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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.
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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

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