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Diffusion of small molecules in edible films: Effect of water and interactions between diffusant and biopolymer

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Abstract

Mass transfers of various molecules in multiphasic food products lead to quality modification and thus require the use of edible films or coatings in-between the foodstuff. Consequently, it is important to assess the barrier properties and efficiencies of edible films as well as to determine the diffusivities of the migrants. Translational diffusion of a reference molecule such as fluorescein, determined by the fluorescence recovery after photobleaching (FRAP) method, displays a threshold of a critical water content inducing an increase of the molecular mobility, and demonstrates that multiple populations of a single molecular specie can be involved in different diffusion kinetics. Further investigations at a molecular scale through high resolution solid state nuclear magnetic resonance (NMR) enables to go deeper into the understanding of the interactions involved in such a system, in particular on the identification of the possible binding sites of the diffusant on the polymer and on the overall effect of interactions on the polymer organization. Therefore, the appraisal of transport properties in foods by means of reference molecules constitutes a relevant approach to use in combination with molecular investigation of physicochemical interactions with the diffusing substances.

Keywords: Diffusion; Carrageenan; Fluorescein; Water; FRAP; Solid state NMR

1. Introduction

The knowledge of small molecule diffusion in biopolymers under various external conditions is of great importance for industrial applications in food domain as well as in pharmaceutical field (Debeaufort, Voilley, & Guilbert, 2002). Their use as protective edible barrier in food domain aims to prevent mass transfer of small molecules (water, solutes, colouring agents, aroma compounds) between the foodstuff and the surrounding medium or between different phases of a composite food product, and therefore to avoid food quality deterioration due to physicochemical and texture changes or chemical reactions. As many other polysaccharides classically used as gelling agent in foods, ι-carrageenan, a water-soluble polymer with a linear chain mainly composed of alternated α(1,3)-D-galactose-4-sulfate and β(1,4)-3,6-anhydro-D-galactose-2-sulfate units, presents a high potentiality as film-forming material. In aqueous solutions, ι-carrageenans produce thermoreversible gels on cooling below the critical temperature, where the conformation changes from random coiled single chains to the formation of double-helices of carrageenan chains (Yuguchi, Thu Thuy, Urakawa, & Kajiwara,

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2002). This three-dimensional network formed by polysaccharide double helices in a gel state is then dried to obtain a compact solid film. Their use as edible films or coatings covers various objectives such as the prevention of mass transfers (Karbowiak, Debeaufort, Champion, & Voilley, 2006), encapsulation (Bartkowiak & Hunkeler, 2001), or support for active molecules (Choi et al., 2005). Transport properties are closely linked to the polymeric network structure, which constitutes the continuous phase of the barrier layer.

However, there is no standard method to determine diffusivities in food systems. Two types of diffusion mechanisms are usually invoked to describe transport phenomena in solids. In porous systems, voids and channels mainly control transfer. In dense systems, diffusion is assumed to follow Fick's laws. Therefore, an apparent diffusivity can be obtained from mass transfer experiments, the solid being considered as a uniform "homogeneous-like" material in which the diffusing substance is dissolved. Several different methods can be used to determine an apparent diffusion coefficient of small molecules in dense food products, with a well-defined geometry under steady or unsteady state conditions. The permeation method is one of the most commonly used. A thin sheet of the solid is placed between two media of different concentrations, and the cumulative amount of the penetrant that has passed through over time under steady state conditions gives an estimation of its diffusivity (Ziegel, Frensdorff, & Blair, 1969). It turned out to be a standardized method to determine the permeability to gas or water vapour transfer through flexible packaging films. Other gravimetric methods such as sorption or desorption are based on the measurement of the mass gain or loss of a volatile compound until equilibrium is reached under dynamic conditions (Felder, 1978). When Fick and Henry laws apply, the permeability coefficient is then described as the product of a thermodynamic parameter, which is the solubility coefficient, and a kinetic parameter, which is the diffusion coefficient. The transport of small molecules through packaging films is described by the sorption–diffusion mechanism in a three steps process: sorption, diffusion and desorption (Rogers, 1985). The diffusant distribution method consists in bringing in contact two cylinders of solid material, each containing a different concentration of the diffusant, in order to measure its concentration profile over time as a function of distance, in one dimension (Voilley & Bettenfeld, 1985). It is thus especially suitable to describe various small molecules (water, solutes) diffusion when direct contact occurs between phases. The combination of the last two methods enabled to build up descriptive and predictive models applied to composite foods (Guillard, Broyart, Bonazzi, Guilbert, & Gontard, 2003). Only few techniques allow the direct measurement of a diffusion coefficient without introducing bulk perturbations to the system. The pulsed field gradient spin-echo nuclear magnetic resonance can give such a non-invasive measurement of small molecules diffusion coefficients (Rondeau-Mouro, Zykwinska, Durand, Doublier, & Buleon, 2004), but only for values above $10^{-14}\,m^2\,s^{-1}$. Another solution to assess the diffusivity of a substance with a given molecular weight in a matrix is to use a reference molecular dye, the diffusion of which can be easily characterized. In this way, diffusion coefficient of fluorescein in polymeric films can be measured by the fluorescence recovery after photobleaching (FRAP) method (Karbowiak, Hervet et al., 2006).

When measuring small molecules diffusivity in a matrix, care must be taken to the assumption underlying the theoretical model applied. In particular, the absence of reaction between solute and the food product is assumed. In solid systems such as food products or polymers, mass transfer processes are generally not only Brownian diffusion, but also include other phenomena due to convection, capillary forces, chemical reactions or transport obstruction, thus giving rise to the measurement of an apparent diffusion coefficient (Masaro & Zhu, 1999). Furthermore, the diffusivity is highly dependant on molecular properties and reactivity of the diffusing substance with the medium. Physicochemical interactions between a macromolecular system and a small diffusing molecule affect its diffusion, as it has been observed in the case of aroma compounds in polymers (Debeaufort, Voilley, & Meares, 1994). Moreover, water plays a key role in the diffusion mechanism for most of food systems, which can then be considered as a ternary mixture: the matrix or the polymer, the solvent and the diffusing solute. As water is a solvent, it can indeed produce a modification of the matrix structure, such as swelling of polymers, and modify diffusion of molecules showing an affinity for water.

If the previously mentioned methods can be used to indirectly demonstrate the existence of molecular interactions between the diffusing substance and the medium, they do not allow to characterize these interactions, in order to understand the mechanisms which control the mass transports in the system. The objectives of this study have thus been to investigate the diffusion of a small reference molecule (fluorescein) in ι-carrageenan films and to go further in understanding the interactions between the polymer and the diffusant, using the highly selective solid state nuclear magnetic resonance (NMR) technique. It must be noted that this technique has already been used for the study of food materials, mainly to investigate the mobility of water and carbohydrates protons in food systems (Kumagai, MacNaughtan, Farhat, & Mitchell, 2002; Sherwin, Labuza, McCormick, & Chen, 2002; Van Den Dries, van Dusschoten, Hemminga, & Van Der Linden, 2000).

2. Material and methods

2.1. Material

ι-Carrageenan was supplied by Degussa Texturant Systems (DTS, Baupte, France) and constituted the film matrix. The diffusing molecule, fluorescein (disodium salt),

was purchased from Kuhlmann, France. This planar, heterocyclic molecule has been chosen in reference to its molecular weight that closely matches that of a sucrose molecule (Champion, Hervet, Blond, & Simatos, 1995). Fluorescein displays therefore a small molecular size compared to the carrageenan polymer. This is consistent with the Stokes–Einstein equation for diffusivity with the assumption that the molecule is a sphere with a hydrodynamic volume proportional to its molecular weight. Another reason for the choice of fluorescein as a tracer is its ability to easily undergo photobleaching under laser irradiation. The various saturated salt solutions used to control water activity were supplied by WVR International (Prolabo, Fontenay sous bois, France).

2.2. Carrageenan film preparation

ι-Carrageenan films were made following the aqueous preparation mode. A ι-carrageenan film-forming solution was first prepared by dispersing 6 g of ι-carrageenan powder in 200 mL of distilled water at 90 °C for 15 min under 700 rpm magnetic stirring. Fluorescein was introduced in this carrageenan solution at a concentration of 3 μM, which corresponds to 0.004% in g per 100 g of carrageenan dry matter, for FRAP experiments. For NMR experiments, a much higher fluorescein concentration, of 10% (g per 100 g of carrageenan dry matter), was used for a better sensitivity of the NMR experiment. The hot solutions were degassed by sonication to remove dissolved air bubbles and poured into a thin-layer chromatography spreader to be spread at a 1 mm thickness onto smooth poly(methyl methacrylate) (PMMA or Plexiglas) plates. These conditions were found to be very convenient for dried films to be peeled intact from the casting surface. To obtain a film, the water was removed by drying in a ventilated chamber (KBF 240 Binder, ODIL, France) for 8 h with temperature and relative humidity fixed at 30 ± 1 °C and 40 ± 2% RH, respectively. The chosen ι-carrageenan concentration is above the critical concentration required for gelation, as reported by Rees, Williamson, Frangou, and Morris (1982). Moreover, the amount of ions already contained in the commercial sample of ι-carrageenan used as film-forming agent (Na^+, 3.2%, K^+, 6.8%, from Degussa) is in accordance with classically observed concentrations for promoting gelation (Hossain, Miyanaga, Maeda, & Nemoto, 2001). Thus, during the film formation, carrageenan polymers, under random coil state in hot solution, undergo on cooling a coil-to-helix transition followed by helices association. Casting and drying are carried out at 30 °C, which is at a temperature below the helix melting point reported for this polymer (Bryce, Clark, Rees, & Reid, 1982). This three-dimensional network formed by polysaccharide double helices is then dried to obtain a compact solid film, the thickness of which, after drying, is about 35 μm. These films have then been stored into controlled humidity chambers at constant temperature of 20 °C until equilibrium, by using saturated salt solutions with different water activities ranging from 0.113 to 0.98. The following ones were used for FRAP experiments: 0.113 (LiCl), 0.231 (CH_3COOK), 0.331 ($MgCl_2$), 0.432 (K_2CO_3), 0.544 ($Mg(NO_3)_2$), 0.591 (NaBr), 0.725 ($SnCl_2$), 0.851 (KCl), 0.90 (BaCl), 0.98 ($K_2Cr_2O_7$). For NMR experiments, the medium 0.544 water activity has been chosen.

2.3. Fluorescence recovery after photobleaching (FRAP)

The experimental procedure concerning the determination of the translational diffusion coefficient of fluorescein in ι-carrageenan edible films by the FRAP method has been detailed in a previous paper (Karbowiak, Hervet et al., 2006). Basically, this technique is based on the photobleaching properties of specific fluorescent molecules under intense light illumination. Experimentally, a partial photobleaching of the fluorescent molecules within a small spot in the sample is achieved by a brief exposure to an intense laser beam. Once this irreversible destruction of fluorescence has occurred, the subsequent recovery of the fluorescence in the bleached area is monitored over time. This recovery is only due to the migration of the dye from the non-bleached areas to bleached ones. For our experiments, the laser beam is generated by an argon ion laser, which emits visible light at a wavelength ($\lambda = 488$ nm) that closely matches the absorption band of the fluorescein. The main laser beam is split into two beams of equal intensity through a play of mirrors. These two Gaussian laser beams converge on the sample, thus creating in the crossing area an interference fringe pattern composed of alternating bright and dark stripes. For photobleaching, a short and intense laser pulse is used. After bleaching, the same light source, but highly attenuated, is used to monitor the recovery of fluorescence due to the diffusion of fluorescent molecules within the bleached areas. The fluorescence of the sample is detected by a photomultiplier tube after passing through an optical bandpass filter centred at about the emission band of fluorescein. In order to increase the signal-to-noise ratio of the experiment, the fringe pattern position is modulated through a piezoelectrically driven mirror giving a resulting signal, which is fed into a lock-in amplifier, the whole system and data collection being computer-controlled. The rate at which the recovery of homogeneous fluorescence process takes place is related to the translational mass diffusion coefficient of the diffusing molecules. The diffusion coefficients were deduced from the fluorescence recovery curves, by fitting to an exponential function according to the description of the concentration of fluorescent molecules in this system by the Fick's equation of unsteady state mass diffusion.

2.4. High resolution solid state NMR

NMR experiments carried on carrageenan films samples with or without fluorescein (previously equilibrated at 20 °C and 0.5 water activity) were run on a Bruker MSL 300 spectrometer operating at frequencies of 300 MHz

and 75.47 MHz for ^{1}H and ^{13}C, respectively, or on a Bruker DSX 400 spectrometer operating at frequencies of 400.1 MHz and 100.6 MHz for ^{1}H and ^{13}C, respectively. All the spectra were acquired with a Bruker double-channel 7 mm MAS probe at spinning speeds of 4 kHz or 5 kHz. For the ^{1}H MAS spectra, the experimental conditions were as follows: a 90° pulse of 5 μs and a recycle delay of 5 s between consecutive pulses. The ^{1}H–^{13}C CP-MAS spectra were obtained by cross-polarization, with proton dipolar decoupling. Hartmann-Hahn matching for the ^{1}H–^{13}C CP-MAS experiments was set on adamantane for ^{1}H and ^{13}C radio-frequency fields of ca. 60 kHz. Chemical shifts for ^{1}H and ^{13}C spectra were referenced to the signal of water (4.87 ppm) and to the methylene signal of adamantane (29.47 ppm), respectively. Other experimental details are indicated in the figure captions.

The ^{1}H–^{13}C WIdeline SEparation experiment (WISE) is a modification of the CP-MAS experiment (Schmidt-Rohr, Clauss, & Spiess, 1992; Tekely, Nicole, Brondeau, & Delpuech, 1986). The corresponding pulse sequence is displayed in Fig. 1. The magnetization of the protons is first submitted to a 90° pulse. Then it is allowed to dephase under the ^{1}H–^{1}H dipolar interaction for a variable delay t_1, followed by the polarization transfer to the ^{13}C nuclei. Consequently, the ^{13}C magnetization will be amplitude-modulated by the ^{1}H magnetization remaining at the end of the t_1 delay. For a sufficiently short contact time to avoid ^{1}H–^{1}H spin diffusion, a plot of the intensity of each resolved ^{13}C signal of the CP-MAS spectrum as a function of t_1 will give a representation of the distinct ^{1}H free induction decay (FID) corresponding to the nearby protons of each carbon atoms (Palmas, Tekely, & Canet, 1995). Therefore, the very high selectivity of NMR allows through this technique to investigate the local ^{1}H–^{1}H dipolar local fields associated with each of the individual carbon atoms. For our results, these local fields can be estimated by fitting

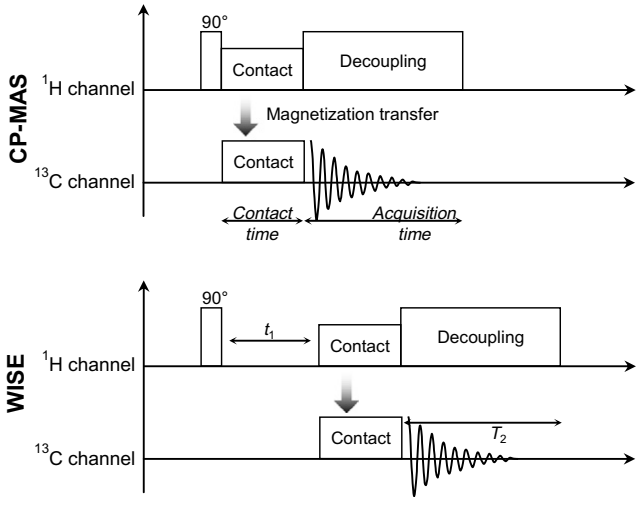

Fig. 1. Pulse sequences used for cross-polarization from protons (CP-MAS) and WIdeline Separation (WISE) experiments.

the t_1-dependant curve to a sum of an exponential and a Gaussian function defined as

$$I = A \times \exp\left(-\frac{t}{T_2}\right) + (1-A) \times \exp\left(-0.5 \times \left(\frac{t}{T_2'}\right)^2\right)$$

where I is the relative intensity and A is the amplitude of the function. For a Gaussian decay, a measure of the strength of the local ^{1}H–^{1}H dipolar interactions is given by the second moment M_2 of the resonance line (Abragam, 1961):

$$M_2 = \frac{1}{(T_2')^2}$$

Raw NMR data were processed using the Win-NMR software (Bruker Biospin) and the WinFit program (Massiot et al., 2002). The analysis of the WISE experiments (fit of the ^{13}C decays) was carried out on MATLAB (version 7.01, The Mathworks, Natick, USA) by minimizing the sum of the square of the differences between the measured and predicted values, using a Levenberg–Marquardt algorithm.

3. Results and discussion

3.1. FRAP investigation of small molecule diffusion in films

This non-invasive technique, first applied to mobility studies in highly hydrated systems (Axelrod, Koppel, Schlessinger, Elson, & Webb, 1976), also appears as a valuable tool to the determination of the diffusion of small molecules in polymer films in order to obtain information on their potential functionality as barrier. The diffusion of fluorescein, a reference molecule of about the molecular size of sucrose, in ι-carrageenan film has thus been investigated in the range of water activities from 0.11 to 0.98. Fig. 2 schemes a typical fluorescence recovery curve obtained for these films for water activity below 0.98. The first part of the graph corresponds to the baseline fluorescence intensity of the sample before bleaching. The fluorescence is decreased to zero during the short bleaching pulse. Then fluorescence recovery is recorded over time. The progressive return to the basis level is due to the diffusion of fluorescent molecules from unbleached regions into bleached ones where photobleaching previously occurred. The rate at which the recovery of fluorescence process takes place is related to the translational mass diffusion coefficient of the diffusing molecules. The value of the diffusion coefficient of fluorescein molecules can be calculated by fitting the recovery curve to an exponential function according to Fick's law applied to unsteady state. The fluorescence recovery was previously checked to be relevant to a diffusive phenomenon, without dependence on the length of the bleaching pattern.

In our case, for carrageenan films at levels of hydration below $a_w = 0.98$, different populations with their proper diffusion kinetics are observed and the experimental curves

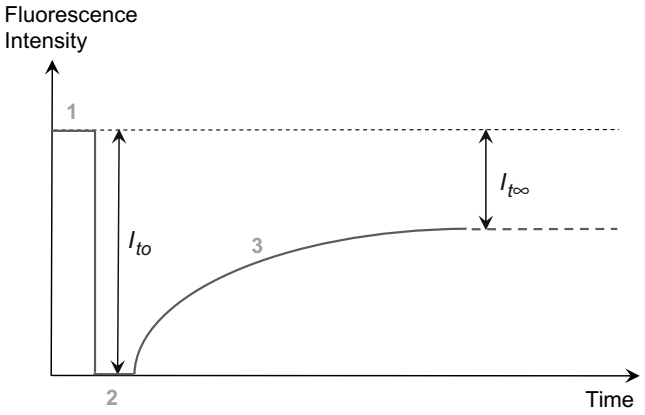

Fig. 2. Typical fluorescence recovery curve obtained after fluorescein diffusion ι-carrageenan films below water activity 0.98. Part 1 corresponds to the baseline fluorescence intensity of the sample before bleaching. In part 2, the fluorescence is decreased to zero during a short bleaching pulse. Then, in part 3, the progressive fluorescence recovery to the basis level is due to the diffusion of fluorescent molecules from unbleached regions into bleached ones where photobleaching previously occurred.

were best fitted to two exponentials, which indicates that the diffusion process is due to a fast diffusing specie, with a diffusion coefficient D1 of about $10^{-13}\,m^2\,s^{-1}$, and a more slower one, with a diffusion coefficient D2 over $10^{-15}\,m^2\,s^{-1}$, the ratio of the two populations being approximately equal to one (Karbowiak, Hervet et al., 2006). The value of D1 when the level of film hydration is increased remains approximately constant in the water activity range 0.1–0.6, corresponding to a film water content of about 0.2 g/g dry matter (with a diffusion coefficient of about $10^{-13}\,m^2\,s^{-1}$). Then for higher hydration levels of the film, corresponding to the water activity range 0.7–0.98, D1 sharply rises up to $10^{-12}\,m^2\,s^{-1}$, while the water content increases up to about 1 g/g dry matter. Clearly, the diffusional process involved in fluorescein transport in the polymer network is related to a water content threshold, of about 0.2 g/g dry matter, below which the diffusion coefficient is nearly constant, and above which it is significantly enhanced. This diffusional breakthrough could be due to a different affinity or a competition between the polymer and the diffusant for the solvent. Above the threshold, water would be available for fluorescein to favour its diffusion, whereas below this threshold, a preferential water sorption by the carrageenan polymer would prevent the fluorescein diffusion.

Moreover, except for 0.98 water activity, fluorescence intensity does not display a complete return to basis intensity, even for long-time experiments. This suggests that a third population of fluorescein is entrapped in the system within the three-dimensional polymer network. The relative proportion of mobile and immobile fluorescein in the sample can then be estimated by the rate of fluorescence intensity recovering from long-time experiments ($I_{t0}/I_{t\infty}$, as displayed in Fig. 2). The estimated fraction of immobilized fluorescein molecules is found to be approximately 50% for water activities below 0.7. Then a decrease is observed to 40% for 0.8 following by 30% for 0.9, and a total release of fluorescein from the polymer network occurred for $a_w = 0.98$. This fluorophore immobilization may arise from either physical (steric hindrance) and/or weak physicochemical interactions (electrostatic, hydrogen bonding). However, these results suggest that the immobilized fraction of fluorescein is not strongly retained by the polymer, as the whole population becomes mobile at the highest level of hydration. Therefore, if interactions between fluorescein and polymer matrix exist, they must be weak, probably lower than hydrogen bonds induced by water.

3.2. Solid state NMR investigation of interactions between the diffusant and the polymer

In order to characterize the interactions which can occur between the carrageenan polymeric matrix and the diffusing fluorescein molecule, we used high resolution solid state ^{13}C NMR as a selective technique for a molecular scale investigation. Fig. 3 shows the ^{1}H–^{13}C cross-polarization magic angle spinning (CP-MAS) NMR spectrum of a carrageenan film containing 10% of fluorescein. This much higher fluorescein concentration was used for a better sensitivity of the NMR experiment. Most of the carbon atoms of each constituting molecules give rise to distinct signals. Except for the region around 105 ppm, the peaks from fluorescein are clearly distinct from those of the carrageenan. All the peaks below 105 ppm (in dark in Fig. 3) correspond to the carrageenan molecule whereas the peaks above 105 ppm (in grey) can be attributed to fluorescein. A scheme of the fluorescein molecule and of the ι-carrageenan dimeric unit (two sulfate ester groups per unit) is shown in Fig. 3. The letter codes refer to the specific carrageenan nomenclature (Knutsen, Myslabodski, Larsen, & Usov, 1994): the idealized ι-carrageenan repeating structure is composed of altering G4S units, which correspond to the 3-linked β-D-galactopyranose 4-sulfate, and DA2S units, or 4-linked 3,6-anhydro-α-D-galactopyranose 2-sulfate. From the literature on solution state NMR of carrageenans (van de Velde, Pereira, & Rollema, 2004), four distinct peaks of our film of ι-carrageenan in a dry state can be identified. In particular, the low frequency peak at 62.6 ppm can be attributed to the carbon number 6 of the G unit, the peak at 69.5 ppm can be tentatively attributed to the carbon number 2 of the G unit, and the peak at 91.7 ppm to the carbon number 1 of the DA unit. The peak at 105.6 ppm is attributed to the carbon number 1 of the G unit, but also corresponds to a carbon group of fluorescein (the NMR spectrum has also been recorded on pure fluorescein and compared separately).

Although not all the peaks can be unambiguously attributed, it is possible to draw information on the interactions between fluorescein and carrageenan, by comparing the relative intensity of a given ^{13}C peak of a molecule in the presence or in the absence of the other molecule (Fig. 4). If we

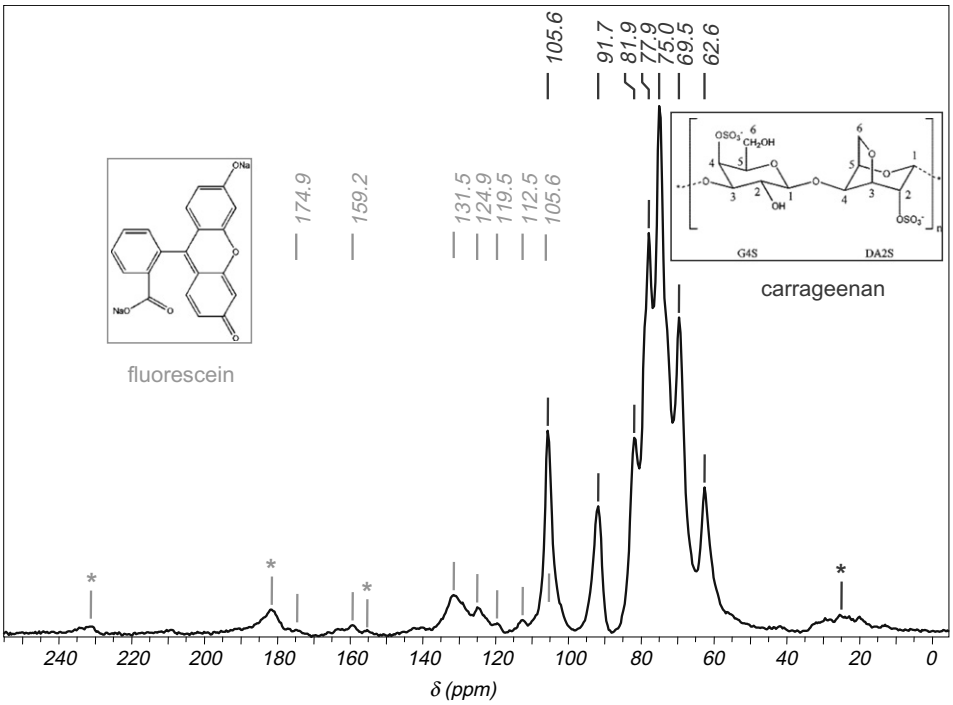

Fig. 3. ^{1}H–^{13}C CP-MAS spectrum of a ι-carrageenan film containing 10% (w/w) fluorescein, with a contact time of 1 ms, a recycle delay of 3 s and 1200 scans. The chemical shifts attributed to the carrageenan molecule are indicated in dark, and those of the fluorescein in grey. Spinning side bands are identified by *.

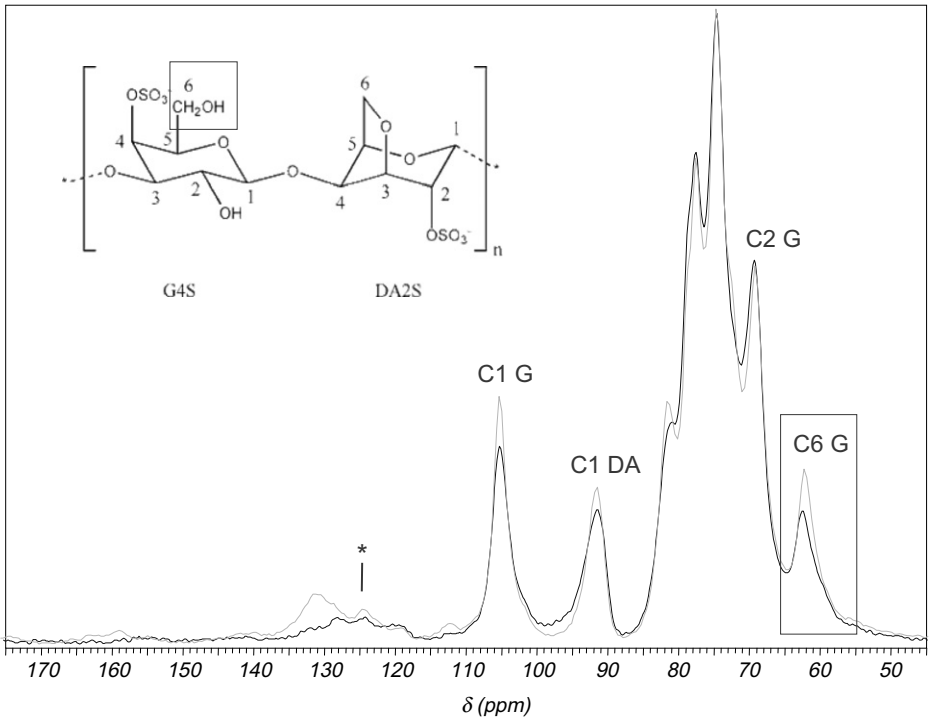

Fig. 4. ^{13}C CP-MAS spectrum of a ι-carrageenan film with 10% (w/w) fluorescein, in grey, and spectrum of a ι-carrageenan film, in dark. Experimental conditions were the same as those indicated in Fig. 3. The identified carbons of the carrageenan dimeric unit are indicated above the corresponding chemical shift. Spinning side bands are identified by *.

compare the spectrum of carrageenan with fluorescein (in grey) and that of carrageenan without fluorescein (in dark), we can see that the relative intensity of both the C1G and the C6G peaks are significantly increased when fluorescein is present. For the C1G peak this increase is due to contributions from both the carrageenan and the fluorescein, and

we cannot discriminate these two contributions. However, for the C6G peak, the only factor that could induce such an increase is a better efficiency of the cross-polarization from nearby protons. Such a better efficiency can result from a reduced mobility of these nearby protons around the C6G atom and/or from an increased density of nearby protons. In the present situation, additional protons would necessarily come from fluorescein molecules immobilized in the vicinity of the C6G carbon. In any case, this suggests that fluorescein interacts specifically with some of the carbon sites of the carrageenan molecules. At this point, we cannot clearly characterize this interaction, but these results support the hypothesis of a specific physicochemical interaction, which could involve hydrogen bonds. Indeed, the three-dimensional structure of ι-carrageenan forms a half-staggered, parallel, threefold, right-handed double helix, stabilized by interchain hydrogen bonds from hydroxyl groups in the G4S units, thus involving the identified carbons C2G and C6G (Janaswamy & Chandrasekaran, 2001). Besides, as shown recently by Exarchou, Troganis, Gerothanassis, Tsimidou, and Boskou (2002), organic molecules such as flavonols and flavones are H-bonding molecules with the unusual ability of retaining it even in aqueous solvents. Fluorescein, which displays a similar chemical nature, could also be involved in hydrogen bond interactions with the ι-carrageenan, and interfere with the native interchain hydrogen bonds.

^{1}H–^{13}C WISE experiments, which allow to measure the selective ^{1}H dipolar second moments (M_2) of the distinct proton groups of the carrageenan, bring additional arguments in favor of specific physicochemical interactions between the polymer and the diffusant (Fig. 5). From the deconvolution of the different spectra in Fig. 5, it is possible to plot the evolution with t_1 of the intensity of all of the distinct ^{13}C peaks. Each of these plots is thus an indirectly reconstructed ^{1}H FID selectively associated with the close protons of each of the distinct ^{13}C signals. As an example, Fig. 6 shows the intensity decay of the 62.6 ppm signal attributed to the C6G carbon of the ι-carrageenan film, with and without fluorescein. All the plots were best fitted to a sum of a Gaussian curve and an exponential curve and the parameters obtained for the three distinct peaks (C1DA, C2G, C6G) are gathered in Table 1. It must be noted that our reconstructed FIDs also display a very weak oscillation. However, none of these curves could be satisfactorily fitted to the Gaussian broadened sinc function used for the FIDs with a beat pattern of concentrated carbohydrate–water solutions (Derbyshire et al., 2004). In our case, this oscillation only occurs after the curve has already leveled off at the baseline. Therefore, it rather corresponds to an artifact of the experiment, which could be, for instance, due to a non-negligible contribution from the C–H dipolar coupling to the proton NMR lineshape (Palmas et al., 1995). Results in Table 1 suggest that, without fluorescein, about 80% of the G4S-DA2S helices are described by an exponential decay of the different ^{13}C signals whereas the remaining 20% are described by a Gaussian decay. In terms of mobility, T_2 values of the order of 5–10 μs (Table 1) clearly indicate that, on the NMR time scale, whether they are related to a Gaussian or a Lorentzian lineshape, all of the carrageenan protons are rigid (Fenwick, Apperley, Cosgrove, & Jarvis, 1999). Therefore, and in order to explain the two contributions to the ^{1}H lineshapes (Table 1 and Fig. 6), it could be hypothesized that this gel is characterized by at least two distributions of local domains with different degrees of

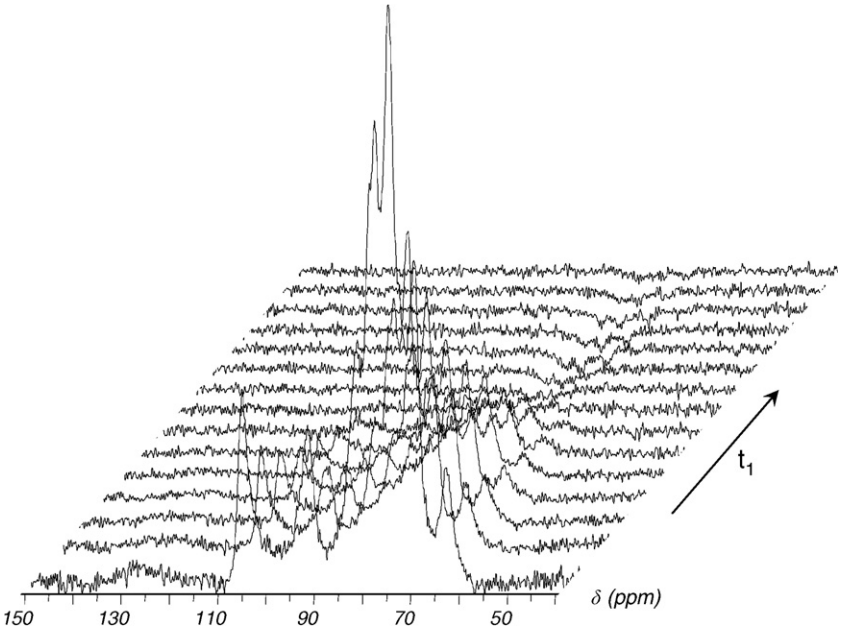

Fig. 5. Stack plot of the ^{1}H–^{13}C CP-MAS spectra of a ι-carrageenan film obtained with the WISE pulse sequence. The contact time was 150 μs, the t_1 delays ranged from 1 μs to 70 μs by step of 3 μs, the number of scans was 800.

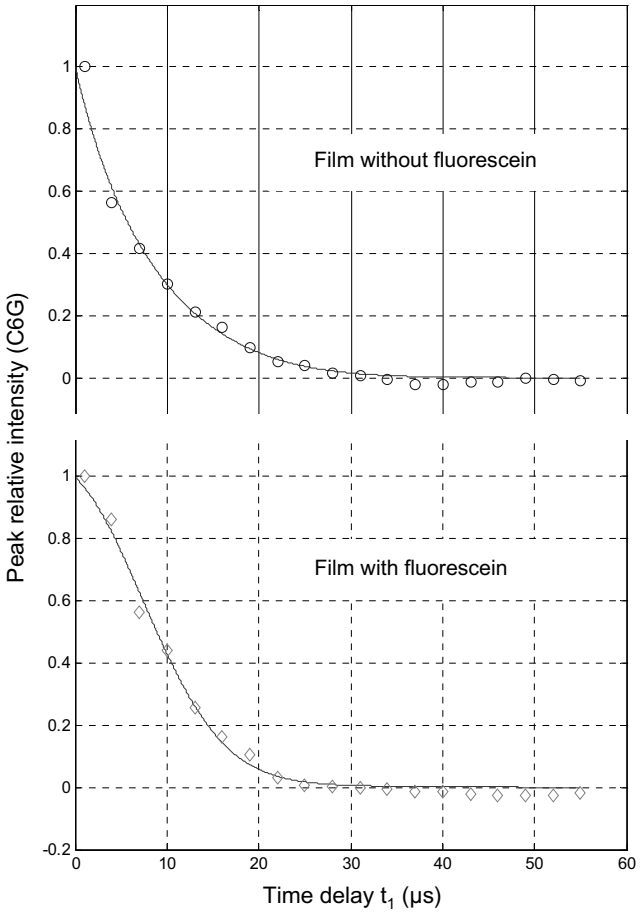

Fig. 6. Plot of the intensity decay of the C6G peak of the ι-carrageenan film, without fluorescein (top) and with fluorescein (bottom), drawn from the ^{1}H–^{13}C WISE experiments. The fit to a sum of a Gaussian curve and an exponential curve is also shown.

local ordering, which can result for instance, from helices arrangements or pore size distributions. Eighty percent of these distributed local domains would then display a higher mean local ordering (exponential decay) than the remaining 20%. As observed in Fig. 6 and Table 1, the decays appear to be undoubtedly different when fluorescein is added prior to the gelation process. Now, about 80% of the local domains are characterized by a Gaussian broadened decay of the ^{13}C signals, with corresponding second moments centered on values that are nearly twice those of the ^{13}C signals without fluorescein. Since the carrageenan protons are already rigid, an increase of M_2 is necessarily due to an increase of the local proton densities around the corresponding carbon atoms (Van Den Dries, Van Dusschoten, & Hemminga, 1998), as a result of the close proximity of fluorescein protons. It must be noted that, in agreement with the above-mentioned results form the ^{1}H–^{13}C CP-MAS spectra, the C6G carbon shows the greatest broadening and seems to be more specifically involved in the interaction with fluorescein. It can therefore be supposed that fluorescein specifically interacts with the carrageenan network and probably hinders the native ordered structure of the pure carrageenan film through the formation of carrageenan–fluorescein hydrogen bonds in competition with native inter-helices hydrogen bonds. Moreover, the decrease in the ordered arrangements of carrageenan helices in the presence of fluorescein could be related to the additional existence of disconnection between carrageenan chains, or "kinks", which can produce a local loss of double helices association property (van de Velde et al., 2002). The addition of fluorescein prior to the gelation process would increase the number of these stopping double helices junction zones, therefore leading to less ordered populations of carrageenan polymer. It also should be noted that the ι-carrageenan helix diameter, which is almost the same as the helix–helix separation, equals 13.9 Å (Janaswamy & Chandrasekaran, 2001), thus offers enough free space for fluorescein diffusion and binding, which hydrodynamic radius is 5 Å (Mustafa, Tipton, Barkley, Russo, & Blum, 1993). Furthermore, different affinity for the binding sites of the carrageenan molecules could explain the existence of various diffusing populations observed with FRAP, especially if there is also a competition between the polymer and the diffusant to bind water. Finally, an additional specific interaction has to be considered for this system, which is related to the great affinity of Pi electrons for alkali cations and especially for Na^+ and K^+ cations (Gokel, De Wall, & Meadows, 2000). As a matter of fact, fluorescein displays several unsaturations, especially with the two aromatic rings, whereas about 10 wt% of the carrageenan film corresponds to Na^+ and K^+ cations, which are directly involved in the long-range ordering of the double helices. Therefore, it is likely that interacting fluorescein molecules also compete with carrageenan helices for the alkali cations, with the consequence that these

Table 1
T_2 values measured from variable t_1 WISE experiment

Carbon atom of the ι-carrageenan molecule	Film without fluorescein					Film with fluorescein				
	Exponential decay		Gaussian decay			Exponential decay		Gaussian decay		
	%	T_2 (μs)	%	T_2' (μs)	M_2 (10^{10} s^{-2})	%	T_2 (μs)	%	T_2' (μs)	M_2 (10^{10} s^{-2})
C1DA	78	5.5	22	8.7	1.3	17	7.6	83	6.7	2.2
C2G	72	5.3	28	9.9	1.0	17	7.5	83	7.6	1.7
C6G	81	6.3	19	12	0.7	24	6.3	76	8.2	1.5

alkali cations are less available for the cohesion of the double helices to compensate the negative charge of the sulfate groups. The Gaussian broadening of the above-mentioned ^{1}H reconstructed FIDs could also probably be a consequence of this disturbance of the long-range ordering of the carrageenan film.

4. Conclusion

The assessment of small molecules mass transfer in food, and particularly in edible barriers, involves generally a macroscopic methodology, giving overall information on transport properties. Among the different methods available to estimate diffusivity, the FRAP technique allows investigation of the diffusion of a reference molecule, fluorescein, introduced in a biopolymer film, of ι-carrageenan, through direct and non-destructive measurements. This reveals the underlying mechanism of diffusion, with the existence of a population of molecules that is retained among the mesh of the film while water concentration remains below a critical level, and above which water becomes sufficient enough in the system to give complete mobility. Further investigations by high resolution solid state NMR can confirm and identify the existence of these physicochemical interactions between the polymer and the diffusant. The diffusion process in solid polymers, in particular the diffusion of solutes in compact polymeric networks, is indeed much more complex than in simple liquids or gels, and the importance of the interactions and their characterization has to be taken into account. The solid state NMR spectroscopy appears as a powerful and sensitive tool for observation of interaction phenomena at a molecular scale and for identification of the potential binding sites between the polymer and the diffusant. It also allows investigation of the modifications of the molecular structures with local ordering changes generated by those interactions. These results provide a deeper knowledge of the polymer structure and of the interactions developed in presence of a specific diffusant, with direct consequences on permeability properties of the polymer to this substance. Therefore, working at a microscopic scale through FRAP analysis and at a molecular scale with high resolution solid state NMR reveals complementary information about the diffusion processes involved in the studied system of carrageenan edible film under specific relative humidity conditions. Moreover, the underlying interactions between the polymer and the diffusant implied in these mechanisms can contribute to improve model of mass transport phenomena. This type of analysis seems thus to be promising for applications to controlled release of drugs from polymer carriers, such as for active packaging or pharmaceutical products. It could also be extended to study interactions between two macromolecules when biopolymers are used in combination.

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