# Europium ion as a probe for binding sites to carrageenans

Ana P. Ramos[a], Rogéria R. Gonçalves[a], Osvaldo A. Serra[a],
Maria Elisabete D. Zaniquelli[a,*], Kenneth Wong[b,1]

[a]Departamento de Química, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo,
Ribeirão Preto, São Paulo 14040-901, Brazil
[b]Laboratório de Físico-Química, Centro de Pesquisas de Paulínia, Rhodia Brasil, Paulínia, São Paulo, Brazil

## Abstract

Carrageenans, sulfated polysaccharides extracted from red algae, present a coil-helix transition and helix aggregation dependence on the type and concentration of counterions. In this study, we focus attention on a mixed valence counterion system: $Eu^{3+}/Na^+$ or $K^+$ with different gel-forming carrageenans: kappa, iota, and kappa-2. Results of stationary and time-dependent luminescence showed to be a suitable tool to probe ion binding to both the negatively charged sulfate group and the hydroxyl groups present in the biopolymer. For lower europium ion concentrations, a single longer decay emission lifetime was detected, which was attributed to the binding of europium ion to the carrageenan sulfate groups. An additional decay ascribed to europium binding to hydroxyl groups was observed above a threshold concentration, and this decay was dependent on the carrageenan charge density. Symmetry of the europium ion microenvironment was estimated by the ratio between the intensities of its emission bands, which has been shown to depend on the concentration of europium ions and on the specificity of the monovalent counterion bound to the carrageenan.

## 1. Introduction

Ion binding operates in biological systems either to effect a structural change (e.g., in a protein, which triggers a reaction) or as an end in itself (e.g., ion transport). Control is carried out by the specificity of the binding; therefore, competition with other ions ineluctably becomes important to determine the system's behavior and tuning. Simple polysaccharides bind metal ions and exhibit some of the binding behavior of more complex systems; so they may be useful as a model system. Furthermore, carbohydrate–metal complexes are important in their own right in analytical chemistry, in the extraction of heavy metal ions and as support materials for solid electrolytes. Polysaccharides thicken and may gel in the presence of cations and, although the mechanisms and specificity of this viscoelastic behavior are not fully understood, these sought-after properties are widely used in food industry [1], cosmetic and pharmaceutical formulations, and in biotechnological applications [2]. Information that helps understand these mechanisms and optimize the performance of these systems is thus desirable.

Carrageenans are one such family of polysaccharides: they are natural, linear, water-soluble, sulfated poly-saccharides consisting [3,4] of disaccharide repeat units of $1 \to 3$ $\beta$-D-galactopyranose and $1 \to 4$ $\alpha$-D-galactopyranose. Some galactopyranoses have an anhydro-bridge, as in the structures depicted in Fig. 1. These disaccharide repeat units correspond to the idealized primary structure of the main gelling carrageenans. By fractional precipitation, one may obtain κ- and ι-carrageenan while a third form named κ-2 is described as being a hybrid species of ι- and κ-carrageenan.

*Corresponding author. Tel./fax: +55 16 3602 4838.
E-mail address: medzaniquelli@ffclrp.usp.br (M.E.D. Zaniquelli).
[1]Present address: Firmenich, Corporate R&D, P.O. Box 239, Route des Jeunes 1, CH-1211 Geneva 8, Switzerland.
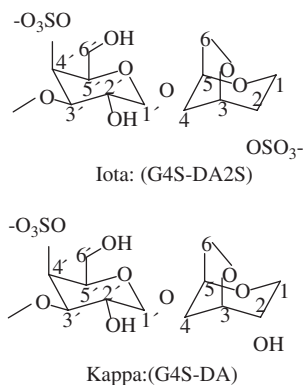
Fig. 1. Ideal primary structures for kappa- and iota-carrageenans (κ- and ι-car) showing the (1,3) and (1,4) alternating glycosidyl linkage of G and D galactopyranose residues (G4S: galactose-4-sulfate and DA: anidrogalactose). The kappa-2 carrageenan is a hybrid species consisting of a copolymer of kappa and iota (50%).

Gel properties, such as gelling time, sol–gel transition temperature, hardness, and syneresis depend [5] on both the supramolecular arrangement of carrageenan helices related to the polysaccharide structure and their aggregation, which is usually promoted by mono and divalent metal cations. It was initially proposed that the gelation of carrageenans was based on the formation of double helices [6] and their aggregation. However, a complete understanding of the helix aggregation for the different carrageenan species has not been achieved yet [7]. Detailed studies [8–14], especially with kappa-carrageenan, show these forms exhibit ion specificity, attested mainly by their different abilities to form gel in specific salt conditions. Studies [15] with purified iota samples have shown that monovalent cations are non-specific to this carrageenan type, which instead demonstrates specificity to calcium [16]. Ion binding has been studied through three different approaches: Mannings's linear charge model [17], simple Poisson–Boltzmann cylindrical cell [13], or connected to Monte Carlo simulations [18]. These models are based on electrostatic interactions and neglect other kinds of interactions. Coulombic attractions between the counterions and the polyanion are in fact the most important interactions. Nevertheless, especially in the presence of d- or f-shell cations like copper, iron, or some other trivalent counterions, complexation with specific ligand groups may occur and can further influence the features of metal–ion binding. The way these specific interactions occur in the presence of mixed counterion systems may elucidate the selectivity of carrageenans for some ions.

Europium is a trivalent rare earth ion that exhibits strong and characteristic emission bands dependent on its microenvironment. Because of its strong affinity for water molecules, few studies have been reported for the complexation of this ion with polysaccharides in aqueous solution. It is reported that the presence of carboxyl groups greatly increases the affinity of carbohydrates for lanthanides [19]. Besides the carboxylate, it was found that the

hydroxyl groups were also bound to the ion. For example, a complex with a uronic acid (GalpA), D-galactopyranosiduronic acid (with four hydroxyl groups) presents [20,21] a stability constant of $350 \, \text{mol}^{-1} \, \text{L}$ for the Eu(III):GalpA = 1:3 species. The formation of europium complexes with polyhydroxyacids has also been reported [22,23]. On the other hand, chirotropical properties for rare earth ion/polysaccharide complexes have been detected [24] for some carboxylated polysaccharides, but not for sulfated ones. Furthermore, europium-linked time-resolved fluoroimmunoassay was developed for the detection of Aspergillus galactomannans [25]. Therefore, the increasing use of europium in fluoroimmunoassays, the apparent lack of data on the interaction of europium with sulfated polysaccharides, the resemblance of carrageenans to DNA regarding the helix formation, and the interest in the fundamental aspect of polysaccharide counterion exchange are enough reasons to justify the investigation of carrageenan–europium systems using the luminescent ion to probe ion exchange in the presence of monovalent counterions that may or may not be specific to the macromolecule.

In this work, we describe the preparation and characterization of sodium- and potassium-kappa, iota, and kappa-2 carrageenan aqueous systems formed in the presence of different europium ion concentrations. Luminescence spectroscopy was used to characterize the obtained gels or thickened solutions.

## 2. Experimental

### 2.1. Purification and characterization of carrageenans

Purified extracts of κ and κ-2 carrageenans were obtained from Gelymar, Puerto Montt, Chile; iota was purchased from Sigma. They were converted into the pure sodium or potassium forms by ion exchange [4] (Amberlite IR120 or Dowex 50 WX4-100 ion exchange resin, Sigma-Aldrich) followed by rotoevaporation and freeze-drying. The ion contents were measured by flame spectrophotometry (Shimadzu AA-680). The molecular weights of the fractions were determined by SEC-MALLS (steric exclusion chromatography HPWaters, USA), using four sequential ultrahydrogel (Waters™ with exclusion limits of $5 \times 10^3 \, \text{g mol}^{-1}$, $8 \times 10^4 \, \text{g mol}^{-1}$, $4 \times 10^5 \, \text{g mol}^{-1}$, and $7 \times 10^6 \, \text{g mol}^{-1}$), coupled with a multiple angle dynamic laser light scattering (Dawn-DSP-Wyatt Technology) and differential refractometer (Waters, model 2410). The aqueous solutions in sodium nitrate $0.1 \, \text{M}$ were injected using a controlled flux of $0.6 \, \text{mL min}^{-1}$. The molecular weights ($M_W$) for κ-, ι-, and κ-2-car in the sodium form were $6.0 \times 10^5 \, \text{g mol}^{-1}$, $2.7 \times 10^6 \, \text{g mol}^{-1}$, and $2.9 \times 10^6 \, \text{g mol}^{-1}$, respectively.

### 2.2. Preparation of the europium chloride solution

The europium chloride solution was prepared from the respective oxide (Sigma-Aldrich, 99% purity) by dissolution

in hydrochloric acid, followed by careful drying and dilution in ion-exchanged MilliQ$^{TM}$ water, to prepare the stock solution (pH 5.5) and further dilutions.

## 2.3. Preparation of the gels

The gels were prepared by adding the appropriate amount of each solid carrageenan to a hot europium ion solution (65 °C, pH 5.5). The resulting mixture was stirred for 15 min by means of a vortex, and then it was taken to a temperature of 85 °C and stirrer for another 25 min. The gels were transferred to fluorescence or absorbance quartz cells and brought to the room temperature. Concentrations are expressed in percentage w/w related to the disaccharidic unity.

The rheology and hence the interactions between the carrageenan rods depend on the history of the sample [26], so the same heating/cooling/aging procedure was used for the preparation of all the solutions. At the working carrageenan concentration, 1.5% w/w, a gel was formed at room temperature in the following conditions: K-κ-car and K-κ-2-car in the entire europium ion concentration range; Na-ι-car and K-ι-car, only for the highest europium ion concentration (1 mmol L$^{-1}$) (see Table 1). Apparently, only thickening occurred in the other conditions. We did not carry out rheometry experiments with these systems. However, the gel rigidities follow the sequence: K-κ-car > K-κ-2-car > K-ι-car ~ Na-ι-car, visually.

## 2.4. Luminescence studies

The room temperature excitation and emission spectra as well as the decay curves were recorded on a spectrofluorometer Spex Triax 550, with a Fluorolog III phosphorimeter accessory and Xe-pulsed lamp. In addition to the monochromator, a filter set was used for selection of the excitation/emission wavelengths, in order to improve the quality of the spectrum. For the emission spectra, the delay time was 0.04 ms and the resolution of the entrance and detection slits were, respectively, 16 and 6 nm. For the time-resolved emission spectra and $^5D_0$ excited state decay curves, the corresponding resolutions of the slits were 16 and 10 nm.

## 3. Results and discussion

### 3.1. Steady state luminescence spectra

The $^5D_0 \rightarrow {}^7F_J$ emission bands [27] for europium ions are intense enough to be visualized for all the studied carrageenan systems containing europium, even at 0.01 mmol L$^{-1}$, especially for $J = 1$, 2, and 4. An example is shown in Fig. 2 for Na-κ-car. The excitation wavelength for all the spectra was 280 nm. It should be observed that this wavelength corresponds to the maximum of a broad band in the excitation spectra of the europium–carrageenan gels, probably due to sulfate groups present in the polysaccharide.

No significant differences in the emission spectra are observed using the 395 nm excitation wavelength, which corresponds to the $^5L_6$ excited level of Eu$^{3+}$ ions. In Fig. 2, the ($^5D_0$–$^7F_2$), or $0\rightarrow 2$ transition and ($^5D_0$–$^7F_1$) or $0\rightarrow 1$ transition are observed at 615 and 590 nm, respectively. The influence of the concentration of the europium ion on the relative intensities of these bands ($I_{0\rightarrow 2}/I_{0\rightarrow 1}$) is clearly shown in Figs. 3A and B. These intensity ratios also show a dependence on the type of carrageenan gel. To better understand this effect, we have to call to mind the selection rules for electronic transitions. Applying group theory following the Racah methodology and Wigner–Eckart theorem [27], we have the $0\rightarrow 2$ transition allowed by electric dipole (ED), which is more sensitive to crystalline field, whereas the $0\rightarrow 1$ transition, allowed by magnetic dipole (MD) moment only, is less sensitive to the vicinity, and can be used as a kind of internal reference. In fact, the intensities for the $0\rightarrow 1$ transition increase linearly with Eu(III) concentration, but this is not the case for the $0\rightarrow 2$ transition. Above 0.5 mmol L$^{-1}$, both transitions show a tendency to decrease, more likely due to changes in the refraction index of the scattering samples. In general [28], when the europium ion is located in the central position of a non-centrosymmetric microstructure, the emission band

Table 1
Lifetime decays ($\tau$, ms) for $0\rightarrow 2$ transition of europium ion in potassium or sodium carrageenan systems (1.5%)

| [Eu$^{3+}$] (mmol L$^{-1}$) | Kappa | | | | Kappa-2 | | | | Iota | | | |
| | K$^+$ | | Na$^+$ | | K$^+$ | | Na$^+$ | | K$^+$ | | Na$^+$ | |
| | $\tau_1$ | $\tau_2$ | $\tau_1$ | $\tau_2$ | $\tau_1$ | $\tau_2$ | $\tau_1$ | $\tau_2$ | $\tau_1$ | $\tau_2$ | $\tau_1$ | $\tau_2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.01 | *0.39* | —* | 0.33 | — | *0.42* | — | 0.40 | — | 0.32 | — | 0.41 | — |
| 0.05 | *0.39* | *0.16* | 0.41 | 0.20 | *0.38* | — | 0.37 | — | 0.30 | — | 0.32 | — |
| 0.1 | *0.38* | *0.15* | 0.35 | 0.17 | *0.37* | *0.18* | 0.35 | 0.11 | 0.26 | — | 0.26 | — |
| 0.5 | *0.40* | *0.16* | 0.34 | 0.14 | *0.34* | *0.15* | 0.43 | 0.17 | 0.32 | 0.15 | 0.30 | 0.15 |
| 1.0 | *0.36* | *0.13* | 0.32 | 0.11 | *0.38* | *0.16* | 0.42 | 0.16 | *0.33* | *0.16* | *0.26* | *0.11* |
| Average | 0.38 | 0.15 | 0.35 | 0.16 | 0.38 | 0.16 | 0.39 | 0.15 | 0.31 | 0.15 | 0.31 | 0.13 |
| SD | 0.02 | 0.01 | 0.04 | 0.04 | 0.03 | 0.02 | 0.03 | 0.03 | 0.03 | 0.01 | 0.06 | 0.02 |

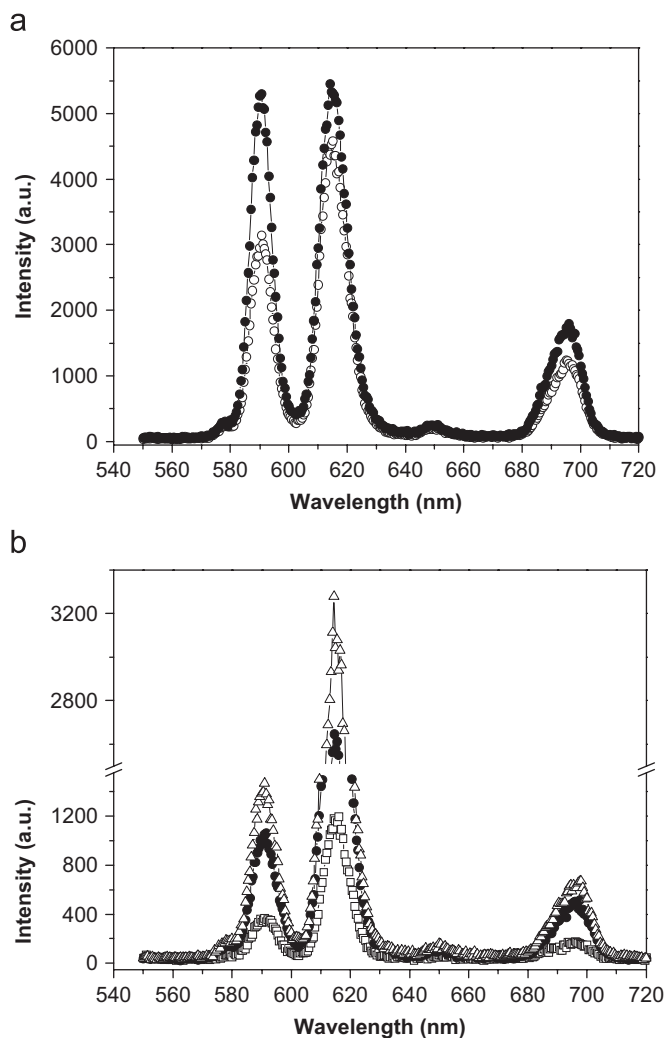*(—) mean the decay is monoexponential and the $\tau_2$ is absent. Italics samples from gel.

Fig. 2. Emission spectra for Na-$\kappa$-car (1.5%) at different europium concentrations: (a) ○: $0.50\,mmol\,L^{-1}$ and ●: $1.0\,mmol\,L^{-1}$. (b) □: $0.01\,mmol\,L^{-1}$, ●: $0.05\,mmol\,L^{-1}$, and △: $0.10\,mmol\,L^{-1}$. The excitation wavelength is 280 nm. Emission is observed even for a $0.01\,mmol\,L^{-1}$ europium aqueous solution.



Fig. 3. Intensity ratio ($I_{0\to2}/I_{0\to1}$) related to europium ion transitions ($^5D_0\to{}^7F_2$) or $0\to2$ transition, and ($^5D_0\to{}^7F_1$) or $0\to1$ transition, for potassium (A) and sodium (B) carrageenans, iota (■), kappa (●), and kappa-2 (▲), as a function of europium concentration. The dashed line corresponds to the average curve (▽) between those obtained for $\kappa$- and $\iota$-car.

of the $0\to2$ transition is more intense than that of the $0\to1$ transition. In this sense, the higher is the $I_{0\to2}/I_{0\to1}$ ratio, the more apart from a centrosymmetric geometry where the europium ion is located. For our particular system, we are considering that the europium ion may be in one of these situations: surrounded by water molecules, coordinated to hydroxyl groups or coordinated to sulfate groups on the polysaccharide. Moreover, these possibilities may not be exclusive. As the polysaccharide is negatively charged, one expects that the Eu$^{3+}$ ions attract the polyions that are going to behave as a macroligand. On the other hand, the coordination with sulfate groups by means of charge transfer to the internal orbitals of the europium ions should demand a determined orientation. However, the different carrageenan fractions used in the present study hold different sulfate contents. We are also postulating that sodium and potassium ions compete in with europium for the sulfate binding in a different way. Nevertheless, this kind of interaction is less probable when
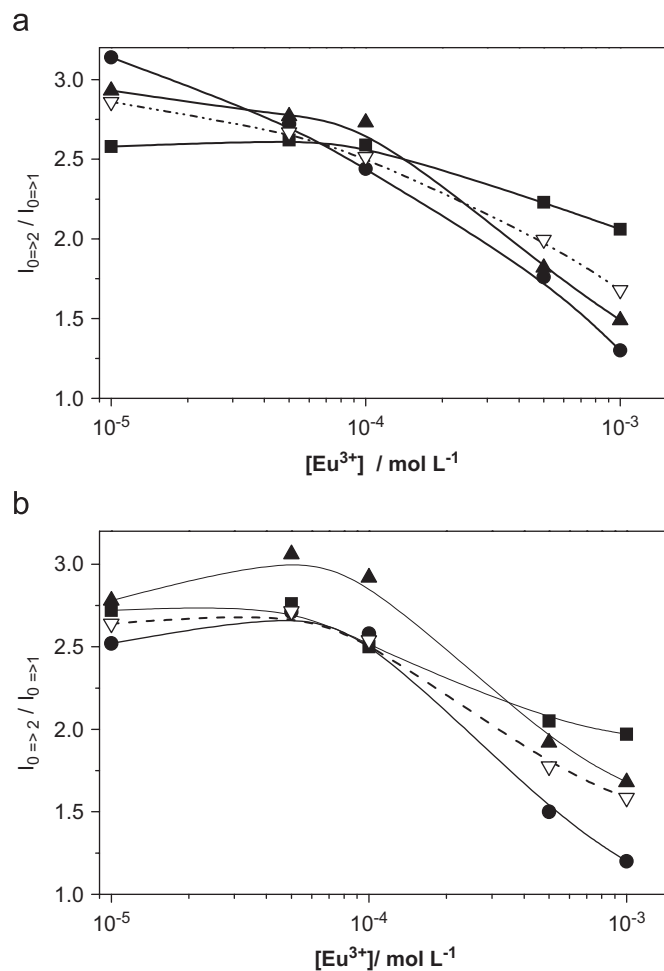
the charge neutralization requirements are fulfiled and in this situation it is very likely that the other europium coordination sites are completed with water molecules or hydroxyl groups of the macroligand. It is known that when europium is completely surrounded by water molecules, which corresponds to a completely isotropic environment, the symmetry of the first coordination sphere is much closer to a centrosymmetric geometry and $I_{0\to2}/I_{0\to1}$ is usually close to one. When a deviation from this behavior occurs, the $I_{0\to2}/I_{0\to1}$ ratio increases [27]. In summary, a higher $I_{0\to2}/I_{0\to1}$ ratio indicates that Eu$^{3+}$ no longer presents a centrosymmetric geometry for its first coordination sphere, which in our context should indicate that the water molecules are being replaced with sulfate or hydroxyl groups on the carrageenans as coordinating ligands. Therefore, different carrageenan types may provide different microenvironments for the europium ion, which should also depend on the displacement of the monovalent counterion by the trivalent ion and its interaction with the polysaccharide.

Figs. 3A and B show the effect of increasing europium concentrations on the relative intensity ratios of the europium transitions $I_{0\to2}/I_{0\to1}$ in systems containing κ-, ι-, or κ-2-car with potassium and sodium as counterions, respectively. A comparison between the two sets of intensity ratios ($I_{0\to2}/I_{0\to1}$) in the presence of $K^+$ and $Na^+$ (Figs. 3A and B) shows that the overall dependence on concentration is related with the sulfation degree of the carrageenans: in both situations, the symmetry conditions for the europium ion changes more abruptly for κ-car, the less sulfated form, followed by κ-2-car, with intermediate content of sulfate groups, and finally for the more sulfated form, ι-car, the intensity ratio changes more gradually. Moreover, this behavior of the intensity ratio is different for carrageenans with different counterions: $Na^+$ or $K^+$, which would not be expected if carrageenans exchanged counterions in an equivalent way. However, analyzing the intensity ratio curves for the three carrageenans having sodium as counterions (Fig. 3B), one observes shallow peaks at about an $Eu^{3+}$ concentration of $0.05\,mmol\,L^{-1}$. The same behavior is recorded for ι-car having $K^+$ as counterion (Fig. 3A). In the presence of potassium ions and for the lowest $Eu^{3+}$ concentration, $0.01\,mmol\,L^{-1}$, an increasing trend of $I_{0\to2}/I_{0\to1}$ ratio values follow the order: iota $<$ κ-2 $<$ kappa. When sodium is the carrageenan counterion, this trend is only observed at a higher concentration, $0.05\,mmol\,L^{-1}$. This tendency is correlated with the decrease from the highest sulfate content—iota, to the lowest one—kappa. The most important differences between sodium and potassium ions are their hydration features: $K^+$ is less strongly hydrated than $Na^+$. Furthermore, carrageenans being polysaccharides with slightly different molecular features may also exhibit different hydration behaviors. Both hydration tendencies result in ionic specificities, as detected from the features of the lanthanide ion emission.

It is known from the literature [29–31] that kappa-carrageenan is potassium specific, whereas iota-carrageenan is not [15]. One should also expect that the hybrid species κ-2 could also display certain specificity toward potassium. The description of gel formation and hardness trend of the gels prepared in this work confirm this observation. On the other hand, the sodium ion is not specific [6,26,29–31] for any of them and in the presence of this ion it is likely that the carrageenans adopt the coil conformation. The picture which develops is that in the course of $Eu^{3+}$ exchanges with cations that bind non-specifically, the $I_{0\to2}/I_{0\to1}$ profile is shallow. However, if the binding is specific, the profile is steep. Noting that the maximum is only present when the counterion is carrageenan-non-specific, we conclude that the displacement of the monovalent counterion by the europium trivalent ion depends on the specificity of the carrageenan ion.

## 3.2. Lifetime measurements

We propose two probable binding sites for $Eu^{3+}$ in the carrageenan structure (Fig. 1): the sulfate groups and the

hydroxyl groups. The sulfate groups participate in long-range coulombic attraction; the hydroxyl groups can coordinate with the europium ions via dipolar interaction.

Which site binds europium ions can be explored through time-resolved emission spectra and $^5D_0$ excited state decay curves. When europium is dissolved in pure water, its emission lifetime decay is $0.10–0.12\,\mu s$, and the coordination number is between 8 and 9 [27].

The systems studied here exhibit one or two lifetime decays ($\tau_1$ and $\tau_2$), depending on the concentration of the europium ion. The data displayed in Fig. 4 for K-κ-car show that the higher the europium concentration, the faster the emission decays. Thus, the emission decay data were adjusted to mono- and double-exponential curves. The choice between these two possibilities was made from the best statistical adjustment of the experimental data. The results are summarized in Table 1.

Two lifetime decays can be attributed to two different europium species or to different binding sites. At the lowest Eu(III) concentration ($0.01\,mmol\,L^{-1}$), the emission decay data are always fitted to a mono-exponential curve (average $\tau_1 = 0.38\,ms$) and these data do not reveal any indication of ion-specificity. In other words, it is not possible to differentiate between the behavior of K-ι-car and K-κ-car from these results. Increasing the Eu(III) concentration, an additional faster decay is identified (average $\tau_2 = 0.15\,ms$). These two lifetime decays can, in fact, be correlated with the differences in the symmetry of the microenvironment or binding site of Eu(III) as evaluated from the intensity ratio $I_{0\to2}/I_{0\to1}$. The concentration ($c_2$) above which two decay lifetimes are observed depends on the surface charge density and sulfate content of the carrageenans. In conditions of gel formation, one may assume that the carrageenan conformation is a double-helix [32–34]. For this condition, the charge separation in the iota chain, with an average of two sulfate
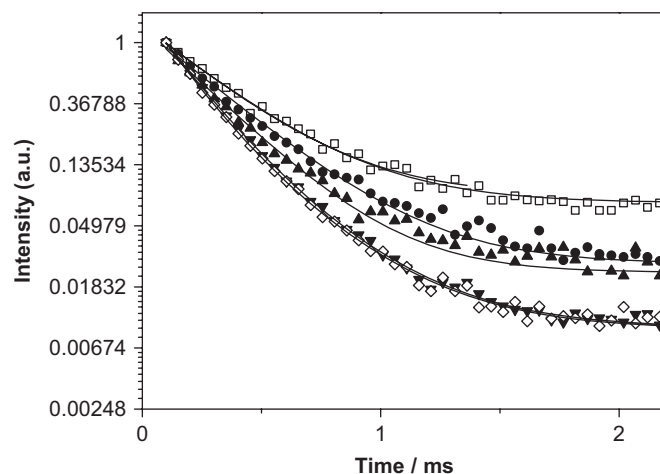


Fig. 4. Emission decay curve for europium-K-κ-car at different europium concentrations: $0.01\,mmol\,L^{-1}$ (□), $0.05\,mmol\,L^{-1}$ (●), $0.10\,mmol\,L^{-1}$ (▲), $0.50\,mmol\,L^{-1}$ (▼), and $1.00\,mmol\,L^{-1}$ (◇). The data were fitted to mono- or bi-exponential curves. Only the best fit for the curves are displayed.

per disaccharide, is half of that found for kappa-carrageenan (average of 0.44 nm), with just one sulfate per disaccharide [35]. From Table 1, $c_2$ is 0.05, 0.1, and 0.5 mmol L$^{-1}$ for kappa, kappa-2, and iota, respectively.

Fig. 5 shows the time-resolved spectra for the Eu$^{3+}$ ions at a concentration of 1 mmol L$^{-1}$, for the three carrageenans combined with the two counterions, Na$^+$ and K$^+$. In all cases, the $I_{0\to2}/I_{0\to1}$ ratio is close to one for the faster delay time after the flash (0.05 ms) and increases for longer delay times, when one should sample the emission for species with longer emission lifetime. Therefore, one can confirm that the faster decay is associated with a higher symmetry binding site, whereas the slower one is due to a site with a less centrosymmetric geometry. As europium binding to an O–H oscillator raises the possibility of non-radiative decays, causing them to be faster, $\tau_2$ is associated with europium–hydroxyl coordination and $\tau_1$ is related to the electrostatic interaction with the sulfate groups. For carboxylated carbohydrates, europium coordination occurs simultaneously via carboxyl and hydroxyl groups [19] and, from the results presented in Table 1 and Fig. 5, it seems this is not the case for carrageenans at low concentration of europium ion, or below $c_2$.

Also, one should consider that the experimental lifetimes ($\tau_{exp}$) comprise radiative (rad) and non-radiative (nrad) processes involved in the $^5D_0$ excited state depopulation

[36,37]. Since the $^5D_0\to{}^7F_1$ transition is MD allowed, with a rate that can be assumed to be site-independent, it can be taken as a reference. The total radiative lifetime can be estimated by considering the intensity ratio between the $0\to1$ transition and the total corrected Eu$^{3+}$ emission from the $^5D_0$ state [36,38].

$$\frac{1}{\tau_{rad}} = A_{01}n^3\left(\frac{I_{tot}}{I_{01}}\right), \tag{1}$$

where $n$ is the refractive index of the medium (1.3345), $A_{01}$ is the spontaneous emission probability for the $^5D_0\to{}^7F_1$ transition in vacuum, and $I_{tot}/I_{01}$ is the ratio of the total area of the corrected Eu$^{3+}$ emission spectrum to the area of the $^5D_0\to{}^7F_1$ transition.

On the other hand, the non-radiative rate for europium in carrageenan gels or solutions can be related mainly to the multiphonon relaxation through OH oscillators of water molecules or hydroxyl coordination in the first coordination sphere of the Eu$^{3+}$ ions. This picture allows the evaluation of the number of hydroxyl oscillators and/or water molecules (named hereafter "$q$ number") coordinated to the Eu$^{3+}$ ions for the different carrageenan systems, by using the Horrocks's model [39,40]:

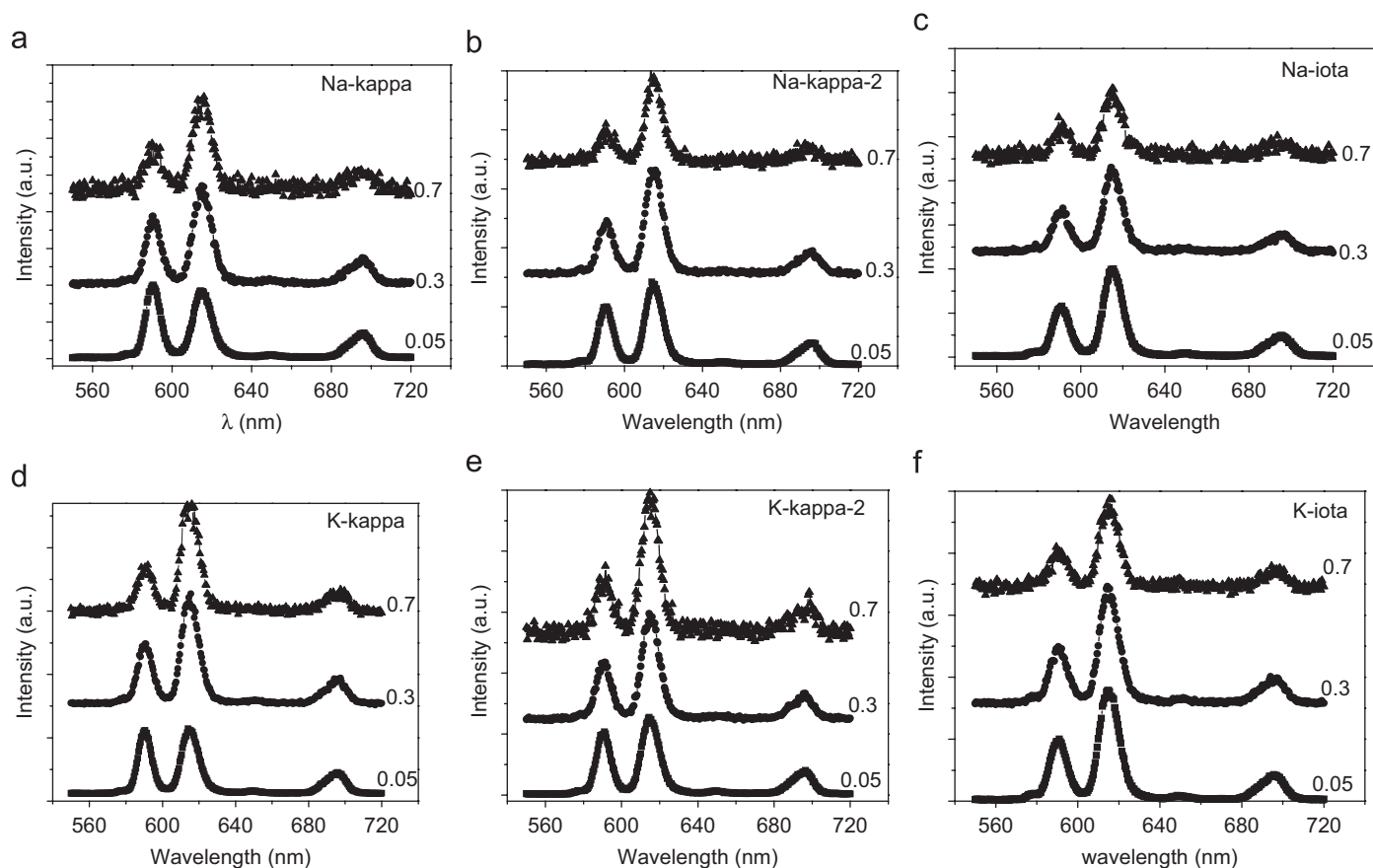$$q = 1.11[\tau_{H_2O}^{-1} - \tau_{D_2O}^{-1} - K_{XH}]. \tag{2}$$



Fig. 5. Time-resolved emission spectra for europium 1 mmol L$^{-1}$ in different carrageenan systems: kappa, kappa-2, and iota with sodium or potassium as the counterions. As faster is the delay after the flash (indicated in the figure, in ms), more symmetrical is the europium binding site for κ-car and κ-2-car, as from the almost identical intensities for $0\to2$ and $0\to1$ bands at 590 and 615 nm, respectively.

As we are looking for comparative results of hydration state or OH binding to europium ions, instead of measuring the lifetimes in deuterated water, we have estimated them considering that they should be equivalent to the contribution of a purely radiative process in accordance with Eq. (1) and Ref. [38]. In Eq. (2), $K_{XH} = \alpha + \beta n_{OH}$ with $\alpha = 0.31$ [40]; the second term was neglected due to the difficulty in establishing the number of OH oscillators ($n_{OH}$) of the macromolecular ligand. A consequence of this approximation may be an overestimation of the $q$ number, especially for the carrageenan with the lowest ether sulfate content or the highest number of hydroxyl groups. The $q$ number was estimated for the lowest and the highest $Eu^{3+}$ concentrations: 0.01 and $1 \, mmol \, L^{-1}$. The former is connected to lifetimes with a highest contribution of sulfate coordination (mono-exponential decay with long decay $\sim 0.38 \, ms$, Table 1). For this lowest concentration, a lower number was estimated, where an average of two OH oscillators were found to be coordinated to the $Eu^{3+}$ ions. This result supports the statement that at low concentration the europium ion binds preferentially to the sulfate groups of the carrageenans, as compared with hydroxyls. For the highest $Eu^{3+}$ concentration, the trivalent ion can be coordinated through the sulfate as well as OH groups. Higher $q$ numbers were estimated for $Eu^{3+}$ $1 \, mmol \, L^{-1}$, with an average equal to $7.2 \pm 1.3$. However, a number well above the mean value was found for Na-κ-car ($q = 9.5$), the carrageenan with the lowest sulfate content and having a non-specific counterion. The $q$ value obtained above the maximum expected for Eu(III) is likely to be attributed to the term ($-\beta n_{OH}$), neglected in the corrected calculations (see paragraph above) since κ-car holds the highest number of hydroxyl groups. Nevertheless, for the same carrageenan type but with different counterion, K-κ-car, we found $q = 7.8$. This result shows a differentiation in the hydration state/ hydroxyl coordination to the europium ion when it is present in the same concentration in two carrageenan systems that differ only by the monovalent counterion. In other words, the $q$ number should be understood as a global measurement of europium coordination to hydroxyl groups and water molecules. Thus, one could expect that for the same number of hydroxyl groups in the carrageenan molecule, as the $q$ number increases, the higher the availability of OH binding sites for the europium ion, and the higher the coordination with water molecules. It is most likely that the structure of the macromolecule in the helix form, promoted by some specificity of the monovalent ion, as in the case of K-κ-car, may decrease the possibility of europium coordination to the hydroxyl groups on carrageenan, since at least some of them may occupy the internal region of the helix. In this sense, europium should be either less tightly bound to the ion-specific K-κ-car or less hydrated when compared to Na-κ-car. This counterion effect could not be distinguished for the other carrageenans from europium lifetime results.

## 4. Conclusion

The results presented here show that the binding of the trivalent, luminescent europium ion to the three different carrageenans, kappa, iota, and kappa-2, having sodium or potassium as counterions, depends on the carrageenan surface charge density: the threshold concentration for which two europium emission lifetime decays are detected increases in the sequence: κ-car < κ-2-car < ι-car, indicating that hydroxyl binding to the polysaccharide becomes important at lower concentration for the carrageenan with lower sulfate content. The specificity toward the monovalent ion was detected in two situations: in the change in the symmetry environment of the trivalent ion, as measured by the intensity ratio of the europium emission bands ($I_{0\rightarrow 2}/I_{0\rightarrow 1}$) at concentrations below the saturation, and from the $q$ number, related to the OH oscillators or water coordination number in the vicinity of the europium ion. This last indicator was only effective for κ-car. This form has the lowest sulfate content and its sodium form presents a $q$ value quite above that of the specific potassium form. In the presence of the non-specific monovalent counterion ($Na^+$ for all carrageenans forms and $K^+$ for iota-car), the emission ratios exhibit a slight maximum in the Eu(III) concentration range of $0.01–0.1 \, mM$, which should be related to the presence of a high centrosymmetric geometry for the first coordination sphere of the europium ion. The set of results presented here gives evidence for the coordination of a rare earth, trivalent ion to both sulfate and hydroxyl groups of the three studied carrageenan forms, despite the currently accepted idea that in aqueous solution it is difficult to dislodge water molecules from the coordination sphere of the metal ion by the un-ionized hydroxyl groups of carbohydrates. Besides the fundamental aspect of this work, it also represents a methodology to obtain a fluorescent carrageenan gel.

## References

[1] M.A. Roberts, B. Quemener, Trends Food Sci. Technol. 10 (1999) 169.
[2] F. van de Velde, N.D. Lourenço, H.M. Pinheiro, M. Bakker, Chem. Inform. 34 (2003) 5.

[3] G.A. De Ruiter, B. Rudolph, Trends Food Sci. Technol. 8 (1997) 389.
[4] F. van de Velde, H.A. Peppelman, H.S. Rollema, R.H. Tromp, Carbohydr. Res. 331 (2001) 271.
[5] I.S. Chronakis, L. Piculell, J. Borgstöm, Carbohydr. Polym. 31 (1996) 215.
[6] E.R. Morris, D.A. Rees, I.T. Norton, D.M. Goodall, Carbohydr. Res. 80 (1980) 317.
[7] Y. Yuguchi, T.T.T. Thuy, H. Urakawa, K. Kajiwara, Food Hydrocolloids 16 (2002) 515.
[8] A.S. Michel, M.M. Mestdagh, M.A.V. Axelos, Int. J. Biol. Macromol. 21 (1997) 200.
[9] Y. Yuguchi, H. Urakawa, K. Kajiwara, Food Hydrocolloids 17 (2003) 485.
[10] V.M.F. Lai, P.A. Wong, C.Y. Lii, J. Food Sci. 65 (2000) 1337.
[11] B. Denef, N. Mischenko, M.H.J. Koch, H. Reynaers, Int. J. Biol. Macromol. 18 (1996) 159.
[12] G. Marcelo, E. Saiz, M.P. Tarazona, Biophys. Chem. 113 (2004) 201.
[13] C. Viebke, J. Borgström, I. Carlsson, L. Piculell, P. Williams, Macromolecules 31 (1998) 1833.
[14] F. Cuppo, H. Reynaers, S. Paolettti, Macromolecules 35 (2002) 539.
[15] L. Piculell, S. Nilsson, P. Muhrbeck, Carbohydr. Polym. 18 (1992) 199.
[16] S. Nilsson, L. Piculell, Macromolecules 23 (1990) 2776.
[17] G.S. Manning, Biopolymers 9 (1970) 1543.
[18] T. Nishio, A. Minakata, J. Phys. Chem. B 107 (2003) 8140.
[19] B. Gyuresik, L. Nagy, Coord. Chem. Rev. 203 (2000) 81.
[20] T. Anthonsen, B. Larsen, O. Smidsrod, Acta Chem. Scand. 26 (1972) 2988.
[21] B.J. Kvam, H. Grasdalen, O. Smidsrod, T. Anthonsen, Acta Chem. Scand. B 40 (1986) 735.
[22] L.I. Katzin, Inorg. Chem. 7 (1968) 1183.
[23] L.I. Katzin, Coord. Chem. Rev. 5 (1970) 279.
[24] V.O. Crescenzi, H.G. Brittain, N. Yoshino, Y. Okamoto, J. Polym. Sci.—Polym. Phys. 23 (1985) 437.
[25] A.J. Paugam, J. Sarfati, R. Romieu, M. Viguier, J. Dupouy-Camet, J.P. Latgé, J. Clin. Microbiol. 36 (1998) 3079.
[26] A.M. Hermansson, E. Eriksson, E. Jordansson, Carbohydr. Polym. 23 (1994) 261.
[27] B.G. Wybourne, Spectroscopic Properties of Rare Earths, Wiley-Intersciences, New York, 1965.
[28] J.C.G. Bünzli, G.R. Choppin, Lanthanide Probes in Life Chemical and Earth Sciences. Theory and Practice, Elsevier, Amsterdam, 1979 Chapter 35.
[29] E.R. Morris, D.A. Rees, G. Robinson, J. Mol. Biol. 138 (1980) 349.
[30] C. Rochas, M. Rinaudo, Biopolymers 23 (1984) 735.
[31] M.R. Mangione, D. Giacomazza, D. Bulone, V. Martorana, G. Cavallaro, P.L. San Biagio, Biophys. Chem. 113 (2004) 129.
[32] C. Rochas, M. Rinaudo, Biopolymers 19 (1980) 1675.
[33] M. Takemasa, A. Chiba, M. Date, Macromolecules 35 (2002) 5595.
[34] L. Piculell, Gelling carrageenans, in: A. Stevens (Ed.), Food Polysaccharides, Marcel Dekker, New York, 2005.
[35] M. Ciszkowska, J.G. Osteryoung, J. Phys. Chem. B 102 (1998) 291.
[36] M.F. Hazenkamp, G. Blasse, Chem. Mater. 2 (1990) 105.
[37] W.T. Carnall, H. Crosswhite, H.M. Crosswhite, Energy structure and transition probabilities of the trivalent lanthanides in LaF₃, Argonne National Laboratory Report, Unnumbered, Argonne National Laboratory, Argonne, IL, 1977.
[38] M.H.V. Werts, R.T.F. Jukes, J.W. Verhoeven, Phys. Chem. Chem. Phys. 4 (2002) 1542.
[39] N. Sabbatini, M. Guardigli, J.M. Lehn, Coord. Chem. Rev. 123 (1993) 201.
[40] R.M. Supkowski, W.D. Horrocks Jr., Inorg. Chim. Acta 340 (2002) 44.