Available online at www.sciencedirect.com
ScienceDirect
European Journal of Pharmaceutics and Biopharmaceutics 69 (2008) 573–581
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

Konjac glucomannan/xanthan gum enzyme sensitive binary mixtures for colonic drug delivery

Felipe Alvarez-Manceñido, Mariana Landin *, Ramón Martínez-Pacheco

Departamento de Farmacia y Tecnología Farmacéutica, Universidad de Santiago de Compostela, Santiago de Compostela, Spain

Received 4 July 2007; accepted in revised form 7 January 2008
Available online 15 January 2008

Abstract

The polysaccharide konjac glucomannan (KGM) is degraded in the colon but not the small intestine, which makes it potentially useful as an excipient for colonic drug delivery. With xanthan gum (XG) KGM forms thermoreversible gels with hitherto unexplored biodegradation properties. In this work, rheological measurements of KGM and KGM/XG systems incubated with and without *Aspergillus niger* β-mannanase (used to mimic colonic enzymes) showed that KGM was degraded by the enzyme even when interacting with XG. Tablets with KGM/XG/sucrose matrices that varied in accordance with a simplex design and bore diltiazem as a typical highly soluble drug load were prepared by wet granulation, and in most cases were found to possess satisfactory mechanical strength and exhibit slow, nearly zero-order drug release. Drug release from these tablets remained zero-order, but was accelerated (presumably due to degradation of KGM), in the presence of *A. niger* β-mannanase at concentrations equivalent to human colonic conditions. However, marked differences between Japanese and American varieties of KGM as regards degree of acetylation and particle size led to significant differences in swelling rate and drug release between formulations prepared with one and the other KGM: whereas a formulation with Japanese KGM released its entire drug load within 24 h in the presence of β-mannanase, only 60% release was achieved under the same conditions by the corresponding formulation with American KGM, suggesting that with this KGM it will be necessary to optimize technological variables such as compression pressure in order to achieve suitable porosity, swelling rate, and drug release. To sum up, the results of this study suggest that sustained release of water-soluble drugs in the colon from orally administered tablets may be achieved using simple, inexpensive formulations based on combinations of KGM and XG that take the variability of KGM characteristics into account.

Keywords: Konjac glucomannan; Xanthan gum; Synergistic interaction; Colonic drug delivery; Enzymatic degradation

1. Introduction

In recent years, drug delivery systems based on polysaccharides have been receiving considerable attention, especially as regards their potential for controlled release [1] and the targeting of specific *in vivo* delivery sites [2]. Colon targeting would not only allow local treatment of colonic diseases, but would also constitute a potential alternative route for systemic absorption of drugs, peptides and proteins [3–6].

Xanthan gum (XG) is a negatively charged microbial exopolysaccharide consisting of a cellulose backbone and trisaccharide side-chains composed of a glucuronic acid residue between two mannose units. XG solutions have high intrinsic viscosity and exhibit weak gel-like properties at low shear rates, but XG does not form true gels at any concentration or temperature [7]. XG is nevertheless an effective excipient for sustained release formulations, achieving near zero-order drug release kinetics [8]. Drug release from XG matrices is a Fickian diffusion process during the first half of the dissolution period, but during the second is mainly due to the erosion or dissolution of

* Corresponding author. Departamento de Farmacia y Tecnología Farmacéutica, Facultad de Farmacia, Universidad de Santiago de Compostela, 15782 Santiago de Compostela, Spain. Tel.: +34 981 563 100; fax: +34 981 547 148.
E-mail address: mlandin@usc.es (M. Landin).

0939-6411/$ - see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2008.01.004

the highly hydrophilic XG [8,9]; it is strongly dependent on the ionic strength of the dissolution medium. The possibility of enhancing the release-control capacity of xanthan gum matrices by strengthening them through interaction with galactomannans, hydroxypropylmethyl cellulose or chitosan has been studied [8,10–12].

Konjac glucomannan (KGM) is a water-soluble polysaccharide used in Asian cuisine. It consists of 1,4-linked β-D-mannose and glucose units in a mole ratio of 1.6:1. It is a slightly branched polymer with acetyl groups on between one-ninth and one-nineteenth of its backbone units [13–15]. These acetyl groups contribute to its solubility and swelling properties and help in making it the soluble fibre with the highest viscosity and water-holding capacity in nature [16–18]. Although unmodified KGM by itself only forms gels that are at best very weak, when modified, or in combination with other polymers, it is of proven efficacy as an excipient for controlled release of hormones [19] and macromolecules such as dextrans, insulin and bovine serum albumin [5,20,21]. In particular, good drug release behaviour may be expected from the thermoreversible gels it forms with polysaccharides such as κ-carrageenan [22], acetan [23] and XG [24–30]. KGM varieties from the three main producing areas for excipient harmonization exhibit significant differences in rheology and capacity to interact with another polysaccharide, apparently because of differences in their degree of acetylation [21,29]. In particular, previous studies of the Japanese and American varieties used in the present work found the Japanese variety, which is the more acetylated, to afford more viscous KGM/XG mixtures than the other [29].

KGM is degraded by the action of β-mannanases produced by colonic flora [31], but is not degraded in the small intestine. This suggests the possibility of its use for colonic drug delivery: a sufficiently strong gel formed by a mixture of KGM and another polysaccharide might retain its integrity and its drug load while passing through the UGIT, but gradually release its load when attacked by colonic flora. In the study described in the remainder of this paper we characterized a range of KGM/XG-based diltiazem formulations [30] only studied tablets with a 1:1 KGM:XG ratio, and we investigated the probable behaviour of such formulations in the colon. Specifically, the goals of this study were (*a*) to check that KGM and KGM/XG gels are susceptible to degradation by *Aspergillus niger* β-mannanase, an enzyme employed in previous studies to mimic the colonic biodegradation of mannose-based polysaccharides [32]; (*b*) to determine the mechanical properties and drug release profiles of a range of KGM/XG-based matrices containing diltiazem as a model of a highly soluble drug; (*c*) to determine, for selected matrix formulations, drug release profiles in simulated colonic medium containing β-mannanase; and (*d*) to evaluate the extent to which the above properties are affected by the above-noted differences among KGM brands. Since the 2005 study [30] found that release from 1:1 KGM/XG-based tablets was faster at pH 1.2 than 7.5 (which suggests that a gastroresistant coating may be necessary to prevent pre-colonic release). In the present study, we only worked at pH 7.5 assuming that the formulations have successfully reached the colon. The addition of enzymes simulates colonic conditions. The effect of pH or ionic medium strength variations on the formulations behaviour is not evaluated in this paper and should be an interesting field for future works.

2. Materials and methods

2.1. Raw materials

Two KGMs with different suppliers and geographical origins were used: a US brand from Triple Crown America Inc. (Lot 3500 C), and the Japanese brand Propol A® (Lot AKG07). These KGMs were characterized in our laboratory as having mean particle sizes of 0.055 mm [standard deviation (SD) 0.049 mm] and 0.176 mm (SD 0.054 mm), respectively, and acetylations of 0.6% and 1.9%, respectively; they were used as received, as was XG, which was supplied by Guinama, Spain (Lot 016). Diltiazem hydrochloride Eur. Ph. was supplied by Roig-Farma, Spain (Lot 0307362), sucrose and magnesium stearate NF by C. Barcia (Spain), and *A. niger* β-mannanase (specific activity 45 U/mL at 40 °C and pH 4.0; Lot 00801) by Megazyme (Ireland). All other reagents were of analytical grade.

2.2. Rheological characterization of the enzymatic degradation of KGM solutions and KGM/XG gels

The degradation of KGM solutions and KGM/XG gels by *A. niger* β-mannanase was evaluated by rheological measurements (when this was possible; see Section 3), the rheological properties of polysaccharides being significantly affected by enzymatic degradation [33,34]. Solutions or gels of KGM and 1:1 KGM/XG mixtures in simulated intestinal fluid (pH 7.5) were prepared at a total polysaccharide concentration of 0.5% (w/v) by mechanical stirring in a hermetic container for 1 h at 85 °C and 400 rpm. For viscosimetry, samples were left to cool and equilibrate overnight, 0, 5.53×10^{-4}, 5.53×10^{-3} or 0.270 U/mL of β-mannanase was added, and viscosity at 37 °C was determined from steady shear measurements carried out over 210 min at a shear rate of $10\ s^{-1}$ in an AR1000-N cone-and-plate rheometer from TA Instruments, Newcastle, UK (cone angle 2°, diameter 60 mm, gap 59 μm). For measurement of complex shear moduli, solutions or gels were prepared by stirring for 1 h at 85 °C as described above, the required amount of enzyme was added at 70 °C, the mixture was left at 37 °C for 210 min, and dynamic oscillatory measurements were performed over a frequency range of 0.05–50 rad s^{-1} at a controlled strain within the linear viscoelastic range. Rheological characterization was performed at physiological temperature (37 °C).

2.3. Preparation and characterization of diltiazem matrices

Tablets 300 mg in weight composed of KGM, XG, sucrose, magnesium stearate (0.5%) and diltiazem HCl (90 mg) were prepared by wet granulation. For each KGM variety, seven formulations differing in the proportions of KGM, XG and sucrose were prepared in accordance with a simplex experimental design [35] with the constraints total polysaccharides >20% and sucrose >20% (Fig. 1). Table 1 describes these formulations and indicates the nomenclature used.

All ingredients except magnesium stearate were passed through 120 μm meshes and mixed for 10 min in a Turbula T2 C blender (WAB, Switzerland). Wet massing was carried out in a Master Chef planetary mixer (Kenwood, UK) using 50:50 water/ethanol as granulating liquid and a liquid:solids ratio of approximately 20%. The wet mass was passed through a 1-mm-mesh sieve using an AR400 oscillating granulator (Erweka, Germany) and was oven dried at 40° C for 24 h. The dried granules were sieved (60 μm–1 mm), mixed with magnesium stearate (0.5%) in a WAB Turbula T2 C, and compressed at a constant pressure of 14 kN to form flat-faced tablets 9 mm in diameter in an eccentric tablet press (Bonals MT, Spain) equipped with pressure and punch-run sensors.

Tablet crushing strength (CS) was measured with an Erweka TB24 durometer (Heusenstamm, Germany) for 10 random tablets of each formulation. Tensile strength (TS) was calculated from the equation [36]

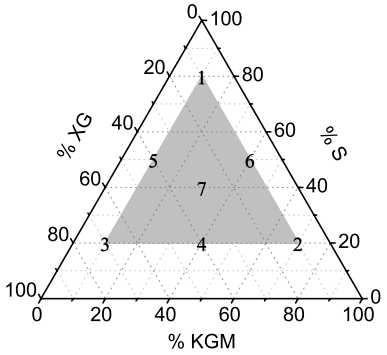

Fig. 1. Simplex experimental design space (grey area), with the test points. S, sucrose.

Table 1
Compositions of the formulations studied, before addition of 0.5% of magnesium stearate

Formulation		Composition (wt%)			
Japanese KGM	American KGM	Diltiazem	KGM	XG	Sucrose
J1	A1	30	7	7	56
J2	A2	30	49	7	14
J3	A3	30	7	49	14
J4	A4	30	28	28	14
J5	A5	30	7	28	35
J6	A6	30	28	7	35
J7	A7	30	21	21	28

$$TS = (2CS)/(\pi D\ E)$$

where D and E are tablet diameter and tablet thickness, respectively.

Tablet swelling rate was measured to relate hydration to drug release. Two tablets of each formulation were each weighed on a platform; platform and tablet were then placed in a bath containing mannanase-free simulated intestinal fluid (pH 7.5; 37 ± 0.5 °C); and at various times over the following 24 h, platform and tablet were withdrawn, drained of excess medium, weighed on an analytical balance (Gibertini, Italy) for calculation of percentage increase in weight, and returned to the fluid bath.

In vitro dissolution tests were performed in a USP type 2 apparatus (Turu-Grau, Spain) in accordance with the requirements of USP 27 (2004) for diltiazem. Six tablets of each formulation were each stirred for 24 h at 75 rpm in 900 mL of simulated intestinal fluid (pH 7.5 ± 0.1, temperature 37.0 ± 0.5 °C) with or without β-mannanase (5.53×10^{-4}, 5.53×10^{-3} or 0.166 U/mL). At predefined times, samples were taken and the volume withdrawn was replaced with the same volume of fresh thermostatted medium. Drug concentrations were determined spectrophotometrically at 236 nm in an Agilent 8453 diode array spectrophotometer (Agilent, Germany) using a validated calibration curve. Dissolved diltiazem cumulative percentages were fitted with zero-order kinetic curves. The physical integrity of the tablets was examined periodically.

2.3. Statistical analyses

The statistical significance of results was estimated by analyses of variance corresponding to the experimental designs employed. The results for the properties of the diltiazem tablets were fitted with polynomial functions of KGM, XG and sucrose content by means of stepwise multiple regression analysis. All statistical calculations were performed using SPSS 14.0 [35].

3. Results and discussion

3.1. Rheological characterization of enzymatic degradation of KGM solutions and KGM/XG gels

Fig. 2 shows viscosity–time curves obtained by steady shear measurements for a solution of the Japanese KGM and a 1:1 KGM/XG gel prepared from the American KGM. Solutions of the American KGM were all of very low viscosity (<0.007 Pa s) even at the start of the experiments, while 1:1 KGM/XG gels prepared with the Japanese KGM were so solid that their viscosity could not be measured by steady shear experiments in our apparatus.

The viscosity of the Japanese KGM solutions (Fig. 2A), which decreased slowly even in the absence of enzyme (probably due to slow reorientation of the KGM chains in the direction of flow), was reduced dose-dependently by added β-mannanase, presumably due to enzymatic

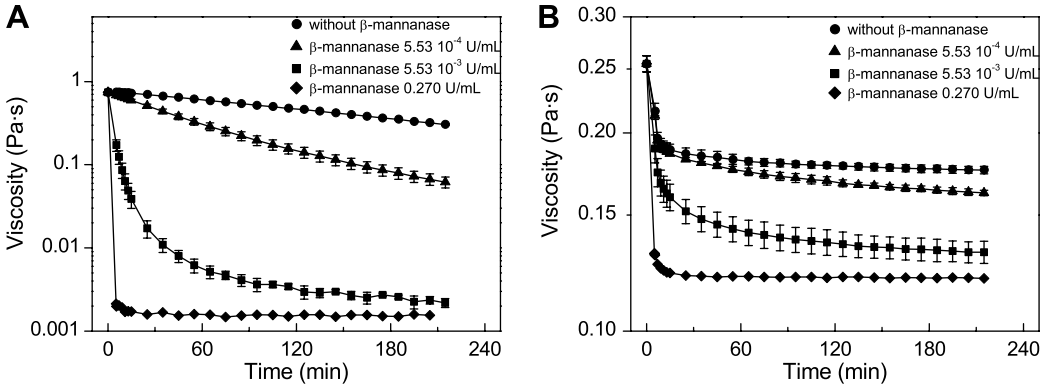

Fig. 2. Evolution of the viscosities of a Japanese KGM solution (A) and a KGM/XG gel prepared with American KGM (B) in steady shear experiments in the presence of various concentrations of *A. niger* β-mannanase. Total polysaccharide concentration was in all cases 0.5% (w/v).

cleavage of the KGM backbones into smaller chains. Note that this occurred at physiological temperature and pH 7.5, even though these conditions differ considerably from those reported to afford peak activity by the manufacturers of the *A. niger* β-mannanase used, 60 °C and pH 3. At the lowest enzyme concentration the time-dependence of the reduction in viscosity was practically linear, and at the highest concentration degradation was practically complete within 10 min, but at the intermediate concentration the viscosity–time profile suggests the action of two distinct processes: a fast process that is complete within about 20 min, and a slower process that continues during the remainder of the experiment. Similar two-stage kinetics was observed by Li and coworkers [37] in experiments in which KGM was degraded by a β-mannanase from *Bacillus* sp.

The viscosity of the American KGM/XG gels (Fig. 2B) exhibited a two-stage decrease even in the absence of enzyme, the initial fast reduction being attributable to disruption of the weak gel structure by shearing. Otherwise these gels behaved like the Japanese KGM solutions as regards the dose-dependence of the enzyme-induced decrease in viscosity and the rapid completion of the enzymatic action at the highest enzyme concentration, although viscosity always remained within a narrower range of values than in the case of the KGM solutions. It may be noted that although access to the KGM backbone must be hindered to some extent by XG in these gels, enzymatic degradation was detectable even at the lowest enzyme concentration and occurred to a marked extent at a concentration 30 times lower than the concentration of 0.166 U/mL reckoned by Burke and coworkers [32] to be most suitable for prediction of the action of colonic β-mannanase *in vivo*.

Fig. 3 shows the mechanical spectra of the KGM/XG gels at 37 °C 210 min after addition of 0, 5.53×10^{-4}, 5.53×10^{-3} or 0.270 U/mL of β-mannanase. In the absence of enzyme, the system with Japanese KGM behaved as a true gel: both the storage shear modulus G′ and the loss shear modulus G″ were almost independent on the three orders of magnitude frequency range, and the loss factor tan δ (= G″/G′) remained well below unity, especially in the low-frequency region of greatest interest for inference of gel structure, where tan $\delta \approx 0.1$. By contrast, the stronger frequency-dependence of G′ and G″ for the system with American KGM, and its larger tan δ values, show it to be a much weaker gel [38]. These differences are in keeping with previous observations of rheological differences between KGM/XG gels prepared with materials from different suppliers [21,29], and as noted in the Introduction are probably due mainly to differences in the degree of acetylation of the KGM.

The addition of β-mannanase at concentrations greater than 5×10^{-3} U/mL reduced G′ and G″, and increased their frequency-dependence, in both KGM/XG systems, especially the system with Japanese KGM, while loss of elasticity (attributable to the cleavage of KGM into smaller molecules; [39]) was shown by significantly increased values of tan δ. With these concentrations of β-mannanase, the system with American KGM ceased completely to behave as a gel, exhibiting tan δ values >1 at low frequencies.

Taken together, the above viscosity–time profiles and mechanical spectra show that both the Japanese and American varieties of KGM are degraded by *A. niger* β-mannanase at 37 °C and pH 7.5, even when they form gels with XG.

3.2. Mechanical properties of KGM/XG-based diltiazem matrices, and drug release profiles in the absence of β-mannanase

Inspection of Table 2, which summarizes the measured tensile strengths of the tablet formulations studied, suggests that for a given XG or sucrose content, tensile strength decreased with increasing KGM content [compare formulations N6 and N5 (N = J or A); N2, N3 and N4; N4 and N5; and N1, N2 and N6], and that the American KGM, the particles of which are on average more than three times smaller than those of the Japanese variety, tended to afford stronger tablets than the Japanese KGM. The existence of significant differences in tensile strength among tablets with different compositions was confirmed by analysis of variance. The formulations with

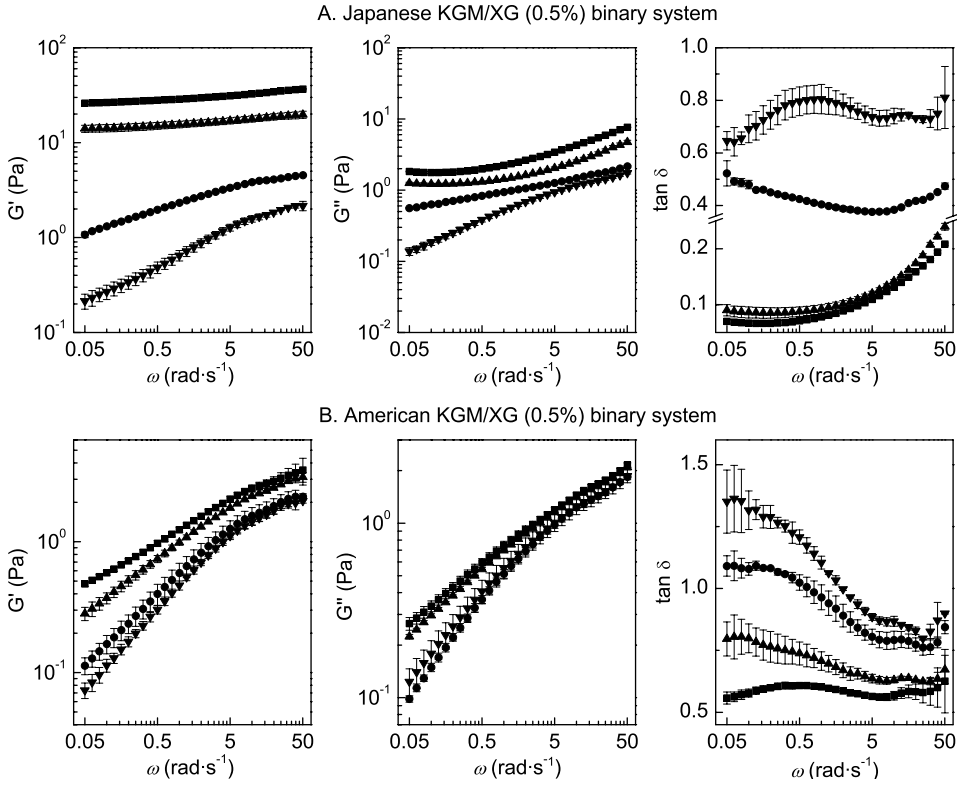

Fig. 3. Plots of storage shear modulus G′, loss shear modulus G″ and loss factor tan δ against frequency ω obtained at 37 °C for 0.5% 1:1 KGM/XG mixtures containing Japanese (A) or American (B) KGM 210 min after the addition of 0 (■), 5.53×10^{-4} (▲), 5.53×10^{-3} (●) or 0.270 (▼) U/mL of β-mannanase.

Japanese and American KGM were, respectively, fitted by the regression equations

$$\mathrm{TS(MPa)} = 2.037 \times 10^{-2}\mathrm{S} + 7.714 \times 10^{-4}\mathrm{S \cdot XG} - 1.733 \times 10^{-4}\mathrm{KGM \cdot S} - 1.313 \times 10^{-4}\mathrm{KGM \cdot XG} \quad (r^2 > 0.99;\ F = 1891.7;\ 4 \text{ and } 3\text{d.f};\ p < 0.01)$$

and

$$\mathrm{TS(MPa)} = 0.028\mathrm{S} + 0.019\mathrm{XG} \quad (r^2 > 0.99;\ F = 135.0;\ 2 \text{ and } 5 \text{ d.f};\ p < 0.01)$$

where XG, KGM and S are, respectively, the quantities of XG, KGM and sucrose in the formulations, expressed as percentages of the combined weight of these excipients.

Table 2
Mean tensile strengths TS of the formulations, with standard deviations SD in parentheses

KGM/XG/S (%)	Japanese KGM		American KGM	
	Formulation	TS (MPa) Mean (SD)	Formulation	TS (MPa) Mean (SD)
10/10/80	J1	2.10 (0.20)	A1	2.55 (0.24)
70/10/20	J2	0.23 (0.03)	A2	0.75 (0.05)
10/70/20	J3	1.41 (0.08)	A3	1.71 (0.08)
40/40/20	J4	0.68 (0.18)	A4	1.52 (0.06)
10/40/50	J5	2.41 (0.18)	A5	2.48 (0.17)
40/10/50	J6	1.03 (0.20)	A6	1.17 (0.08)
30/30/40	J7	1.36 (0.16)	A7	1.50 (0.06)

In both cases, tensile strength can be described as mainly determined by the percentages of XG and sucrose, both of which are materials known for their good compression properties. The grey areas in Fig. 4 show the proportions of KGM, XG and sucrose in formulations that the above equations predict to have tensile strengths greater than 1 MPa, a value that may be regarded as the minimum required for (9 mm) tablets of acceptable mechanical properties.

The matrices swollen process was studied by the evaluation of the simulated intestinal fluid (SIF) uptake as a function of time for a 24 h period. Results are presented in Fig. 5. In agreement with previous authors findings [11,40] on contact with aqueous medium, the hydrophilic matrix gradually begins to hydrate from the periphery towards the centre, forming a gelatinous swollen mass, the thickness and strength of which control the drug release.

The amount of SIF uptake by these types of systems is related to the total percentage of polysaccharides in the formulation, but as it can be seen for the same system composition, the American and Japanese KGM formulations present very different swollen profiles. Japanese KGM formulations take a higher total amount of water and at faster rate than the American variety formulations with identical composition. These differences can be explained, firstly by the differences in the acetylation degree of KGMs [29] which determines the interaction polysaccharide/water

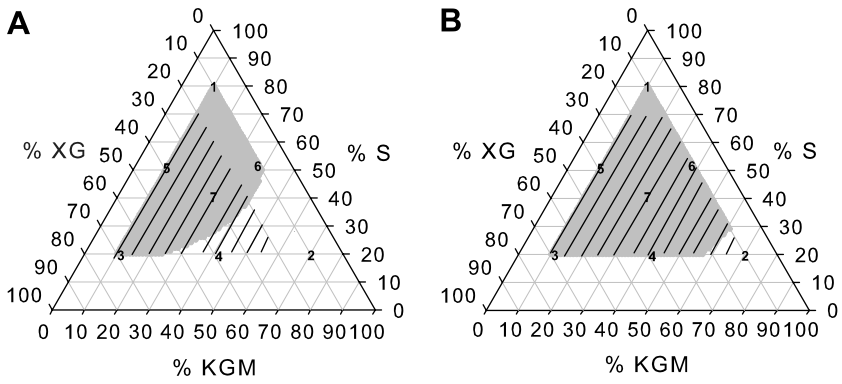

Fig. 4. KGM:XG:sucrose excipient ratio space. Grey areas show regions where formulations with Japanese KGM (A) or American KGM (B) have tensile strengths greater than 1 MPa, and striped areas regions where the formulations have quasi-zero-order diltiazem release kinetics in simulated intestinal fluid with no mannanase.

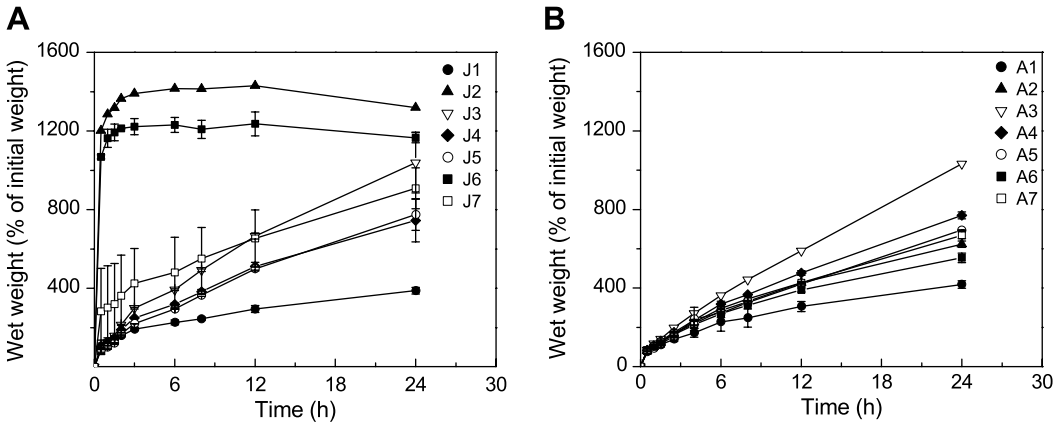

Fig. 5. Uptake of simulated intestinal fluid over 24 h by tablets prepared with Japanese KGM (A) and American KGM (B).

and secondly, by variations in particle size distribution of both products. The big particles of Japanese KGM absorb water and swell quickly making this material act as a disintegrant. As a consequence some formulations including high proportions of Japanese KGM (J2 and J6), disintegrate and do not perform as matrices. Formulations elaborated with the American KGM variety, of smaller particle size, lower porosity and stronger from a mechanical point of view, experience a progressive swollen process (fitting nearly zero-order kinetics) maintaining their integrity along the experimental time except for the formulation A1, whose main component, sucrose, is dissolved.

Fig. 6 shows the diltiazem release profiles recorded in simulated intestinal fluid with no mannanase. Like the fluid

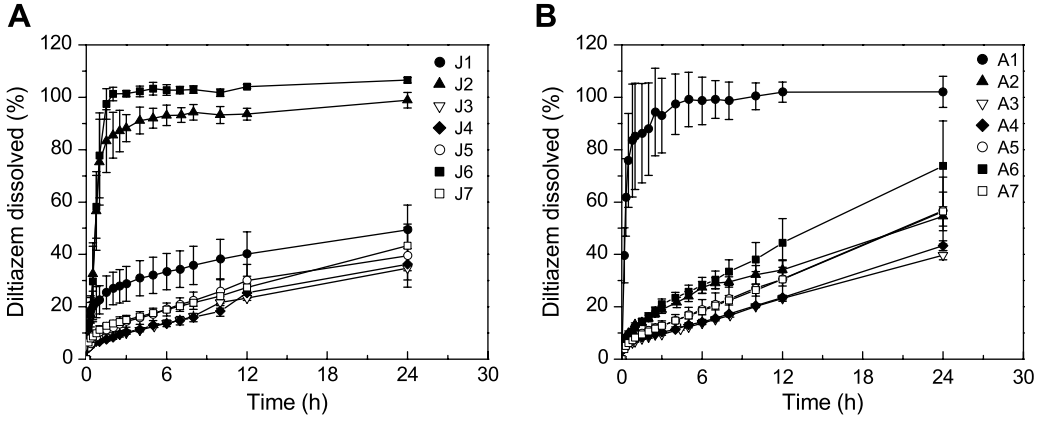

Fig. 6. Dissolution profiles for release of diltiazem into simulated intestinal fluid from formulations prepared with Japanese KGM (A) and American KGM (B).

Table 3
Drug release rate constants (K_0) of zero-order kinetic curves (% diltiazem dissolved = K_0 t) fitted to the drug release profiles, with the corresponding determination coefficients (r^2)

Ternary mixture % KGM/XG/S	Japanese KGM			American KGM		
		K_o (% h^{-1})	r^2		K_o (% h^{-1})	r^2
10/10/80	J1	0.023	0.83	A1	0.026	0.32
70/10/20	J2	0.043	0.23	A2	0.032	0.95
10/70/20	J3	0.021	0.98	A3	0.024	0.99
40/40/20	J4	0.022	0.98	A4	0.026	0.99
10/40/50	J5	0.025	0.94	A5	0.036	0.99
40/10/50	J6	0.049	0.28	A6	0.046	0.98
30/30/40	J7	0.024	0.98	A7	0.035	0.99

sorption profiles, most reflect almost zero-order kinetics (with $r^2 \geqslant 0.94$; see Table 3), suggesting that release is limited by the tablet swelling process. The main exceptions were again A1, J2 and J6 (and for the same reasons as before); and J1, which is mostly sucrose, unsurprisingly released a relatively large proportion of its drug load at a very early stage, as the result of the dissolution of the sucrose.

Table 3 shows a tendency for formulations with American KGM to release their drug load faster than those with Japanese KGM. This is in keeping with the lower viscosity of KGM/XG mixtures when American KGM is used [29].

The synergistic effect of interaction between KGM and XG on the rheology of their mixtures has been reported to be greatest at a KGM:XG ratio of 1:1 [27,28]. Although drug release might therefore be expected to be slowest with this KGM:XG ratio (since release is in principle slower, the more consistent the matrix; [11]), the virtually identical release curves of formulations J4 (1:1) and J3 (1:7), and of A4 and A3, show that the rheological properties of gels do not completely explain diltiazem release from these matrices. Other characteristics, such as porosity or swelling properties, must also have significant influence.

The striped areas in Fig. 4 correspond to formulations with approximately zero-order release kinetics. When considered jointly with the grey areas corresponding to formulations with acceptable tensile strength, they show that the maximum proportions of KGM that can usefully be incorporated in formulations of this kind is 60% for American KGM but only 40% for the Japanese variety. To study the effect of β-mannanase on drug release, we chose formulations A7 and J7, which are both near the centres of the regions corresponding to useful formulations.

3.3. Drug release profiles in the presence of β-mannanase

Fig. 7 shows that zero-order drug release kinetics was maintained by formulations A7 and J7 in the presence of β-mannanase ($r^2 > 0.98$). The rate of release from both formulations increased with mannanase concentration, but this effect was much more marked for the formulation with Japanese KGM, from which almost all diltiazem had been released within 24 h when the mannanase concentration was that estimated by Burke and coworkers [32] as most appropriate for estimation of *in vivo* behaviour, 0.166 U/mL. That the mannanase had a greater effect with Japanese than with American KGM seems likely to be due to the slower swelling of the latter (see Fig. 4), which would have hindered the access of mannanase to the KGM backbone. The development of matrices including American KGM variety should be designed taking into account such variables as compression pressure to modulate porosity and swelling properties to achieve 100% drug dissolution at 24 h. Results indicate that matrices containing KGM/XG mixtures, for both Japanese and American KGM, maintain the biodegrability by colonic enzymes which is the most important fact in the development of polysaccharides colonic formulations [41].

4. Conclusions

In this work, rheological measurements showed that KGM is susceptible to degradation by *A. niger* β-mannanase even when it forms binary systems with XG, and hence that these systems may also be degraded by β-mannanases produced the flora of the human colon. Assays of the ten-

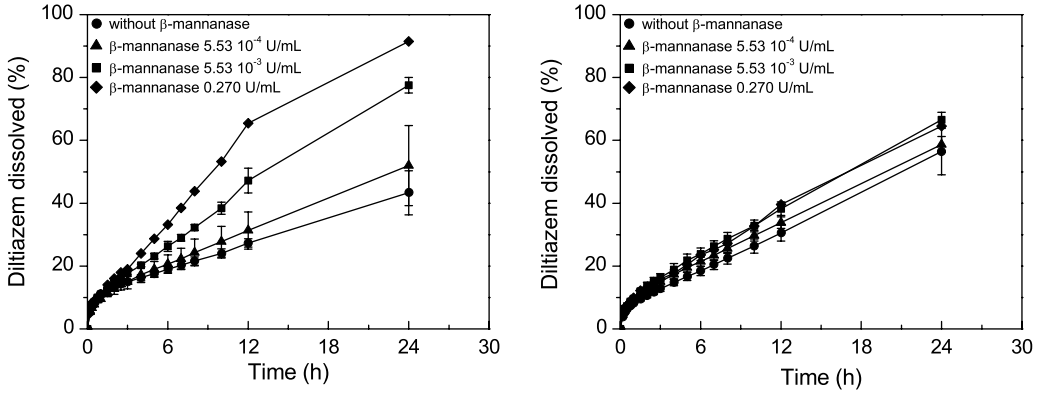

Fig. 7. Dissolution profiles for release of diltiazem into simulated intestinal fluid containing various concentrations of β-mannanase, for formulations J7 (A) and A7 (B).

sile strength and drug release profiles of diltiazem tablets prepared with KGM, XG and sucrose as excipients showed that for both Japanese and American KGMs there are significant regions of (KGM, XG, S) space in which the tablets are both satisfactorily strong and exhibit slow, nearly zero-order release of this highly soluble drug. With the Japanese KGM, drug release was virtually complete within 24 h in the presence of a concentration of *A. niger* β-mannanase equivalent to colonic conditions. The smaller effect of the enzyme on release from a formulation with American KGM, and the consequent failure of these tablets to release their entire drug load within 24 h, is attributed to their slower swelling, and suggests that for tablets prepared with this KGM it will be necessary to optimize technological variables such as compression pressure in order to achieve suitable porosity, swelling rate, and drug release.

To sum up, the results of this study suggest that sustained release of water-soluble drugs in the colon from orally administered tablets may be achieved using simple, inexpensive formulations based on combinations of KGM and XG that take the variability of KGM characteristics into account.

Acknowledgments

Authors thank the Xunta de Galicia for the financial support PGIDIT05BTF20301PR. Felipe Alvarez Manceñido thanks the Xunta de Galicia for his predoctoral grant. Authors gratefully acknowledge Shimizu Chemical Corporation (Japan) for the Propol A® sample.

References

[1] T. Coviello, P. Matricardi, C. Marianecci, F. Alhaique, Polysaccharide hydrogels for modified release formulations, J. Control. Release 119 (14) (2007) 5–24.
[2] A.W. Basit, Advances in colonic drug delivery, Drugs 14 (65) (2005) 1991–2007.
[3] T.F. Vandamme, A. Lenourry, C. Charrueau, J. Chaumeil, The use of polysaccharides to target drugs to the colon, Carbohyd. Polym. 3 (48) (2002) 219–231.
[4] M.K. Chourasia, S.K. Jain, Polysaccharides for colon targeted drug delivery, Drug Deliv. 2 (11) (2004) 129–148.
[5] Z.L. Liu, H. Hu, R.X. Zhuo, Konjac glucomannan-graft-acrylic acid hydrogels containing azo crosslinker for colon-specific delivery, J. Pol. Sci. Pol. Chem. 17 (42) (2004) 4370–4378.
[6] L.G. Chen, Z.L. Liu, R.X. Zhuo, Synthesis and properties of degradable hydrogels of konjac glucomannan grafted acrylic acid for colon-specific drug delivery, Polymer 16 (46) (2005) 6274–6281.
[7] R.P. Millane, B. Wang, A cellulose-like conformation accessible to the xanthan backbone and implications for xanthan synergism, Carbohyd. Polym. 1 (13) (1990) 57–68.
[8] M.M. Talukdar, R. Kinget, Swelling and drug-release behavior of xanthan gum matrix tablets, Int. J. Pharm. 1 (120) (1995) 63–72.
[9] V. Dhopeshwarkar, J.L. Zatz, Evaluation of xanthan gum in the preparation of sustained-release matrix tablets, Drug Dev. Ind. Pharm. 9 (19) (1993) 999–1017.
[10] M.M. Talukdar, A. Michoel, P. Rombaut, R. Kinget, Comparative study on xanthan gum and hydroxypropylmethyl cellulose as matrices for controlled-release drug delivery. 1. Compaction and in vitro drug release behaviour, Int. J. Pharm. 129 (1–2) (1996) 233–241.
[11] C.W. Vendruscolo, I.F. Andreazza, J.L.M.S. Ganter, C. Ferrero, T.M.B. Bresolin, Xanthan and galactomannan (from M-scabrella) matrix tablets for oral controlled delivery of theophylline, Int. J. Pharm. 1–2 (296) (2005) 1–11.
[12] M. Fukuda, N.A. Peppas, J.W. McGinity, Properties of sustained release hot-melt extruded tablets containing chitosan and xanthan gum, Int. J. Pharm. 1–2 (310) (2006) 90–100.
[13] K. Maekaji, Determination of acidic component of konjac mannan, Agric. Biol. Chem. 1 (42) (1978) 177–178.
[14] M. Maeda, H. Shimahara, N. Sugiyama, Studies of mannan and related-compounds 5. Detailed examination of the branched structure of konjac glucomannan, Agric. Biol. Chem. 2 (44) (1980) 245–252.
[15] M.A.K. Williams, T.J. Foster, D.R. Martin, I.T. Norton, M. Yoshimura, K. Nishinari, A molecular description of the gelation mechanism of konjac mannan, Biomacromolecules 3 (1) (2000) 440–450.
[16] E.M. Ozu, I.C. Baianu, L.-S. Wei, Physical and chemical properties of glucomannan gels and related polysaccharides, in: Ion C. Baianu, H.P., T.F.K. (Eds.), Physical Chemistry of Food Processes, New York, (1993) 487–517.
[17] C. Shinzato, A.M. Broussalis, G.E. Ferraro, Glucomanano: un aporte a su control de calidad, SAFYBI 95 (35) (1996) 26–31.
[18] E.I. Yaseen, T.J. Herald, F.M. Aramouni, S. Alavi, Rheological properties of selected gum solutions, Food Research International 2 (38) (2005) 111–119.
[19] A. Gonzalez, N. Fernandez, A. Sahagun, J.J. Garcia, M.J. Diez, L.J. Castro, M. Sierra, Effect of glucomannan and the dosage form on ethinylestradiol oral absorption in rabbits, Contraception 5 (70) (2004) 423–427.
[20] K. Wang, Z.M. He, Alginate-konjac glucomannan-chitosan beads as controlled release matrix, Int. J. Pharm. 1–2 (244) (2002) 117–126.
[21] F. Alvarez-Mancenido, K. Braeckmans, S.C. De Smedt, J. Demeester, M. Landin, R. Martinez-Pacheco, Characterization of diffusion of macromolecules in konjac glucomannan solutions and gels by fluorescence recovery after photobleaching technique, Int. J. Pharm. 1–2 (316) (2006) 37–46.
[22] P. Penroj, J.R. Mitchell, S.E. Hill, W. Ganjanagunchorn, Effect of konjac glucomannan deacetylation on the properties of gels formed from mixtures of kappa carrageenan and konjac glucomannan, Carbohyd. Polym. 3 (59) (2005) 367–376.
[23] M.J. Ridout, G.J. Brownsey, V.J. Morris, Synergistic interactions of acetan with carob or konjac mannan, Macromolecules 8 (31) (1998) 2539–2544.
[24] F.M. Goycoolea, R.K. Richardson, E.R. Morris, M.J. Gidley, Stoichiometry and conformation of xanthan in synergistic gelation with locust bean gum or konjac glucomannan – evidence for heterotypic binding, Macromolecules 24 (28) (1995) 8308–8320.
[25] E. Miyoshi, T. Takaya, P.A. Williams, K. Nishinari, Effects of sodium chloride and calcium chloride on the interaction between gellan gum and konjac glucomannan, J. Agric. Food Chem. 9 (44) (1996) 2486–2495.
[26] E. Miyoshi, T. Takaya, P.A. Williams, K. Nishinari, Rheological and DSC studies of mixtures of gellan gum and konjac glucomannan, Macromol. Symp. (120) (1997) 271–280.
[27] G. Paradossi, E. Chiessi, A. Barbiroli, D. Fessas, Xanthan and glucomannan mixtures: synergistic interactions and gelation, Biomacromolecules 3 (3) (2002) 498–504.
[28] M. Tako, Binding sites for mannose-specific interaction between xanthan and galactomannan, and glucomannan, Colloid. Surface. B 2 (1) (1993) 125–131.
[29] F. Alvarez-Mancenido, I. Lacik, M. Landín, R. Martínez-Pacheco, Konjac glucomannan and konjac glucomannan/xanthan gum mixtures as excipients for controlled drug delivery systems. Diffusion of small drugs, Int. J. Pharm. 349 (1–2) (2008) 11–18.
[30] M. Landín, F. Álvarez-Manceñido, R. Martínez-Pacheco, Macro and microviscosity in konjac glucomannan/xanthan gum mixtures: effects on drug release process, Abstract of second EUFEPS conference on optimising drug delivery and formulation: evaluation of drug delivery systems issues and perspectives. Versailles (2005) 91–92.

[31] N. Nakajima, Y. Matsuura, Purification and characterization of konjac glucomannan degrading enzyme from anaerobic human intestinal bacterium, *Clostridium butyricum Clostridium beijerinckii* group, Biosci. Biotechnol. Biochem. 10 (61) (1997) 1739–1742.
[32] M.D. Burke, J.O. Park, M. Srinivasarao, S.A. Khan, A novel enzymatic technique for limiting drug mobility in a hydrogel matrix, J. Control. Release 1 (104) (2005) 141–153.
[33] A. Tayal, R.M. Kelly, S.A. Khan, Rheology and molecular weight changes during enzymatic degradation of a water-soluble polymer, Macromolecules 2 (32) (1999) 294–300.
[34] A. Tayal, V.B. Pai, S.A. Khan, Rheology and microstructural changes during enzymatic degradation of a guar-borax hydrogel, Macromolecules 17 (32) (1999) 5567–5574.
[35] G.A. Lewis, D. Methieu, R. Phan-Tan-Luu, Mixtures in a constrained region of interest. Screening, defining the domain, and optimizing formulations, in: J. Swarbrik (Ed.), Pharmaceutical Experimental Design, Marcel Dekker Inc., New York, 1999, pp. 413–454.
[36] M.P. Summers, R.P. Enever, J.E. Carless, Influence of crystal form on tensile-strength of compacts of pharmaceutical materials, J. Pharm. Sci. 8 (66) (1977) 1172–1175.
[37] G.J. Li, L. Qi, A.P. Li, R. Ding, M.H. Zong, Study on the kinetics for enzymatic degradation of a natural polysaccharide, Konjac glucomannan, Macromol. Symp. (216) (2004) 165–178.
[38] K. Almdal, J. Dyre, S. Hvidt, O. Kramer, Towards a phenomenological definition of the term gel, Poly. Gels Netw. 1 (1) (1993) 5–17.
[39] H. Zhang, M. Yoshimura, K. Nishinari, M.A.K. Williams, T.J. Foster, I.T. Norton, Gelation behaviour of konjac glucomannan with different molecular weights, Biopolymers 1 (59) (2001) 38–50.
[40] P. Colombo, R. Bettini, P. Santi, N.A. Peppas, Swellable matrices for controlled drug delivery: gel-layer behaviour, mechanisms and optimal performance, Pharm. Sci. Technol. Today 6 (3) (2000) 198–204.
[41] M.K. Chourasia, S.K. Jain, Pharmaceutical approaches to colon targeted drug delivery systems, J. Pharm. Pharm. Sci. 1 (6) (2003) 33–66.