Sulfation of arabinogalactan with sulfamic acid under homogeneous conditions in dimethylsulfoxide medium

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Abstract

Sulfation of larch wood arabinogalactan (AG) with sulfamic acid in dimethylsulfoxide (DMSO) medium in the presence of urea was studied for the first time. The use of DMSO as a solvent instead of more toxic 1,4-dioxane allows to sulfate AG under homogeneous conditions. The sulfated AG with a high sulfur content (12.0–12.5 wt %) was produced by sulfation at a temperature of 85–90 °C, the molar ratio of AG / sulfating agent equal to 1:0.85 during 2–3 h. The introduction of sulfate groups into the structure of arabinogalactan was confirmed by the appearance of new absorption bands in FTIR and FT Raman spectra, characteristic for the vibrations of the sulfate groups. It was proved by ^{13}C NMR spectroscopy that the predominant substitution of the primary hydroxyl groups at C6 carbon atoms of the terminal galactose units of main and side chains of arabinogalactan takes place. Simultaneously, the hydroxyl groups associated with C2 and C4 carbon atoms of galactose unit of the main chain are only partially sulfated. According to results of GPC study, the sulfated AG is characterized by a narrow molecular weight distribution with an average molecular weight of 18.8 kDa and a polydispersity of 1.3.

Introduction

Larch is a widespread species in the boreal forests of Siberia and North America. Larch wood is valued for its tough, waterproof and durable properties (Koizumi et al. 2003). A characteristic feature of larch wood is the high content of arabinogalactan (AG), a water-soluble biologically active polysaccharide, which can be easily

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extracted by water. The products of AG modification are of considerable interest to the medicine, veterinary, pharmaceutical and cosmetic industries (Heinze 2005; Kuznetsov et al. 2018; Medvedeva et al. 2003).

Sulfated derivatives of AG have anticoagulant, hypolipidemic and membrano-tropic activities (Ciancia et al. 2007; Kostyro and Stankevich 2015; Medvedeva et al. 2003). These useful biological properties, along with good solubility of AG in water makes it possible to consider AG sulfates as a promising alternative to heparin in the prevention and treatment of thromboembolic diseases (Kostyro and Stankevich 2015; Mestechkina and Shcherbukhin 2010). Heparin can cause side effects, being a drug produced from animal tissue, it may contain pathogens that are dangerous to humans (Melo et al. 2004). Recently, Liu et al. (2020) reported that thrombocytopenia-induced heparin represents a high mortality risk in critical COVID-19 patients, treated with heparin. For these reasons, researches aimed at creating new anticoagulants are very relevant and practically important.

Current methods of AG sulfates production are based on the use of aggressive and environmentally hazardous sulfating agents, such as sulfuric anhydride and chlorosulfonic acid (Fang and Ma 2007; Kostyro et al. 2008; Tang et al. 2019; Vasilyeva et al. 2013). To overcome these disadvantages, Kostyro and Stankevich (2015) proposed a method of AG sulfation based on the interaction of AG with dry potassium persulfate in dimethyl sulfoxide (DMSO) medium. However, this method requires the use of complex multi-stage procedures of ultrafiltration and sublimation or sputtering drying for purification and isolation of final products.

Earlier, it was found possible to sulfate AG by sulfamic acid—urea mixture in 1,4-dioxane medium (Vasilyeva et al. 2015). This new method allows to produce sulfated AG which is a simpler and more environmentally friendly way compared to the previously used methods. However, the sulfation of AG in 1,4-dioxane medium proceeds under heterogeneous conditions, since AG is insoluble in dioxane, creating diffusion restrictions that reduce the efficiency of the sulfation process.

To carry out the sulfation of polysaccharides under homogeneous conditions, ionic liquids (ILs) (Gericke et al. 2009; Wang et al. 2009) or organic solvents with the assistance of lithium chloride (LiCl) (Cheng et al. 2017; Daus et al. 2011; Qin et al. 2014) are usually used. Gericke et al. (2009) successfully prepared cellulose sulfates with a degree of substitution (DS), that ranged from 0.14 to 1.46, using sulfuric anhydride/pyridine, sulfuric anhydride/N,N-dimethylformamide (DMF) and chlorosulfonic acid in 1-butyl-3-methylimidazolium chloride (BMIMCl)/DMF. The sulfation reaction proceeds with the predominant substitution of the primary hydroxyl groups. The disadvantage of this method is the rather long time (up to 24 h) of the cellulose dissolution in the ionic liquids at high temperature (80 °C). The use of DMF/LiCl solvent system for sulfation of xylan under homogeneous conditions was reported by Daus et al. (2011). Xylan sulfates with a maximum DS of 1.90 have been prepared with the use of sulfuric anhydride/pyridine complex. As was reported by Qin et al. (2014), the bacterial cellulose sulfates with DS values that range from 0.10 to 1.50 were prepared by direct homogeneous sulfation of bacterial cellulose with sulfuric anhydride/pyridine complex in N,N-dimethylacetamide (DMAc)/LiCl solution. The major disadvantage of this method is the use of toxic chemicals (DMF, pyridine, sulfuric anhydride).

Compared to solvents which are traditionally used for the sulfation of polysaccharides (Kuznetsov et al. 2015), DMSO has several significant advantages. Firstly, it is not toxic, and secondly, it is capable to dissolve the AG, and the sulfation reaction proceeds under homogeneous conditions with less diffusion limitations.

In this work, a new environmentally safe method of AG sulfation in DMSO medium by sulfamic acid—urea mixture is suggested and optimized.

Materials and methods

AG from the wood of Siberian larch (*Larix sibirica Ledeb.*) produced by OOO "Khimiya drevesiny" ("Chemistry of wood", Irkutsk, Russia) under the trade name "FibrolarS" was used as the initial material.

Sulfation of arabinogalactan with sulfamic acid in DMSO in the presence of urea

AG sulfation was carried out in a 3-neck flask (50 ml), equipped with a thermometer, mechanical stirrer and a glycerin bath, using the modified procedure, described in Vasilyeva et al. (2015) for dioxane solvent. AG (2.5 g) and freshly distilled DMSO (7 ml) were put into a flask, and the mixture was stirred at 75 °C until the AG was completely dissolved. Then, urea (1.5–3.0 g, 25–50 mmol) and sulfamic acid (2.4–4.9 g, 25–50 mmol) were added to the resulting solution and stirred at a temperature of 75–90 °C for 0.25–4.0 h (according to the sulfation conditions shown in Table 1). After that, the reaction mixture was cooled to room temperature, neutralized by 25% ammonia solution (1.5–2.5 ml) to neutral reaction and poured into 96% ethanol (150 ml). The resulting viscous product was washed three times with 96% ethanol (each portion consisted of 10 ml) to form a solid precipitate. The obtained precipitate (sulfated derivative of AG in the form of ammonium salt) was filtered, washed on the filter with 96% ethanol (15 ml) and air-dried.

The ammonium salt of sulfated AG was purified by the dialysis on cellophane against distilled water. The product was dialyzed for 10–15 h; water was changed at 1–2-h intervals. Dialysis bag MF-5030–46 MFPI (USA) with a pore size of 3.5 kDa and width of 46 mm was used.

The sodium salt of sulfated AG was obtained by gradually adding a 10% aqueous solution of sodium hydroxide (1.5–4.0 ml) to a solution of ammonium salt of sulfated AG (2.5 g) in water (10 ml) under heating at 70–75 °C during 0.5 h. Then, the volume of water was diminished on a rotational evaporator up to a residual volume of 10–15 ml. The residue was poured into ethanol (50 ml), and the precipitated sodium salt of sulfated AG was filtered, washed on a filter with 96% ethanol (10 ml) and air-dried. Transparent films of the sulfated AG samples were obtained by evaporation of their 6–7% aqueous solutions on a smooth glass surface in the air at room temperature.

Numerical optimization of the process of arabinogalactan sulfation by sulfamic acid was carried out with the use of Statgraphics Centurion XVI software (Design of Experiment) as described in Sudakova et al. (2013). Temperature, duration and amount of the

Table 1 Effect of the conditions of AG sulfation by sulfamic acid—urea mixture in DMSO medium on the yield of sulfated AG and sulfur content

Temperature (°C)	Time (h)	AG[a]/SC[b] molar ratio	Content of sulfur (wt %)	Yield (%)	Conversion rate (% S)	Degree of substitution (DS)[c]
75	1.0	1:0.59	4.1	92.8	14.6	0.23
75	2.0	1:0.59	5.4	94.0	20.1	0.32
75	3.0	1:0.59	6.3	94.3	24.3	0.39
75	1.0	1:0.71	6.2	93.1	19.9	0.38
75	2.0	1:0.71	7.1	94.7	23.6	0.45
75	3.0	1:0.71	7.4	94.7	24.9	0.48
75	1.0	1:0.85	6.4	94.0	17.2	0.40
75	2.0	1:0.85	8.6	95.1	25.4	0.58
75	3.0	1:0.85	8.9	95.5	26.6	0.61
75	1.0	1:1.19	7.0	94.8	13.9	0.44
75	2.0	1:1.19	8.8	95.2	18.9	0.60
75	3.0	1:1.19	9.1	95.2	19.8	0.63
80	1.0	1:0.59	5.9	93.8	22.4	0.36
80	2.0	1:0.59	6. 2	94.9	23.8	0.38
80	3.0	1:0.59	7.1	95.4	28.3	0.45
80	0.25	1:0.71	3.9	91.1	11.5	0.22
80	0.5	1:0.71	4.6	92.9	13.9	0.27
80	1.0	1:0.71	7.6	94.9	25.8	0.50
80	2.0	1:0.71	8.2	95.2	28.5	0.55
80	3.0	1:0.71	8.4	95.0	29.5	0.57
80	0.25	1:0.85	4.2	91.7	10.4	0.24
80	0.5	1:0.85	5.0	92.0	12.7	0.29
80	1.0	1:0.85	7.8	94.1	22.2	0.51
80	2.0	1:0.85	10.1	95.2	31.9	0.74
80	3.0	1:0.85	10.7	94.8	34.8	0.80
80	4.0	1:0.85	11.0	94.5	36.3	0.84
80	1.0	1:1.19	9.1	95.3	19.8	0.63
80	2.0	1:1.19	10.3	95.0	23.7	0.76
80	3.0	1:1.19	11.0	94.1	26.1	0.84
85	1.0	1:0.59	6.6	94.8	25.8	0.41
85	2.0	1:0.59	7.3	95.2	29.4	0.47
85	3.0	1:0.59	7.8	94.5	32.0	0.51
85	0.25	1:0.71	4.1	91.2	12.1	0.23
85	0.5	1:0.71	5.1	91.5	15.7	0.30
85	1.0	1:0.71	7.5	94.8	25.3	0.49
85	2.0	1:0.71	8.9	95.4	32.0	0.61
85	3.0	1:0.71	8.8	94.4	31.5	0.60
85	0.25	1:0.85	5.2	91.4	13.4	0.31
85	0.5	1:0.85	5.6	92.8	14.6	0.34
85	1.0	1:0.85	10.6	94.9	34.3	0.79

Table 1 (continued)

Tempera-ture (°C)	Time (h)	AG[a]/SC[b] molar ratio	Content of sulfur (wt %)	Yield (%)	Conversion rate (% S)	Degree of substitution (DS)[c]
85	2.0	1:0.85	12.0	93.1	41.6	0.96
85	3.0	1:0.85	12.5	92.6	44.5	1.03
85	4.0	1:0.85	12.5	89.7	44.5	1.03
85	2.0	1:1.19	12.3	94. 9	31.2	1.00
85	3.0	1:1.19	12.4	93.8	31.6	1.01
90	1.0	1:0.59	6.9	95.2	27.3	0.44
90	2.0	1:0.59	7.4	95.0	29.9	0.48
90	3.0	1:0.59	7.9	93.5	32.6	0.52
90	0.25	1:0.71	4.8	94.0	14.6	0.28
90	0.5	1:0.71	5.9	94.6	18.7	0.36
90	1.0	1:0.71	8.3	95.4	29.0	0.56
90	2.0	1:0.71	8.9	95.3	32.0	0.61
90	3.0	1:0.71	9.1	94.0	33.0	0.63
90	0.25	1:0.85	5.5	91.0	14.3	0.33
90	0.5	1:0.85	6.3	93.2	16.9	0.39
90	1.0	1:0.85	9.8	95.1	30.5	0.70
90	2.0	1:0.85	12.2	94.2	42.8	0.99
90	3.0	1:0.85	12.5	93.4	44.5	1.03
90	4.0	1:0.85	12.4	90.4	43.9	1.01
90	2.0	1:1.19	12.3	95.7	31.2	1.00
90	3.0	1:1.19	12.6	94.2	32.5	1.04

[a]Amount of free hydroxyl groups in monosaccharide unit of AG is 2.7 according to data (Medvedeva et al. 2003)

[b]Sulfating complex (SC): equimolar mixture of sulfamic acid and urea

[c]Degree of substitution is amount of sulfated moieties per one monosaccharide units of AG (Mark 2011)

sulfating complex as independent variables are included in the numerical optimization. The sulfur content in sulfated arabinogalactan is taken as the output parameter.

Analytical methods

The sulfur content in the sulfated arabinogalactan was determined by elemental FlashEA™ 1112 analyzer (Thermo Quest, Italia).

The yield of sulfated arabinogalactan (%) was determined in accordance with the equation for cellulose (Mark 2011) modified for arabinogalactan:

$$\text{Yield}(\%) = \frac{m_{SAG} \times (A_r(S) - 102 \times W(S))}{A_r(S) \times m_{AG}} \tag{1}$$

where m_{SAG}–mass of sulfated AG (g); m_{AG}–mass of initial AG (g); S–sulfur content in sulfated AG (%).

The degree of substitution (DS) of sulfated arabinogalactan is defined as the average number of sulfate groups per one monosaccharide unit, and was calculated from the mass fraction of sulfur, using the following equation:

$$DS = \frac{158 \times W(S)}{A_r(S) - 102 \times W(S)} \tag{2}$$

Conversion rate (% S) was calculated according to the DS equation (Mark 2011) and divided by SC and 2.7:

$$Conversion\ rate(\%S) = \frac{DS}{SC \times 2.7} = \frac{158 \times W(S)}{(A_r(S) - 102 \times W(S)) \times SC \times 2.7} \times 100\% \tag{3}$$

where W(S)–mass fraction of sulfur; A_r(S)–atomic weight of sulfur; 102—increasing weight of AG unit resulting from the introduction of one SO_3Na group; 158—average molecular weight of one AG unit according to the data (Babkin et al. 2016; Medvedeva et al. 2003); SC—amount of sulfating complex (mol) per one mol of monosaccharide unit; 2.7—amount of free hydroxyl groups in monosaccharide unit of AG according to the data (Babkin et al. 2016; Medvedeva et al. 2003).

FTIR spectra were recorded with the use of Tensor-27 FTIR spectrometer (Bruker, Germany) in the wavelength range of 400–4000 cm^{-1}. Spectral data were processed by program OPUS (version 5.0). Solid samples were in tablets with matrix KBr (2 mg of a sample/1000 mg of KBr).

FT Raman spectra of the samples were recorded, using Bruker RFS 100/S spectrometer with a liquid-nitrogen cooled Ge diode, as detector. A cw-Nd:YAG-laser with an exciting line of 1.064 nm was applied as light source for the excitation of Raman scattering. The Raman spectra were recorded within a range of 200–3500 cm^{-1} using an operating spectral resolution of 2 cm^{-1}. A laser power output of 100 mW was used. Spectral data were processed with the use of the operating spectroscopy software OPUS Ver. 6.0 (Bruker Optik GmbH, Ettlingen Germany).

Proton-decoupled ^{13}C NMR spectra were recorded at 25 °C, using a Bruker Avance III spectrometer (600 MHz (^1H) and 155 MHz (^{13}C)) in D_2O. Acquired spectrum was processed after accumulation of 4096 transients with 10 s relaxation delay. 2D HSQC spectra were acquired, using edited-HSQC pulse sequence from Bruker library (hsqcedgp). About 80 mg of sample was dissolved in 0.6 ml of D_2O during 12 h.

The average molecular weight (M_w), number average molecular mass (M_n) and polydispersity (D) of the arabinogalactan and sulfated arabinogalactan samples were determined by gel permeation chromatography, using an Agilent 1260 Infinity II Multi-Detector GPC/SEC System chromatograph with triple detection: refractometer, viscometer and light diffusion. The separation was performed on two Aquagel-OH columns at 35 °C, using the solution 0.1 M $LiNO_3$ in water (pH = 7), as a mobile phase. The column was calibrated by the polyethylene glycol standards (Agilent, US). The eluent flow rate was 1 ml/min, and the sample volume was 100 µl. Prior to the analysis, the samples were dissolved in the
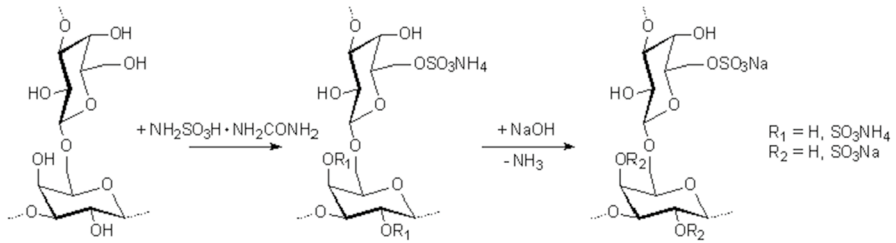
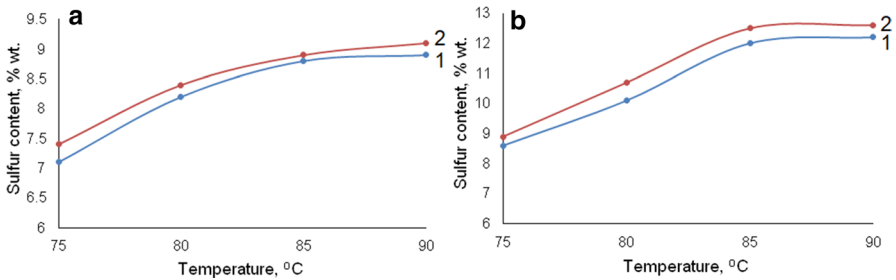**Fig. 1** Scheme of the arabinogalactan sulfation in DMSO medium with sulfamic acid in the presence of urea

Fig. 2 Effect of temperature of arabinogalactan sulfation in DMSO medium on the sulfur content in sulfated arabinogalactan: **a**—molar ratio AG/SC = 1:0.71; **b**—molar ratio AG/SC = 1:0.85 (1:2 h, 2:3 h)

mobile phase (~ 5 mg/ml) and filtered through a 0.22-µm PES membrane filter (Agilent). Data collection and data processing were performed, using the Agilent GPC / SEC MDS software.

Results and discussion

Sulfation of arabinogalactan with sulfamic acid in the presence of urea in DMSO medium

The sulfation of arabinogalactan in DMSO with sulfamic acid in the presence of urea and the subsequent isolation of sulfated arabinogalactan in the form of ammonium and sodium salts were carried out in a similar way (Fig. 1), as in the case of AG sulfation in dioxane medium (Vasilyeva et al. 2015).

Arabinogalactan is soluble in DMSO medium. Therefore, unlike dioxane solvent, the sulfation of AG in DMSO medium proceeds under homogeneous conditions. To optimize the process of AG sulfation by sulfamic acid—urea mixture in DMSO medium, the temperature, time and composition of reaction mixture were varied (Table 1, Fig. 2).

As follows from the obtained data, the sulfur content in AG increases with the increase in the sulfation process temperature, time and sulfating reagent amount (Fig. 2). The highest degree of AG sulfation (sulfur content of 12.0–12.5 wt %)
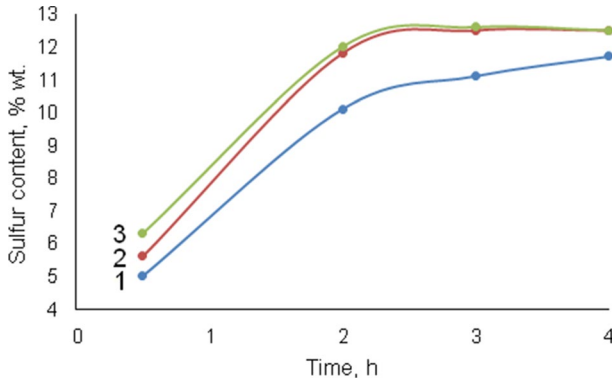
Fig. 3 Effect of time of arabinogalactan sulfation in DMSO medium on the sulfur content in sulfated AG at different temperatures and molar ratio AG/SC=1:0.85 (1–80 °C, 2–85 °C, 3–90 °C)
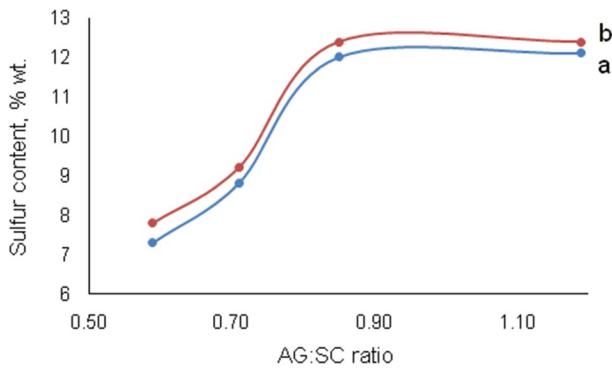

Fig. 4 Effect of molar ratio AG/SC on the sulfur content in the products of AG sulfation in DMSO medium at a temperature of 85 °C during 2 h (**a**) and 3 h (**b**)

is reached after 2–3 h sulfation at temperatures of 85–90 °C and a molar ratio AG/SC equal to 1:0.85 (Fig. 3). At the same time, an increase in the molar ratio AG/SC from 0.85 to 1.19 does not have a significant effect on the sulfur content in the product (Fig. 4). It should be noted that the sulfation of AG under these conditions proceeds with a maximum conversion rate (40–42%).

The sulfation of AG at temperatures of 85–90 °C during 4 h leads to a decrease in the yield of the sulfated product (Table 1) due to the formation of low-molecular weight fractions of the sulfated polysaccharides, which are removed at the stage of product dialysis.

Mathematical optimization of arabinogalactan sulfation process

The task of mathematical optimization was to calculate the optimal conditions for sulfation of arabinogalactan with sulfamic acid–urea mixture in DMSO medium, ensuring the production of sulfated AG with a maximum sulfur content (wt %).

When constructing mathematical models, the following factors were used as independent variables: temperature (X_1), duration of the sulfation process (X_2) and molar ratio AG/SC (X_3). The result of the optimization of the sulfation process was characterized by one output parameter: the sulfur content in sulfated arabinogalactan (Y_1).

The results of analysis of variance are shown in Table S1 (in Electronic Supplementary Material).

Analysis of variance showed that within the accepted experimental conditions the greatest contribution to the total variance of the output parameter is made by two factors – the duration of the arabinogalactan sulfation process and molar ratio AG/SC. This is indicated by the high values of the dispersion coefficients F for the main effects, also called the efficiencies.

The dependence of the sulfur content in arabinogalactan sulfates (Y_1) on variable process factors is approximated by the regression equation:

$$\begin{aligned} Y_1 = & -86.0898 + 1.73944 \times X_1 + 1.32013 \\ & \times X_2 + 26.4069 \times X_3 \\ & -0.0104456 \times X_1^2 + 0.0264927 \times X_1 \times \\ & \times X_2 + 0.132832 \times X_1 \times X_3 - 0.768574 \\ & \times X_2^2 + 1.2737 \times X_2 \times X_3 - 18.8043 X_3^2 \end{aligned} \quad (4)$$

The predictive properties of Eq. (4) are shown in Fig. S1 (in ESM), which compares the values of the output parameter Y_1 obtained in the experiment with the values calculated using Eq. (4).

The value of coefficient of determination $R^2_{adj} = 90.0\%$ indicates a good quality of approximation.

The mathematical model is used to graphically display the dependence of the output parameter Y_1 on the variable factors in the form of a response surface (Fig. 5).

The maximum predicted sulfur content 13.0% wt. in the studied area of the factor space is achieved, at the point corresponding to the following values of the variable factors: process temperature 85.6 °C, process duration 3.7 h and molar ratio AG/SC 1:1.08.

Kinetics of arabinogalactan sulfation process

The kinetics of the process of arabinogalactan sulfation by sulfamic acid—urea mixture was studied in the temperature range of 75–85 °C with variation in the ratio of arabinogalactan and sulfating complex (Fig. S2 in ESM).
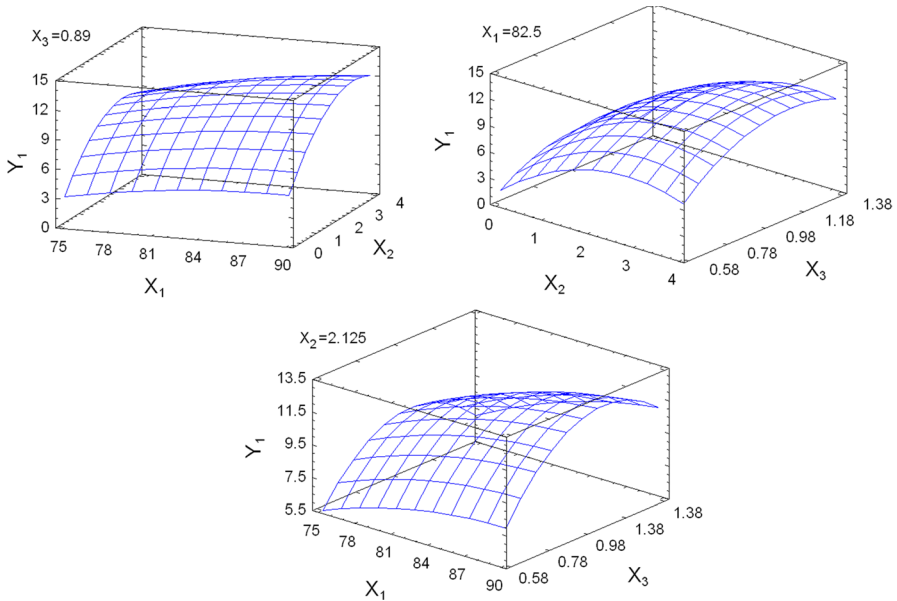
Fig. 5 Response surface of the output parameter Y_1 (sulfur content, % wt) depending on variable factors

The rates of AG sulfation process were calculated based on the change in the sulfur content in sulfated arabinogalactan. The reaction rate constants were calculated using the first order equation (Laidler 1987):

$$Lnk = LnC - \frac{E_a}{R} \times \frac{1}{T} \tag{5}$$

The value of activation energy of the sulfation process was determined using temperature dependence of rate constants in the Arrhenius coordinates (Fig. S3 in ESM).

The calculated rate constants and activation energies of the process of arabinogalactan sulfation at different molar ratios AG/SC are given in Table 2.

The low values of the activation energy probably indicate that the process of arabinogalactan sulfation is carried out in the diffusion region (Karger et al. 2005).

Table 2 Rate constants and activation energies of the arabinogalactan sulfation in DMSO medium at different molar ratios AG/SC

Molar ratio AG/SC	T (°C)	$k*10^{-5}$ (s^{-1})	E_a (kJ/mol)
1:0.71	75	1.8	13.1
	80	1.9	
	85	2.0	
1:0.85	75	2.0	10.2
	80	2.1	
	85	2.3	

Low activation energy values were also observed when abies ethanol lignin was sulfated with a mixture of sulfamic acid—urea in 1,4-dioxane medium (Kuznetsov et al. 2020) and starch was sulfated in an eutectic solvent—a mixture of sulfamic acid and urea (Akman et al. 2020). These results suggest that the sulfation of natural polymers under the studied conditions mainly occurs in the diffusion region.

Characterization of sulfated arabinogalactan samples

The structure of sulfated AG samples was studied by FTIR, FT Raman, ^{13}C NMR and GPC methods.

In the FTIR spectra of sulfated arabinogalactan (Fig. 6), in contrast to the initial AG, the new absorption bands at 820 cm^{-1} and 1260 cm^{-1} are present that evidences the introduction of the sulfate groups into AG structure. The high-intensity absorption band at 1260 cm^{-1} belongs to asymmetric stretching vibrations $\upsilon_{as}(O{=}S{=}O)$ of sulfate group. The specific absorption band at 820 cm^{-1} corresponds to the stretching vibrations $\upsilon(C{-}O{-}S)$ of the sulfated arabinogalactan sodium salt (Pereira et al. 2003).

The intense absorption band at 3423 cm^{-1} assigned to the stretching vibrations of hydrogen-bonded OH groups is observed in the spectrum of the initial AG. However, in the spectrum of sulfated AG its intensity decreases, and the band is shifted to the high-frequency region of 3450 cm^{-1} due to substitution with sulfate groups. In addition, a fall in the intensity and a shift from 1376 to 1385 cm^{-1} of
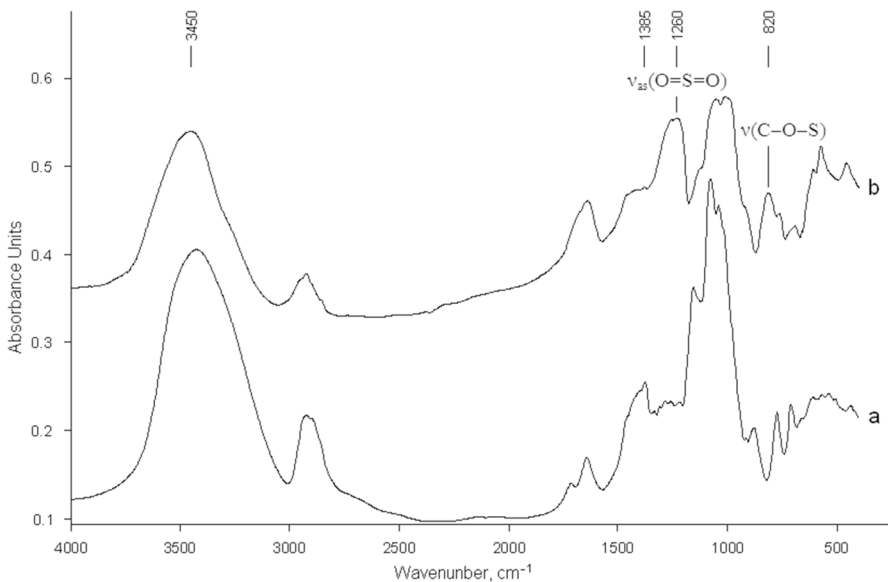

Fig. 6 FTIR spectra of AG (**a**) and sulfated AG (sulfur content 12.4 wt %) (**b**)
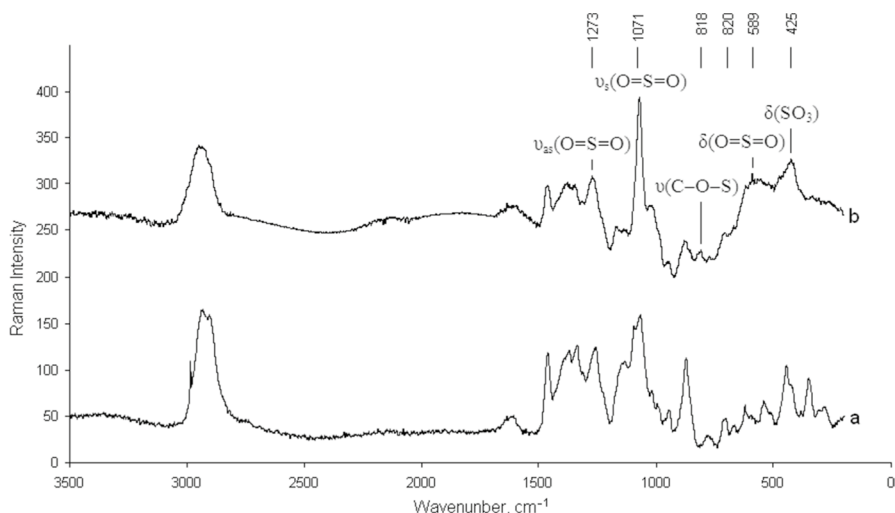
Fig. 7 FT Raman spectra of AG (**a**) and sulfated AG (sulfur content 12.4 wt %) (**b**)

the absorption band belonging to in-plane bending vibrations of OH groups were observed.

The introduction of the sulfate groups into the AG structure was also confirmed by the appearance of new absorption bands at 425, 589, 818, 1071, 1273 cm^{-1} in the Raman spectra of the sodium salts of sulfated AG samples (Fig. 7).

The absorption band at 425 cm^{-1} corresponds to the deformation vibrations of $\delta(SO_3)$, while the band at 589 cm^{-1} refers to the deformation vibrations $\delta(O=S=O)$. The absorption band at 818 cm^{-1} was attributed to the C–O–S stretching vibrations $v(C$–O–$S)$. The absorption bands of stretching vibrations $v(C$–$O)$, $v(C$–$C)$ and in-plane bending vibrations of COH groups dominate in the region of 1050–1150 cm^{-1} of Raman spectrum of the initial arabinogalactan (Wiercigroch et al. 2017). Whereas, due to the introduction of sulfate groups, the absorption band at 1071 cm^{-1} attributed to symmetric stretching vibrations $v_s(O=S=O)$, dominates in the spectrum of sulfated AG. The peak at 1273 cm^{-1} corresponds to asymmetric stretching vibrations $v_{as}(O=S=O)$.

FTIR and FT Raman spectra of sulfated AG samples, synthesized in the medium of DMSO with the use of sulfamic acid—urea mixture were similar to spectra of samples, prepared by AG sulfation in dioxane medium (Kuznetsov et al. 2017; Vasilyeva et al. 2015), AG sulfation by chlorosulfonic acid (Tang et al. 2019; Vasilyeva et al. 2013, 2014) and potassium persulfate (Kostyro and Stankevich 2015). This indicates a similar structure of sulfated AG samples prepared by all these methods.

It is known (Babkin et al. 2016) that AG isolated from the larch wood has a highly branched structure. The main chain consists predominantly of 3,6-di-O-substituted β-D-galactopyranose fragments, while the side chains consist of 6-O-substituted β-D-galactopyranose fragments and terminal β-D-galactopyranose fragments which have no substituents (Fig. S4 in ESM).
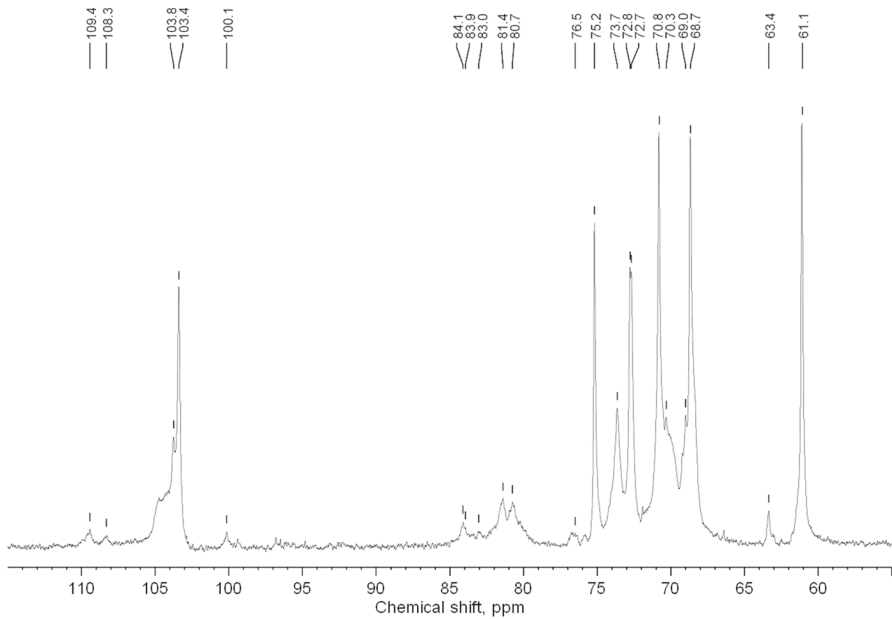
Fig. 8 ^{13}C NMR spectrum of AG isolated from *Larix sibirica* wood

Table 3 Assignment of signals in ^{13}C NMR spectrum of *Larix sibirica* AG to the corresponding carbon atoms

Structural unit	Chemical shifts (ppm)					
	C1	C2	C3	C4	C5	C6
β-D-Galp-(1→	103.4	70.8	72.8	68.7	75.2	61.1
→6)-*β*-D-Galp-(1→	103.4	70.8	72.7	68.7	73.7	69.3
→3,6)-*β*-D-Galp-(1→	103.8	70.3	81.4	68.7	73.7	69.8
α-L-Araf-(1→	109.4	80.7	76.5	83.9	61.5	
β-L-Arap-(1→	100.1	68.4	68.8	69.0	63.4	
→3)-*α*-L-Araf-(1→	108.3	80.3[a]	84.1	83.0	61.5	

[a]Assignment of some minor arabinose peaks was carried out according to Ponder and Richards (1997a)

Arabinose units are included in the AG macromolecule mainly as side chains consisting of 3-O-substituted L-arabinofuranose fragments and terminal fragments of *β*-L-arabinopyranose, *β*-D-arabinofuranose and α-arabinofuranose (Ponder and Richards 1997b). The arabinose units may also be present in the main chain of the AG macromolecule (Medvedeva et al. 2003).

The additional data about the structure of AG isolated from *Larix sibirica* wood were obtained from ^{13}C NMR (Fig. 8 and Table 3) and HSQC (Fig. S5 in ESM) spectra.

The structure of AG isolated from wood of Siberian larch (*Larix sibirica*) is similar to arabinogalactans isolated from *Larix occidentalis* (Ponder and Richards 1997a) and American larch *Larix laricina* (Goellner et al. 2011).

The reactions of AG sulfation can proceed with the participation of various hydroxyl groups of the main structural units of AG macromolecules (Fig. S4 in ESM).

The sulfation of *Larix sibirica* AG with SO_3–dimethylformamide complex in dimethyl sulfoxide (DMSO) medium and the sulfation of AG with potassium persulfate in DMSO medium were described earlier by Ganenko et al. (2015), Kostyro and Stankevich (2015) and Medvedeva et al. (2014). It was found that the substitution of hydroxyl groups by sulfate groups leads to a downfield shift of the signals of associated carbon atoms by ~6 ppm in the ^{13}C NMR spectrum (α-effect of sulfation), while the signals of neighboring carbon atoms shifted upfield by ~2 ppm (β-effect). Similar changes in the chemical shifts were observed, when comparing ^{13}C NMR spectra of the initial and sulfated AG samples, obtained using the SO_3–pyridine complex in pyridine solvent (Vasilyeva et al. 2013) with those obtained with the use of sulfamic acid—urea mixture in dioxane solvent (Vasilyeva et al. 2015). Taking these experimental data into account, the presence and location of sulfate groups in the sulfated arabinogalactan sample, obtained with the use of sulfamic acid—urea mixture and DMSO solvent were proved from ^{13}C NMR spectrum (Fig. 9). The assignment of signals to the corresponding carbon atoms in the polysaccharide units is given in Table 4. It should be noted that due to the overlapping of some peaks the analysis based on ^{13}C chemical shifts was not enough to reliably assign them to
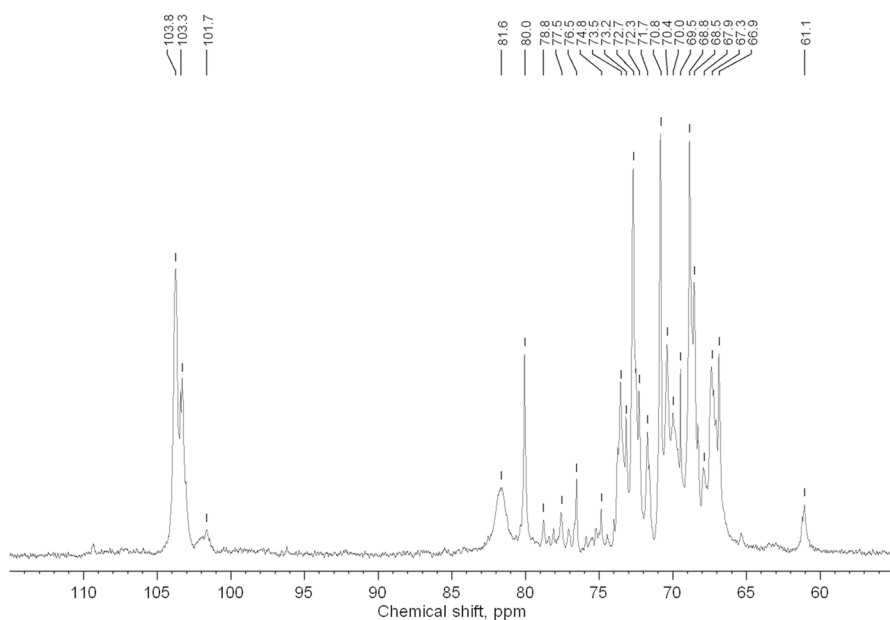

Fig. 9 ^{13}C NMR spectrum of sulfated AG sample obtained with the use of sulfamic acid—urea mixture and DMSO solvent (sulfur content 12.4 wt%)

Table 4 Assignment of signals in ^{13}C NMR spectrum of sulfated AG to the corresponding carbon atoms (C-sulfation in the corresponding position, C'-under the influence of sulfation to the adjacent position)

Structural unit	Chemical shifts (ppm)					
	C1/C1'	C2/C2S	C3/C3'	C4/C4S	C5/C5'	C6/C6S
β-D-Galp-(1→	103.3/–	70.8/–	72.7/–	68.8/–	–/72.3	61.1/67.3
→6)-β-D-Galp-(1→	103.3/–	70.8/–	72.7/–	68.8/–	73.5/–	69.5/–
→3,6)-β-D-Galp-(1→	103.8/101.7	70.4/76.5	81.6/78.8	68.8/74.8	73.5/71.7	70.0/–

specific carbon atoms. Thus, some of the information was obtained from the HSQC spectrum with DEPT editing (Fig. S6 in ESM).

As follows from the data, given in Table 4, some well-identified signals of carbon atoms of β-D-galactopyranose units of the initial AG macromolecule disappear in the ^{13}C NMR spectrum of the sulfated AG and the new additional signals of carbon atoms appear. This indicates that the structural changes take place in the AG macromolecule during the sulfation process. The signal of average intensity at 67.3 ppm, which is absent in the spectrum of the initial AG, was assigned to C6 atoms, associated with a sulfate group in the terminal β-D-galactopyranose units. This is evidenced by a noticeable decrease in the signal intensity at 61.1 ppm of the C6 atoms, associated with primary hydroxyl groups in these units. This signal may also be attributed to C5 carbon atoms of α-L-arabinofuranose, which signals are also located in this region (Ponder and Richards 1997a).

The predominant sulfation of the primary hydroxyl groups at C6 carbon atoms of the terminal β-D-galactopyranose units is confirmed by the complete disappearance of the signal of the neighboring C5 carbon atom at 75.2 ppm, accompanied by the appearance of a low-intensity signal at 72.3 ppm. At the same time, the signal of C5 carbon atoms undergoes a shift by 2.9 ppm in a high field.

The presence of sulfate groups at C2 and C4 carbon atoms in some part of the β-D-galactopyranose units of the main chain is proved by the appearance of additional weak signals of these carbon atoms at 76.5 and 74.8 ppm. The chemical shifts of nonsulfated C2 and C4 carbon atoms of the β-D-galactopyranose units are of 70.4 and 68.8 ppm, respectively.

Similar changes in chemical shifts caused by sulfation of hydroxyl groups of neighboring carbon atoms are also observed for C1, C3 and C5 atoms of the β-D-galactopyranose units of the main chain. The signals of C1, C3 and C5 atoms at 103.8, 81.6 and 73.5 ppm are partially shifted by 1.8–2.8 ppm (β-effect) and in the spectrum of sulfated AG are assigned to peaks at 101.7, 78.8 and 71.7 ppm, respectively.

It should be noted that the ^{13}C NMR spectrum of sulfated AG also contains signals of low intensity corresponding to the carbon atoms of the arabinose units. However, due to the rather weak intensity of these signals because of the low content of arabinose units in AG (Babkin et al. 2016) and due to the overlapping of some signals, it is difficult to assign them.
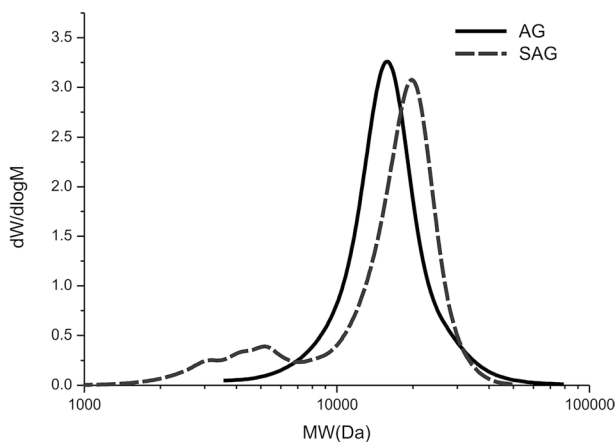
Fig. 10 Molecular weight distribution (MWD) curves of AG and sulfated AG (sulfur content 12.4 wt%)

Table 5 Molecular mass characteristics of AG and sulfated AG samples

N	Sample	M_p (kDa)	M_n (kDa)	M_w (kDa)	D
1	Initial AG	15.6	14.4	16.8	1.17
2	Sulfated AG	19.7	14.5	18.8	1.30

Comparison of the results of ^{13}C NMR study of sulfated AG samples, prepared by described method, with those prepared under the heterogeneous conditions (Vasilyeva et al. 2013, 2015) shows that the sulfation in DMSO solvent under the homogeneous conditions promotes a predominant substitution of the primary hydroxyl groups at C6 carbon atoms of the terminal galactose units of main and side AG chains. Meanwhile, the hydroxyl groups, associated with C2 and C4 carbon atoms of the main galactan chain, undergo partial sulfation. These results are in full agreement with the data of ^{13}C NMR study of sulfated AG samples obtained under the homogeneous conditions of DMSO medium with the use of SO$_3$–dimethylformamide complex (Medvedeva et al. 2014).

Molecular mass is one of the most important characteristics of polymers, especially biologically active ones. The curves of the molecular weight distribution (MWD) of both, the initial AG and its sulfated derivative, obtained using the gel permeation chromatography method with PEG/PEO as standard (Fig. 10) have a monomodal distribution, with a clear symmetric peak, which indicates a high homogeneity of the samples, as described by Tang et al. (2019). At the same time, the main peak of the MWD of sulfated AG shifts to the region of high-molecular weights, and the appearance of a low-intensity region with a molecular mass of 2–7 kDa is observed. The weak change in the MWD profile indicates that only small degradation of polymer chains takes place during the sulfation process. Some destruction of polymer chains of AG under experimental conditions can be caused by acid hydrolysis and thermal breaking of weak bonds (Xu et al. 2016). The molecular weight characteristics of the initial AG and sulfated AG samples

are given in Table 5. The average molecular weight of sulfated AG increases from 16.8 to 18.8 kDa, which is consistent with the data, reported in Tang et al. (2019) and Ganenko et al. (2015).

The experimentally determined value of the average molecular weight of sulfated AG turned out to be less than that calculated, taking into account the degree of substitution of sulfated AG. This is probably due to the different DS of sulfated low-molecular and high-molecular fractions of arabinogalactan. A higher DS should have low-molecular weight fractions in which hydroxyl groups are more accessible for interaction with a sulfating agent. Due to the appearance of low-molecular weight fractions, the polydispersity of sulfated AG increases from 1.17 to 1.30. Thus, the MWD of sulfated AG is determined by two opposite factors—an increase in the molecular weight due to the introduction of OSO_3Na groups into the polymer chain and its decrease as a result of destruction. A similar polydispersity value was observed for a sample of AG sulfate, obtained by sulfating with sulfamic acid under heterogeneous conditions in a 1,4-dioxane medium. Moreover, a decrease in the sulfating temperature contributed to the formation of AG sulfate with a higher average molecular weight (Kuznetsova et al. 2020).

Conclusion

A new method of arabinogalactan sulfation with low-toxic sulfamic acid—urea mixture in DMSO medium under homogeneous conditions is suggested. Optimal conditions for the production of sulfated AG with a high yield and sulfur content (12.0–12.5 wt %) and high conversion rate (41.6–44.5%) are established: temperature of 85–90 °C, molar ratio of AG to sulfating agent 1:0.85, sulfation time 2–3 h.

The composition and structure of sulfated arabinogalactan were proven by FTIR, FT Raman, ^{13}C NMR, GPC methods and by elemental analysis.

The presence of sulfate groups in the resulting AG sulfates was confirmed by FTIR and FT Raman spectroscopy. It was shown by ^{13}C NMR spectroscopy that the predominant part of sulfate groups in sulfated arabinogalactan is located at the C6 carbon atoms of the galactose terminal units of the main and side chains. The hydroxyl groups, associated with C2 and C4 carbon atoms of the main galactan chain, are partially sulfated. The sulfation of AG with sulfamic acid under homogeneous conditions, thanks to the use of DMSO, as a solvent, instead of 1,4-dioxane contributes to a more complete substitution of the primary hydroxyl groups at C6 carbon atoms of the galactose terminal units of the main and side AG chains.

The absence of significant depolymerisation of AG during its sulfation with sulfamic acid—urea mixture in DMSO is indicated by GPC data. The sample of sulfated AG is characterized by a rather narrow molecular weight distribution with an average molecular weight of 18.8 kDa and a polydispersity of 1.3.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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