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Impact of fine structure of galactomannans on their interactions with xanthan: Two co-existing mechanisms to explain the synergy

Michel Grisel, Youssef Aguni, Frédéric Renou*, Catherine Malhiac*

University of Le Havre, URCOM, EA 3221, FR CNRS 3038, 25 rue Philippe Lebon, B.P. 1123, 76063 Le Havre Cedex, France

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ABSTRACT

The objective of the study is to investigate the role of fine structure of galactomannan (guar and locust beam gum) on the molecular interactions occurring in the presence of xanthan in aqueous solution. First, 2 Locust bean and 2 guar gums were characterized from a structural point of view thus pointing out some differences. Both Intrinsic viscosity and retention of hydrophobic probe were determined for pure polysaccharide solutions and mixtures. Results clearly indicate the crucial role of galactomannan fine structure on the synergy with xanthan in the case of guar gums. More precisely, the impact of the galactose units distribution along the main chain is linked to the synergetic mechanism. For high M/G only junction zone associations govern the synergy. Oppositely, for low M/G, segregative and junction zone associations co-exist and depend on the fine structure of the galactomannan.

1. Introduction

Among the numerous hydrocolloids commonly utilized at industrial scale, both well-known xanthan and galactomannan (guar, locust bean and tara gums) polysaccharides are of great interest due to their stabilization and textural properties. Xanthan gum (XG) is an anionic polysaccharide produced by bacteria *Xanthomonas campestris*. Its structure consists in a β-(1 → 4)-D-glucopyranosyl backbone substituted on every second unit with a charged trisaccharide side-chain composed of a glucuronic acid residue between two mannose units. The terminal mannose unit may be substituted by a pyruvic acid residue, and an O-acetyl group is frequently present at the inner mannose (Jansson, Kenne, & Lindberg, 1975; Rinaudo, Milas, Lambert, & Vincendon, 1983). Xanthan polysaccharide presents a secondary "ordered" structure that still remains controversial as it might correspond to a single or to a double stranded helix at low temperature and/or in the presence of electrolytes. Nevertheless, the last proposal is the most accepted (Liu & Norisuye, 1988; Sato, Norisuye, & Fujita, 1984). In aqueous media, Xanthan presents a conformational transition temperature (Tm) corresponding to an ordered-to-disordered conformation transition; Tm depends on the ionic strength, the nature of electrolyte and the pH of the media; it is also related to acetyl and pyruvate rate (Rocks, 1971).

Galactomannans (GAL) are plant storage polysaccharides consisting in a main chain of (1 → 4) linked β-D-mannopyranosyl residues with single unit α-D-galactopyranosyl side-chain residues. The mannose to galactose ratio (M/G) depends on both the plant source and the extraction method used. For instance, Guar galactomannans (GG) usually own an M/G around 1.3 while locust bean (LBG) are less substituted, with M/G close to 3. Surprisingly, only few researchers have investigated the galactomannan fine structure and the distribution of the galactosyl along the main chain. In 1985, McCleary, Clark, Dea, and Rees (1985) used an enzymatic cleavage (*β-mannanase*) to determine the fine structure of galactomannans. Even if the action of the *β-mannanase* can bring structural information, to our knowledge, only Daas, Schols, and de Jongh (2000) compared the M/G and the galactose distribution along the chain of commercial galactomannans. In this work, authors also used the action of *β-mannanase* to determine the nature and the quantity of hydrolyzed oligosaccharides; On the basis of quantitative results, they defined the degree of blockiness (DB) which is determined as the ratio between the released free mannose and the total unsubstituted mannose. The DB reflects the presence of both unsubstituted mannose blocks (or smooth block) and substituted blocks (or hairy block). Nevertheless, this approach is limited since two galactomannans with very different M/G (as

* Corresponding authors. Tel.: +33 2 32 74 44 00.
E-mail addresses: frederic.renou@univ-lehavre.fr (F. Renou), catherine.malhiac@univ-lehavre.fr (C. Malhiac).

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LBG and Guar) may exhibit quite similar DB values, while owning completely different galactose distribution.

As first reported by Rocks (1971), synergy occurs when mixing xanthan and galactomannan gums thus involving a sharp viscosity enhancement or even gel occurrence. Many researchers have been working to understand this phenomenon, and different mechanisms have been proposed that are still subject to discussion.

Few authors explained the synergy as the consequence of mutual gum incompatibility (Kovacs, 1973) and volume exclusion (Schorsch, Garnier, & Doublier, 1995). Among the different scenarii evoked, xanthan chains may interact together and form crystalline mesophase without affecting molecule helical structure. Other authors suggested the lonely occurrence of topological entanglements without any specific intermolecular interactions but these hypotheses are poorly argued (Shatwell, Sutherland, & Ross-Murphy, 1990).

Most of researchers explain the synergy as a consequence of cooperative interactions between Xanthan and Galactomannan (Dea et al., 1977; Goycoolea, Richardson, & Morris, 1995). Three main mechanistic models have been proposed. One model explains binding between both polysaccharides as a lock and key mechanism (Cuvelier, 1988; Cuvelier & Launay, 1986). In this case, side chains of xanthan helices interact with non-substituted regions of galactomannan backbone. The second model consists in cooperative interactions between non-substituted regions of galactomannan chains and ordered helices of xanthan (Cheetham & Mashimba, 1988; Morris, Rees, Young, Walkinshaw, & Darke, 1977). The third model firstly proposed by Cairns (Cairns, Miles, & Morris, 1986; Cairns, Miles, Morris, & Brownsey, 1987) assumed that interactions occur between disordered segments of xanthan molecules and LBG. They suggest that denaturation of xanthan helical structure is necessary for the occurrence of intermolecular association and thus gelation.

Morris and Foster (1994) demonstrated that xanthan denaturation is a prerequisite to observe gel formation between xanthan and LBG, thus confirming the model proposed by Cairns. This model is the most commonly accepted mechanism nowadays.

To better understand the origin of the synergy, one needs to take into account the fine chemical structure of the polysaccharides; however, surprisingly, only few authors focused on that parameter. Recently, Renou et al. have studied the effect of xanthan structure on its interactions with locust bean gum and particularly the effect of pyruvate and acetate rate on the rheological parameters (Renou, Petibon, Malhiac, & Grisel, 2013). They evidenced that smooth areas of LBG and disordered segments of Xanthan are involved in the synergistic mechanism, and also demonstrated the respective impact of both acetate and pyruvate content on the viscoelasticity enhancement of XG/LBG mixtures.

Concerning the effect of galactomannan structure, Cheetham and Mashimba (1988) demonstrated that the less substituted is the galactomannan the more it interacts with xanthan. As reported in literature (Mazeau & Rinaudo, 2004; Wu, Li, Cui, Eskin, & Goff, 2012), even if the effect of the M/G on the interaction with xanthan is well established, the distribution of the galactosyl units along the chain and its impacts on the XG/GAL interactions still remain misunderstood.

The present work aims to better understand the interactions between Xanthan gum and galactomannans; to this end, the fine structure of galactomannan samples was characterized in terms of M/G ratio, and also by determination of smooth and substituted chains distribution. Then, on the one hand, the xanthan/galactomannan solution viscometric properties in the dilute regime were investigated as it represents an efficient tool to investigate ternary polymer–polymer–solvent systems (Cragg & Bigelow, 1955), as described by several authors (Cuvelier, Tonon, & Launay, 1987; Goycoolea, Morris, & Gidley, 1995; Higiro, Herald, & Alavi, 2006; Wang, Wang, & Sun, 2002). On the other hand, the behaviour of ethyl decanoate within the same gum solution and mixtures was investigated and expressed in terms of retention partition coefficient. The whole results allowed determining the impact of galactosyl content and distribution on the XG/GAL interactions.

2. Materials and methods

2.1. Materials

Polysaccharide powders were kindly given by Danisco (Portugal) (Xanthan XG), SBI (France) (Locust bean LBG1 and Guar GG1) and Alland & Robert (France) (LBG2 and GG2). NaCl was provided by Merck (Merckeurolab, France) and the bactericide (NaN_3) by Prolabo (Rhône-Poulenc, France). Pure water was obtained from an Easypure UV Compact ultrapure water system (Barnstead). Ethyl decanoate (99%) was provided by Aldrich France. Mannobiose, mannotriose and mannotetraose β-(1 → 4)-linked were purchased from Megazyme International, Bray, Ireland (Purity 95%). The *endo-β-Mannanase* (EC 3.2.1.78), a commercial enzyme from *Aspergillus niger* was provided by Megazyme International, Bray, Ireland.

2.2. Preparation of the polysaccharide solutions

Xanthan powder (0.5%; w/w) was first dispersed in water at room temperature in the presence of 0.01 M NaCl in order to keep the molecule in its ordered conformation (Capron, Brigand, & Muller, 1997). NaN_3 (400 ppm) was added to prevent bacteria proliferation. The solution was mechanically stirred during 6 h at room temperature. Solutions were then kept at low temperature (4 °C) overnight prior to utilization without further purification.

Galactomannan powders (0.5%; w/w) were dispersed at 85 °C for 3 h in pure water under mechanical stirring then was stopped after an additional 2 h at 20 °C. Then, the galactomannan solutions were centrifuged at 12 000 rpm for 30 min to remove insoluble residues. Dry extracts allowed the determination of the final polysaccharide concentration of supernatant. Finally, the appropriate amounts of salt and bactericide were added to reach the final concentration of 0.01 M and 400 ppm respectively.

2.3. Preparation of mixed solutions of xanthan and galactomannan gum

Xanthan and galactomannan gums are high molecular weight polysaccharides, above 10^6 Da that can be evaluated using a variety of methods such as Size Exclusion Chromatography or viscosimetry (see (Holzwarth, 1978) for xanthan, and (Pollard et al., 2008) for galactomannans). For viscosimetry, as the study of the interaction between two polymers needed to keep the solution in the dilute regime, previous viscometric experiments were performed to check the linear dependence of the reduced viscosity versus polymer concentration, thus illustrating the lonely effect of intrinsic hydrodynamic volume of polymer chains, without intermolecular interactions (Lapasin & Pricl, 1995), in the gum concentration ranges used for the present work. The gum solutions were diluted with salted water to obtain a final polymer concentrations ranging from 0.002 g/dL to 0.02 g/dL (all concentrations are below $C^*/20$ thus excluded volume interactions are negligible). Mixtures of the Xanthan with Galactomannan at the desired concentration of total polysaccharide were obtained with 60% xanthan/40% galactomannan ratio; the solution was then stirred for 1 min at 2000 rpm by means of a vortex at room temperature before storage at 4 °C overnight prior to any measurement. We have demonstrated