

The Raman Spectroscopy, XRD, SEM, and AFM Study of Arabinogalactan Sulfates Obtained Using Sulfamic Acid

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Received November 18, 2016; in final form, November 21, 2016

Abstract—The structure of sodium salts of arabinogalactan (AG) sulfates obtained by sulfating AG of larch wood with a sulfamic acid–urea mixture in 1,4-dioxane was studied by the methods of Raman spectroscopy, X-ray diffraction (XRD) phase analysis, scanning electron microscopy (SEM), and atomic force microscopy (AFM). The introduction of sulfate groups into the structure of arabinogalactan was confirmed by the appearance in the Raman spectra of new absorption bands related to the deformation vibrations δ (SO_3) at 420 cm^{-1} and δ ($\text{O}=\text{S}=\text{O}$) at 588 cm^{-1} , stretching vibrations ν ($\text{C}-\text{O}-\text{S}$) at 822 cm^{-1} , symmetrical stretching vibrations ν_s ($\text{O}=\text{S}=\text{O}$) at 1076 cm^{-1} , and asymmetric stretching vibrations of ν_{as} ($\text{O}=\text{S}=\text{O}$) at 1269 cm^{-1} . According to the XRD data, the amorphization of arabinogalactan structure occurs during the sulfation process. The SEM method revealed a significant difference in the morphology of the sulfated and starting arabinogalactan. The starting AG consists of particles of predominantly globular shape with a size of 10 to 90 μm ; arabinogalactan sulfates, of particles of various shapes with sizes of 1–8 μm . According to the AFM, the surface of sulfated arabinogalactan film consists of rather homogeneous spherical particles about 70 nm in size. The root-mean-square value of the surface roughness is 33 nm. The surface of sulfated AG film does not contain impurities.

Keywords: physicochemical investigation, sulfated arabinogalactan, “green” synthesis, sulfamic acid, urea

DOI: 10.1134/S106816201707010X

INTRODUCTION

Sulfated derivatives of arabinogalactan (AG) are analogues of heparin, a substance of animal origin possessing anticoagulant activity [1]. However, heparin can be infected with pathogens and viruses, which leads to the need to search for its synthetic and natural analogues. Sulfated derivatives of polysaccharides exhibit a high anticoagulant and hypolipidemic activity, which makes their use promising for the prevention and treatment of cardiovascular diseases [2].

AG sulfates can be prepared using various sulfating agents: SO_3 –DMF complex in DMSO [3], SO_3 –pyridine complex in DMF [4], SO_3 –pyridine complex in pyridine [5]. In all the above processes for preparation of sulfated derivatives of AG, toxic or environmentally hazardous sulfating reagents and solvents are used.

We have previously found the possibility of arabinogalactan sulfation with sulfamic acid in dioxane in the presence of a basic catalyst, urea [6]. This allowed

the development of a new method, simpler and more environmentally friendly than the known ones, for the synthesis of sulfated arabinogalactan. AG sulfates obtained by this method were studied by the IR and ^{13}C NMR spectroscopy methods [6].

In this work, we performed an in-depth physicochemical study of AG sulfates by the methods of Raman spectroscopy, X-ray diffraction (XRD) phase analysis, scanning electron microscopy (SEM), and atomic force microscopy (AFM).

EXPERIMENTAL

Arabinogalactan (AG) of the Siberian larch (*Larix sibirica* Ledeb.) wood produced by Khimiya Drevesiny company (Irkutsk, Russia) under the trade name FibrolarS was used as the starting material.

Sulfation of AG was carried out with sulfamic acid in dioxane in the presence of a basic catalyst, urea, according to the procedure [6]. Then, the resulting ammonium salt of AG sulfate was converted into the sodium salt (sulfur content 13.0 wt %). Transparent

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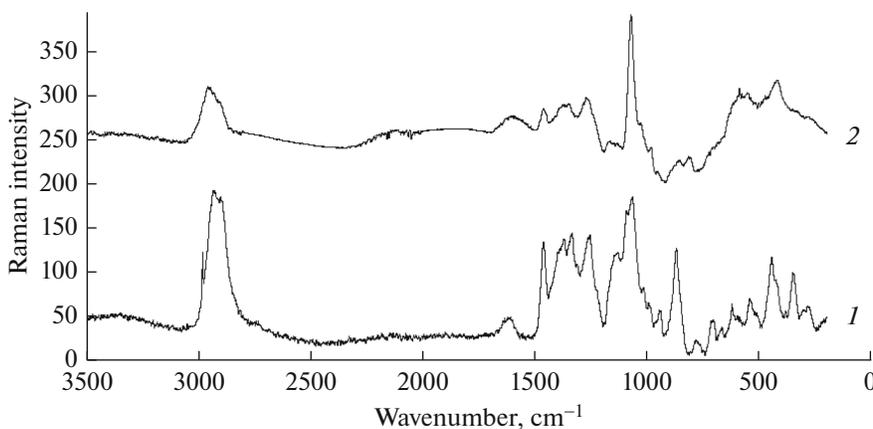


Fig. 1. Raman spectra of samples of (1) the starting AG and (2) sulfated AG.

films of the sulfated AG samples were obtained by evaporation of 6–7% aqueous solutions on a smooth glass substrate.

The Raman spectra of the samples were recorded on a Bruker RFS 100/S spectrometer with Ge diode as a detector cooled with liquid nitrogen. The CW Nd:YAG laser with an excitation line of 1.064 μm was used as a light source for the Raman scattering excitation. The Raman spectra were recorded in the range of 200–3500 cm^{-1} using an operating spectral resolution of 2 cm^{-1} . Data analysis was performed using OriginPro 7.0 (OriginLab Corporation, United States).

The X-ray diffraction (XRD) phase analysis was carried out on a DRON-3 X-ray diffractometer using $\text{CuK}\alpha$ monochromatized radiation ($\lambda = 0.154 \text{ nm}$), voltage 30 kV, current 25 mA. The scanning step is 0.02 deg; intervals for 1 s per data point. The measurement was carried out in the interval of the Bragg angles 2θ from 5.00 to 70.00 deg.

The electron microimages were obtained with a TM-1000 HITACHI scanning electron microscope (Japan) with an accelerating voltage of 15 kV and a magnification from 100 to 10000 \times with a resolution of 30 nm.

Study of the sulfated AG films by AFM in a semi-contact mode was carried out using a Solver P47 multi-mode scanning probe microscope (NT-MDT, Moscow). Scanning was performed at no less than 3–4 points at several sites. Scan speed was 1.5–2.0 Hz, the resolution of the resulting image was 256 \times 256 pixels.

RESULTS AND DISCUSSION

In continuation of the work on studying the AG sulfates obtained by sulfation with sulfamic acid in the

presence of urea in dioxane [6], we examined the sodium salts of AG sulfates by the methods of Raman spectroscopy, XRD, SEM, and AFM.

The introduction of the sulfate group into the AG structure was confirmed by the appearance of new absorption bands in the regions of 420, 588, 822, 1076, and 1269 cm^{-1} in the Raman spectra of sodium salts of sulfated AG samples (Fig. 1).

The absorption band at 420 cm^{-1} corresponds to the deformation vibrations of the SO_3 groups $\delta(\text{SO}_3)$, and the band at 588 cm^{-1} can be attributed to the deformation vibrations $\delta(\text{O}=\text{S}=\text{O})$. The absorption band at 822 cm^{-1} is inherent in the C–O–S stretching vibrations $\nu(\text{C}–\text{O}–\text{S})$. The character of absorption in the region of 1050–1150 cm^{-1} of sulfated arabinogalactan differs significantly from the character of absorption of the initial AG. In this region in the Raman spectrum of polysaccharides, there can be the absorption bands of stretching vibrations $\nu(\text{COC})$ of glycosidic bonds and pyranose cycles, and for sulfated polysaccharides symmetric stretching vibrations $\nu_s(\text{O}=\text{S}=\text{O})$ [7]. In the spectrum of sulfated AG, the absorption band at 1076 cm^{-1} that belongs to the symmetric stretching vibrations $\nu_s(\text{O}=\text{S}=\text{O})$ dominates. The peak at 1269 cm^{-1} is a signal of asymmetric stretching vibrations $\nu_{as}(\text{O}=\text{S}=\text{O})$.

Samples of AG isolated from larch wood have an amorphous structure [8]. A comparison of the X-ray diffraction patterns of the samples of the initial AG and AG sulfated with sulfamic acid in the presence of urea in dioxane (Fig. 2) showed that further amorphization of the material structure occurred during the sulfation process. In the X-ray diffraction pattern of the sample of sulfated AG, we observed peak smoothing in the range of angles from 15 $^\circ$ to 25 $^\circ$ θ .

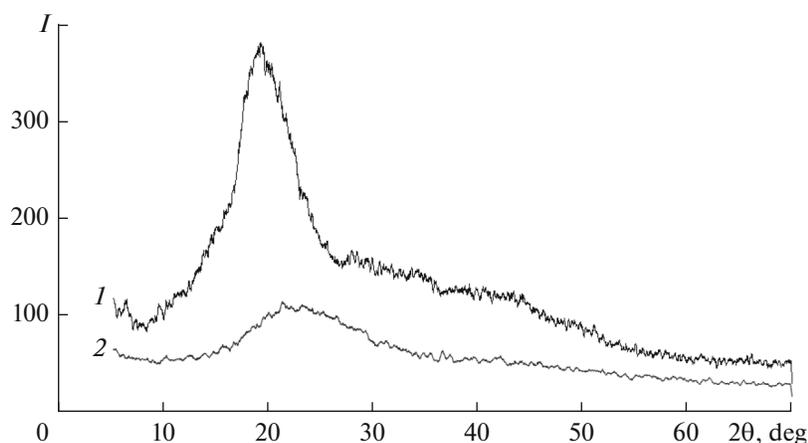


Fig. 2. X-ray diffraction patterns of samples of (1) the starting AG and (2) sulfated AG.

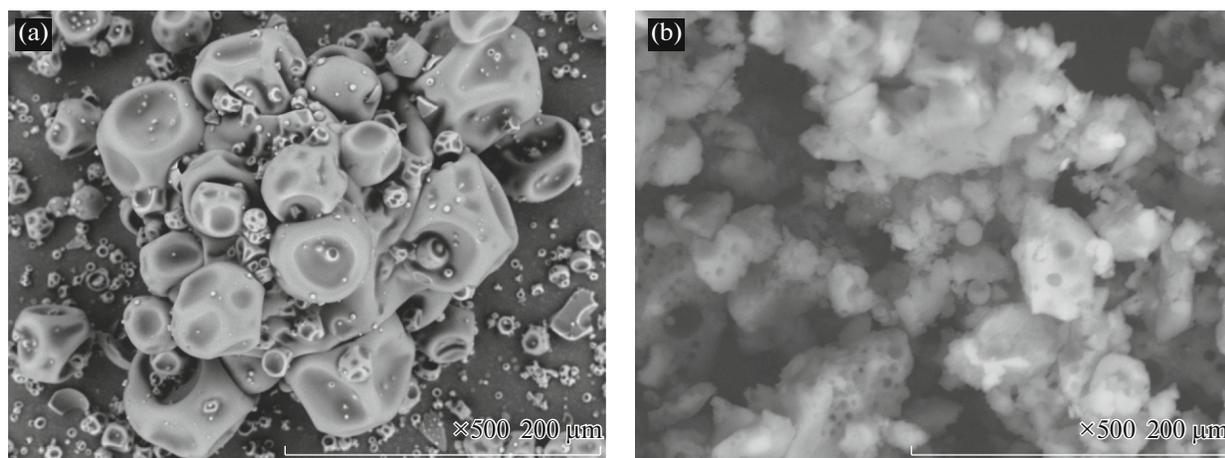


Fig. 3. SEM images of samples of (a) the starting AG and (b) sulfated AG.

The study of AG samples before and after sulfation was carried out by the method of scanning electron microscopy (Fig. 3). The initial AG consists of aggregated and single particles of various shapes and sizes. Particles of a globular shape with a diameter of 10 to 90 μm predominate. There are also particle agglomerates, the size of which reaches 300 μm.

After sulfation, the samples have morphology somewhat different from that of the starting AG (Fig. 3). Sulfated AG consists of particles of different shapes with prevailing sizes of 1–8 μm; in this case, some particles form agglomerates with sizes of 12–20 μm.

The synthesized films of sulfated AG were studied by the AFM method. Unlike SEM, the AFM method allows measuring not only the lateral dimensions of

nanoscale objects, but also their height with high accuracy up to 0.1 nm [9].

According to the AFM data (Figs. 4a and 4d), the surface of the sulfated AG film is formed by sufficiently homogeneous spherical particles with an average diameter of 70 nm. The value of the root-mean-square roughness calculated from the cross-section profile of the surface (Fig. 4c) is 33 nm.

According to the phase contrast images of (Fig. 4b), the surface of the sulfated AG film is sufficiently homogeneous and does not contain impurities.

CONCLUSIONS

Arabinogalactan sulfates obtained by a new method, sulfating of AG from larch wood with sulfamic acid in dioxane in the presence of urea, were

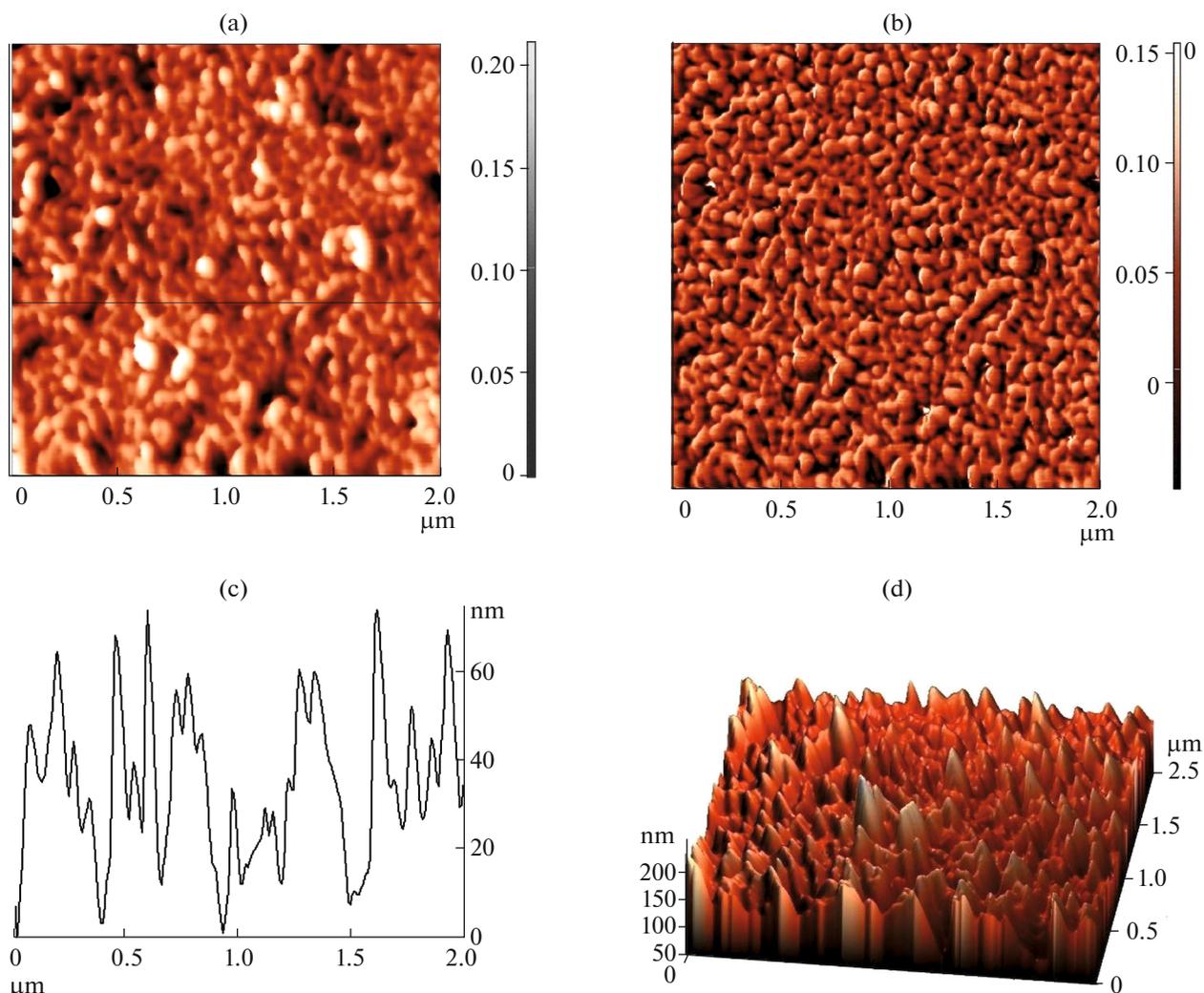


Fig. 4. Typical AFM image of the sulfated AG film: (a) relief; (b) phase contrast; (c) cross-sectional profile along the line in image (a); (d) relief in 3D representation.

studied by the methods of Raman spectroscopy, XRD, SEM, and AFM. The introduction of sulfate groups in the polysaccharide structure was confirmed by the appearance of new absorption bands characteristic of the vibrations of the sulfate group bonds in the Raman spectra.

It has been shown that a change in the AG morphology and further amorphization of the structure occurs during AG sulfation. In contrast to the starting AG that mainly consists of spherical particles ranging in size from 10 to 90 μm , the sulfated AG is formed by particles of various shapes with sizes of 1–8 μm .

According to the AFM data, the surface of the sulfated AG film is formed by sufficiently homogeneous crystallites of spherical shape with an average diameter of 70 nm and does not contain impurities.

ACKNOWLEDGMENTS

The work was financially supported by the Russian Foundation for Basic Research and the Government of the Krasnoyarsk krai (project no. 16-43-242083).

The instruments of the Krasnoyarsk Regional Center for Collective Use of the Siberian Branch of the Russian Academy of Sciences were used.

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Translated by G. Levit