polysaccharide chain, affect the shape, size, and relative area of the pores of the formed IPECs and, in consequence, lead to differences in swelling values and in drug release profiles. The more regular shape and smaller pores observed in the SEM images of IPEC EE-SC could be originated by a more evenly distributed diad sequence.

The comparison of release values for the first 30 min with the subsequent values indicates the presence of a low and similar "burst release" effect for both IPECs. As burst release may occur in monolithic hydrophilic matrices obtained by direct compression in the presence of drug in the external parts of the delivery system, our results suggest that the hydrogel structure is rapidly and efficiently formed preventing the instantaneous release of an important amount of drug (Huang and Brazel 2001).

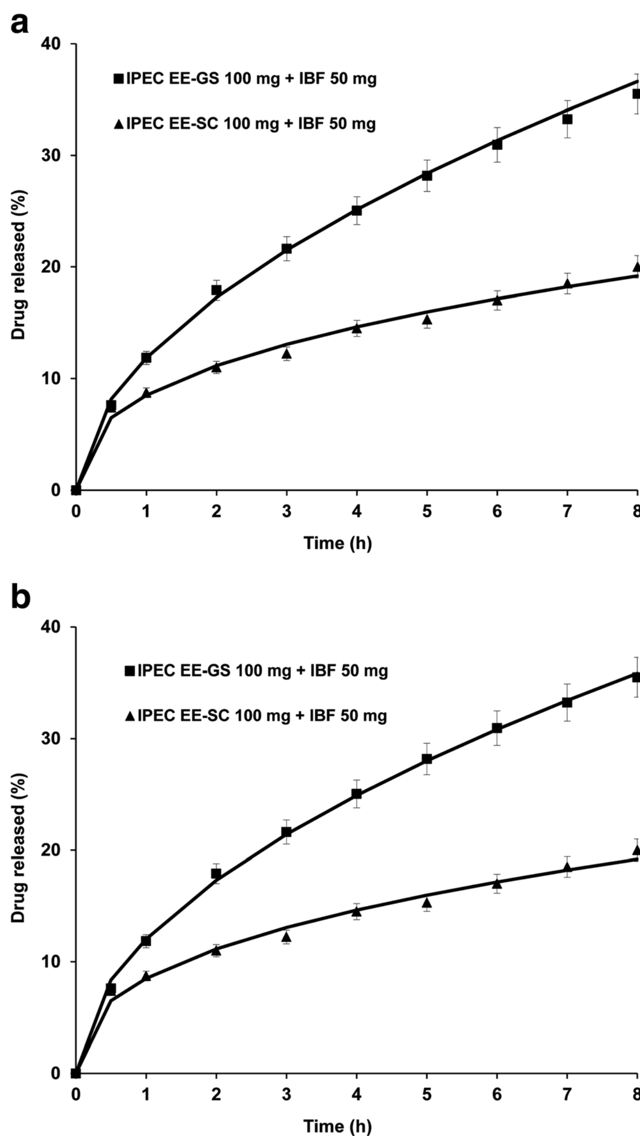

Fig. 5 Comparison between the experimental (points) and predicted (solid lines) release profiles of ibuprofen by the Peppas model (**a**) and by the Peppas and Sahlin model (**b**). Data represent the mean ± SD for $n = 3$

The Peppas model (Peppas 1985) was used to fit release profiles. The mathematical equation of the model is as follows:

$$\frac{M_t}{M_\infty} = kt^n$$

where M_t/M_∞ is the fraction of drug released at time t (in hours), k is the apparent release constant related to geometrical and structural characteristics of the system (its unit is hours^{-n}), and n the release exponent.

The characteristic parameters of the Peppas model are depicted in Table 6. Fitting of the model to experimental data is denoted by high R^2 values (≥ 0.998) and graphically in Fig. 5a.

Table 6 Model characteristic parameters for the IPECs of *S. crispata* and *G. skottsbergii*

Composition of the tablets	Peppas model			Peppas and Sahlin model			
	k (h^{-n})	n	R^2	k_1 (h^{-m})	k_2 (h^{-2m})	m	R^2
IPEC EE-SC 100 mg + IBF 50 mg	8.51	0.39	0.998	243.11	0.01	0.39	0.998
IPEC EE-GS 100 mg + IBF 50 mg	11.87	0.54	0.999	114.66	0.01	0.52	0.998

Considering cylindrical geometry for both systems, a release exponent of 0.39 for the IPEC EE-SC points to Fickian diffusion transport, whereas a value of 0.54 for the IPEC EE-GS suggests non-Fickian transport (Table 6).

In order to obtain a closer insight on the mechanism involved in drug release, we also applied the Peppas and Sahlin model (Peppas and Sahlin 1989), which uses the following equation:

$$\frac{M_t}{M_\infty} = k_1 t^m + k_2 t^{2m}$$

M_t/M_∞ and t have the same meaning as in the previous model, k_1 and k_2 are the diffusion and relaxation constants (the unit of k_1 is hours^{-m} and the unit of k_2 is hours^{-2m}), and m is the purely Fickian diffusion exponent for a system of any geometrical shape. Thus, the first term of the equation indicates the Fickian diffusion contribution to the total release (F), whereas the second one represents the case II matrix relaxation (R) which corresponds mainly to swelling and erosion phenomena. The good fitting of the Peppas and Sahlin model to experimental data is also denoted by high R^2 values ($\geq$0.998) and shown in Fig. 5b and Table 6. A ratio of both contributions can be expressed by the following equation:

$$\frac{R}{F} = \frac{k_2 t^m}{k_1}$$

The R/F ratios versus the drug released for both IPECs are shown in Fig. 6, which clearly indicates that Fickian contribution largely surpasses matrix relaxation for both IPECs. The relative contribution of Fickian diffusion is slightly lower for the IPEC EE-GS compared to the IPEC EE-SC, for the release time evaluated; the relative contribution of relaxation increases with the advance of the release experiment for both IPECS. It should be kept in mind that IPEC EE-GS presents larger pores.

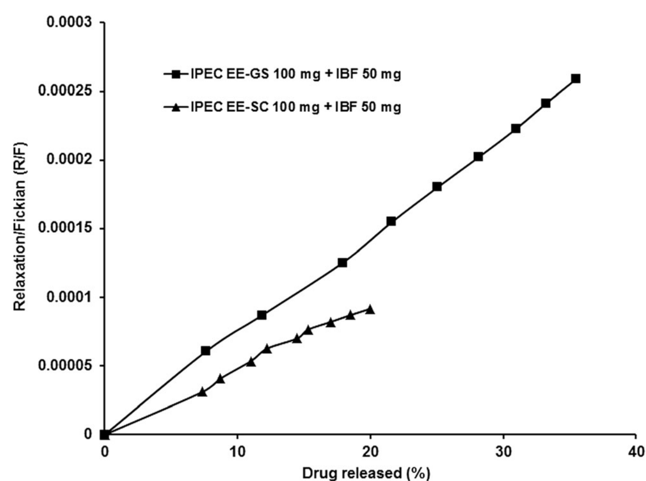

Fig. 6 Rate of contribution of relaxation and Fickian diffusion to the release of ibuprofen from IPEC EE-GS (black squares) and IPEC EE-SC (black triangles)

In a previous study (Prado et al. 2008a), we reported the preparation, characterization, and drug release behavior of a stoichiometric IPEC formed by Eudragit E and a commercial κ-carrageenan. Ibuprofen release was tested from a matrix tablet containing 100 mg IPEC and 50 mg ibuprofen, which is equivalent to the one herein reported. The experiment was carried out first in an acid medium for 2 h (where very low release values were found) and then in 0.05 M phosphate buffer of pH 7.2. The latter conditions are identical to the ones employed in the present study, where we found similar final release values for the GS-based IPEC and the commercial κ-carrageenan-based IPEC. This could be due to the higher 3,6-anhydrogalactose content in GS in comparison to that found for SC, closer to the content in commercial κ-carrageenan. It is noteworthy that the galactose:3,6-anhydrogalactose:sulfate molar ratio of an ideal κ-carrageenan is 1:1:1.

The effect of crosslinking degree of hydrogels on drug release profiles has been reported in scientific literature and, in general, release profiles are slower for more crosslinked hydrogels, due to increased microstructural tortuosity and decreased space between macromolecular chains (Martínez et al. 2014). However, the effect of the homogeneity in crosslinking density has hardly been studied, possibly due to the technical difficulties such a task involves. As a result, most drug release models assume a homogeneous matrix (Siepmann and Siepmann 2012). One of the few reports found account differences in release profiles from poly(oligoethylene glycol methacrylate) hydrogels, where homogeneous or microheterogeneous crosslinked systems were tested. Interestingly, minor differences were observed in the swelling determinations of those hydrogels (Bakaic et al. 2015). The comparison of SEM images obtained for IPEC EE-SC and IPEC EE-GS, the pronounced differences detected in ibuprofen release profiles and the small differences in the swelling studies, suggests the possibility of microheterogeneity in the IPEC EE-GS matrix. In this IPEC, the ionic crosslinks seem to be

less evenly distributed originating microdomains with more open or more closed channels, in the three-dimensional net of the hydrogel, leading to differences in their interaction with the model drug molecules.

Conclusions

We conclude that the polysaccharide from *Sarcothalia crispata* (SC) and *Gigartina skottsbergii* (GS) presents a similar (but not equal) diad composition, mainly κ-diads and lower but significant amounts of ι- and ν-diads. However, IPECs prepared from Eudragit E and SC or GS showed particular characteristics by SEM, and also in swelling and controlled release experiments. These could be due to differences in the diad composition and in the sequence in which the diads are distributed along the polysaccharide chain. IPEC EE-SC with more regular and smaller pores also exhibited smaller final swelling values and lower drug release profiles, possibly due to an even diad sequence, in comparison with IPEC EE-GS.

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Compliance with Ethical Standards

Conflict of Interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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