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Interaction of Charged Nitroxyls with Some High-polymer Membranes. A Spin Label Study

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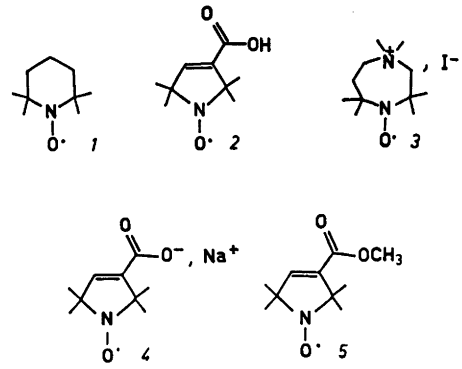
The bonding and immobilization of a neutral, an anionic and a cationic nitroxyl radical to some neutral and negatively charged high-polymer membranes have been investigated. ESR measurements show that the mobility and the diffusion of the radicals in neutral cellulose membranes are related to the degree of swelling and to the nature of the radical. Additional hydrogen bonding, electrostatic interaction and stereochemical interaction between nitroxyls and cellulose may exist. The latter types of interaction are strong between cellulose and *4* in acetone, and exist also between *5* and cellulose in water. In the charged membranes the charge of the radical has a much greater influence on the form of the ESR spectra than has the degree of swelling. It was shown that sulfonated polysulfones bind the cation radical stronger than carboxymethylcellulose, even if the sulfonated polysulfone has larger pores. A neutral radical is also bound and immobilized in the sulfonated polysulfone. Increasing ionic strength in the membrane decreases the mobility of the radical. The possibility of stereochemical and electrostatic interactions between membranes and nitroxyls is discussed.

The purpose of this work is to extend previous results[1] on the non-covalent spin labelling of polycarboxylic acid membranes. The use of nitroxyl radicals has recently been introduced in the field of ion exchange membrane chemistry;[2–4] the aim in this case is to characterize the interactions inside a mechanically ordered matrix, which are dependent on the porosity of the support or specifically due to the nature of the functional groups of the radical and the support, with solvent effects taken into account.

Cellulose membranes were studied for two reasons: (1) because of its neutrality the membrane serves as model of the backbone of polymeric ion exchange membranes, and (2) to determine the adsorption and desorption of neutral, positively charged and negatively charged small molecules in the cellulose in water, acetone, and acetone/water, respectively. The choice of nitroxyl spin labels was made accordingly; the nitroxyls *1* – *5* are of similar molecular size, their spectroscopic behaviour is similar, the only variable is the nature of the non-paramagnetic part of the molecule.



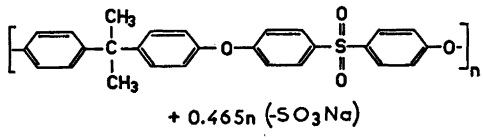
Cation exchange membranes of different chemical nature (polycarboxylic and sulfonated polysulfonic) and with varying porosity were chosen to determine the nature of the electrostatic attraction and repulsion in the membrane using the spin labels *1*, *3* and *4*. Provided there are no stereochemical interactions or hydrogen bonds present the neutral spin label would provide information on the electrostatic interaction between the membrane and the

counterion, and the anion spin label on the activity of the anions in the membrane, respectively.

EXPERIMENTAL

Nitroxyls. The syntheses of the radicals *1* – *5* have been described previously.[5–8] The radicals were dissolved in water or the appropriate solvent to a concentration of 1 mg/ml.

Membranes. The polymeric membranes consisted of regenerated cellulose, carboxymethylcellulose and of sulfonated polysulfone (structural unit *6*).



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The films of regenerated cellulose are from a tube for dialysis manufactured by the Union Carbide Corporation, U.S.A., treated with a boiling solution of sodium bicarbonate and subsequently washed with water. The pore size for the water swollen cellulose membrane is 2 nm (estimated by the manufacturer).

The films of carboxymethylcellulose (CMC) were prepared as described in Ref. 9; they are polycarboxylic ion exchange membranes obtained by the reticulation of CMC with formaldehyde. Pore size in water swollen membranes is 0.9 nm.[10]

The membranes of the sulfonated polysulfone are manufactured by Rhône-Poulenc, France; they are membranes for hyperfiltration (dense film (PSS) or asymmetric membrane (TR) and for ultrafiltration (IRIS)). The pore size increases from PSS to IRIS.[11]

The degree of swelling, τ, of the membrane is expressed by the weight of solvent in the membrane to the weight of dry membrane.

Preparation of samples. The cellulose films were equilibriated in water, acetone, and water/acetone, the ionic membranes were equilibriated in water or in water solutions of high ionic strength (1 M with respect to NaCl or $CaCl_2$). The effect of the counterion was studied by stabilizing the membranes in Na^+ and Ca^{2+} forms, respectively.

2 – 5 mg of the membranes were equilibriated during at least 24 h in 1 ml of the solvent containing 0.1 ml of the solution of the radical. Before measuring the spectra the membranes were washed rapidly in an excess of the proper solvent to eliminate radicals absorbed to the surface of the membrane, and rapidly dried between sheets of filter paper.

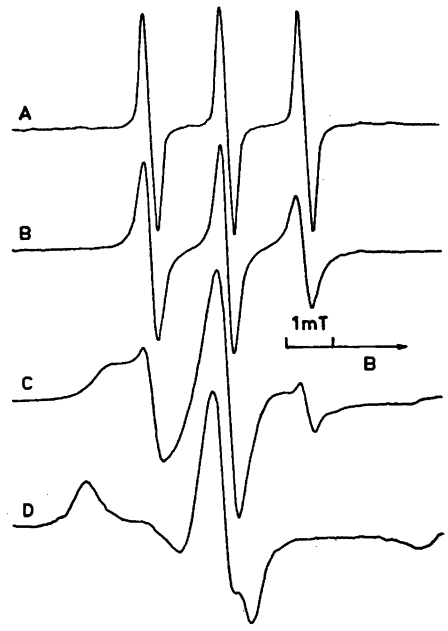

Fig. 1. ESR spectra of nitroxyl radicals. A, *3* in water solution; B, *3* in a Na-CMC membrane in water; C, *3* in a Ca-TR membrane in 1 N $CaCl_2$ water solution; D, *4* in a cellulose membrane after 2 h washing in acetone.

Mobility and diffusion of nitroxyls by ESR. The spectra were measured at 20 °C with an X-band Varian E 3 spectrometer; the films were placed in a quartz plate sample tube S806A from J. F. Scanlon & Co.

The different types of spectra obtained are collected in Fig. 1.

The hyperfine coupling constant of the nitrogen nucleus ^{14}N,a_N, expresses the separation between the lines in spectra of types A and B. It is supposed to be the isotropic value and is a measure of the polarity of the environment of the nitroxyl. Spectra of types A and B represent cases of rapid rotational diffusion; the width of such a spectrum, L_1, is determined from the first to the last maximum of the spectrum. When the nitroxyl is immobilized the spectrum is characterized by its total width, L_2, from the first maximum to the last minimum. L_2 gives an estimate of the anisotropic contributions to the linewidths in cases where molecular tumbling makes the angular components of the hyperfine tensor a function of time.

The mobility of the spin label is expressed by the ratio of the height of the high field line I_{-1} and the center line I_0 in the limit of rapid rotation ($10^{-11} - 5 \times 10^{-9}$ s).[13] In the range of slow diffusion ($> 5 \times 10^{-9}$ s) it is difficult to

Table 1. Data from ESR-spectra of *1*, *3* and *4* in water and acetone ($\sim 10^{-3}$ M) and of *4* in glycerol solution (10^{-3} M).

Radical	Solvent	t °C	a_N/mT	I_{-1}/I_0	L_1/mT	L_2/mT
1	Acetone	+20	1.60 ± 0.02	0.98	3.23	
	Water	+20	1.73 ± 0.02	0.94	3.21	
3	Acetone	+20	1.54 ± 0.02	0.95	3.05	
	Water	+20	1.54 ± 0.02	0.94	3.23	
4	Acetone	+20	1.53 ± 0.02	0.94	3.28	
	Water	+20	1.63 ± 0.02	0.95	3.36	
	Glycerol	+80	1.58 ± 0.02	0.68	3.11	
	Glycerol	+27	1.56 ± 0.02	0.39	3.12	
	Glycerol	0	1.55 ± 0.02	0.23	3.12	
	Glycerol	−35				6.68
	Glycerol	−70				7.10

estimate correlation times only from experimental spectra, since the little motion the spin labels have relative to the macromolecular structure is anisotropic. A complete lineshape analysis would yield details concerning the nature of the motion in addition to the apparent correlation time. Work on this matter is in progress.

The interaction between the membrane and the radical is evidenced by the change in mobility of the radical on introducing a portion of solvent into the sample cell. The spectra were measured from time to time in order to estimate the rate of diffusion of the radical from the membrane into the solvent. Finally the membrane was stored in pure solvent, and spectra of the films were measured after different periods of washing.

Radical *4* has been used as a model, and its spectra in glycerol solutions at different temperatures have been measured and compared to results of measurements with labelled membranes. The ESR characteristics of *1*, *2* and *4* are collected in Table 1.

RESULTS AND DISCUSSION

Interactions in cellulose membranes. The nitroxyls *1*, *3* and *4* are all incorporated into cellulose. However, the amount of adsorbed radical is different in the three cases. From the intensity of the spectra the conclusion is drawn that the anion is adsorbed in greatest concentration and the neutral radical is adsorbed in smallest concentration. Radicals *1* and *4* show similar spectral patterns both in glycerol solution and in the cellulose matrix, respectively. The spectral width L_1 for *1* is larger in cellulose than in solution. The environment of the radicals *1* in the cellulose is therefore to be considered very strongly polar. The radicals *3* and *4* are adsorbed in an environment resembling water solution since the spectral widths L_1 are equal in cellulose and in water. The mobility of *3* in cellulose is greater than that of *1* and *4*. The results of the measurements are collected in Table 2.

Of particular interest is the very strong interaction between the cellulose and *4* in acetone. In order to investigate the influence of chemical nature of the radical on the form of ESR spectrum, the results of measurements with *4* were compared to spectra of cellulose labelled with the corresponding free acid *2*, its methyl ester *5*[8] and with the anion *4* at pH = 9. The anion radical was prepared by neutralizing solutions of *2* in water and acetone to pH = 7 and to pH = 9 with a solution of sodium hydroxide in water.

From acetone *2* is incorporated to a smaller degree than the anion form *4*. The latter is very strongly associated and does not diffuse from the cellulose. At pH = 9, *4* is very easily incorporated from water, probably due to extensive swelling of the macromolecular structure at this pH value. The radical *5* is incorporated to a very low degree; in acetone a fraction of it stays immobilized in the membrane over a long period, probably due to hydrogen bond formation. The acid form of *2* and the anion form *4* are strongly associated to the cellulose in acetone, on addition of water to the samples they are very rapidly liberated.

The radicals *1*, *3* and *4* were kept in contact with samples of the cellulose membrane in

Table 2. Incorporation and diffusion of the radicals *1*–*5* in cellulose membranes.

Radical	Solvent	I_{-1}/I_0	a_N/mT	L_1/mT	L_2/mT	Effect of addition of solvent into sample tube	Ratio [a]	Ratio [b]
1	Acetone	0.33±0.03	1.68±0.2	3.48±0.02		+ Acetone: slow diffusion	3:1	0.34
	Water	0.55±0.05	1.75±0.02	3.48±0.02		+ Water: rapid diffusion	∞	
2	Acetone	Partly incorporated and completely immobilized			7.0±0.2	+ Acetone: partial diffusion		
	Water	0.33±0.03	1.58	3.19±0.02		+ Water:		
	+ water	0.99±0.03	1.61	3.15±0.02		rapid diffusion		
3	Acetone	0.48±0.01	1.53	3.02±0.05		+ Acetone: slow diffusion of free radical	2:1	0.33
	Water	0.86±0.02	1.60	3.23±0.05		+ Water: slow diffusion of free radical	20:1	0.77
4	Acetone	Very large fraction incorporated and strongly immobilized				+ Acetone: no diffusion	1:1	No change
		0.20			6.9±0.1	+ Water: rapid diffusion		
	Water	0.69±0.02	1.66	3.25±0.05		+ Water:		
	+ water	0.91±0.04	1.66	3.25±0.05		rapid diffusion	100:1	0.60
	Water/NaOH pH = 9	0.58±0.02	1.60	3.20±0.05		+ Water: rapid diffusion		
5	Acetone	Small fraction incorporated and immobilized			6.7±0.2	+ Acetone: no diffusion		
	Water	0.42±0.06	1.58	3.15±0.05		+ Water: partial diffusion		

[a] I_0(initial)/I_0(after 1.5 h). [b] I_{-1}/I_0(after 1.5 h).

mixtures of acetone and water. This allows for comparison with respect to the swelling of the membrane. In the solvent mixtures the spectra show the three line pattern of the diffusing radical, but the relative intensities of the lines are dependent on the degree of swelling of the cellulose in the same way as the spectra of *4* in glycerol are temperature dependent. The spectrum of *4* in cellulose is a superposition of the spectra of free and associated radicals in acetone-water mixtures; the spectra are of type C, Fig. 1. After washing with acetone an immobilized portion of the radical stays within the membrane (spectrum of type D in Fig. 1). This is probably explained by interaction between the anionic group of *4* and functional groups in the cellulose.

The mobility of the spin label is directly related to the porosity of the film, determined as the degree of swelling, τ, Fig. 2. To the value $\tau \equiv 0.3$ the mobility of the radical increases rapidly, at higher values the changes are small. At values $\tau > 0.3$ the radicals show a nearly constant I_{-1}/I_0 ratio which is supposed to be dependent only on the nature of the radical and its interaction with the support.

Interactions in the ion exchange membranes. The radicals *1*, *3* and *4* are incorporated in the ion exchange membranes, except for *1* in CMC-membranes, where the concentration of radical in the membrane is too low to give

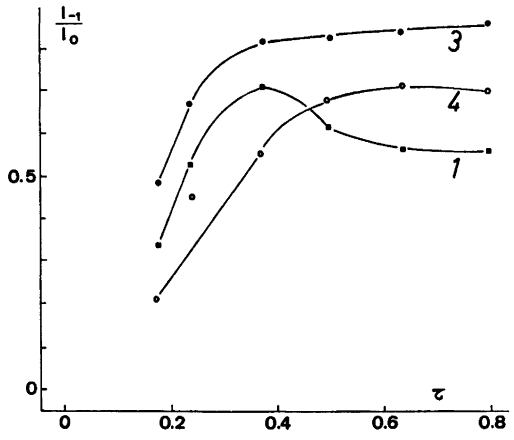

Fig. 2. The dependence of the ratio I_{-1}/I_0 for the nitroxyl radicals *1*, *3* and *4* on the degree of swelling τ of a cellulose membrane. The τ values correspond to the swelling of the membrane in (from left to right) pure acetone, acetone/water 80/20 (w/w), acetone/water 60/40 (w/w), acetone/water 40/60 (w/w), acetone/water 20/80 (w/w), and pure water, respectively.

Table 3. Characteristics of the interaction of *1*, *3* and *4* with ion exchange membranes from ESR-spectra. A, type of incorporation; B, spectrum.

Membrane	τ	*1*[a] A	B	L_2/mT[d]	*3*[a] I_{-1}/I_0[e]	A	B	(L_1 or L_2)/mT	*4*[c] I_{-1}/I_0	L_1/mT
NaCMC/H_2O	0.758	N			0.6			$L_1 = 3.3$	0.35	3.27
NaCMC/NaCl	0.576	N			0.4			$L_1 = 3.3$	0.25	3.27
CaCMC/H_2O	0.468	N			0.4				Very low incorporation	
CaCMC/$CaCl_2$	0.410	[b]			[b]					
NaPSS/H_2O	0.160						D	$L_2 = 6.2$		
NaPSS/NaCl	0.154	L	D	6.3		S	D	$L_2 = 6.3$	0.83	3.27
CaPSS/H_2O	0.154	L	D	6.3		S	D	$L_2 = 6.2$	Very weak signal	
CaPSS/$CaCl_2$	0.150	S	D	6.3		S	D		Very weak signal	
NaIRIS/H_2O	1.91	F	C			F	C		0.91	3.27
NaIRIS/NaCl	1.65	F	C	6.4		F	C		0.86	3.27
CaIRIS/H_2O	1.81	F	C			F	C		0.88	3.27
CaIRIS/$CaCl_2$	1.66	F	C	6.2		F	C		0.84	3.27
NaTR/H_2O	1.64	S	D			S			0.94	3.27
NaTR/NaCl	1.62	S	D			F	C		0.88	3.27
CaTR/H_2O	1.52	S	D			S	D		0.88	3.27
CaTR/$CaCl_2$	1.49	S	D			F	C		0.69	3.27

[a] N, no incorporation; L, low incorporation; S, strong association; F, fixation of two types. [b] Too low incorporation to give significant results. [c] Spectra of type B. [d] ± 0.2 mT. [e] ± 0.1 mT.

significant results. The radical concentration in membranes in sodium form is lower than in membranes in calcium form. Results of the ESR measurements are collected in Table 3.

The sulfonated polysulfonic membranes immobilize *1* and *3*, the measured spectra are of type C or D, Fig. 1. The immobilization of *3* is explained by interactions with negatively charged groups of the membrane. However, this explanation is not valid for *1* which shows a spectrum of type D also for membranes with a high degree of swelling (IRIS and TS). This may be taken as evidence for electrostatic interaction between *1* and PSS. Furthermore, *1* is not incorporated in the CMC membrane in spite of the relatively high porosity. Likewise, *3* is less immobilized in CMC than in IRIS and TR with a higher degree of swelling.

These results permit the following conclusions of the influence of the charged $-SO_3H$ and $-COOH$ groups on the fixation of *3* and hence the form of the spectra; the $-SO_3^-$ group binds *3* more strongly than the $-COO^-$ group. The above conclusion is valid also when stereochemical interactions between the radical and the polysulfone and CMC matrices are taken into account.

The role of the ionic strength and the nature of the cation on the mobility of the radical can be estimated by the use of *4*. It is notable that the ionic strength which decreases the degree of swelling also diminishes the mobility of the radical. Consequently, in the Ca-form the swelling is the least which is reflected in a low value of the ratio I_{-1}/I_0 in the spectra.

Spectra of *4* in the three sulfonated polysulfone membranes indicate the difference in the morphology of the three membranes (PSS, TR and IRIS): (1) in the dense film (PSS) there is a low degree of incorporation and the radical is totally immobilized, (2) in the asymmetric membranes (TR)[11] and the ultrafiltration membranes (IRIS) the measured I_{-1}/I_0 ratios are almost identical. In the asymmetric membrane the influence of the skin is not measurable; this shows that the macroporous phase is modified by the influence of the conditions in the environment.

Diffusion of the radicals from cellulose. The radical labelled, washed and dried films were kept in contact with the pure solvent during 1 h 30 min after which they were dried and spectra measured. The results are collected in Table 2.

The diffusion rate from the membrane into water decreases for the radicals in the order *1* > *4* > *3*. On acetone treatment the radicals stay largely immobilized in the membranes; the diffusion into water is rapid in all the cases except for a small portion of radical which probably is immobilized in pores of minor dimension in the membrane. The diffusion of *1*, which is very easily soluble in water and has somewhat smaller dimensions than the other radicals, is very rapid on comparison with the more polar radicals.

The electrostatic interaction between *4* and the membrane in acetone solution is confirmed as *4* does not diffuse into acetone. The spectrum of this complex is comparable to the spectrum of dry covalently labelled cellulose.[12]

The distribution of radicals between pores of varying sizes gives a possibility to explain the results of Fig. 2 in view of the diffusion from the membrane: in the cases of *3* and *4* the contribution of a small immobilized fraction of radical does not change the ratio I_{-1}/I_0 significantly, whereas for *1* the mobility decreases while the radical still shows a line spectrum of type B; consequently the ratio I_{-1}/I_0 diminishes more than for *3* and *4*.

Diffusion of the radicals from ion exchange membranes. In all the cases the diffusion of *4* into solvent is total in one hour. The radical anion is thus excluded from the negatively charged membrane.

It was not possible to measure the diffusion from the CMC membranes for *1*; the fraction of radical incorporated was too small. From the sulfonated polysulfone membranes the diffusion of *1* is very slow. After 2 h of washing 4/5 of the radical remain incorporated in the membrane, and the spectral pattern remains the same (type D). Neither the presence of salt, nor the elevated swelling of the IRIS and the TR membranes alter this result. It is probable that there is a stereochemical or electrostatic interaction in these cases.

The diffusion of *3* from the CMC membrane is more rapid for the membrane in calcium form than in sodium form. The amplitude of the center line has decreased to 1/10, and 1/2 respectively, during 2 h washing. The same phenomenon is observed for the sulfonated polysulfone membranes, although the difference

is smaller. Thus, after 2 h washing the amplitude of the signal from the TR membrane in its sodium form has not changed while the signal from the membrane in its Ca^{2+} form has decreased to 2/3. This difference was not found in the presence of sodium chloride or calcium chloride; after 2 h washing the signal had decreased with 1/3 in both cases. The presence of salt suppresses the difference between the sodium and the calcium forms by rapid exchange between bound cations and those unbound in the membrane.

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