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NMR investigations of the 4-ethyl guaicol self-diffusion in iota (ι)-carrageenan gels

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Abstract

Self-diffusion coefficient of an aroma molecule (4-ethyl guaicol) was measured using the pulsed field gradient spin echo NMR (PGSE-NMR) method in order to investigate the influence of a macromolecular matrix on its diffusion and release processes. Iota (ι)-carrageenan was used for its ability to form thermoreversible gels in aqueous salt solutions. Variations of the ι-carrageenan and the salt concentrations permitted various gels with different thermal and rheological properties to be obtained. These latter were modified by an isotope effect obtained by preparing gels in D_2O. The NMR self-diffusion measurements realised for water and the aroma molecules indicated neither chemical interactions with ι-carrageenan, nor obstruction effects from the polysaccharide chains. In ι-carrageenan gels, the diffusional phenomenon was highly dependent on the heterogeneous gel structure and controlled by hydrodynamic interactions due to frictional drag between each molecule of the system and water microviscosity changes.

Keywords: ι-carrageenan; Aroma; Diffusion; Guaicol; PFG-STE; Isotope

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1. Introduction

Diffusion of solutes in polymeric hydrogels is of interest for many biological and synthetic systems in various industrial applications. In the pharmaceutical, cosmetic and food fields, the release of molecules as drugs or aroma is influenced by diffusion processes, which in turn are supposed to be governed by the physical and chemical properties of the diffusive molecules and by the structure of the matrix. The latter could be composed of synthetic polymers, proteins and/or polysaccharides able to form gels in aqueous environment. It is believed that the diffusion of a solute is decreased by polymers in solutions or gels, depending on the size of the solute, the structure and the concentration of the polymer (Amsden, 1998; Masaro & Zhu, 1999; Muhr & Blanshard, 1982). The diffusion processes in polysaccharide gels generally follow the same tendency (Farhat, Loisel, Saez, Derbyshire, & Blanshard, 1997; Furukawa, Arauz-Lara, & Ware, 1991; Kwak & Lafleur 2003; Ohtsuka, Watanabe, & Suzuki, 1994). This phenomenon could originate from various factors including hydrodynamic, chemical interactions or obstruction effects related to the structure of the system, its ability to bind solute or to interact to form an overlapping network (Amsden, 1998; Masaro et al., 1999; Muhr et al., 1982; Farhat et al., 1997; Furukawa et al., 1991; Kwak & Lafluer, 2003; Ohtsuka et al., 1994).

Carrageenans are polysaccharides extracted from red seaweed forming a family of polymeric sulfated galactans (Doublier & Thibault, 2002; Piculell, 1995; Rees, 1972; van de Velde & de Ruiter, 2002). They are used for their gelling ability in aqueous salt solutions. They are composed of alternating 3-linked β-D-galactopyranose (G units) and 4-linked α-D-galactopyranose (D-units) or 4-linked 3,6-anhydro-α-D-galactopyranose (DA-units) (Knutsen, Myslabodski, Larsen, & Usov, 1994). Three major types of carrageenan exist: ι-, κ- and λ-carrageenan depending on the position and the number of sulfated groups. In ι-carrageenan, galactans are sulfated on the C2 and C4 carbons of the DA and G units, respectively, (Fig. 1).

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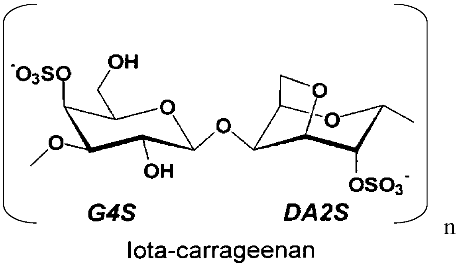

Fig. 1. Repeating units of ι-carrageenan.

This carrageenan type is extensively used as gelling agent in food and pharmaceutical formulations (Doublier & Thibault, 2002; van de Velde, Knutsen, Usov, Rollema, & Cerezo, 2002; van de Velde, Rollema, Grinberg, N.V., Burova, Grinberg V., Tromp, 2002). It is well known that ι-carrageenan forms a three-dimensional network constituted of polysaccharide chains structured as double-helices. This gelation is induced by cooling heated suspensions of the polysaccharide in water under appropriate salt conditions resulting in a coil–helix conformational transition (Abeysekera, Bergström, Goodall, Norton, & Robards, 1993; Morris, Rees, & Robinson, 1980; Tako, Nakamura, & Khoda, 1987). The role of cations is thought to shield the negative charges of the polysaccharide sulfated groups, reducing the repulsion effects between the ι-carrageenan chains and promoting formation of associations between double helices (Morris et al., 1980; Tako et al., 1987). However, little is known about the relationship between the network structure formed by ι-carrageenan gels and their potential of incorporating and retaining small molecules.

Over the large variety of additive compounds found in many formulations, the aroma molecules are widely used in order to impart a specific organoleptic impression determining the odor and the taste of foods or beverages (Kinsella, 1989). Their efficiency is largely influenced by the rate of their release from the matrix which itself can interact with small molecules by chemical or frictional effects (Amsden, 1998; Masaro & Zhu, 1999; Muhr et al., 1982). The result of these interactions as observed in the case of obstruction effects is a retardation of the diffusion process (Amsden, 1998; Masaro et al., 1999; Muhr & Blanshard, 1982).

Diffusion of ions and small molecules in polymeric systems has been studied using various methods based on concentration gradient profiles (radioactive tracer and gravimetric methods) or instrumental techniques as Fluorescence or Nuclear Magnetic Resonance (NMR) spectroscopy (Westrin, 1991; Westrin, Axelsson, & Zacchi, 1994). The latter has been shown to be resolutive and precise in addition to its rapidity (Special issue in Magn. Res. Chem. 2002; Price, 1997; Stilbs, 1987). As a non-destructive method, NMR is a tool of choice for a vast number of diffusion investigations in gels. Use of NMR to measure diffusion processes has been suggested since 1950 when Hahn (1950) discovered the advantages of the magnetic field inhomogeneity (field gradient). Several variations of the method have been explored since the development by Stejskal and Tanner (1965) of the Fourier Transform Pulsed field Gradient Spin Echo sequence (FT-PGSE), but the principle remains the same with application of two gradient pulses separated with a diffusion delay of a few ten of milliseconds, in order to label magnetisation of the diffusing molecules on distances of few micrometers. The method requires the system to be at equilibrium, stable in time, with no volume variation and no mass transfer. In this case, a self-diffusion coefficient is obtained (Crank, 1975). Several studies concerned the use of NMR in investigating the water or solute diffusion processes in biopolymers such as starch or dextran (Farhat et al., 1997; Furukawa et al., 1991; Kwak & Lafleur, 2003 Ohtsuka et al., 1994). Electrochemical and gravimetric methods have already been used in studies of ions release from ι-carrageenan gels or tablets (Ciszkowska & Guillaume, 1999; Picker, 1999), but no experiment has been reported using the NMR method which probes microscopic domains by discriminating between chemical interactions, obstruction or hydrodynamic effects.

We report here the use of NMR spectroscopy for the investigation of the diffusion phenomenon of an aroma molecule in ι-carrageenan gels. As aroma compound, we chose 4-ethyl guaicol copying the coffee or fruit odor in wine or beer, and used as flavour agent in pharmaceutical formulations (Cerdan, Mozaz, & Azpilicueta 2002; Licker, Acree, & Kling, 1998). The influence of the gel structure on the aroma diffusion was analysed by varying the concentration of ι-carrageenan (1, 2 and 3% (w/w)) and the NaCl content (0.1 and 0.3%), the latter being known to play an important role in gelation. To complement the diffusion-based NMR studies, rheological and calorimetric investigations permitted to get macroscopic informations about the gelation transition temperatures and viscoelastic properties of the ι-carrageenan gels. Some isotope effects was observed by comparing gels prepared in H_2O with those obtained in D_2O.

2. Materials and methods

2.1. Carrageenan gels preparation

Iota (ι)-carrageenan was a commercial preparation (Rhodia, France, MEYPRO-SOL 02.2001) with intrinsic viscosity of 430 ml/g measured at 60 °C in 0.2 M NaCl. The 4-ethyl guaicol was a gift from the Unité Mixte de Recherches sur les Arômes INRA-ENESAD of Dijon-France. Each deuterated solution was purchased from Euriso-top (CEA, Saclay-France).

The various ι-carrageenan gels were prepared using the same method with adequate proportions of each ingredient. For instance, the preparation of the 1% ι-carrageenan gel with 0.1% NaCl was achieved by homogenising 0.01 g of carrageenan with 0.001 g of NaCl in D_2O. The mixture

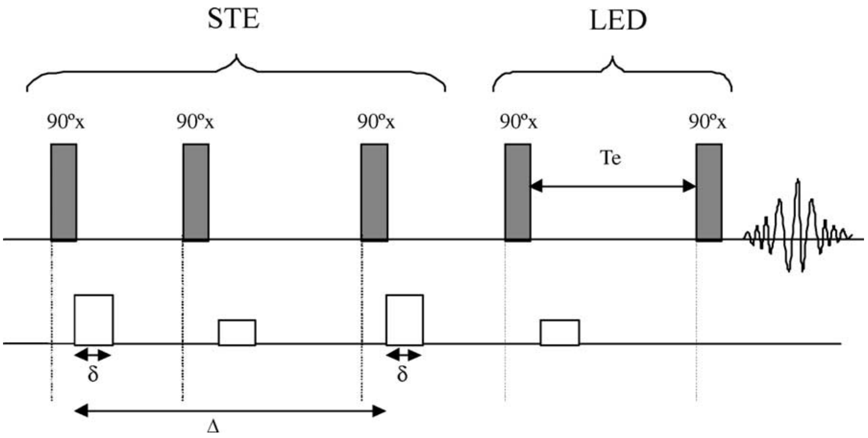

Fig. 2. Pulsed field gradient-stimulated echo longitudinal eddy current delay (PFG-STE LED) sequence (Gibbs et al., 1991).

was then heated at 90 °C for 30 min. The 4-ethyl guaicol (diluted in deuterated methanol) was finally added at 90 °C just before the introduction of the mixture in a liquid NMR tube (5 mm) in which gelation occurred by cooling. Two concentrations of aroma were used: 6.89 and 0.689 mM corresponding to dilution of the pure solution 1000 and 10000 times, respectively.

2.2. Rheology

All rheological measurements were carried out with a AR2000 rheometer (T.A. Instruments) using the cone and plate geometry (diameter of 4 cm, angle 4°). Samples were placed directly onto the plate at 60 °C and recovered with paraffin film oil to prevent evaporation of water. The following in-sequence protocol was used for each preparation: (1) a temperature evolution of the G' and G'' moduli at the frequency of 1 rad/s between 60 and 25 °C at 1 °C/min, and (2) a mechanical spectrum with frequency varying between 100 and 0.02 rad/s at 25 °C and at a constant strain of 1%.

2.3. Differential scanning calorimetry

Differential Scanning Calorimetry (DSC) thermograms were recorded using a Setaram micro DSC III apparatus operating at a scan rate of 1 °C/min. The cells were filled with about 0.8 ml of the hot solution of polysaccharide with 0.1 or 0.3% of NaCl in H_2O or D_2O. Two successive scans were performed within the range 20–70 °C. As a rule, the data of the second scan were used. Every thermogram was characterized by the maximum of the peak, either endothermic on heating or exothermic on cooling, and by the transition enthalpy from the area under the peak.

2.4. Nuclear magnetic resonance

NMR spectra were recorded on a Bruker ARX 400 spectrometer operating at a proton frequency of 400.13 MHz. The aroma chemical shifts were calibrated from internal DMSO with methyl assigned to 2.71 ppm. The polysaccharide signals chemical shifts were determined by using the HDO peak at 4.79 ppm as an internal reference. The NMR proton experiments were realized using a 90° proton pulse of 7 μs and a 75 s recycling time for an acquisition of 1.64 s.

The strength of the static gradient was estimated at 54.1 (±0.2) G/cm for an amplifier output at 10A. For these studies, the PFG-STE LED sequence (Gibbs & Johnson, 1991) shown in Fig. 2 was used with a delay diffusion Δ varying between 30 ms and 1 s. Thirty-two transients were acquired for each of the 16 values of the gradient strength (between 1 and 95% of 54.1 G/cm) with a gradient duration δ of 2 ms and a gradient recovery of 250 μs. Duration of the homospoil gradient pulses and of the LED was, respectively, 1 and 5 ms.

Calibration of the temperature unit was carried out using a sample of deuterated methanol (Eurisotop, CEA Saclay, France) containing 4% of dried protonated methanol. Each NMR experiments was carried out at 298 K.

3. Results and discussion

3.1. DSC measurements

Thermograms recorded for the different gels prepared in D_2O with 1, 2 and 3% of ι-carrageenan (0.1% NaCl) are shown in Fig. 3a for the heating and in Fig. 3b for the cooling processes. By convention, the transition temperature was taken as the minimum (heating) or the maximum (cooling) of the curves. It is interesting to note the quite unusual width of the melting and the cooling peaks which reflect the helix–coil (heating) and coil–helix (cooling) transitions. Similar breadth which has been also reported for ι-carra-carrageenan at a lower concentration (0.2%) appears typical of ι-carrageenan (Grinberg, V. Ya, Grinberg, N.V., Usov, Shusharina, Khokhlov, & de Kruif, 2001; van de Velde, Rollema, Grinberg, Burova, Grinberg, & Tromp, 2002).

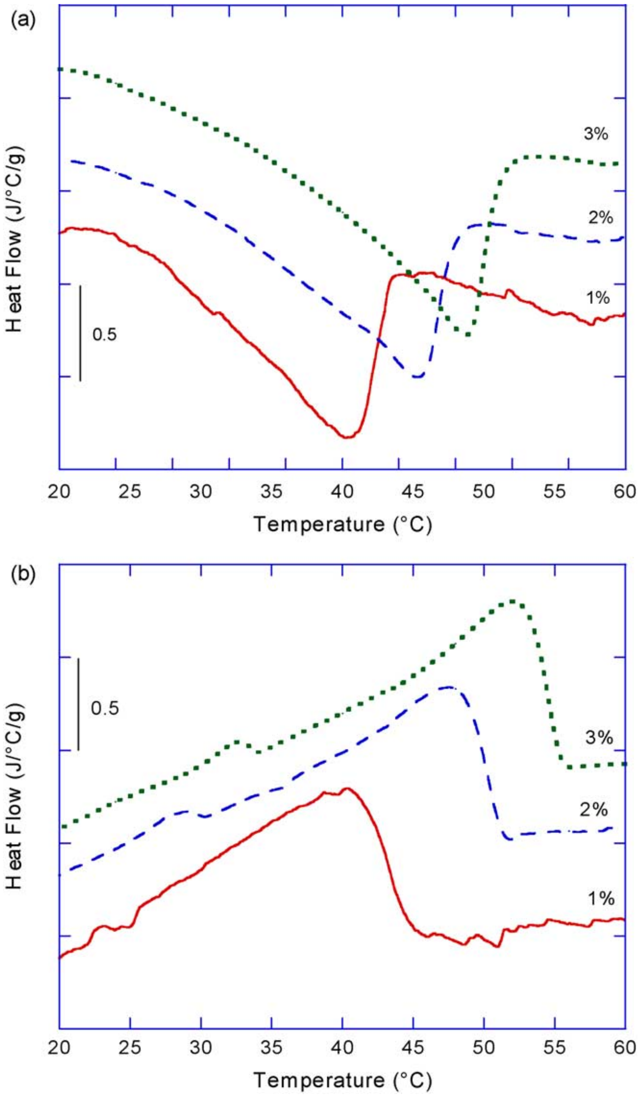

Fig. 3. Thermograms obtained when heating (a) and cooling (b) the 1, 2 and 3% ι-carrageenan gels prepared in D_2O with 0.1% NaCl.

An additional small peak was observed on the heating curves at around 30–35 °C which may be ascribed to the presence of a kappa (κ)-carrageenan fraction. The overall DSC results in D_2O are summarised in Table 1 together with those obtained in H_2O (thermograms not shown). As seen in Fig. 3 for measurements in D_2O and 0.1% NaCl, the transition temperature increased with the polysaccharide concentration. Indeed, in these conditions, the transition temperature upon heating varied from 45.3 to 55.8 °C between 1 and 3% of ι-carrageenan. The cooling process showed the same trends (40.3, 47.5 and 51.8 °C for, respectively, 1, 2 and 3% of ι-carrageenan in 0.1% NaCl and 49.3, 54.0 and 58.2, in 0.3% NaCl). As shown in Table 1, the transition temperature in H_2O was lower than in D_2O, particularly upon cooling, which reflects that more energy is necessary to build up the polymeric network when prepared in D_2O. This statement is also supported by the enthalpy value (ΔH) which was slightly higher in D_2O than in H_2O.

Table 1
Coil→helix and helix→coil transition temperatures (°C) and transition enthalpy (J/g) of the 1, 2 and 3% ι-carrageenan prepared in H_2O and D_2O

		$T_{\text{helix}\rightarrow\text{coil}}$[a] (°C)	$\Delta H_{\text{helix}\rightarrow\text{coil}}$ (J/g)	$T_{\text{coil}\rightarrow\text{helix}}$[a] (°C)	$\Delta H_{\text{coil}\rightarrow\text{helix}}$ (J/g)
ι-carrageenan + 0.1% NaCl					
1%	H_2O	39.9	−8.2	33.3	6.1
	D_2O	45.3	−9.8	40.3	7.8
2%	H_2O	47.5	−10.5	43.4	7.4
	D_2O	51.4	−11.5	47.5	8.2
3%	H_2O	51.7	−11.6	48.4	7.9
	D_2O	55.8	−12.9	52.1	8.1
ι-carrageenan + 0.3% NaCl					
1%	H_2O	47.6	−10.4	42.6	10.0
	D_2O	53.6	−14.6	49.3	12.0
2%	H_2O	53.3	−14.9	49.7	10.5
	D_2O	57.9	−14.4	54.0	10.9
3%	H_2O	57.2	−15.8	53.9	10.6
	D_2O	61.8	−16.9	58.2	11.1

[a] Temperature taken at the maximum of the peak.

3.2. Rheological studies

Measurements at 1 rad/s during the cooling step from 60 °C down to 25 °C allowed the gelling process to be monitored through the G' and G'' variations as a function of temperature (results not shown). Classical patterns were seen with $G'' > G'$ at high temperature reflecting the behaviour of a macromolecular solution and a $G' - G''$ cross-over as the temperature decreased, due to the gelling process. We arbitrarily defined this cross-over as the gelling temperature. As expected, this parameter increased with the concentration. Also, this temperature laid several degrees lower than the DSC maxima upon cooling (coil to helix transition; Table 1) which may suggest that a certain amount of helices are required in order to the three-dimensional network is to be developed.

Fig. 4a, b and c show the G' and G'' variations as a function of frequency at 25 °C for the gels prepared in D_2O with, respectively, 1, 2 and 3% ι-carrageenan (0.1 and 0.3% NaCl). For each sample, the modulus G' was always higher than G'' and increased as the ι-carrageenan concentration increased. The 2 and 3% gels yielded a mechanical spectrum typical of a true gel with G' essentially independent of frequency, $G' > 10\ G''$ at low frequency and G'' displaying a slight dependency with frequency. Furthermore, the G' values at 1 rad/s (Table 2) reflected the properties of relatively rigid gels: 80 and 237 Pa for 2 and 3% in D_2O, respectively. A similar behaviour was exhibited by the 1% ι-carrageenan system in 0.3% NaCl but the gel was much softer ($G' \sim 33$ Pa) while in 0.1% NaCl, the system displayed the properties of a weak gel with $G' \sim 5G''$ at 0.1 rad/s and $G' \sim 5$ Pa. Furthermore, there was evidence that the 1% systems system slightly evolved with time suggesting that a complete equilibrium was not reached immediately as it was for 2 and 3% ι-carrageenan gels. All the data from rheological measurements are given in Table 2.

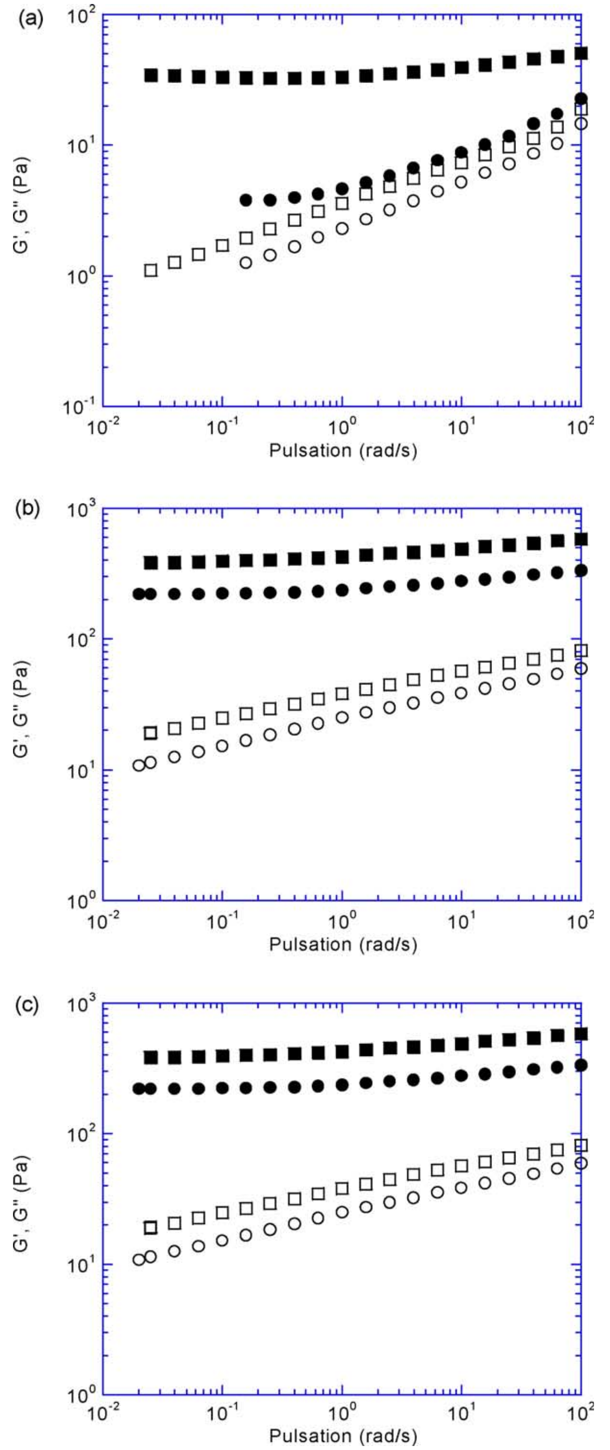

Fig. 4. Mechanical spectra of the 1% (a), 2% (b) and 3% (c) ι-carrageenan gels prepared in D_2O with 0.1% NaCl (G'(●), G''(○)) and 0.3% NaCl (G'(■), G''(□)). (25 °C; strain amplitude: 1%).

Table 2
G' and G'' moduli (Pa) at 25 °C (frequency: 1 rad/s) and sol–gel transition temperatures (°C) of the 1, 2 and 3% ι-carrageenan prepared in H_2O and D_2O

		Moduli for a frequency of 1 rad/s		Gelling temperatures[a] (°C)
		G' (Pa)	G'' (Pa)	
ι-carrageenan + 0.1% NaCl				
1%	H_2O	4.6	2.3	33
	D_2O	9.2	3.0	34
2%	H_2O	53.2	10.7	39
	D_2O	80.1	10.9	43
3%	H_2O	76.5	12.9	42
	D_2O	237.1	24.9	53
ι-carrageenan + 0.3% NaCl				
1%	H_2O	14.6	2.6	35
	D_2O	33.4	3.6	42
2%	H_2O	112.9	13.0	45
	D_2O	140.4	13.3	49.5
3%	H_2O	316.5	32.0	51
	D_2O	427.7	38.0	56

[a] Defined by the G'–G'' cross-over during the gelling experiment (see text).

The G' values obtained for the 2 and 3% gels prepared in H_2O were significantly lower than those measured in D_2O. This observation is in agreement with the DSC results. Recent observation on gelatin (Oakenfull & Scott, 2003) showed a similar trend; this confirms that gels formed in D_2O are more rigid than in H_2O. The fact that the gelling temperature was higher in D_2O than in H_2O is consistent with this conclusion.

3.3. NMR measurements of diffusion

Spectra of the different gels of ι-carrageenan (0.1% NaCl) acquired in D_2O at 25 °C in the presence of diluted 4-ethyl guaicol are displayed in Fig. 5. As a comparison, the spectrum obtained in the same conditions for the aroma molecules is shown with assignment of the chemical groups. Whatever the ι-carrageenan concentration, spectra display the same signals with equivalent chemical shifts. Additional peaks likely originating from fractions of κ-carrageenan, glucose and sucrose could be detected and assigned according to the literature (Günther, 1993; Ferreira, Vidal, Geraldes, & Gil, 2000; Tojo & Prado, 2003; van de Velde, Knutsen, Usov, Rollema, & Cerezo, 2002) (Table 3). Other unknown impurities pollute each sample and make the relative quantification of the assigned fractions impossible.

The self-diffusion coefficient measurements performed on the three gels prepared in D_2O in the presence of 4-ethyl guaicol with 0.1 or 0.3% NaCl are summarised in Table 4. We added the calculated values of the averaged distance of travel R of the aroma molecules during the experimental delay Δ (50 ms) using the following Einstein equation (Einstein, 1956):

$$R = \sqrt{2D\Delta} \qquad (1)$$

The free solution of aroma, whatever the salt concentration, shows the expected self-diffusion coefficient of the residual water known to be equal to 2.29×10^{-9} m^2 s^{-1} at 25 °C (Holz, Heil, & Sacco, 2000). Considering the standard deviation of measurements around 3%, equivalent

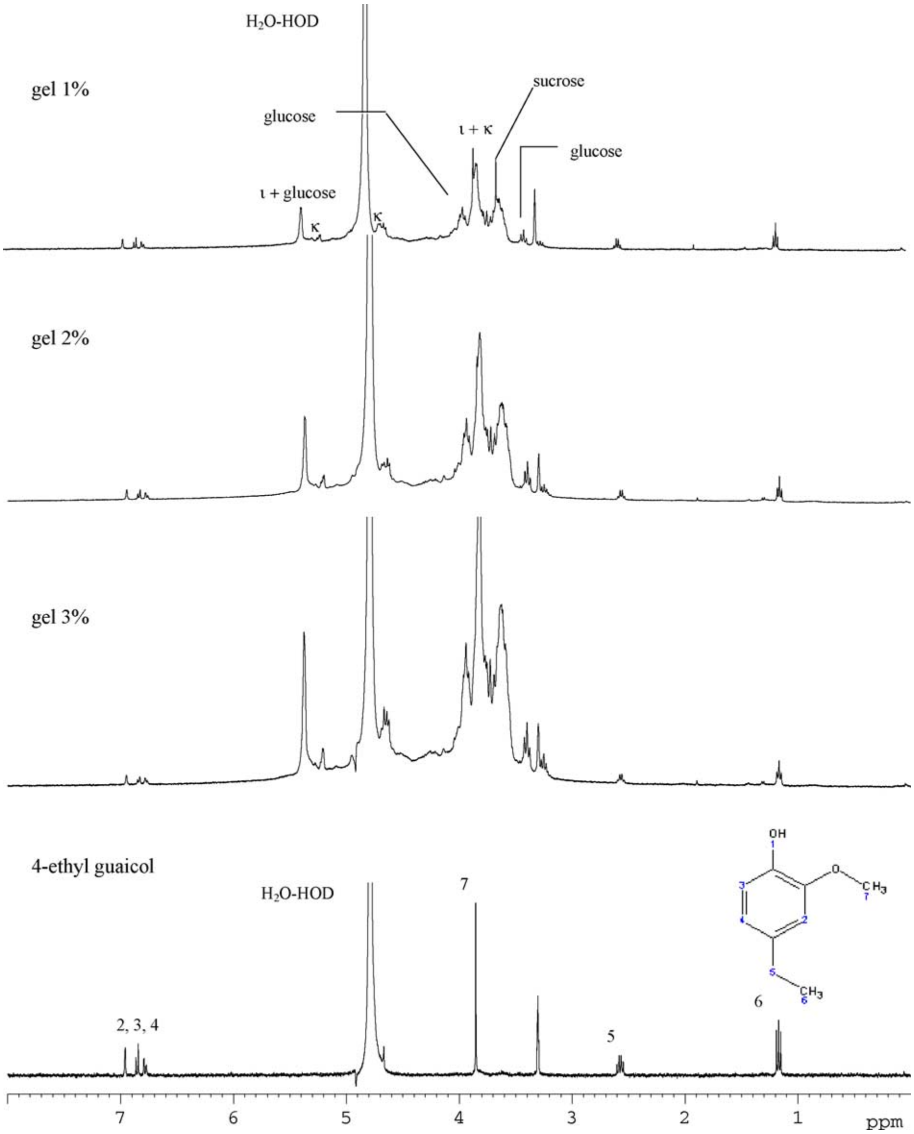

Fig. 5. Proton NMR spectra at 25 °C of the aroma free solution (0.689 mM) and for the 1, 2 and 3% ι-carrageenan gels containing the same amount of aroma, prepared in D_2O with 0.1% NaCl. Chemical structure of the aroma molecule in the inset.

self-diffusion coefficients were measured for the free aroma molecules in the presence of 0.1 and 0.3% of NaCl (0.82×10 and $0.76\times10^{-9}\ \mathrm{m^2\,s^{-1}}$, respectively). A significant decrease of the water and aroma molecules self-diffusion values was observed in the ι-carrageenan gels.

By comparison between gels prepared with 0.1 and 0.3% NaCl, no changes has to be noted for the water and aroma self-diffusion coefficients measured in the 1 and 2% ι-carrageenan gels (Table 4). The values measured for the 1% gels were reduced by 1.7 times with respect to the value in the free solution ($0.48\times10^{-9}\ \mathrm{m^2\,s^{-1}}$ compared to $0.82\times10^{-9}\ \mathrm{m^2\,s^{-1}}$, respectively). On the other hand, the diffusion coefficients obtained in the 2% gels (with an aroma dilution of 10000 times) was higher than for the 1% gels. No significant changes was observed when increasing the aroma concentration to 6.89 mM (1000 times dilution) in the 2% gel prepared with 0.1% NaCl, showing that no chemical interaction occurs between 4-ethyl guaicol and the ι-carrageenan chains. Indeed, association of the aroma molecules with polysaccharide would yield an increase of D_{aroma} with increased aroma concentration. Moreover, as shown in Fig. 5, no change in the chemical shifts was detected for the aroma molecule between the free solution and the various gels.

Concerning the 3% gels, a clear difference of the diffusion behaviour has to be noted for water and the aroma molecules, between the 0.1 and 0.3% NaCl preparations. A significant increase is observed for the diffusion coefficients in the 3% gel obtained with 0.3% NaCl, compared to the one prepared with 0.1% NaCl. Indeed, the aroma self-diffusion coefficients were estimated at $0.48\times10^{-9}\ \mathrm{m^2\,s^{-1}}$ in the 3% ι-carrageenan gel with 0.1% NaCl compared to $0.62\times10^{-9}\ \mathrm{m^2\,s^{-1}}$ in 0.3% NaCl. The same tendency was observed for the water molecules.

Table 3
Proton NMR assignments of the various fractions contained in the ι-carrageenan gels at 25 °C

$\delta \pm 0.01$ (ppm)	Assignments	Position of proton	Reference
1.17	4-Ethyl guaicol	CH_3-6	
2.57	4-Ethyl guaicol	$-CH_2-5$	
3.39	Glucose	H-2	Günther, 1993
3.63	Sucrose	H-6	Ferreira et al., 2000
3.82	ι- and κ-Carrageenan	H-5 (*G*)	Tojo et al., 2003; van de Velde et al., 2002
3.85	4-Ethyl guaicol	CH_3-7	
3.94	Glucose	H-6, 6′	Günther, 1993
4.68	κ-Carrageenan	H-5 (DA)	Tojo et al., 2003; van de Velde et al., 2002
4.79	Residual water		
5.20	κ-Carrageenan	H-1 (DA)	Tojo et al., 2003; van de Velde et al., 2002
5.37	κ-Carrageenan + glucose	H-1 (DA)	Tojo et al., 2003; van de Velde et al., 2002
6.77|6.95	4-Ethyl guaicol	Aromatic protons (2, 3, 4)	Tojo et al., 2003; van de Velde et al., 2002

Numeration of the aroma protons according to the inset of Fig. 5.

Calculation of the averaged displacement during the NMR experiment ($\Delta = 50$ ms) indicates that the aroma molecules diffuse in gels over distances equal to around 7 μm whereas they travel over 9 μm in the free solution. Images acquired in electron (Brigham, Gidley, Hoffmann, & Smith, 1994) and in atomic force microscopy (McIntire & Brant, 1999), show that the gel mesh size (estimated to be of the order of 200–250 nm) is much smaller than the aroma displacements in ι-carrageenan gels. Theoretically, for concentrated polymer systems in which the chains start to overlap, the hydrodynamic theory can be used to explain the diffusion process. However, in ι-carrageenan gels known to be heterogeneous systems, it is difficult to discriminate between the different physical models (chemical interactions, obstruction effects, hydrodynamic or free volume theory) usually applied for diffusion phenomenon. A way to check if the ι-carrageenan chains retard by obstruction the diffusion of the water and aroma molecules in gels is to increase the diffusion delay (Δ) of the NMR sequence (Stejskal & Tanner, 1965; Woessner, 1963). Fig. 6 shows the variations of the water and aroma self-diffusion coefficient as a function of Δ in gels prepared with 0.1% NaCl (same tendency for the 0.3% NaCl gels—not shown). No important change as a function of Δ was found for the diffusion coefficients of aroma molecules while a very slight increase was displayed for the water diffusion. Slight enhancement of temperature in samples could produce an acceleration of the molecules but should also be observed for the aroma molecules.

Without any chemical interaction or obstruction effect, the retardation of the water and aroma molecules in ι-carrageenan gels should originate from frictional effects based on the hydrodynamic theory (Amsden, 1998; Masaro et al., 1999; Muhr et al., 1982). The hydrodynamic description of solute transport in gels is related to the Stokes-Einstein equation (Einstein, 1956; Stokes, 1952):

$$D = \frac{k_B T}{f} \tag{2}$$

where k_B is Boltzmann's constant, T is the temperature and f is the frictional drag coefficient and depends on the medium viscosity and the molecule size, considering it as a hard sphere with radius A, according to this relation: $f = 6\pi\eta A$. Within the hydrogel, the polymer chains are considered to be fixed in place relative to the moving solute and to enhance the frictional drag of the solute by slowing down the fluid near the polymer chains and by inducing impacts between molecules of various size (Amsden, 1998; Masaro et al., 1999; Muhr et al., 1982).

The rheological studies showed that the 1% ι-carrageenan gel is softer than the 2 and 3% gels. The decrease of the aroma diffusion coefficient in the 1% gels (0.1 or 0.3% NaCl) as compared to the free solution could be explained by the presence of a partial network formed by chain segments intertwined in double helices dispersed in water. However, it is likely that gelation is incomplete. In this medium, frictional interactions between each molecule of

Table 4
Self-diffusion coefficients D (10^{-9} m^2 s^{-1}) and theoretical displacement R (μm) at 25 °C for the water and aroma molecules in the free solution and in the 1, 2 and 3% ι-carrageenan gels prepared in D_2O with 0.1 and 0.3% NaCl

	0.1% NaCl					0.3% NaCl			
	Free solution (10000th)	Gel 1% (10000th)	Gel 2% (10000th)	Gel 2% (1000th)	Gel 3% (10000th)	Free solution (10000th)	Gel 1% (10000th)	Gel 2% (10000th)	Gel 3% (10000th)
D_{H_20} (10^{-9} m^2/s)	2.29	1.61	1.79	1.80	1.66	2.27	1.56	1.74	1.90
D_{aroma} (10^{-9} m^2/s)	0.82	0.48	0.58	0.56	0.48	0.76	0.48	0.56	0.62
R_{aroma} (μm)	9	7	7.6	7.5	7	8.7	7	7.5	7.8

Standard deviations were estimated for the diffusion coefficients between 0.01 and 0.05.

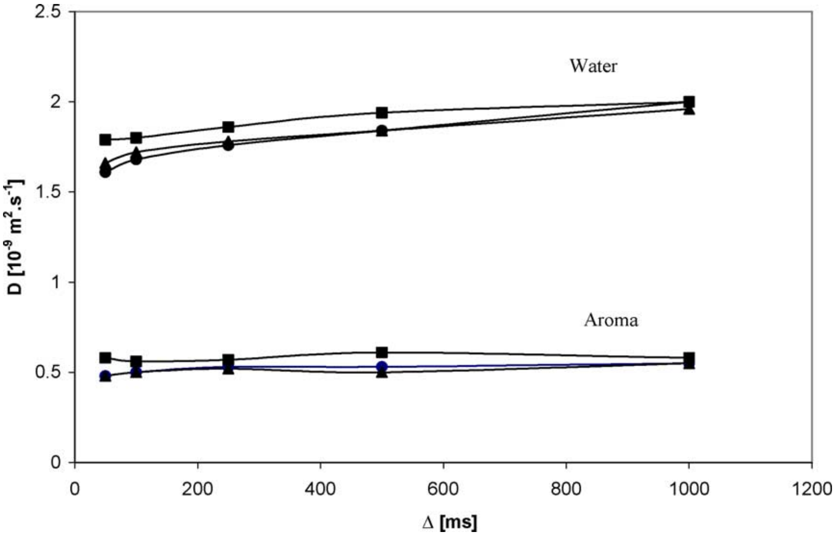

Fig. 6. Self-diffusion coefficients D (10^{-9} m^2 s^{-1}) as a function of the diffusion delay Δ (ms) for the water and aroma molecules in the 1, 2 and 3% ι-carrageenan gels prepared in D_2O with 0.1% NaCl.

the system would be very important and induce a slowing down of aroma molecules.

On the other hand, in a stronger gel like in the 2% gels, the network is better structured with more intertwined chains; the free space would be enhanced and molecular movements of the free molecules (aroma and water) would be favoured. Indeed, it has been shown by transmission electron microscopy that the mesh size in 2% ι-carrageenan gels is around 200 nm (Brigham et al., 1994). The fact that no obstruction effect is evidenced supports that the network remains large enough compared to averaged displacement and the hydrodynamic radius of the 4-ethyl guaicol. The latter was estimated at 0.42 nm using the Stokes–Einstein relation (Einstein, 1956; Stokes, 1952) (Eq. (2)) with a water viscosity of 0.9×10^{-3} Pa s at 25 °C and a diffusion coefficient of 0.58×10^{-9} m^2 s^{-1} in a 2% ι-carrageenan gel with 0.1% NaCl. The larger *D* value measured for the 2% gels argues for a better structured network but with remaining dispersed coils slowing down the aroma diffusion compared to the free solution by frictional interactions with the other molecules. These hydrodynamic effects should be enhanced in the 3% gels due to the higher polysaccharide concentration. In fact, depending on the salt concentration (0.1 or 0.3% NaCl), two different diffusion processes occur.

In the 3% gel 0.1% NaCl, the network is denser than in the 2% gel with locally different water microviscosity and so retards the diffusion of the water and aroma molecules compared to the 2% gel. In the 0.3% NaCl gels, increase of the diffusion with the polysaccharide concentration could be attributed to an improvement of the network order. It has to be noted that few percent of polysaccharide in more than 95% of water could not form an homogeneous network composed of long chains of polysaccharide without slowing down the small molecules diffusion by obstruction effects. On the other hand, high salt concentrations (0.3% NaCl) could induce some clusters or aggregates dispersed in water. This gelation phenomenon with formation of packed double helices should produce an enhancement of the free volume of solvent where small molecules can diffuse more rapidly. This result is similar to the diffusion studies realised on κ-carrageenan gels known to involve subsequent aggregations of the helices, particularly in high potassium concentrations (Johansson, Skantze, & Löfroth, 1991). The authors had showed some increase in the solute diffusion in gelled structures compared to solutions of the polysaccharides. These effects had been attributed to a gain of spaces between helices compared to the solutions containing randomly distributed polysaccharide chains that hinder the pathways of small molecules by obstruction effects. In the 3% ι-carrageenan gel containing 0.3% NaCl, the aroma and water self-diffusion coefficients increase, reaching a value for the aroma of 0.62×10^{-9} m^2 s^{-1}, closer to the value obtained in the aroma free solution (0.76×10^{-9} m^2 s^{-1}). The small decrease of the self-diffusion values, compared to those obtained in the free solution, argues for hydrodynamic interactions between the molecules of the system. In this kind of heterogeneous gel, in addition to impacts between molecules, the water microviscosity is influenced by the presence of the polysaccharide clusters, which modifies the frictional drag of the solute by slowing down the fluid near the polysaccharide chains. Nevertheless, it seems that more ordered the system is, less influenced aroma diffusion is.

4. Conclusion

The present study shows the feasibility of the PGSE-NMR method for investigating the diffusion process of small molecules as aroma in a polysaccharide gel matrix. Using supporting evidence from calorimetric and rheological analyses, we demonstrated that in ι-carrageenan gels, the 4-ethyl guaicol diffusion is influenced by the heterogeneous structure of the network which induces hydrodynamic interactions between the aroma molecules and the polysaccharide chains. In such systems, impacts between molecules and water microviscosity changes modify the friction forces and retard the diffusional movements. However, as already shown for κ-carrageenan gels (Johansson et al., 1991), in well ordered and structured ι-carrageenan gels, the 4-ethyl guaicol self-diffusion process is less disturbed and close to the free solution diffusion. In our case, no obstruction has been detected in ι-carrageenan gels whatever the polysaccharide concentration. This result is not consistent with some admitted rules for diffusion processes in polymeric systems, but is supported by an improvement of the network organisation under higher salt concentrations as observed for κ-carrageenan gels. This work could be applied to more and more complex systems modelling a food product, the aim being to elucidate, which are the molecules or the phenomenon responsible for the alteration of the flavour equilibrium partition coefficient and then of its perception.

As already shown in the case of several protein systems (Lefebvre, Popineau, & Deshayes, 2002; Oakenfull & Scott, 2003), the present rheological and calorimetric studies on ι-carrageenan have demonstrated that gels formed in D_2O had greater rigidity with higher melting temperatures than those prepared in H_2O. This isotope effect not so far demonstrated in the case of polysaccharides, is generally ascribed to larger strength of deuterium bonds and deuterium bridges compared to hydrogen analogues (Kresheck, Schneider, & Scheraga, 1965; Henderson, R.F., Henderson, T.R., & Woodfin, 1970).

Finally, we showed that the PGSE-NMR method should help in the classification of aroma molecule, which are likely to be retained by controlled-release biopolymeric matrices. Investigation of the self-diffusion process of other aroma molecules owing to various chemical family (aldehydes, ketones, etc.) should give interesting informations about the capacity of the ι-carrageenan gels in releasing or retaining small molecules.

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