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Probing cellulose wettability by electron paramagnetic resonance

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Carboxymethyl-cellulose has been labelled with a stable free radical by reaction with 4-aminotempo in the presence of a coupling agent (EEDQ). The spin-labelled cellulose is highly stable, no leaking being noticed after months at room temperature. EPR spectroscopy was used as a main tool to study the wettability of such a material. The EPR spectrum of the dry spin-labelled cellulose shows the expected anisotropy, while addition of several solvents (acetone, ethyl acetate, DCM, methanol, toluene, PEG 200) induces the splitting of the spectrum into a two component system. Thus, the EPR spectrum is composed of a mobile component superimposed onto an immobilized one. Addition of water leads to a mono-component isotropic spectrum. These data clearly indicate the presence of two types of sites to which the spin-label is attached. Variable temperature EPR spectra showed that an increase of temperature results in an increase in the mobility of the spin-label. Deposition of plain or spin-labelled gold nanoparticles on the cellulose fibres also affects the structure of the polymeric chain, as seen by changes in the EPR spectra.

Key words: *cellulose; EPR; radical; tempo; spin label*

1. Introduction

Cellulose, one of the oldest known and used materials in mankind history [1], is a natural carbohydrate (polysaccharide) consisting of glucose units, linked together through 1–4 glycosidic bond, forming in this way a linear molecular chain [2]. Cellulose forms fibres with porous structure, arranged into amorphous and crystalline regions [3]. However, despite many investigation methods and various approaches to characterize such a natural material, there are unknown or very little understood data regarding cellulose, mainly its interaction with liquids, like organic solvents and water. It is well known that cotton can absorb and retain large amounts of solvents.

A detailed investigation of the structure and of the wettability of cellulose may be performed using electron paramagnetic resonance spectroscopy (EPR or ESR) because

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EPR can provide information on the micro-environment of the spin-probe (a stable free radical) [4]. The EPR parameters are dramatically affected by the polarity, viscosity and dynamics of the surrounding space next to the spin-probe; major changes are noticed for the recorded EPR parameters [5]. Nitroxide spin probes (stable free radicals, like tempo) can monitor structural and chemical changes occurring during various physicochemical processes. The spin-labelling technique, in which the stable free radical (the spin-probe) is covalently bonded to a material (like cellulose), provides useful information on the flexibility and the mobility of the spacer which links together the solid support (cellulose) and the reagent (spin-probe).

The attachment of a spin-label to cellulose, known since '70s [6], requires certain conditions; several successful strategies have been employed, first derivatizing cellulose (i.e. *via* cyanogens bromide or tosylation), and then reacting it with 4-aminotempo. There is also a recent paper about the effect of humidity on the supramolecular structure of cotton, studied by quantitative spin probing; in this case, the free radicals 4-hydroxytempo were just deposited in cotton at different loadings [7].

2. Materials and method

All materials and solvents were purchased from Aldrich. Carboxymethyl-cellulose (CM-Cel), in the form of fibres, as sodium salt, has been used as a starting material; the labelling was performed as follows: to 1 g of CM-Cel suspended in 50 ml of dry methanol, 1 g of 4-aminotempo and 2 g of EEDQ (2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline) was added, and the mixture was stirred at room temperature for 24 h, then it was centrifuged and the supernatant removed. This step was followed by addition of methanol and re-centrifugation; the process was repeated till no EPR signals were detected in methanol (about 15 times). The solid spin-labelled cellulose (SL-Cel) was then dried *in vacuo* overnight. The average loading was determined by careful double integration of the EPR signal, compared with a tempo standard. For EPR measurements, a very small amount of SL-Cel (around 1 mg) was suspended in various solvents (0.1 ml) and the EPR spectra were recorded at room temperature, or at selected temperatures. Typical settings for the EPR spectra (recorded on a Jeol Jes FA100 spectrometer): centre field 3360 G, sweep field 150 G, frequency 9.50 GHz, power 1 mW, sweep time 60 s, time constant 0.03 s, modulation frequency 100 kHz, gain 50, and modulation width 1 G. Plain and spin-labelled gold-nanoparticles (SL-Au NPs, nanoparticles which contain attached tempo moieties to the Au surface) were synthesized as previously described [8]. The deposition of gold nanoparticles on SL -Cel was performed as follows: 50 mg of SL-Cel were added to 1 mL of gold nanoparticles in toluene (10^{-4} M) and the mixture was stirred for 24 h, after that the solid was filtered off and dried. TEM pictures were taken using a Jeol 200 CX microscope operated at 200 kV.

3. Results and discussion

3.1. Synthesis

Our strategy involved a direct reaction between CM-Cel and 4-aminotempo, in the presence of EEDQ as a coupling agent (Fig. 1).

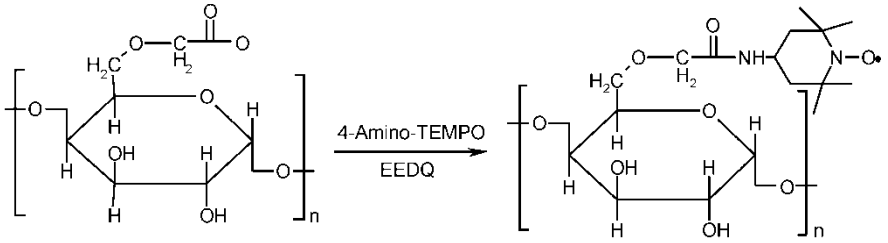

Fig. 1. Spin-labelling of CM-Cel

The obtained material is stable for months at room temperature, and no leaking of the spin-label has been noticed. The loading with tempo moiety was estimated from double integration of the signal arising from a known amount of sample compared with a pure tempo standard: about 10% of the carboxyl groups were labelled.

3.2. EPR spectra at room temperature

The EPR spectrum of the dry SL-Cel showed the well known pattern of an immobilized radical (Fig. 2a). Addition of various solvents (ethyl acetate, acetone, methanol, DCM, water, PEG 200) produces visible changes in EPR spectra (Figs. 2b–g). The spectra (Figs. 2b–f) are characterized by two components, one arising from immobilized tempo moieties (slow motion), while the other one arising from the mobile tempo moieties (fast motion), all of them being linked to the cellulose chains. Such two component spectra are easily characterized by the order parameter *S,* which has values from 0 (isotropic motion) to 1 (non-isotropic motion):

$$S = \frac{A_{\parallel} - A_{\perp}}{A_{ZZ} - \dfrac{A_{xx} + A_{yy}}{2}} \times \frac{A_{xx} + A_{yy} + A_{zz}}{A_{\parallel} + 2A_{\perp}} \tag{1}$$

where A_{zz}, A_{xx}, A_{yy}, $A_{\perp}$, and $A_{\parallel}$ are hyperfine components of the EPR spectra; the factor on the right is a polarity correction to the hyperfine tensor element [9–11].

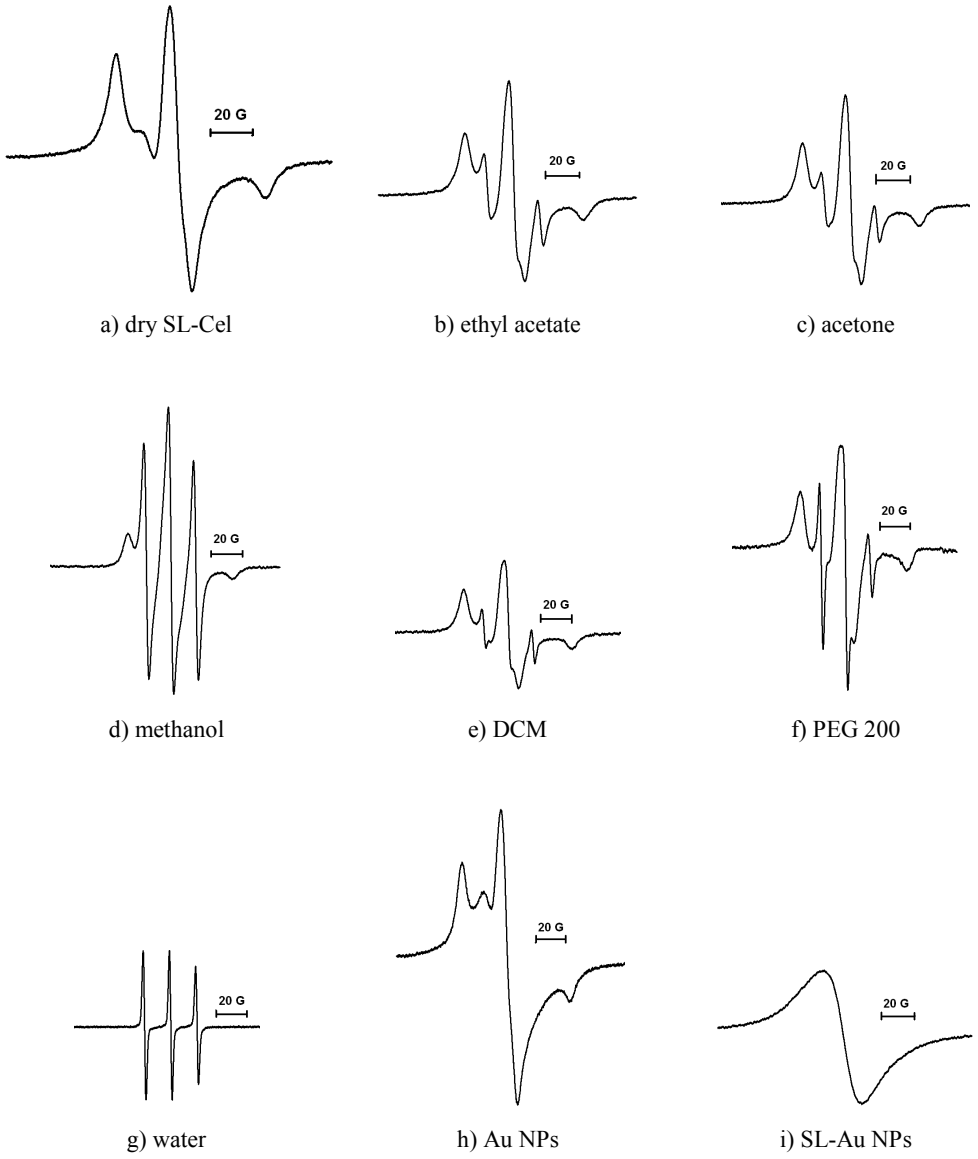

Fig. 2. EPR spectra of SL-Cel, dry (a), in various solvents (b–g), and in the presence of plain (h) and SL-gold nanoparticles (i)

As we mentioned before, the spectra change from the well known type of an immobilized spin-label (Fig. 2a, dry SL-Cel) to the same very well known type of a complete mobile one (Fig. 2g, in the presence of water). These results are not unexpected, as water has the highest E_T value (polarity), and therefore most strongly interacts with cellulose chains. Other solvents (acetone, ethyl acetate, DCM, methanol, toluene, PEG 200) clearly induce the appearance of a mobile component into the re-

corded EPR spectra (Figs. 2b–f), characterized by the hyperfine coupling constants a_N. These data mean that there are two types of sites on the cellulose chains (which are spin-labelled) acting differently; it is well known that cellulose fibres contain crystalline and amorphous domains (the amorphous domains are porous, being easily accessible to external agents like solvents). The order parameter S is high for all samples (besides dry SL-Cel, Table 1), meaning that the spins are likely to be arranged into supramolecular architectures. The cellulose molecules might be as well responsible for the arrangement of the solvent molecules into a certain structural way, which can lead to a highly ordered structure around the spin-labels.

Table 1. EPR and solvent data

Solvent	$2a_{\parallel}$ [G]	$2a_{\perp}$ [G]	S	$2a_N$ [G]	E_T [kcal/mol]	Viscosity at 25 °C [cP]
Dry SL-Cel	69	41	0.481	–	–	–
Ethyl acetate	70	28	0.722	32	38.9	0.441
Acetone	69	28	0.705	31	42.2	0.306
DCM	70	30	0.688	31	41.1	0.404
Water	–	–	–	34	63.1	0.890
Methanol	68	30	0.654	33	55.5	0.547
PEG 200	69	29	0.688	32	–	–
Toluene (300 K)	70	25	0.774	31	33.9	0.552
Toluene (320 K)	69	25	0.757	32	–	–
Toluene (340 K)	68	25	0.740	32	–	–
Toluene (360 K)	68	25	0.740	32	–	–
Toluene (380 K)	68	26	0.722	32	–	–

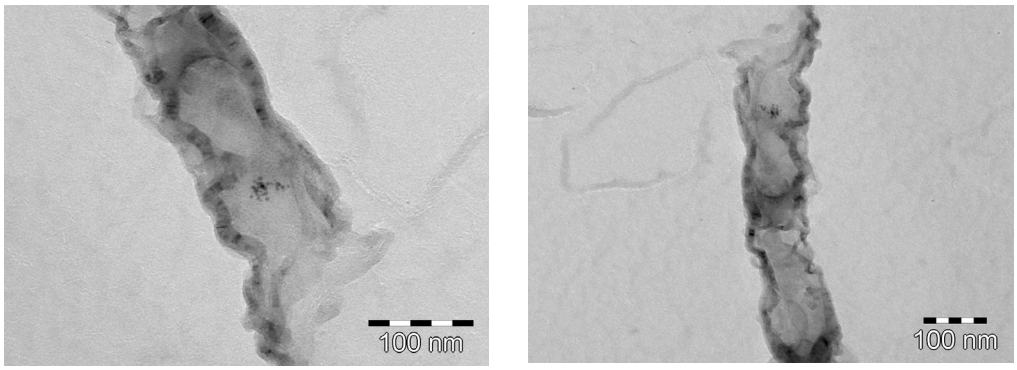

Fig. 3. Typical TEM pictures of SL-Cel with deposited Au NPs

An interesting feature has been noticed when plain gold nanoparticles (Au NPs) were deposited on the cellulose fibres. The EPR spectrum changes, as if the spin-labels became closer (Fig. 2h); this behaviour may be explained by the fact that Au NPs prefer to attach to the amorphous domain rather than the crystalline one, as has

been shown in literature for the direct synthesis of Au NPs on cellulose fibres [12]. In the case of SL-Au NPs, an interaction between the spins from cellulose with the spins from Au NPs is clearly noticed, leading to a single broad line in EPR spectrum (Fig. 2i). Typical TEM pictures of cellulose which contain deposited Au NPs are shown in Fig. 3; Au NPs deposited on the cellulose fibres are easily visible. Table 1 compiles the EPR values of all the recorded spectra, together with the E_T and viscosity values of the solvents used.

3.3. EPR spectra at variable temperatures

Toluene has been chosen as the solvent in which the variable temperature experiments may be performed, due to its high boiling point and also due to the fact that at room temperature a very little amount of the spin-labels are solvated (Fig. 4, 300K). Upon rising the temperature gradually to 380 K, one can easily notice that the mobile component increases with temperature (Fig. 4). This might be explained by the increased mobility of the spin-labels, from the amorphous domains of the cellulose fibres, which can move at higher temperature more freely; moreover, the order parameter S decreases slightly with temperature (Table 1). Due to the complexity of the system, other interaction may be triggered by increasing temperature, leading to a similar effect.

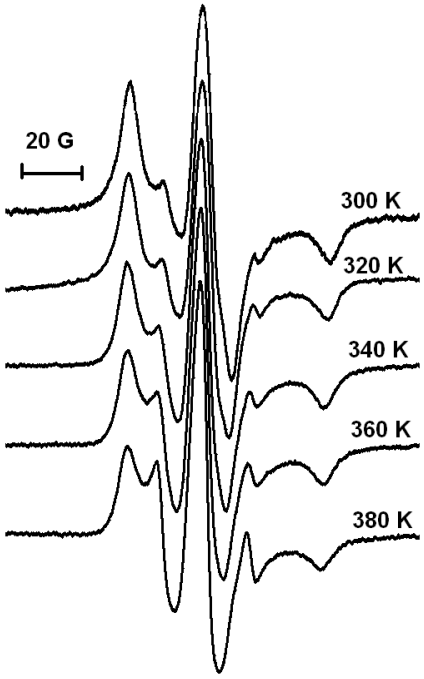

Fig. 4. EPR spectra of SL-Cel in toluene at selected temperatures

4. Conclusions

CM-cellulose has been spin-labelled with a free stable radical, and the wettability (with acetone, ethyl acetate, DCM, methanol, toluene, PEG 200, and water) of the material was studied by EPR spectroscopy. The results show that there are two different labelled sites, which probably belong to amorphous and crystalline domains of cellulose. Only the spin-labels from amorphous domains are easily solvated by different organic solvents, while water solvates the spin-labels from crystalline domain as well. The interaction of the spin-labelled cellulose with gold nanoparticles also leads to changes in the EPR spectra.

Acknowledgements

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