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PLANT BIOPOLYMERS

Investigation of the Gelation of Galactomannan Isolated from the Seeds of *Styphnolobium japonicum* (Fabaceae)

A. V. Filatova[a], L. B. Azimova[a, 1], and A. S. Turaev[a]

[a] *Institute of Bioorganic Chemistry, Uzbekistan Academy of Sciences, Tashkent, 100125 Uzbekistan*

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Abstract—This article is devoted to the study of the structuring process of galactomannan isolated from Japanese Sophora seeds with borate ions. Based on galactomannan isolated from seeds of Japanese Sophora (*Styphnolobium japonicum*), a basis for a soft dosage form has been developed. The strength properties of the formed gel structures based on the formed galactomannan gels were evaluated depending on the nature of the crosslinking agent introduced into the systems. It is assumed that the network structures of gel systems are formed due to the formation of bridging bonds between the functional groups of galactomannan and boron ions. The rheological properties of the formed jellies have been studied. The study of the galactomannan gelation process showed that the dynamic viscosity depends on pH, the concentration of the crosslinking agent and the concentration of the galactomannan solution. Plasticization of the gel was carried out and it was assumed that the presence of glycerol destroys chemical crosslinking due to the formation of a complex compound with sodium tetraborate. A soft dosage form has been developed with the addition of 10% propylene glycol as a plasticizer and a 0.1% preservative solution. As a result of further studies of the developed soft dosage form, it was revealed that after 6–12 months of storage under natural conditions at a temperature of +20 ± 5°C, the studied gel remained stable. These studies made it possible to use the investigated gel in medicine as a basis for a soft dosage form.

Keywords: galactomannan, gelling base, sodium tetraborate complex, bridging, crosslinking

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INTRODUCTION

The development of research in the field of studying the specific properties of natural polysaccharides opens up wide opportunities for the creation of biologically active substances or additives for food products or medicines on their basis [1]. Polysaccharides of higher plants, including galactomannans, have immunomodulatory properties.

Galactomannans are plant polysaccharides, which are polymeric compounds of mannose and galactose [2, 3]. Depending on the physicochemical properties and concentration, it is possible on their basis to create a soft dosage form in the form of a gel [4, 5]. The choice of one or another gelling agent is determined by its compatibility with other components and cost.

Galactomannans, due to the properties of their aqueous solutions and the absence of toxicity, are used in the food, textile, pharmaceutical industry and medicine as food additives, stabilizers, flocculants, thickeners and gelling agents (in binary mixtures) [6–8].

Pharmacological studies have shown the presence of anticoagulant [9, 10], hepatoprotective and analgesic properties in galactomannans and their derivatives [11, 12]. It was found that the use of partially hydrolyzed galactomannan has a positive effect on lipid and carbohydrate metabolism, and also normalizes the intestinal microflora [13, 14].

The most important property of many natural polysaccharides, including galactomannans, is the formation of highly viscous gels, which is explained by the structure of their macromolecules, which are highly hydrophilic. The process of gelation in aqueous solutions must be considered as a sequence of chemical and physical processes [15].

The methods of obtaining gelation of solutions of natural and synthetic polymers and the features of these processes are the subject of numerous studies [16].

The task of finding and synthesizing biologically active natural and synthetic compounds and their derivatives, as well as studying the processes of structuring their aqueous solutions and developing the technological features of these processes, establishing possible areas of their application, is relevant in medical practice.

The purpose of this work is to study the process of gelation of galactomannan (GM) isolated from seeds

[1] Corresponding author: e-mail: luiza8181@mail.ru.

Styphnolobium japonicum, with borate ions in order to obtain a soft dosage form.

EXPERIMENTAL

Object of study. GM isolated from Japanese Sophora seeds (*Styphnolobium japonicum* (Fabaceae)).

As crosslinking agents used: aluminum sulfate GOST 3758-75, iron (II) sulfate GOST 6981-94, calcium chloride GOST 450-77, sodium tetraborate GOST 8129-77.

Isolation of a water-soluble polysaccharide. To separate the endosperm, whole seeds were placed in a flask with a reflux condenser, boiled water was added until the seeds were completely covered, and heated in a boiling water bath for 20 min. The swollen seeds were divided into three parts, including the seed coat, the embryonic part, and the endosperm. Then the separated endosperm was crushed, extracted by boiling with 80% ethanol (at a ratio of 1 : 4) for 1 h. The alcoholic extract was decanted and the crushed endosperm was air dried.

Cold water extraction was carried out at room temperature; for this, 500 mL of water was added to the crushed endosperm (10 g) and placed on a magnetic stirrer for 10 h. The extract was filtered off and precipitated with 2 volumes of 96% ethyl alcohol. The white precipitate was filtered off and dried.

Then the endosperm meal after cold extraction was placed in a glass and distilled water was added with stirring at 80°C in three to four times the volume. As the extract thickened, hot water was added in portions to the original volume. The viscous extract was decanted, and a new portion of hot water was added to the residue, and the extraction was continued under the same conditions for another 3 h. The resulting extracts were combined and 2 volumes of 96% ethanol were added. The white precipitate was filtered off, washed with ethanol, and dried.

Rheological research. Investigations of the rheological properties of the GM solution and gel were carried out on a Reotest-2 rotational viscometer (Mettingen, Germany) with a working unit of coaxial cylinders in the stress range ((1.6–3) × 10^3) Pa and shear rates ((0.2–1.3) × 10^3) s^{-1} at temperatures of 25, 40, 55, and 70°C [17]. From the measurements of the shear stress and shear rate, the dynamic viscosity is calculated (η) according to the formula: $\eta = \tau_2/D_2 \times 100\%$, where η is the dynamic viscosity (Pa s), τ_2 the shear stress (10^{-1} Pa s), and D_2 the shear rate (C^{-1}).

IR spectroscopy. IR spectra were recorded on an IR Fourier spectrometer 2000 (PerkinElmer, United States) in the frequency range 400–4000 cm^{-1} in a tablet with KBr (1 : 100).

Determination of pH of gels. The hydrogen index was determined in a solution with a mass fraction of galactomannan gel in accordance with the requirements of GOST 29188.2 [18], potentiometrically at a temperature of 25°C, using a pH/mv/TEMPMeterP25 instrument (Gain express holding Limited, Hong Kong, China) from 5.0 to 8.0.

Shelf life of the gel in vivo was established by monitoring quality indicators after six months during the first year of storage and 12 months thereafter.

DISCUSSION

We have previously investigated the isolation of galactomannan from seeds of Japanese Sophora (*Styphnolobium japonicum* (Fabaceae) [19].

The structuring of aqueous solutions of GM in the presence of polyvalent metal ions was studied at various ratios of metal : GM, pH of the medium, which made it possible to characterize the structuring process as a reaction of macromolecules with low-molecular-weight compounds. Calcium, iron, magnesium, and aluminum ions are often used as structuring agents for crosslinking polysaccharides.

Table 1 shows the results of the gelation reaction of a 0.5% GM solution at different pH of structuring agents.

As follows from Table 1, chemical crosslinking of GM occurs only with sodium tetraborate in an alkaline medium.

Figure 1 shows the IR spectra of the original GM and borate-crosslinked GM.

As follows from Fig. 1, the IR spectrum of the GM crosslinked with borate ions differs from the IR spectrum of the original GM. In the IR spectrum of the cross-linked GM, absorption bands appear at 1078.2 and 1244.0 cm^{-1} that are absent in the original GM, in the range 900–1100 cm^{-1} there are bands related to the stretching vibrations of B-O bonds in the BO_4 tetrahedron [20]. In addition, in the IR spectrum of the cross-linked GM, absorption bands of deformation vibrations of CH_2- and CH-groups (1354.0–1438 cm^{-1}) and water (1630–1660 cm^{-1}), absorption bands in the region of 3220–3460 cm^{-1} refer to hydrogen bonds [20].

GM has hydroxyl groups in the cis position, which is favorable for the formation of borate complexes, since the hydroxyl groups are located on one side of the plane of the carbon ring [21, 22].

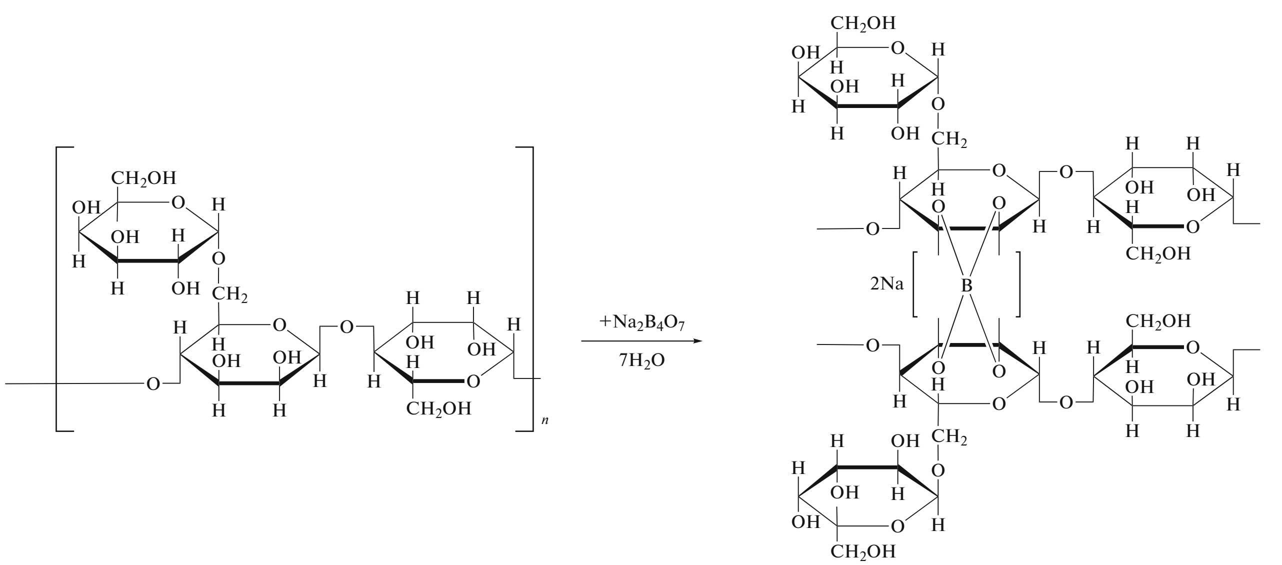
It can be assumed that structuring proceeds according to the following scheme:

Table 1. Gelling of GM solution at different pH and concentration of metal ions

Salt	Concentration of GM solution, %	pH of metal salt solution	Concentration of metal ions, %	Observed effect
Aluminum sulfate	0.5	3	0.3	No gelation
Aluminum sulfate	1	7.5	0.5	No gelation
Iron(II) sulfate	0.5	3	0.3	No gelation
Iron(II) sulfate	1	7.5	0.5	No gelation
Calcium chloride	0.5	4	0.3	No gelation
Calcium chloride	1	6	0.5	No gelation
Sodium tetraborate	0.5	3	0.3	No gelation
Sodium tetraborate	1.0	8.6	0.5	Gelation reaction proceeds

Table 2. Rheological properties of solutions of crosslinked GM with sodium tetraborate

GM: tetraborate, mol: mol	Dynamic viscosity of the gel, Pa s	Observed effect
1 : 0.5	102.3	Gelatinous mass
1 : 0.1	88.74	–
1 : 0.05	35.7	Formation of strong gel
1 : 0.01	28.95	Formation of strong gel
1 : 0.001	19.86	Stretching gel formation
1 : 0.0001	3.1	Solution



Further, the rheological properties of cross-linked solutions of galactomannan with sodium tetraborate were investigated at various molar ratios (Table 2).

From Table 2 it follows that when sodium tetraborate solution is added to the GM solution, the viscosity increases (3.1–102.3 Pa s) and a strong gel is formed.

Our goal was to obtain a soft plastic gel that spreads well, retains its shape when smeared, and does not spread over the surface, so we plasticized the gel. For this, various plasticizers were added to the gel, and the gel samples were homogenized (Table 3).

When a certain amount of glycerin is added to the gel, the crosslinking disappears (gelation disappears), since, apparently, glycerol interacts with sodium tetraborate, forming a complex compound [20], and gels with propylene glycol did not have such a disadvantage.

Based on the above studies, a soft dosage form was developed based on a 1% solution of GM crosslinked

Table 3. Rheological properties of plasticized gel samples

Dynamic viscosity of the gel, Pa s	Glycerin, %	Propylene glycol, %	Observed effect
3.9	5		Durable gel
1.5	10		Gelation disappears
5.8		5	Durable gel
4.4		10	Plastic gel
2.8		15	The gel does not hold its shape, it flows

with sodium tetraborate with the addition of 10% propylene glycol as a plasticizer and a 5% solution of ethyl alcohol, in which 0.1 g of *para*-hydroxybenzoic acid methyl ester is dissolved as a preservative. As a result of further studies of the developed soft dosage form, it was revealed that after 6–12 months of storage under natural conditions at a temperature of +20 ± 5°C, the studied gel remained stable. These studies made it possible to use the gel in medicine as a basis for a soft dosage form.

CONCLUSIONS

(1) GM was isolated from the seeds of *Styphnolobium japonicum*, the stitching of which resulted in a strong gel, on the basis of which a soft form was developed.

(2) The strength properties of the formed gel structures based on the formed GM gels were evaluated depending on the nature of the crosslinking agent introduced into the systems. It is assumed that the network structures of gel systems are formed due to the formation of bridging bonds between the functional groups of galactomannan and boron ions.

(3) The study of the process of GM gelation showed that the dynamic viscosity depends on pH, the concentration of the crosslinking agent and the concentration of the GM solution.

(4) Plasticization of the gel was carried out and it was assumed that the presence of glycerin destroys chemical crosslinking due to the formation of a complex compound with sodium tetraborate.

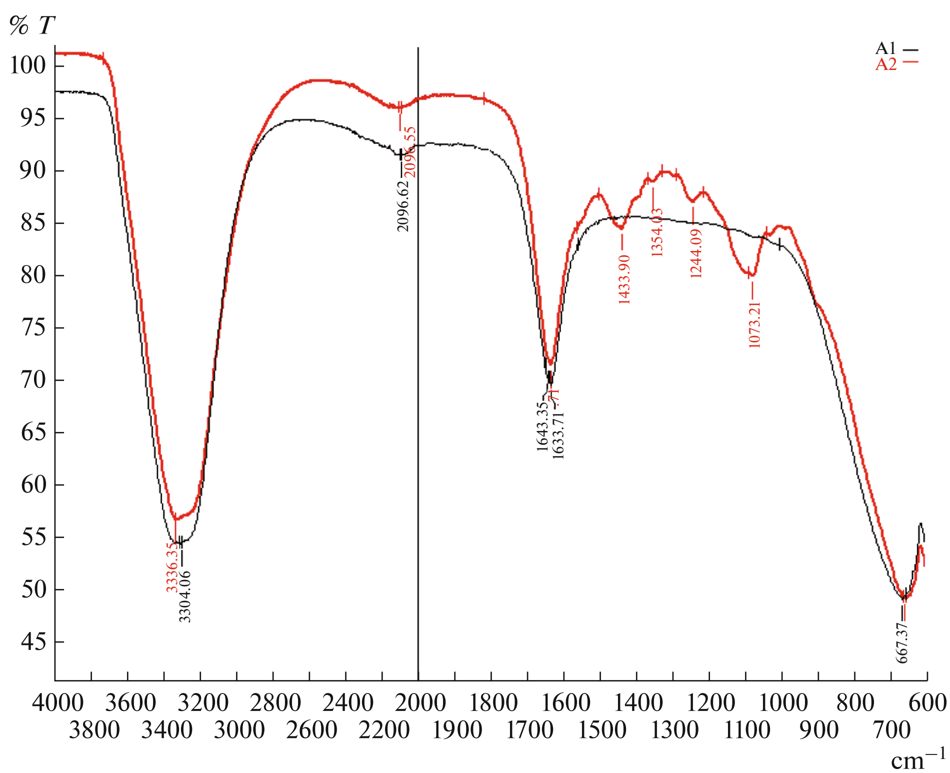


Fig. 1. IR spectra of the original GM (A1), crosslinked GM (A2).

(5) On the basis of GM and borate, it is possible to obtain a soft dosage form that meets all the requirements of medicine.

COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any studies involving animals or human participants performed by any of the authors.

Conflict of Interests

The authors declare that they have no conflicts of interest.

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