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Solid-phase mechanochemical incorporation of a spin label into cellulose

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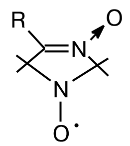
Samples of cellulose labeled with stable nitroxyl radicals were prepared by mechanochemical synthesis. The samples were studied by IR and EPR spectroscopies, X-ray phase analysis, and electron microscopy. The EPR spectral patterns indicate a uniform distribution of "grafted" paramagnetic centers over the cellulose macromolecular chains. X-Ray diffraction patterns obtained and the results of crystallinity index calculations for the samples showed that strong bonding of spin labels causes changes in the cellulose structure up to nearly complete amorphization of the material.

Key words: mechanochemistry, cellulose, imidazoline nitroxyl radicals, spin label.

The mechanochemical investigations of high-molecular-weight compounds cover, in particular, cellulose and its derivatives.[1] The subjects of research include the mechanical degradation and changes in the macrostructure of compounds, the synthesis of copolymers, and the preparaton of intermolecular complexes including the complexes with pharmaceuticals.[2—9] Cellulose samples mechanically treated in Bridgeman anvils were studies by EPR spectroscopy.[10] In this case, paramagnetic centers (PCs) are generated radiochemically or by treating the sample with a solution of nitroxyl radical (the case for incorporation of a spin label).

In this work we studied a solid-phase mechanochemical procedure for incorporation of spin labels (stable imidazoline nitroxyl radicals,[11] namely, 1-oxyl-4R-2,2,5,5-tetramethyl-3-imidazoline-3-oxides (**1—3**)) into cellulose.



1—3

R = Me (**1**), COOH (**2**), CH_2Br (**3**)

The aim of the work was not only to prepare spin-labeled cellulose samples but also evaluate the possibility of solid-phase mechanochemical "grafting" of low-molecular-weight organic residues to cellulose using highly sensitive EPR spectroscopy.

To this end, the following samples were mechanically treated: **I**) mixture of microcrystalline cellulose (MCC) and powdered radical **1** (in order to obtain an intercalate or prepare intermolecular complexes of the components), **II**) mixture of MCC and powdered radical **2** (in order to form an ester bond between the radical carboxyl group and cellulose hydroxyls), and **III**) mixture of MCC and powdered radical **3** in the presence of NaOH (in order to form an ether bond between the radical methylene group and cellulose hydroxyls).

Experimental

Experiments were carried out using microcrystalline cellulose (MCC TU-6-09-10-1818-87, purchased from MC Center, Dzerzhinsk, Russia), corresponding imidazoline-*N*-oxyl derivatives synthesized at the N. N. Vorozhtsov Novosibirsk Institute of Organic Chemistry, Siberian Branch of the Russian Academy of Sciences, and commercially available reactants.

Mechanical treatment was carried out in an AGO-2 planetary ball mill designed and manufactired at the Institute of Solid-State Chemistry and Mechanochemistry, Siberian Branch of the Russian Academy of Sciences (Novosibirsk, Russia) at an acceleration of 60g. The drum volume was 40 mL, the total weight of steel balls of diameter 6 mm was 75 g, and the overall loading of the material was 2.5—3.5 g.

Samples **I** and **II** were prepared from 0.05—0.2 g of radicals **1** and **2**, respectively, and MCC (2.4g). The duration of mechanochemical treatment was 1, 3, and 10 min.

Sample **III** was prepared as follows. First, the mill was loaded with MCC (2.4 g) and NaOH (6.6 g) and the mixture was treated for 3 min to obtain alkali cellulose. Then, radical **3** (0.05—2 g) was added to the reaction mixture and the resulting mixture was treated for 1, 3, and 10 min.

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Mechanically treated materials were successively washed with water, acetone, and isopropyl alcohol. Each time the material was suspended in corresponding solvent (×50 mL), filtered off, and dried on the filter. The samples were washed until a constant EPR spectrum.

EPR spectra in the 3-cm band were recorded on a SE/X-2544 spectrometer (Poland). Powder X-ray diffraction patterns were obtained on a Dron-4-07 diffractometer (Russia) with a Co cathode in the angle interval from 0 to 60°.

IR spectra of samples (KBr pellets) were recorded on a Specord-IR spectrometer.

Electron micrographs were obtained using a Tesla BS-242E electron microscope with a maximum magnification up to ×1500.

Results and Discussion

The EPR spectra of the starting powdered radical **2**, mechanically treated mixture **2**+MCC, and "cleaned" samples are shown in Fig. 1. The EPR spectra of powdered radicals **1** and **3** are qualitatively similar, being mainly different in signal amplitude. All EPR spectra of the starting radicals are singlets of width up to 10 G, due to the exchange interaction between neighboring spins in the crystal. At the same time the EPR spectra of mechanically treated, "cleaned" cellulose samples obey a pattern characteristic of matrix-isolated nitroxyl radicals with random orientations.[10,11] Such PCs must be separated by rather long distances (in the length scale of the exchange and dipole-dipole interactions)[12] and uniformly distributed over the bulk of the cellulose sample. The EPR spectra of the "non-cleaned" mechanically treated samples also revealed the presence of the solid phase of the crystalline radical. Washing with solvents led to disappearance of the signals of the crystalline radicals and to a decrease in the intensities of the spectra of isolated radicals. This means that the mechanically treated samples contain two types of "isolated" radicals, which are bound loosely (probably, through adsorptive forces or intercalation) and strongly (probably, through covalent bonds). The parameters of the EPR spectra of the "cleaned" spin-labeled cellulose samples are given below.

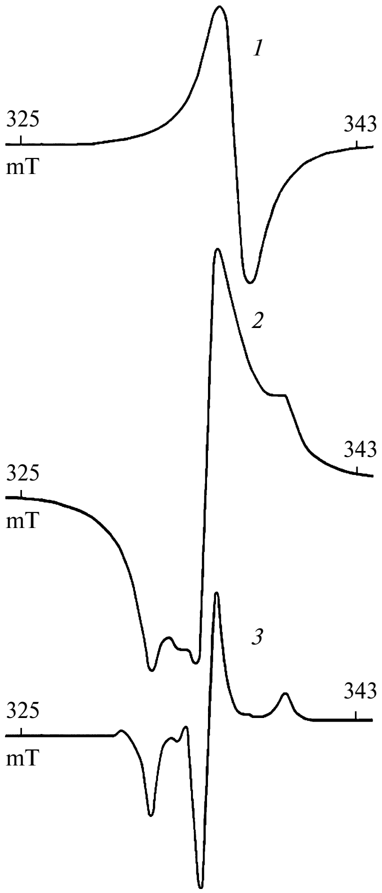

Fig. 1. EPR spectra of starting powdered radical **2** (*1*); mechanically treated mixture of cellulose with radical **2** (*2b*), and mechanically treated mixture of cellulose and radical **2** washed successively with water, acetone, and isopropyl alcohol (*3*).

Radical	**1**	**2**	**3**
A_{zz}/G	35.35	34.32	34.43

The PC concentration in the "cleaned" samples depended on the mechanochemical treatment and the weight of the radical and was calculated by the double integration of the spectra relative to a reference sample. The maximum concentrations were $7 \cdot 10^{18}$ (sample **I**), $1.4 \cdot 10^{21}$ (**II**), and $3.5 \cdot 10^{20}$ spin g^{-1} (**III**). The lowest concentration achieved for radical **1** even more decreased on further washing with solvents. The highest concentration of the "grafted" spin labels was attained under conditions suitable for covalent bonding between the stable radical and cellulose (radicals **2** and **3**). Further washing caused no decrease in the EPR signal, which also remained unchanged over a period of a year (samples were stored in air at room temperature). After suspension of these samples in a 10^{-2} *M* ethanol solution of $CuCl_2$ the observed EPR signal of the spin label decreased by ~50%, which can probably due to a dramatic spectral broadening caused by the "superficial" PCs accessible to spin exchange with Cu^{2+} ions.[10] Therefore, the proportion of the bound PC in the bulk of the material was also ~50%.

According to simple calculations, the maximum content of strongly bonded PC in sample **II** is similar in order of magnitude to the number of monomer units in the chain cellulose molecule, *i.e.*, the spin label is on the average attached to each monomer unit. Sample **III** is modified to a lesser extent. An increase in the concentration of the "grafted" label causes broadening of the EPR spectra, probably, due to the dipole-dipole interaction.[10] At low "label" concentrations ($<10^{20}$ spin g^{-1}), the shape of the EPR spectrum (see Fig. 1, *c*) indicates a uniform distribution of the "grafted" PCs over the cellulose macromolecular chains.

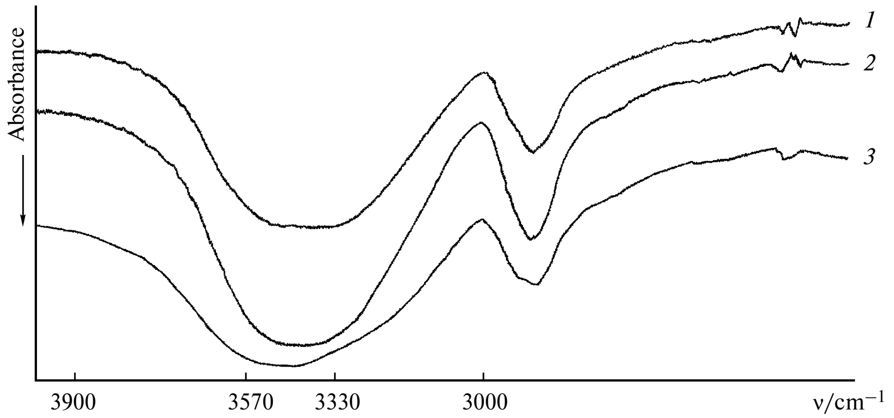

Fig. 2. IR spectra of samples in the region of the OH stretching vibrations of the starting cellulose (*1*) and the mechanically treated and "cleaned" samples **I** (*2*) and **II** (*3*).

Apparently, the introduction of bulky substituents must violate the network of hydrogen bonds formed by the cellulose hydroxyls. Indeed, the IR spectra (Fig. 2) exhibit corresponding changes in the frequency range of the hydroxyl stretching vibrations (3000—3900 cm^{-1}). For instance, the band maximum in the IR spectrum of sample **I** (see Fig. 2, curve *2*) is not shifted but the bandshape changes significantly, which indicates violation of the system of intermolecular hydrogen bonds formed by the cellulose hydroxyls. The IR band maximum in the spectrum of sample **II** (Fig. 2, curve *3*) is shifted toward higher frequencies (3400 to 3500 cm^{-1}), the bandshape being also changed. A number of quantitative criteria for estimating the degree of substitution of cellulose hydroxyls includes,[13] in particular, calculations of the ratio of the absorption densities at 3570 and 3330 cm^{-1} (D_{3570}/D_{3330}), the ratio of the integrated absorption densities of the high-frequency and low-frequency band components (S'/S''), and the band asymmetry relative to the 3460 cm^{-1} frequency (a/b) (a and b are the widths of the high-frequency and low-frequency components of the absorption band). The results obtained are listed in Table 1. As can be seen, the mechanochemical interaction of cellulose with radical **1** (sample **I**, "cleaned") only leads to violation of the network of hydrogen bonds, whereas introduction of the strongly bonded substituent — radical **2** (sample **II**, "cleaned") causes a decrease in the number of hydroxyl groups involved in the strong hydrogen bonds, as indicated by the increase in the intensity of the high-frequency component of the band and by the shift of its maximum toward high-frequency region.

Table 1. Changes in the parameters of IR spectra and X-ray diffraction patterns of cellulose and mechanically treated mixtures of cellulose with radicals **1** and **2**

Sample	D_{3570}/D_{3330}	S'/S''	a/b	K
Microcrystalline cellulose	0.594	0.83	0.79	0.901
"Cleaned" sample **I**	0.457	0.84	0.83	0.312
"Cleaned" sample **II**	0.843	1.11	1.03	−3.66

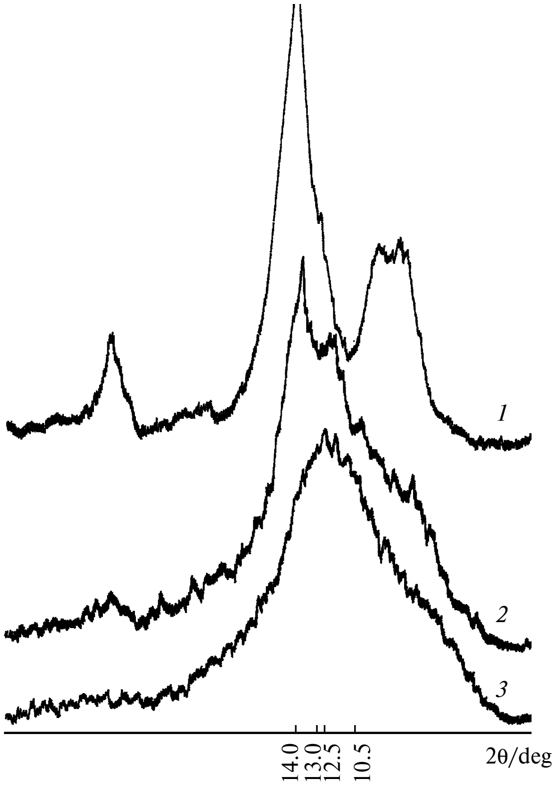

Fig. 3. X-ray diffraction patterns of the starting microcrystalline cellulose (*1*) and the mechanically treated and "cleaned" samples **I** (*2*) and **III** (*3*).

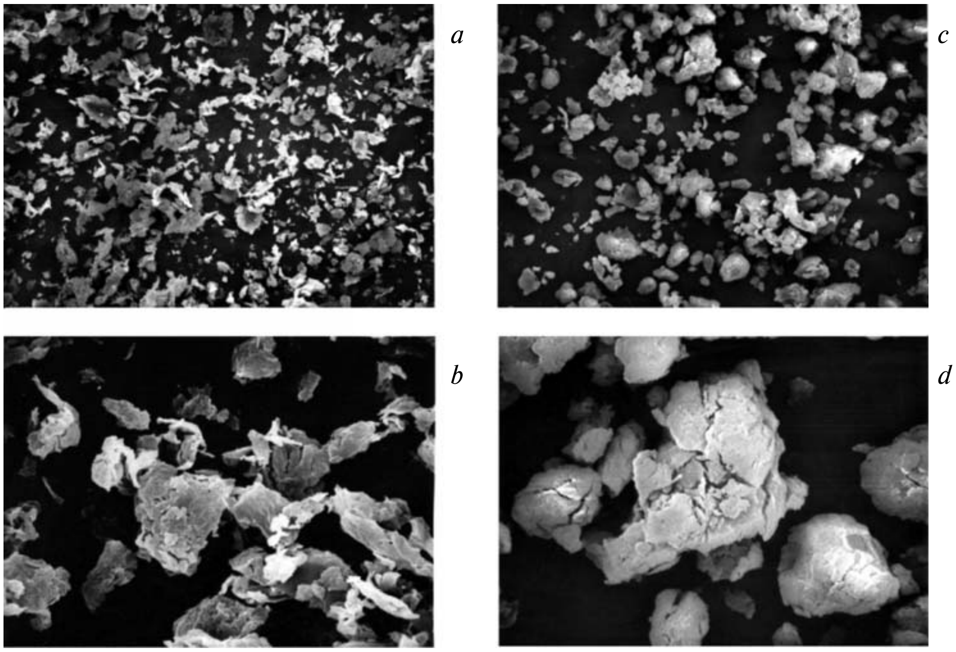

Fig. 4. Electron micrographs of mechanically treated and "cleaned" samples **I** (magnifiied, ×350 (*a*) and ×1500 (*b*)) and samples **II** (magnifiied, ×350 (*c*) and ×1500 (*d*)).

The X-ray diffraction patterns of the starting MCC and the mechanically treated samples are shown in Fig. 3. Comparison of these patterns shows that mechanical treatment causes partial amorphization of cellulose. Besides, the "non-cleaned" samples contain traces of the crystalline phase of the starting radicals, which is consistent with the EPR spectra described above. To evaluate changes in the cellulose structure, we calculated the crystallinity index[13] $K = 1 - h_1/(H - h_1)$, where h_1 is the distance between the minimum of the diffraction pattern in the range 10.5—12.5° and the base line and H is the distance between the maximum of the diffraction pattern between 13—14° and the base line. The crystallinity index values (see Table 1) show that mechanical treatment with radical **1**, which does not react with MCC, leads to a decrease in the degree of crystallinity of cellulose. However, the greatest changes in the structure of the material (up to nearly complete amorphization) occur when the spin labels are strongly bonded to cellulose.

We also obtained electron micrographs of mechanically treated and "cleaned" samples **I** and **II** (see Fig. 4). Sample **I** is comprises finely dispersed (2—10 μm) crystalline particles with well-defined boundaries, while sample **II** is comprised of larger (6—25 μm) and water-worn particles, which indicates a greater degree of amorphization of the material. By and large, the results of our studies of the electron micrographs are in agreement with the data obtained by X-ray analysis and IR spectroscopy.

* * *

Thus, we can draw the following conclusions.

1. Mechanical treatment of a mixture of solid reactants afforded cellulose samples labeled with stable nitroxyl radicals.
2. There are two types of the bonding of radicals with respect to "washing out" from cellulose with solvents.
3. Strong bonding of a radical can be due to the formation of covalent bonds between the radical residue and cellulose.
4. The results of X-ray diffraction, IR spectroscopy, and electron microscopy studies indicate that the strong bonding is accompanied by significant distortions of the cellulose structure, namely, by violation of the network of hydrogen bonds and amorphization of the material.

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