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Characterization of diffusion of macromolecules in konjac glucomannan solutions and gels by fluorescence recovery after photobleaching technique

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Abstract

Konjac glucomannan (KGM) is a neutral polysaccharide with interesting properties as gelling agent and thickener. Its peculiar biodegradability, being not degradable in the small intestine but degradable by the anaerobic human intestinal bacteria, turn it into a promising candidate for colonic drug delivery systems. In this study aqueous systems (0.5%, w/v,) of KGM from three different origins and their mixtures with xanthan gum (XG) (1:1) were evaluated as regards their rheological properties and the diffusion coefficients and mobile fraction of macromolecules (dextrans of different molecular weight). Rheological data illustrate the synergism between KGM and XG at a stoichiometric relationship 1:1. Moreover, fluorescence recovery after photobleaching (FRAP) data indicate that diffusion of probes through the polysaccharide systems cannot be completely explained by the macroscopic properties of the medium but it is related to their molecular size and as a consequence to a sieving mechanism. The strong differences between KGM from different suppliers suggest the convenience of establishing specifications for this material in order to use it as pharmaceutical excipient.

Keywords: Konjac glucomannan; Xanthan gum; Fluorescence recovery after photobleaching; Colonic delivery; Diffusion coefficient; Rheology

1. Introduction

In the last years an important work has been carried out within the pharmaceutical field directed to establish the utility of various natural polysaccharides as base excipients for the elaboration of specific drug delivery systems (Kumar and Kumar, 2001). Particularly challenging is the delivery of drug macromolecules like peptides or protein to the systemic circulation through colonic absorption which is unfeasible until now.

Among the various approaches used for colon drug targeting it is remarkable the use of natural polysaccharides, modified natural polysaccharides or its mixtures. These materials have been traditionally used both in food and pharmaceutical industries and are considered non-toxic, low cost and available in a variety of structures and interesting properties. Large number of polysaccharides has already been tried for their potential as colon-specific drug carrier systems, such as chitosan, pectin, chondrotin sulphate, cyclodextrins, dextrans, guar gum, inulin, pectin, locust bean gum and amylose (Sinha and Kumria, 2001).

Konjac glucomannan (KGM) is a natural neutral water-soluble polysaccharide obtained from the tubers of *Amorphophallus konjac*. It is composed of a backbone chain of β-1,4 linked D-mannose and D-glucose with a low degree of acetyl groups related to its gel formation properties (Williams et al., 2000; Katsuraya et al., 2003; Gao and Nishinari, 2004). This soluble fibre has an extraordinarily high water-holding capacity, forming highly viscous solutions when dissolved in water. It has the highest viscosity at lowest concentration of any known dietary fibre (Ozu et al., 1993; Yaseen et al., 2005).

While the use of KGM as a gelling agent, thickener, film former and emulsifier has been receiving considerable attention in the food area, little attention has been paid to its possible use in the pharmaceutical area. However, the interest of researchers for KGM has increased recently because it has been demonstrated that this polysaccharide has the ability to lower blood cholesterol

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and sugar level, help with weight loss, promote intestinal activity, immune function, etc. (Vuksan et al., 1999, 2000; Fang and Wu, 2004).

Due to its gel-forming and biodegradability, being not degradable in the small intestine but degradable by the anaerobic human intestinal bacteria (Nakajima and Matsuura, 1997; Nakajima et al., 2002), can be considered a promising candidate for colonic drug delivery systems. In fact, studies on KGM have been carried out in recent years in order to establish its utility as colon-specific delivery excipient for hormones (Gonzalez et al., 2004) and some proteins as insuline or bovine serum albumin (Wang and He, 2002; Liu et al., 2004).

Specially interesting it is the synergistic interaction between KGM and other polysaccharides, such as xanthan gum pointed out by different authors. (Goycoolea et al., 1995a, 1995b; Paradossi et al., 2002).

Xanthan gum (XG) is an exopolysaccharide from *Xanthomonas campestris* that can play a successful role in matrix formulations for oral controlled-release drug delivery (Talukdar and Kinget, 1995; Santos et al., 2005).

Xanthan solutions exhibit weak gel-like properties at low shear rates, but it does not form true gels at any concentration or temperature (Millane and Wang, 1990).

Mixtures of KGM and XG, even at extremely low concentration, produce strong and elastic gels (Goycoolea et al., 1995a) the utility of which as drug delivery systems has not been yet established.

When a solid dosage form based on KGM–XG is placed into an aqueous environment it absorbs water from the medium and forms a gel through which drug is released. A key aspect from a pharmaceutical point of view if we like to be able to modulate drug delivery is to know and understand how diffusion of the drug it is influenced by the structure of the polymer network (DeSmedt et al., 1997).

Several techniques like nuclear magnetic resonance, light scattering or fluorescence recovery after photobleaching (FRAP) have been proved very useful for the characterization of macromolecules diffusion within hydrogels (Gumbleton and Stephens, 2004; Burke et al., 2005; Van Tomme et al., 2005).

FRAP denotes a method for measuring the motion of fluorescently labelled molecules. A microscopic small area of the fluorescent sample is photobleached by a brief exposure to an intense focused laser beam. Recovery occurs by replenishment of intact fluorophore in the bleached area by diffusion from the surrounding medium. Interesting information obtainable from FRAP experiments includes determination of the diffusion coefficient and total fraction of fluorophore which is mobile (Axelrod et al., 1976; Perry et al., 2006).

Nowadays, most confocal scanning laser microscopes (CSLM) are equipped with the feature to bleach user-defined regions within fluorescent samples. This allows FRAP experiments to be easily carried out.

The goals of the present study were:

(a) to evaluate the utility of CSLM in conjunction with FRAP for measuring diffusion coefficients in konjac glucomannan solutions and the intermanufacturer variability of KGM;
(b) to assess the influence of molecular weight macromolecules on their diffusion coefficients and mobile fraction;
(c) to study the effect of the interaction between KGM and xanthan gum on diffusion coefficients and total mobile fraction of macromolecules.

2. Materials and methods

2.1. Raw materials

Konjac glucomannans from different suppliers and geographical origins: American (Triple Crown America Inc., Lot: 3500C), European (Escuder, Spain, Lot: 019) and Japanese (Propol A®, Lot: AKG07) and Xanthan gum (Guinama, Spain, Lot: 016) were studied as received.

Fluorescein isothiocyanate dextran (FITC-dextran) probes of different averaged molecular weights (M_w): 7.7×10^4, 1.3×10^5, 5.11×10^5 g/mol and approximate $M_w = 2 \times 10^6$ g/mol and 4-(2-hydroxyethyl) piperazine-1-ethanesulphonic acid, (HEPES) were obtained respectively from Sigma Aldrich and Fluka Biochimika.

2.2. KGM or KGM/XG systems preparation and rheological characterization

Polysaccharide systems in distilled water at a concentration of 0.5% (w/v) were prepared by mechanical stirring for one hour at 85 °C in an hermetic container. When KGM/XG mixture systems were elaborated a ratio of 1:1 in weight was used and total polysaccharide concentration maintained. Solutions were left to cool and equilibrate overnight and its rheological properties characterized using a rheometer AR1000 (TA Instruments, Newcastle, UK) fitted with a cone- and plate geometry (2° cone angle, 60 mm diameter, 59 μm gap). For the gel systems preheating of the rheometer peltier plate above gel temperature was needed to avoid the appearance of harmonic signals.

Steady shear measurements and dynamic rheological characterization were carried out at least in triplicate at 25 °C.

Steady shear measurements were made using a logarithmic torque ramp in order to decrease the initial acceleration and the effects of inertia.

Dynamic rheological characterization started with torque sweeps to ensure operation within the linear viscoelastic region of the viscoelastic samples. The extension of the linear viscoelastic regime has been determined under oscillatory shear conditions at a frequency of 1 rad/s. Dynamic frequency sweep experiments have been carried out at constant strain amplitude within the limits of the linear viscoelastic region in the range of 0.1–100 rad/s.

Samples were covered with a thin layer of paraffin oil to limit evaporation.

2.3. Laser scanning confocal microscope experiments

Fluorescein isothiocyanate dextran (FITC-dextran) probe solutions with a concentration range from 0.45 to 20 mg/mL were prepared in 20 mM HEPES buffer pH 7.4. Solutions or

gels of the three KGM, XG or KGM/XG mixtures (1:1) were prepared in 20 mM HEPES buffer pH 7.4 at a total polysaccharide concentration of 0.5% (w/v) by mechanical stirring for 1 h at 85 °C in an hermetic container.

The fluorescent probes were added to an aliquot of the KGM solutions or KGM/XG gels, stirred and left to cool at room temperature to obtain homogeneous samples. Concentration of the sample probes was always 1.5 mg/mL.

FRAP experiments were performed on a CSLM (model MRC1024 UV, Bio-Rad, UK) modified to be able to bleach arbitrary regions (Wedekind et al., 1994; Braeckmans et al., 2003). Bleaching experiments have been performed with the 488-nm line of a 4 W Ar-ion laser (model Stabilite 2017; Spectra-physics, Germany). A 10× objective lens (CFI Plan Apochormat; Nikon, The Netherlands) with a numerical aperture of 0.45 was used. Typical photobleaching and measuring powers in the sample were 2 mW and 5.5 μW, respectively.

The sample was positioned on the microscope stage and a location of interest brought into focus. After a disk of a particular diameter (25 μm) has been drawn in the bleaching software a time-series is recorded with the CSLM resulting in a stack of images. The first image of the series shows the sample before bleaching, the second one shows the disk at the time of bleaching and the subsequent images show the recovery process after bleaching (see Fig. 3). Usually we have recorded stacks of 30 images with a time interval between 2.5 and 30 s depending on the recovery speed.

To extract the experimental recovery curve from the stack of images, at first the centre of the bleached disk is accurately calculated by a centre of mass algorithm. Secondly, each of the recovery images is normalized to the first image, the prebleach image. Next the mean fluorescence intensity inside the disk is calculated for each image and normalized to one background user-defined region on the same image to correct for possible bleaching during the recording process. Finally the experimental parameters – effective diffusion coefficient (D) and mobile fraction (k) – are determined by a least-squares fit of the equations, as described in Braeckmans et al. (2003), to the experimental recovery curve.

2.4. Statistical analysis

The statistical package SPSS 12.0S for Windows was used to study the variability among group means by analysis of variance (ANOVA) and post hoc comparisons among pairs by Scheffe test (Walpole et al., 1998).

3. Results and discussion

It is well documented that the structure and the percentage of glucomannan in the *A. konjac* roots vary considerably depending on the cultivars, strains, time or producing areas of tubers (Takigami and Phillips, 1996; Fang and Wu, 2004). The variations in the methods of extraction and purification increase the rheological and biochemical differences inherent to this type of products meaning high intermanufacturer variability in the same way as other natural products. There are not yet pharmaceutical specifications for this material, therefore it is necessary to consider the intermanufacturer variability in order to establish its utility as raw material in the development of dosage forms.

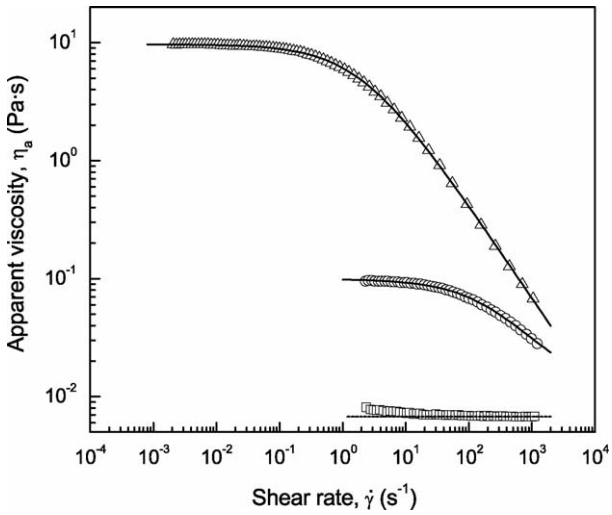

Fig. 1. Flow curves for 0.5% konjac glucomannan solutions at 25 °C. Symbols: Japanese KGM (△), American KGM (□) and European KGM (◯). Full lines represent cross-model predictions and dashed line Newtonian model prediction.

Fig. 1 presents the typical flow curves at 25 °C for the solutions at 0.5% (w/v) for the three KGM from different origins studied showing marked differences between them.

American-KGM solution (0.5%, w/v) shows a Newtonian behaviour being the shear rate proportional to the shear stress. The shear viscosity is low (7 mPa s) and does almost not vary with the shear rate. This could be related to a lower molecular weight for the American-KGM and as a consequence, at a concentration of 0.5% the number of entanglements created between molecules and disrupted by the shear is the same, so the viscosity is constant.

European and Japanese KGM show a clearly shear thinning behaviour at high shear rates after a first Newtonian region at low shear rates, in agreement with other authors for the KGM (Jacon et al., 1993) and other polysaccharides as galactomannans, chitosan or gellan (Miyoshi and Nishinari, 1999; Hwang and Shin, 2000; Oblonsek et al., 2003). At low shear rates the disruption of entanglements imposed by the shear is balanced by the formation of new ones, so no net change in the entanglements occurs and the viscosity is maintained constant. The constant viscosity value is called zero-shear rate apparent viscosity η_0. As the shear rate increases the disruption of entanglements overcomes the formation of the new ones and as a consequence apparent viscosity decreases.

Many equations have been proposed to describe the shear thinning behaviour of polysaccharide solutions (Lapasin and Pricl, 1995). Cross model has provided the better fit to describe accurately the flow properties of the European and Japanese KGM (0.5%, w/v) solutions and predict zero-shear rate viscosity values. Cross model may be defined in the equation:

$$\frac{\eta_a - \eta_\infty}{\eta_0 - \eta_\infty} = \frac{1}{1 + (K\dot{\gamma})^m}$$

Table 1
Magnitudes of de Newtonian and cross model parameters for steady shearing, obtained for American KGM, European KGM, Japanese KGM and XG solutions

Samples	Model	η (Pa s)	K (s)	m	S.E.[a]
American KGM	Newtonian	0.007 (0.000)	–	–	3.985
European KGM	Cross	0.103 (0.007)	0.005 (0.000)	0.810 (0.024)	3.834
Japanese KGM	Cross	9.471 (0.338)	0.500 (0.013)	0.792 (0.002)	2.374
XG	Cross	32.250 (1.456)	39.217 (1.670)	0.829 (0.002)	3.307

[a] S.E. $= \frac{\left[\left(\sum_{i=1}^{i=n}(x_{\exp,i}-x_{\mathrm{cal},i})^2\right)/(n-2)\right]^{1/2}}{\mathrm{range}(x_{\exp})} \times 1000 \times \mathrm{mean(S.D.)}$.

where $\dot{\gamma}$ is the shear rate (s^{-1}), η_a the apparent viscosity (Pa s), η_0 the zero-shear rate viscosity (Pa s), η_∞ the infinite shear rate viscosity (Pa s), K a time constant (s) and m is a dimensionless constant which dictates the degree of shear thinning. Values of m tending to zero describe more Newtonian liquids while the most shear thinning liquids have a value of m tending to unity.

Table 1 presents the parameters derived from Cross model for European and Japanese KGM and the viscosity value for the American KGM. Very good agreement between experimental data and model predictions can be observed in Fig. 1 and also is illustrated by the low magnitudes of the standard error and allowed to an accurate determination of the zero-shear rate viscosity.

The magnitude of zero-shear rate viscosity is a macroscopic representation of microstructural nature of biopolymers indicating that the Japanese KGM establish a higher number of entanglements than the European KGM.

Results demonstrate clear flow rheological differences among KGMs from different suppliers that could be a priori determinant of its behaviour as excipient in drug delivery systems.

Fig. 2 corresponds to the mechanical spectra of the KGM or KGM/XG systems at a 0.5% (w/v) concentration.

Despite of the visual appearance of the systems elaborated with the Japanese and the European KGM (Fig. 2A), from a rheological point of view, neither of them can be classified as a gel but viscous solutions.

Almdal et al. (1993) have established two conditions to distinguish between viscous solutions and gels according to which: (a) a gel is a soft, solid or solid-like material of two or more

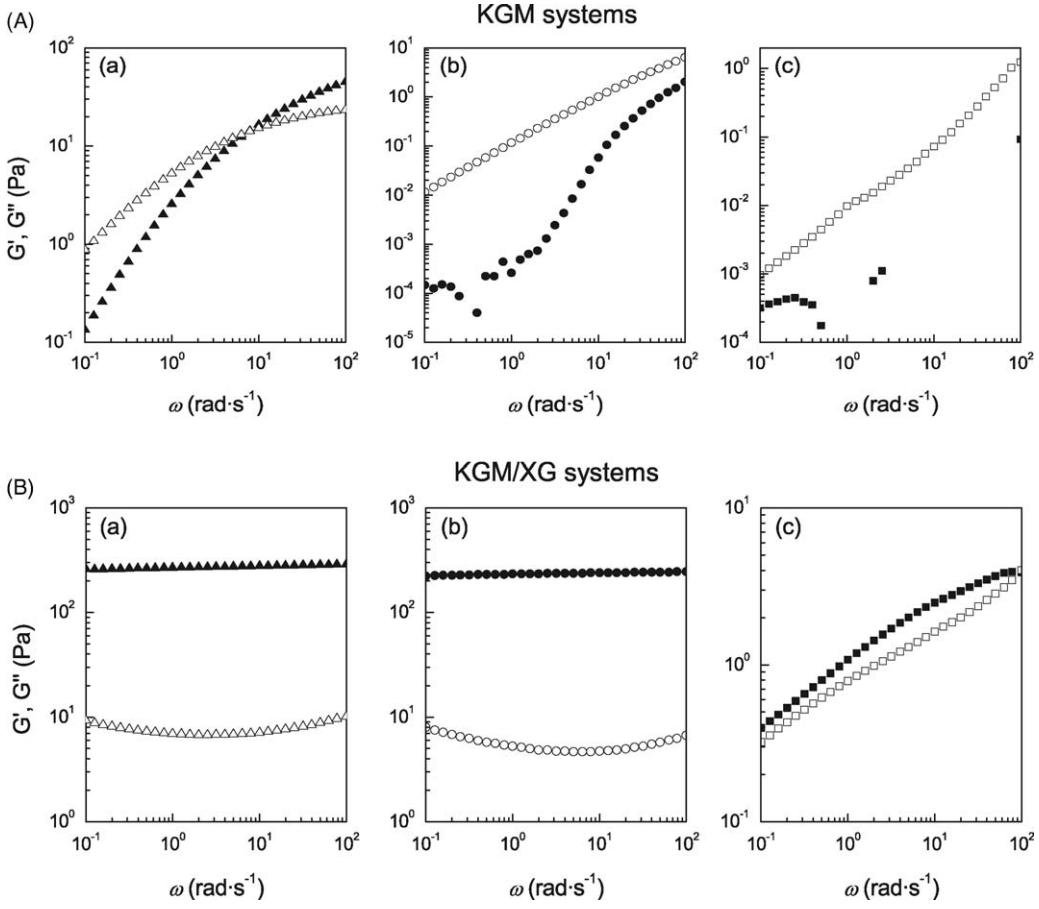

Fig. 2. Dynamic elastic (G') and viscous (G'') moduli as function of frequency. (A) 0.5% (w/v) KGM systems. (B) 0.5% (w/v) KGM/XG (1:1) aqueous systems. (a) Japanese KGM, (b) European KGM and (c) American KGM (closed symbols: G'; open symbols: G'').

components one of which is a liquid, present in substantial quantity and (b) solid-like gels are characterized by the absence of an equilibrium modulus, by a storage modulus, $G'(\omega)$, which exhibits a pronounced plateau extending to times at least of the order of seconds, and by a loss modulus $G''(\omega)$, which is considerably smaller than the storage modulus in the plateau region.

The rheological spectra obtained for all 0.5% KGM alone (Fig. 2A) are typical of polysaccharide solutions at a concentration well below the minimum critical gelling concentration, with $G'' \gg G'$ and strong frequency dependence in both moduli. It is also evident from Fig. 2A that at this concentration neither of the polysaccharides fulfill the requirement of a pronounced plateau with G' over G'' and therefore cannot be considered as gels.

When XG is present in the polysaccharide system composition, the rheological response undergoes a great change and shows for all the KGM/XG mixtures gel-like characteristics (Fig. 2B). It can be seen that G' exceeds G'' throughout the accessible frequency range, being the relative magnitude of the two moduli roughly constant and higher than ten times for the Japanese and the European KGM. Those systems have a definite shape and can be considered as true gels. For American KGM however can be observed that both moduli have a lower magnitude and a much greater frequency dependence. Similar behavior is observed for single–polymer gelling systems close to gel point, and is characteristic of a very sparingly cross-linked network.

In brief, rheological properties of KGM or KGM/XG systems indicate a high intermanufacturer variability in glucomannans from Konjac. Moreover, all KGM (0.5%) water systems can be considered viscous solutions from a macroscopic and rheological point of view. The mixture of KGM with XG (1:1) at the same concentration (0.5%) gives strong gels with a defined shape for the Japanese and European varieties. Data suggest that their three-dimensional structure can be described as a mesh, with spaces between the polymer chains filled with water being the cross-links formed by physical entanglements.

Gel rheological properties has been pointed out as determining factor in controlling drug diffusion process in matrices of mixtures of polysaccharides (Vendruscolo et al., 2005) suggesting that both the Japanese and the European KGM should have better control release ability than the American KGM variety. Moreover, the synergistic effect on rheological properties observed when KGM and XG are mixed should have a consequence on the diffusion of molecules through the gel structure. In order to study molecular diffusion through those KGM and KGM/XG systems fluorescence recovery after photobleaching measurements were carried out.

Before performing FRAP measurements on KGM solutions or gels, the concentration range in which a linear relation exists between the observed fluorescence and the fluorophore concentration has to be determined. FITC-dextran solutions with a concentration range from 0.45 to 20 mg/mL were prepared and its fluorescence measured. A linear region was found for concentrations up to 2 mg/mL ($r^2 > 0.9947$) for all the FITC-dextran probes. For higher concentrations there is a deviation from the linear relation due to the well-known inner-filter effect (DeSmedt et al., 1997).

Diffusion coefficients (D) and mobile fractions (k) of FITC-dextran probes in Konjac, xanthan gum or KGM/XG systems were measured by recording stacks of approximately 30 images with a CSLM at regular time intervals. Fig. 3 shows the first six images of a typical experiment.

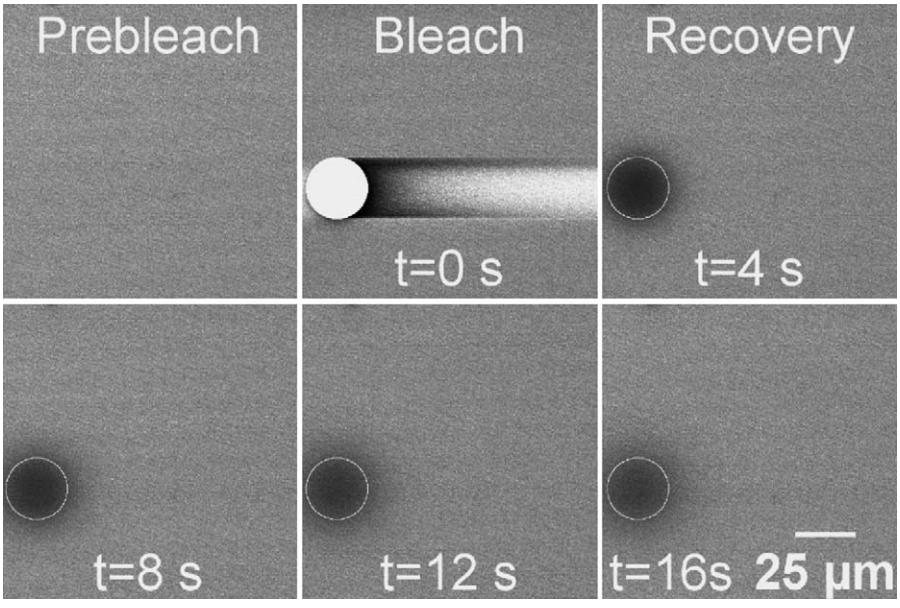

Fig. 3. Sequence of the first six images of the experiment with 51 1000 g/mol FITC-dextran in the Japanese KGM solution. The first image (prebleach), acquired with a highly attenuated laser bean, shows an homogeneous distribution of fluorophore through out the KGM/XG system. When the second image is scanned laser power is increased at the region of interest and a user-defined 25 μm diameter disk is uniformly bleached. The white circles traced onto the following images mark the 2D size and position of the original bleach disk and indicate the area over which fluorescence recovery is measured during the recovery phase with the laser intensity switched back to the prebleach level.

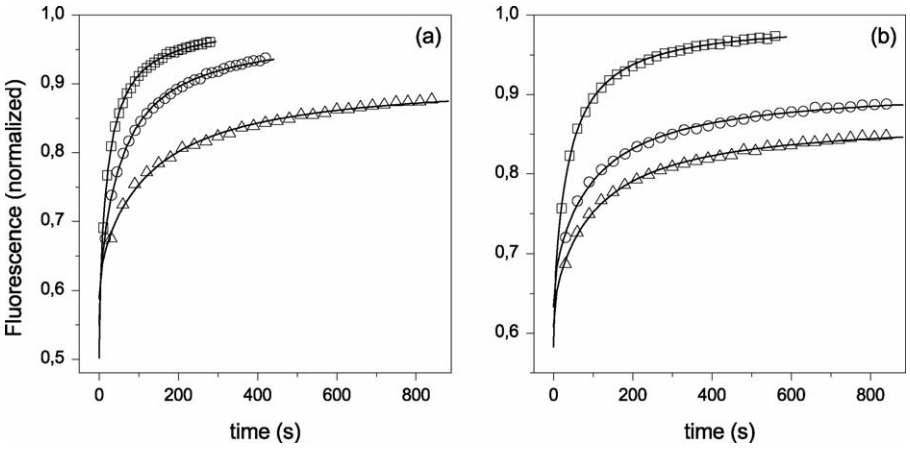

Fig. 4. Fluorescence recovery curves for the 2 000 000 g/mol FITC-dextran in KGM solutions (a), and KGM/XG gels (b). Symbols: Japanese KGM (△), European KGM (◯) and American KGM (□). Solid line corresponds to the fit of the uniform disk model (Braeckmans et al., 2003).

The first image corresponds to the FITC-dextran/KGM solution before bleaching, the second image was taken at the moment of bleaching the region of interest (disk), and subsequent images show the recovery of the fluorescence in the bleached area of the KGM system. Images were processed to obtain the fluorescence recovery curve.

Fig. 4 shows an example of the obtained recovery curves corresponding to the 2×10^6 g/mol FITC-dextran in the KGM solutions and KGM/XG gels. The solid lines are the fit of the uniform disk model (Braeckmans et al., 2003) to the experimental data. Three different phenomena can explain the probe diffusion process: fluid phase viscosity, binding and crowding. For unrestricted diffusion of single fluorescent species in an homogeneous unhindered environment, just limited by fluid phase viscosity, fluorescence recovers to the initial (pre-bleached) level (Verkman, 2002). However, fluorescence recovery can be incomplete if the probe diffusion is restricted by binding or a crowding phenomena. Fluorescence recovery profiles showed a total fluorescence recovery after several minutes for most probes (data not shown) but for the 2×10^6 g/mol FITC-dextran probe incomplete recovery was observed in the Japanese and the European KGM systems. As no reason exists for binding of FITC-dextran in KGM or KGM/XG systems, incomplete recovery that gives a reduction in the calculated mobile fraction (k) suggests a possible crowding or sieving mechanism for the diffusion of the macromolecules.

Mean and standard deviation of at least five diffusion coefficient measurements in different sample locations as a function of the probe molecular weight are presented in Fig. 5.

Presence of a 0.5% KGM in the HEPES buffer has a large effect on the FITC-dextran diffusion. Polysaccharides act as a barrier to the probe diffusion producing reductions in the D values of more than 40% compared to the buffer solution. Variations in diffusion coefficients between KGM solutions for a specific probe are statistically significant showing the Scheffé test four subsets. The following represents diffusion coefficient means found to be significant different using Scheffe's procedure:

HEPES buffer ≠ American KGM ≠ European KGM ≠ Japanese KGM

The results could be explained according to Stokes–Einstein equation in which diffusion coefficient D and dynamic viscosity η are indirectly related (Cheng et al., 2002):

$$D = \frac{kT}{6\pi\eta r_{\mathrm{H}}}$$

Despite of linear correlation between viscosity and diffusion coefficients could not be established, a clear effect of viscosity on diffusion coefficients can be noticed. The variety with the lowest viscosity, the American KGM, shows the lowest reduction for all the probes and the Japanese KGM with the highest viscosity presents the greatest reductions.

FITC-dextran chains diffuse slower as increasing its molecular size. Dependence of diffusion coefficient as a function of FITC-dextran molecular weight in KGM solutions from different origins was analysed and found that it can be described the

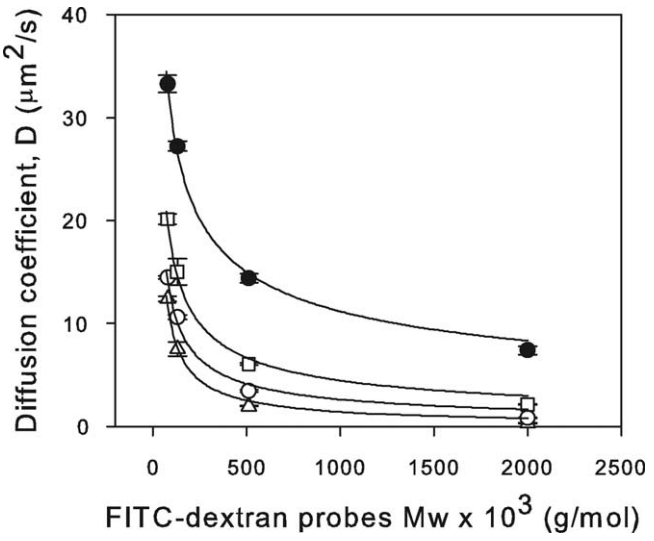

Fig. 5. Diffusion coefficients (D) of FITC-dextrans in 0.5% KGM solutions from different origin as a function of its molecular weight and fitting to $D \sim M_{\mathrm{w}}^{a}$ (solid lines). Symbols: HEPES buffer (●), Japanese KGM (△), European KGM (◯) and American KGM (□).

Table 2
Exponents and correlation factors for the $D \sim M_w^a$ fitting to the experimental data for FITC-dextrans in buffer and 0.5% KGM solutions

	a	R^2
HEPES buffer	−0.46	0.9997
Japanese KGM	−0.97	0.9998
European KGM	−0.74	0.9979
American KGM	−0.64	0.9994

potential relationship:

$$D \sim M_w^a$$

Data of the fitting obtained are summarized in Table 2.

As can be derived from the exponent values the dependence is not the same for the KGM varieties studied. If the increasing in viscosity produced by KGM was just the mechanism involved in the reduction of diffusion coefficients, having in mind Stokes–Einstein equation, the same exponent for FITC-dextran in buffer and KGM solutions and the same relative reduction at a given molecular weight probe should be expected. On the contrary, reduction percentages of the FITC-dextrans diffusion coefficients in the varieties of KGM with respect to FITC-dextrans diffusion coefficients in buffer (Table 3) are different as a function of its molecular weights indicating an additional effect of the polymer network (Braeckmans et al., 2003). Konjac network sterically hinders the diffusion of FITC dextrans: the larger the molecule, the stronger the sterical hindrance.

Furthermore, by analysing the mobile fraction (k) data obtained by the uniform disk model (Fig. 6), it can be seen that this parameter also reduces as dextran probe molecular weight increases. The ANOVAs show statistically significant differences among probe molecular weights for a specific KGM variety. For all the KGM solutions k values are close to 1, except for FITC-dextran Mw 2×10^6 in the Japanese KGM solution. Reduction in this value is important (20.8%) and shows that bigger molecules will be entrapped in the Japanese KGM chains, conditioning its release.

When the analysis of the dependence of FITC-dextran diffusion coefficient as a function of probe molecular weight for the KGM/XG gels was performed, the same relationship $D \sim M_w^a$ was found. Data of the fitting obtained are summarized in Table 4.

Exponents for the European and American KGM varieties are similar and close to the value obtained for the xanthan gum (exponent and correlation factor for the $D \sim M_w^a$ fitting of the experimental data for FITC dextrans in 0.5% XG solution; $a = -0.84$, $R^2 = 0.9987$) at the same conditions suggesting that FITC-dextran probes diffusion in the KGM/XG gels is determined by the xanthan gum structure.

Table 3
Reduction percentages of diffusion coefficients and standard deviations (S.D.) of FITC-dextrans in the varieties of KGM with respect to diffusion coefficients in buffer

FITC-dextran M_w	Reduction ± S.D. (%)		
	Japanese KGM	European KGM	American KGM
77000	62.7 ± 3.1	56.5 ± 3.0	39.4 ± 3.1
130000	72.3 ± 3.2	61.0 ± 2.1	44.9 ± 5.0
511000	86.2 ± 4.4	76.0 ± 4.2	57.8 ± 3.9
2000000	95.2 ± 7.1	88.7 ± 6.8	71.0 ± 6.3

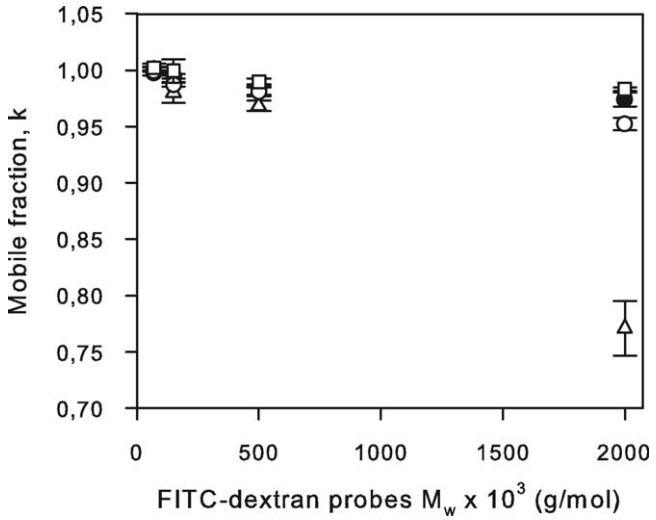

Fig. 6. Mobile fraction (k) of FITC-dextrans in 0.5% KGM systems as a function of its molecular weight. Symbols: HEPES buffer (●), Japanese KGM (△), European KGM (◯) and American KGM (□).

Fig. 7 shows FITC-dextran probes diffusion coefficients versus percentage of KGM in the KGM/XG mixtures. It can be observed differences in the behaviour of the KGM from different origin suggesting the need of specifications for this material. The variations in diffusion coefficients among KGM/XG gels for a given probe are once more statistically significant showing the Scheffé test three subsets. The following represents diffusion coefficient means found to be significant different using Scheffe's procedure:

American KGM/XG ≠ European KGM/XG ≠ Japanese KGM/XG

However, the synergistic effect deduced from the rheological measurements cannot be stated from the dextran probe diffusion coefficients. Diffusion coefficients in the KGM/XG mixtures were not significantly lower than D values in the single polysaccharide solutions as expected indicating that the interactions between KGM and XG supported by rheological studies do not influence translation diffusion coefficient of probes.

On the contrary, a synergistic effect can be observed when the FITC- dextran mobile fraction is analysed (Fig. 7). While a complete fluorescence recovery ($k \to 1$ for $t \to \infty$) was observed for the smaller FITC-dextran probe (7.7×10^4 g/mol) with no differences between KGM/XG systems, reductions of 25% and

Table 4
Exponents and correlation factors for the $D \sim M_w^a$ fitting of the experimental data for FITC-dextrans in 0.5% KGM/XG gels

	a	R^2
Japanese KGM/XG	−0.94	0.9996
European KGM/XG	−0.80	0.9976
American KGM/XG	−0.81	0.9992

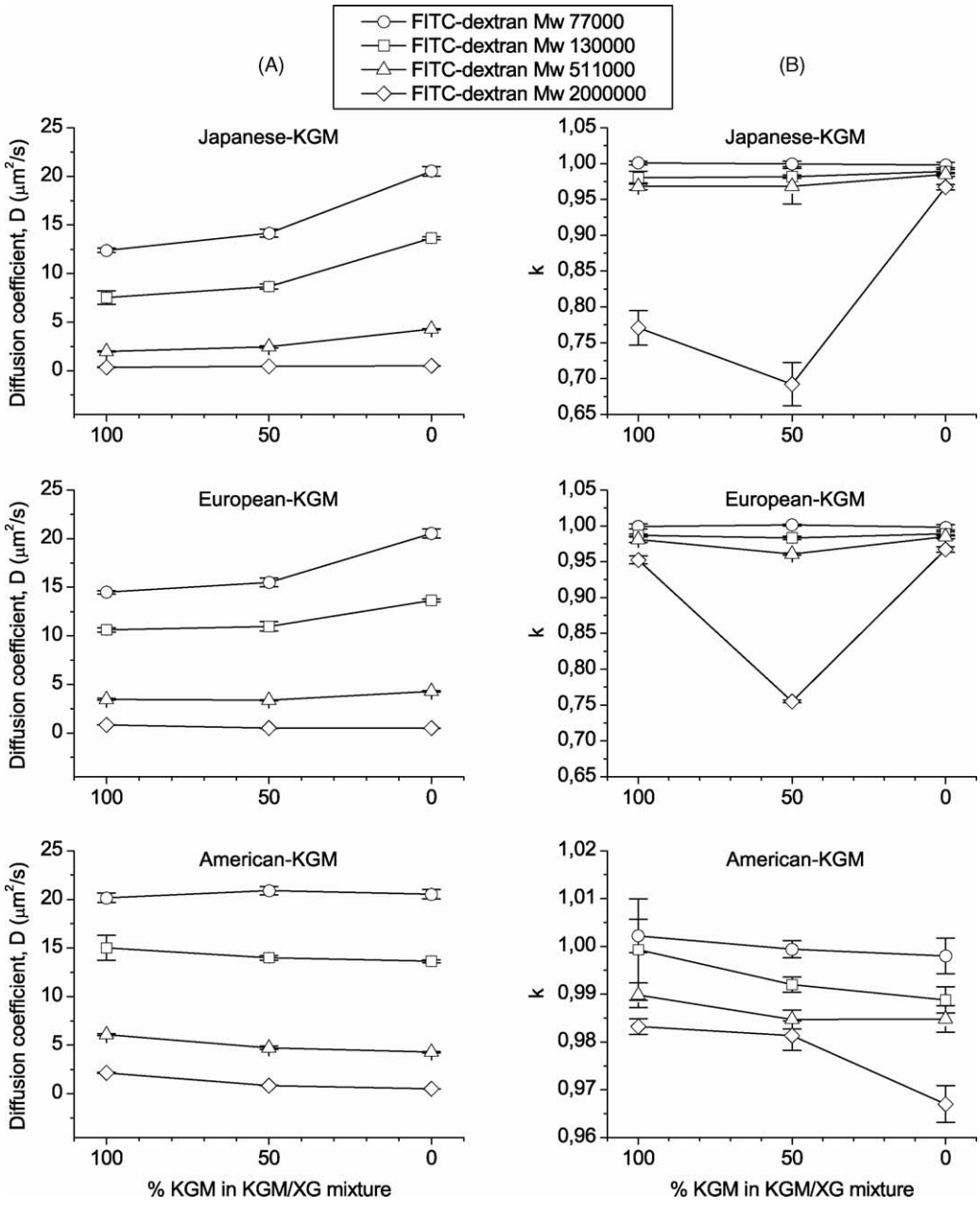

Fig. 7. Diffusion coefficient (D) and mobile fraction (k) of FITC-dextrans as a function of 0.5% (w/v) KGM/XG system composition.

30% for the European and Japanese varieties, respectively, were found for bigger probe molecules. The fluorescence for the biggest probes recovered only partially in the KGM/XG gels indicating that its mobility is restricted to a greater extent.

It highlighted that diffusion of probes through the polysaccharide system cannot be completely explained by the macroscopic properties of the medium but it is related to a sieving mechanism as it has been found previously for other polymers (DeSmedt et al., 1997).

In conclusion, we have characterized KGM from different suppliers showing strong differences that suggest the necessity of establishing specifications for this material. Rheological data illustrate the synergism between KGM and XG at a stoichiometric relationship 1:1. At 0.5% systems prepared with KGM or XG can be considered from a rheological point of view solutions, but systems elaborated with mixtures 1:1 KGM/XG in the same proportion and conditions form true gels.

Results also have shown the utility of FRAP in conjunction with CSLM in order to characterize diffusion of different molecular weight macromolecules through KGM systems. FRAP has been shown as a very sensitive technique allowing differentiation between varieties of KGM.

The determination of the diffusion coefficients of small and large solutes measured in KGM and KGM/XG systems indicates that rheological properties of the gels cannot be used to predict changes in the diffusivities of FITC-dextran chains through the

network and that the size of the solutes is an important factor in controlling the diffusion. This technique seems very promising for characterizing diffusion through polysaccharide solutions and gels based on konjac glucomannan polysaccharide.

The combination of rheological and FRAP measurements allows the selection of KGM/XG systems with physical strength and limited solute diffusion which could be potentially used as colonic drug delivery systems.

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