

Free-Radical and Singlet-Oxygen Mobility in Cotton Probed by EPR Spectroscopy

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Spin probe EPR experiments using TEMPO and TEMPOL are reported on dry cotton. The spectra are in the slow tumbling region with $\tau_{\text{rot}} \sim 1$ ns, the line width is found to be affected by Heisenberg spin exchange and aggregation of the radical. To probe the entire amorphous zone of cotton, ethanol and water are used to deposit the radicals; to probe the fiber surface specifically toluene was used. Analysis shows that in both regions the viscosity is 30 cP and that the fiber surface is an amorphous layer with a depth of approximately 2 nm. The radical motion follows normal liquid theory with a diffusion rate constant of $2 \times 10^8 \text{ mol}^{-1} \text{ L s}^{-1}$. A small proportion of the radicals deposit to an area of much higher viscosity. The classical spin probe method is extended via a simple electron spin polarization experiment. This involved irradiation of the sample and analysis of the radical–triplet mechanism polarization of TEMPOL/ $^1\text{O}_2$, which indicates $^1\text{O}_2$ diffuses with a similar rate constant.

Introduction

Cotton is one of the most widely used fibers and has been the subject of much research.¹ It is constructed of polycarbohydrate chains in amorphous and crystalline zones. Surprisingly little information is available on the mobility and local environment of molecules absorbed into the structure or on the surface, although it is clear that only the amorphous zones are readily accessible. Recently this point has been addressed by elegant diffuse reflectance experiments on microcrystalline cellulose,^{2–9} (which is structurally related to cotton) and cotton itself.¹⁰ These studies used triplet–triplet energy, electron-transfer, and hydrogen-transfer reactions to probe the structure, alongside fluorescence/absorption. The work showed the high viscosity of the environment, the importance of hydrogen-bonding interactions, aggregation effects, and interesting differences in behavior when substrates were adsorbed onto the surface or into the structure. Unfortunately, little quantitative kinetic information could be obtained due to difficulties in calibrating the concentration of reacting species.

In this context spin probe experiments appear helpful, which involve measuring the electron paramagnetic resonance (EPR) spectrum of stable nitroxide radicals absorbed on or in the substrate. Analysis of the resonance line shape and its dependence on radical concentration then gives quantitative information on the local environment. Such studies are reported here, using 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) and 4-hydroxy-2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPOL) radicals as spin probes. Cotton is used as the substrate, and the probes are applied from water, ethanol, and toluene solutions. Previous work has shown that water and ethanol swell the entire amorphous zone while toluene wets only the surface of the fiber, allowing both area to be investigated.^{1,5}

It is remarkable that such simple studies have not been previously reported. Reference to the literature shows some work by Russian authors;¹¹ however, these involved spin probes chemically bonded to cotton where the motion is severely restricted. During the experiments it was found that the

TEMPOL radicals could be polarized via the radical triplet pair mechanism with singlet oxygen, $^1\text{O}_2$.¹² Analysis of this effect allowed the diffusion rate constant for $^1\text{O}_2$ in cotton to be estimated.

Experimental Section

Bleached cotton sheeting which had not been mercerized or finished was obtained from several suppliers and used as received. No significant differences in results were found between the different suppliers of cotton. It was cut into $0.5 \times 10 \text{ cm}^2$ strips and then placed in water, ethanol, or toluene solutions of known radical concentration. After 30 s the cloth was withdrawn, excess solvent was removed, and the remainder evaporated by leaving at 40°C in air for 60 min. Separate experiments showed that this delivered a known weight ($\pm 20\%$) of solvent per gram of cotton. In the TEMPOL case, this allowed radical concentrations on the dry cotton ($\rho = 1.25 \text{ kg L}^{-1}$) to be calculated, but for TEMPO sublimation of the radical from the cloth prevented this. The dried cotton was placed in an NMR sample tube (0.5 cm diameter), and the EPR spectrum was recorded using an X-band spectrometer (Bruker ER200 with TE102 cavity). Care was taken to ensure that the modulation frequency and microwave power did not distort the line shape.

TEMPO and TEMPOL were obtained in their purest commercial form and the solvents in HPLC grade; all were used as supplied. Samples were not deoxygenated.

For the spin polarization experiments, irradiation of the sample was achieved using the focused light of a 200W Hg–Xe lamp. To avoid sample degradation effects, spectra were recorded within 60 s of irradiation beginning. Degradation was monitored by measuring the EPR spectrum in the dark before and after irradiation. Cotton dyed with Remazol Blue was obtained from a commercial supplier without a finish.

Results and Discussion

(i) Conventional Spin Probe Experiments. EPR spectra obtained with TEMPOL applied from ethanol are shown in Figure 1. At the lowest concentration a slow tumbling spectrum

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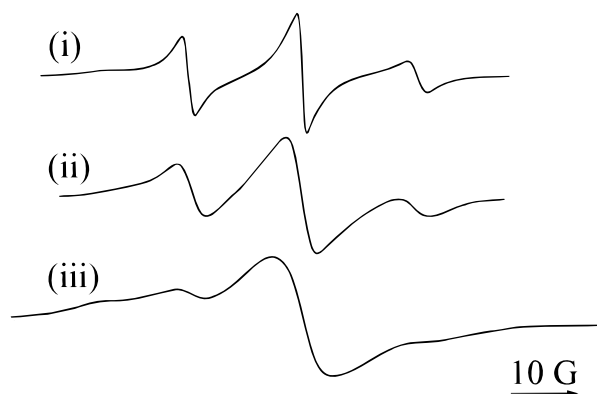


Figure 1. EPR spectra obtained from TEMPOL on dry cotton, deposited using ethanol. The concentration of the radicals on the dry cotton is (i) 9.7×10^{-3} M, (ii) 0.014 M, (iii) 0.042 M. (1.25 kg of cotton = 1 L).

TABLE 1: Experimental Values of A and C_{\max} for TEMPOL and the Values of k_d Extracted from Them^a

solvent	A/G $\text{mol}^{-1} \text{L}$	$C_{\max}/\text{mol L}^{-1}$	% volume occupied	$k_d/10^8 \text{ mol}^{-1}$ L s^{-1}
water	100	1.5×10^{-2}	42	1.9
ethanol	123	1.4×10^{-2}	42	2.4
toluene	2810	6.4×10^{-4}	1.5	(2)

^a At least six radical concentrations were used to determine each value

is observed typical of radicals in highly viscous environments, Figure 1(i).¹³ As the radical concentration is increased the lines broaden, Figure 1(ii), then the central line increases in intensity, Figure 1(iii), until it is all that is seen, (not shown). To the left of the low field line an additional resonance is found. When the spin-labeled cotton was wetted with water or ethanol, the spectrum of TEMPOL found in low-viscosity solvents¹³ was observed. However, if toluene was used no difference was seen in the spectrum, illustrating the different regions of cotton accessible to the solvents. The N hyperfine coupling in dry cotton, [Figure 1(i)] was 16.9 G, irrespective of the deposition solvent. When wet with water and ethanol, the couplings were 16.9 and 16.2 G, respectively, compared to values in the pure solvents of 16.9 and 15.6 G, respectively.

Similar results were observed using water and toluene to deposit TEMPOL, though for the latter the concentration dependence was different. In all cases, the average initial peak-to-peak broadening was proportional to the radical concentration until a certain concentration, C_{\max} , was reached. Table 1 gives the proportionality constants, A , and C_{\max} . The spectra from TEMPO matched TEMPOL's, but due to radical sublimation from the dry cotton A and C_{\max} values could not be measured.

Analysis of the spectra obtained at the lowest radical concentrations gives the rotational correlation times of the radical, τ_B and τ_C . These were calculated from standard formulas:

$$\tau_B = 6.6 \times 10^{-10} \Delta H_0 \left[\sqrt{\frac{V_0}{V_{+1}}} - \sqrt{\frac{V_0}{V_{-1}}} \right] \quad (1)$$

$$\tau_C = 6.6 \times 10^{-10} \Delta H_0 \left[\sqrt{\frac{V_0}{V_{+1}}} + \sqrt{\frac{V_0}{V_{-1}}} - 2 \right] \quad (2)$$

where ΔH_0 is the peak-to-peak line width of the central line in Gauss and V_{+1} , V_0 , and V_{-1} are the height of the low, central,

TABLE 2: Values of the Rotational Correlation Times Extracted from Spectra and the Viscosities Calculated from Them

solvent	TEMPO			TEMPOL		
	$\tau_C/10^{-9} \text{ s}$	$\tau_B/10^{-9} \text{ s}$	η/cP	$\tau_C/10^{-9} \text{ s}$	$\tau_B/10^{-9} \text{ s}$	η/cP
water	1.1	0.54	21	2.0	1.1	37
ethanol	1.3	0.60	24	1.3	0.74	24
toluene	2.3	1.2	44	1.7	1.02	32

and high field line, respectively.¹³ The values are given in Table 2 and within error are similar for the three solvents and two radicals. Accordingly, the fiber surface (probed using the toluene solution) appears to be an amorphous layer, equivalent to the amorphous region within cotton. This is not unexpected, as both areas are at the edge of the crystalline zone.

It is interesting that the 4-hydroxy group of TEMPOL has little effect on the rotation, although it might be expected to increase interactions with the polycarbohydrate chains. Interaction of the nitroxide moiety may override any such effects. The correlation times are unequal, with $\tau_C \approx 1.9\tau_B$, showing that the preferred rotation axis is one perpendicular to the NO bond.¹⁴ Unfortunately, on the basis of the current data a more accurate assignment cannot be given. Sterically it might be expected that the radicals would predominately rotate in the plane of the polycarbohydrate chains.

Using a mean value of the correlation times an approximate value of the microviscosity may be calculated via the Debye formula:

$$\tau = \frac{4\pi r^3 \eta}{3kT} \quad (3)$$

where r is the radical's radius, calculated from increments¹⁵ ($r_{\text{TEMPO}} = 0.341 \text{ nm}$, $r_{\text{TEMPOL}} = 0.346 \text{ nm}$) and η the solvent viscosity. The results are given in Table 2 and give an average value of 30 cP. There is little literature data available for comparison; however, diffusion coefficients for TEMPOL have been measured in the amorphous polymers, poly(dimethylsiloxane), natural rubber, and a butadiene-styrene copolymer SKS-30 which correspond to viscosities of 630, 310, and 68 cP, respectively.¹⁶ The cotton value appears reasonable in comparison and may be lower due to the presence of approximately 10% water even in dry cotton.¹

The signal observed to the left of the low field resonance, Figure 1, is part of the rigid limit spectrum of TEMPOL¹⁷ and was confirmed by observation of a weak resonance 41 G high field from the central line (not shown). It arises from radicals in much more viscous environments with $\tau \approx 10^{-6} \text{ s}$. These are presumably lodged much closer to the crystalline region, where the viscosity rapidly rises. Hence, there appear to be two distinct areas for radical absorption, with the more mobile area favored, shown by the stronger signal, Figure 1.

The initial increase in line width with radical concentration is due to Heisenberg spin exchange and/or the dipole-dipole interaction between electron spins on neighboring radicals. Graphical data in previous work^{16,18} shows that the latter should become important at 300 K for $\eta > 40 \text{ cP}$. Consequently, the diffusion-dependent spin exchange should dominate here and its contribution to the line width, ΔH_{ex} , given by¹⁹

$$\Delta H_{\text{ex}} = 3.6 \times 10^6 k_d [\text{radical}] \quad (4)$$

where ΔH_{ex} is the excess line width in Gauss and $k_d (= 8RT/3\eta)$ is the diffusion rate constant. The measured A values are equal to $\Delta H_{\text{ex}}/[\text{radical}]$ and therefore to obtain k_d the effective

radical concentrations in the amorphous zones are required. Deuteration experiments have shown that 42% of the total volume of cotton is accessible to water.¹ Using this figure for the amorphous volume fraction in cotton gives $k_d = 2 \times 10^8 \text{ mol}^{-1} \text{ L s}^{-1}$ for water and ethanol, Table 1, corresponding to $\eta = 31 \text{ cP}$. The result confirms the η value obtained above, the amorphous fraction, and the assumption that the broadening is predominately due to spin exchange. That such good agreement is found between methods demonstrates that the radical motion in the mobile regions may be adequately described using simple liquid theory. This is contrary to results for transient radicals produced from benzophenone in microcrystalline cellulose, where nonhomogeneous distribution of adsorption site was thought to effect the encounter rate.^{4,5} However, the complex chemistry of carbohydrate radicals may also have played a role in the kinetics.²⁰

Jansen¹⁰ reported the lifetime of the triplet state of aluminum phthalocyanine chloride in cotton wetted with water as 238, 154, and 60 μs , when under vacuum, air, and oxygen atmospheres, respectively. Although not calculated in the paper, these values correspond to an oxygen quenching rate constant of the triplet state on wet cotton of $2 \times 10^6 \text{ mol}^{-1} \text{ L s}^{-1}$, assuming the concentration of oxygen is similar to that in water ($1.4 \times 10^{-3} \text{ mol L}^{-1}$ under air). The reaction will be diffusion controlled, yet the rate constant is 2 orders of magnitude smaller than the k_d found here in dry cotton. In wet cotton, k_d will certainly be larger, and assuming $k_d > 10^9 \text{ mol}^{-1} \text{ L s}^{-1}$ gives a triplet lifetime of $< 0.5 \mu\text{s}$, too fast for the equipment used to detect.¹⁰ Thus the signal observed must arise from those phthalocyanine molecules in much less mobile environments with much slower diffusion. This agrees with the results presented here where some nitroxides deposit to a much more viscous environment.

When toluene is used to deposit TEMPOL, the radicals are only in the surface amorphous layer. Since the local environment is similar to that in the bulk amorphous region, vide supra, the diffusion rate constant should be similar. To obtain $k_d = 2 \times 10^8 \text{ mol}^{-1} \text{ L s}^{-1}$ the surface amorphous layer must occupy 1.5% of the total volume. Experiments with benzene vapor¹ show that a maximum of 1.2 g per 100 g cotton can be adsorbed. Assuming this forms a unimolecular layer and with $r_{\text{benzene}} = 0.27 \text{ nm}$ ¹⁵ then a surface area of $6.6 \text{ m}^2 \text{ g}^{-1}$ is calculated. The surface area multiplied by the average surface amorphous layer depth, d , is equal to 1.5% of the total cotton volume. For this to transpire then $d \sim 2 \text{ nm}$ corresponding to 3–4 lengths of the carbohydrate base unit, a physically reasonable value.

As the radical concentration is raised above C_{max} , aggregation of the radicals occurs, attested by the development of a broad central resonance, Figure 1(iii), typical of the crystalline radical. The ratio of $C_{\text{max}}(\text{water/ethanol})/C_{\text{max}}(\text{toluene})$ is 23 which agrees with the ratio of the volumes occupied above, 42%/1.5% = 28. This supports the conclusion that molecules adsorbed onto the surface behave similarly to those in the amorphous zones within cotton. Furthermore, the total surface area of cotton, i.e., the crystal surface area within and on the surface of the fiber, has been reported as $180 \text{ m}^2 \text{ g}^{-1}$.¹ This is 27 times the value obtained with benzene vapor, in excellent agreement with the above figures, if the amorphous layers are all an average 2 nm deep.

(ii) Electron Spin Polarization Experiments. During the spin probe study it was discovered that under irradiation the EPR signal size increased by approximately 20%. When the light was extinguished the signal returned to its original size. Observation of such absorptive polarization from nitroxide radicals is unusual. Prolonged exposure ($> 5 \text{ min}$) led to the

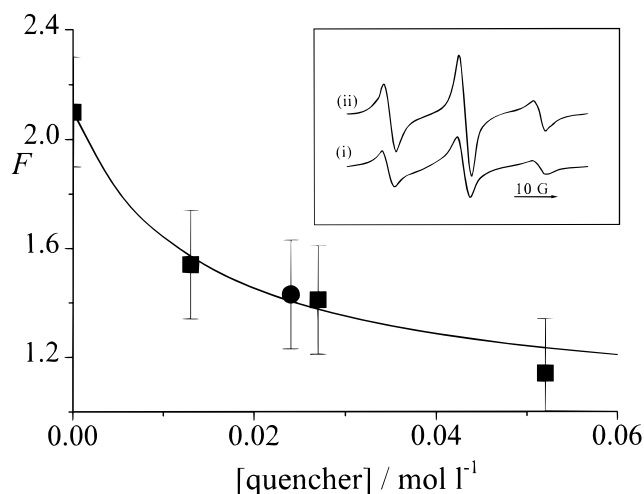


Figure 2. Variation in the polarization of the TEMPOL EPR signal under irradiation on dry cotton containing 0.12 mol L^{-1} Remazol Blue, with the concentration of quencher in the amorphous zone (square = DABCO, circle = Trolox). Insert shows the EPR spectrum, (i) in the dark and (ii) under irradiation without the quencher. The concentration of TEMPOL in all experiments was $10^{-3} \text{ mol L}^{-1}$.

irreversible decay of the radical signal due to photoreaction. Recent liquid-phase studies have shown that the EPR signal size of nitroxide radicals is increased in the presence of singlet oxygen, $^1\text{O}_2$, due to the radical triplet pair mechanism of polarization.^{12,21} $^1\text{O}_2$ could be produced in white cotton under irradiation due to the presence of impurities such as lignin, explaining the current results. To investigate the effect further, cotton containing 0.12 M of the $^1\text{O}_2$ generator, Remazol Blue,^{22,23} and $0.3\text{--}4.4 \times 10^{-3} \text{ M}$ Tempol was measured. Under irradiation the mean peak-to-peak height of the three resonances, F , increased by a factor of 2.1, independent of the TEMPOL concentration. However, when the singlet oxygen quencher DABCO or Trolox was added the polarization became much smaller, Figure 2. All chemicals were applied from ethanol or water. This shows that the radical triplet pair mechanism involving $^1\text{O}_2$ and TEMPOL is responsible for the polarization.

In liquids the phenomenon may be accurately described using a modified Bloch equation approach.¹² Under steady-state conditions and on irradiation, the signal increase F is given by the formula:

$$F = 1 + P(k_q [\text{Quencher}] + \tau(^1\text{O}_2)^{-1})^{-1} \quad (5)$$

where P is a constant dependent on the system and light intensity, k_q is the $^1\text{O}_2$ quenching rate constant, and $\tau(^1\text{O}_2)$ is the lifetime of $^1\text{O}_2$ without the quencher. Best fits to the experimental data are obtained using $P/\tau(^1\text{O}_2) = 1.1$ and $k_q\tau(^1\text{O}_2) = 71 \text{ mol}^{-1} \text{ L}$, Figure 2. As with spin exchange, liquid phase theory may be used to describe the reaction.

Both DABCO and Trolox are fitted with the same values indicating that on dry cotton their k_q 's are identical. The amorphous zones in cotton contain considerable amounts of water, vide supra. For DABCO in water $k_q = 2.8 \times 10^8 \text{ mol}^{-1} \text{ L s}^{-1}$,²⁴ unfortunately k_q has not been measured for Trolox in water but by analogy with α -tocopherol, which has a similar structure and quenching constants,^{25,26} $k_q \sim 2.6 \times 10^9 \text{ mol}^{-1} \text{ L s}^{-1}$. That both DABCO and Trolox quench with the same rate constant in cotton indicates the reaction is diffusion controlled with $k_d(^1\text{O}_2) < 2.8 \times 10^8 \text{ mol}^{-1} \text{ L s}^{-1}$. It is unlikely that $^1\text{O}_2$ would diffuse at a slower rate than TEMPOL and thus $k_d(^1\text{O}_2) \sim 2\text{--}3 \times 10^8 \text{ mol}^{-1} \text{ L s}^{-1}$ may be estimated.

For the high concentrations of Remazol Blue, RB, used here, $\tau(^1\text{O}_2)$ will be controlled by quenching with RB and thus $\tau(^1\text{O}_2)^{-1} = k_q^{(\text{RB})}[\text{RB}]$. From the fit, Figure 2, and previous discussion $\tau(^1\text{O}_2) = 71/2.5 \times 10^8 = 280$ ns, which when combined with the concentration of RB in the amorphous zone gives $k_q^{(\text{RB})} = 1.3 \times 10^7 \text{ mol}^{-1} \text{ L s}^{-1}$. No values have been measured for this compound in solution, but by comparison with similar chemicals a value in the range 10^7 – $10^9 \text{ mol}^{-1} \text{ L s}^{-1}$ would be expected.^{27,28}

Conclusion

The behavior of nitroxide radicals on dry cotton may be explained using a simple liquid model with a microviscosity of 30 cP in the amorphous regions. Consistent with this model the radicals may be polarized by the radical–triplet pair mechanism with singlet oxygen. This provides an interesting extension to classical spin probing. It is hoped that the current work will encourage others to investigate cotton as a host for radical reactions and also aid the interpretation of previous results.^{2–10}

Note Added in Proof. Prof. Bredereck (Stuttgart) has pointed out to the author that the estimate of the amorphous layer depth is in good agreement with results from exclusion column chromatography (Bredereck, K.; Gruber, M.; Otterbach, A.; Schulz, F. *Textilveredlung* **1996**, 31, 194).

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