Local Environment of Cations and Ionomer Morphology from ^{14}N Superhyperfine Interaction. ESR of Cu^{2+} in Nafion/Acetonitrile

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Abstract: The local environment of cupric ions in Nafion salts swollen by CH_3CN and CD_3CN has been investigated by electron spin resonance (ESR) spectroscopy. The advantage of this method is the ability to detect and analyze superhyperfine (shf) splittings from the nitrogen ligands. The number and relative intensity of these shf signals is a direct indication of the number of ^{14}N ligands closest to the central cation. The results indicate preferential solvation of the cation to the oxygen ligands from the water remaining after one cycle of drying of the membranes to constant weight, even though the water/Cu^{2+} and CH_3CN/Cu^{2+} molar ratios are approximately 2.5 and 118, respectively. Only after an additional cycle of drying are all four equatorial ligands replaced by four nitrogen ligands. A clear isotope effect on the solvation process is detected for CH_3CN and CD_3CN, with the latter replacing the water less effectively. Changes in the local environment of the cation over several weeks are detected at room temperature and are thought to arise from the presence of different axial ligands and different degrees of tetrahedral distortions of the tetragonal symmetry of the Cu^{2+} complexes.

Ionomers are amphiphilic polymers that contain up to about 10 mol % of ionic groups. In the ionomers formation of ionic domains, where the charges are located, is due to preferential solvation of the ions by a polar solvent.[1,2] The size of the ionic domains and the distribution of the ionic charges have been extensively studied by small-angle X-ray scattering (SAXS), where an extra peak is detected in ionomers, compared to the polymer backbone without ionic groups.[3] The quantitative interpretation of SAXS results is model dependent and is still a question of debate.[4] The absence of the ionic peak in some ionomers is often justified by a fortuitous cancellation of electron density contributions from the organic and ionic portions.[5] Small-angle neutron scattering (SANS) and a variety of spectroscopic techniques have also been useful for the study of ionomer morphology.[1,2,5]

The local environment of the ionic charges can be studied directly with electron spin resonance (ESR) spectroscopy of ionomers whose pendant, sulfonic or carboxylic, groups have been neutralized by paramagnetic cations such as Cu^{2+}, Mn^{2+}, and Ti^{3+}.[6-8] In recent years we have obtained detailed information on the specific ligation of the cations as a function of solvent structure and amount, on the ionic mobility as a function of temperature, and on the distribution of and distance between the cations. This information has been deduced from a variety of electron magnetic resonance methods such as continuous wave (cw) ESR and electron nuclear double resonance (ENDOR).[9-12] In cw ESR experiments the most important information is derived from an analysis of the **g**-tensor components, which reflect the molecular symmetry and the crystal field parameters around the paramagnetic cations; of the hyperfine splittings from the central cation and interacting nuclei; and of the line widths and line shapes of the spectral lines, which reflect unresolved interactions with neighboring nuclei. In addition, reversible changes in the ESR spectra as a function of temperature have been analyzed to provide data on the motional mechanism and energetics.

In the ESR spectra of Nafion perfluorinated membranes neutralized by Cu^{2+} ions, the **g**-tensor components and the hyperfine interaction of the unpaired $3d^9$ electron with nuclei, enriched in the ^{63}Cu isotope ($I = {}^3/_2$), have been detected and analyzed. In all previous experiments the ligands were oxygens from the solvents used to swell the ionomers: water, methanol, water/methanol mixtures, dimethylformamide (DMF), and tetrahydrofuran (THF). The concept of "strain" has been very important in the analysis of the results and in the computer simulation of the spectra. Strain is expressed as a distribution of ESR parameters of the solvent ligated paramagnetic cations; the width of the distribution reflects the heterogeneity in the cation site.[11,13]

Because in previous studies we were unable to observe directly interaction of central cations with surrounding ligands, we initiated an ESR study of Cu^{2+} in Nafion swollen by acetonitrile, CH_3CN, and CD_3CN. Indirectly the presence of ^{14}N ligands can be detected from the well-documented effect on the $g_\parallel$ values and on $A_\parallel$, the parallel hyperfine splitting from the ^{63}Cu nucleus.[14] The main advantage of this study is the ability to *directly* deduce the local structure around the cation by an analysis of the superhyperfine splitting from ^{14}N nuclei ($I = 1$) ligated to the central cupric ion. In some systems the ^{14}N superhyperfine splittings are not resolved, and broader lines are observed, compared to cations ligated to oxygen ligands. In favorable cases, however, the superhyperfine splittings are resolved, and the number and relative intensities of these splittings reflect the number of ^{14}N ligands.[15]

Because oxygen ligands from the sulfonic groups are also available in addition to nitrogen ligands, the method we report can give a general picture of the ligation and structure around the cations. Although not all results are completely understood at this stage, this study indicates that the analysis of nitrogen ligation is a method of unsurpassed sensitivity, which can provide directly a detailed picture of the effect of various parameters on the local environment of the cation.

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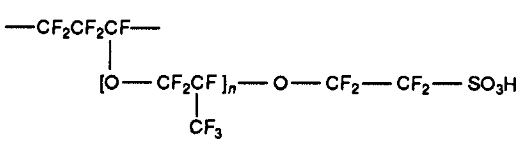
Table I. ESR Parameters for Various Sites of Copper Ions in Nafion Membrane Soaked with Acetonitrile[a]

system	$g_\parallel$	$g_\perp$	$A_\parallel$, G	$A_\parallel$(shf), G	$A_\perp$(shf), G	assignments comments or remarks
Site I						
Cu/Nafion/CD_3CN	2.3931	2.074	129.7			Cu^{2+} ligated to $4H_2O$, $2SO_3^-$
1st soaking with dried solvent			(0.01449 cm^{-1})			(ref 16)
Site II						
Cu/Nafion/CD_3CN	2.4201	2.074	119.2			Cu^{2+} ligated to $6H_2O$
1st soaking with dried solvent			(0.01347 cm^{-1})			(ref 16)
Site a						
Cu/Nafion/CD_3CN	2.3491	2.0745	144.6	12.7	15.9	4 N ligands
2nd soaking with dried solvent			(0.01586 cm^{-1})	(0.00140 cm^{-1})	(0.00154 cm^{-1})	
Site b						
Cu/Nafion/CH_3CN	2.3581	2.0785	142.2		12.3	2 or 3 N ligands
1st soaking with dried solvent			(0.01566 cm^{-1})		(0.00120 cm^{-1})	
Site a′						
Cu/Nafion/CH_3CN	2.3490	2.0742	145.2	12.4	15.5	4 N ligands
2nd soaking with dried solvent			(0.01593 cm^{-1})	(0.00140 cm^{-1})	(0.00150 cm^{-1})	
Site c						
Cu/Nafion/CH_3CN	2.3082	2.0569	156.9		13.4	4 N ligands
1st soaking with fresh CH_3CN (not dried)			(0.01691 cm^{-1})		(0.00129 cm^{-1})	not easy to reproduce this spectrum
Site d						
Cu 4%/Zn 96%/Nafion/CH_3CN	2.3436	2.0719	143.2		13.4	2 N ligands
(dried) 1st soaking			(0.01567 cm^{-1})		(0.00129 cm^{-1})	
Site a″						
Cu 4%/Zn 96%/Nafion	2.3348	2.0743	148.2	12.4 G	15.83	4 N ligands
CH_3CN dried, 2nd soaking			(0.01616 cm^{-1})	(0.00135 cm^{-1})	(0.00153 cm^{-1})	
Site b′						
Cu 100%/Nafion	2.3520	2.0794	140.4		12.7	1 N or 2 N ligands
CH_3CN, dried 1st soaking			(0.15472 cm^{-1})		(0.00123 cm^{-1})	
Site e						
Cu 4%/Nafion/CH_3CN	2.3191	2.0709	146.8		15.1	4 N ligands
2nd soaking with dried solvent, after 12 days			(0.01589 cm^{-1})		(0.00146 cm^{-1})	two sites, leading to loss of shf resolution
Site e′						
Cu 4%/Nafion/CH_3CN	2.3168	2.0708	146.9		15.0	4 N ligands
2nd soaking with dried solvent, after 30 days			(0.01588 cm^{-1})		(0.00145 cm^{-1})	two sites

[a] Details are given in the text. The nomenclature used for the different sites is as follows. Sites I and II represent ligation of Cu^{2+} to oxygen ligands. Sites of type a (a, a′, and a″) represent ligation to four nitrogen ligands and have essentially the same g, $A(^{63}Cu)$, and shf(^{14}N) values. Sites of type b (b, b′) represent ligation to two, and possibly three, nitrogen ligands. Site c indicates four nitrogen ligands, but the spectral parameters are different from those of type a. Site d represents ligation to one or two nitrogen ligands. Sites e (e, e′) describe the change of the spectra with time and most likely represent a superposition of two types of ligation for Cu^{2+}.

Experimental Section

The Nafion 117 sulfonated membrane, with an equivalent weight of 1100 g/mol of SO_3H and a thickness of 0.13 mm, were obtained from DuPont. The formula is given below.



$$-CF_2CF_2CF- \quad [O-CF_2CF(CF_3)]_n-O-CF_2-CF_2-SO_3H$$

Nafion, n = 1

When stored for several weeks Nafion membranes become slightly brown. The color was removed by soaking small pieces of the membrane (suitable for ESR experiments, about 1 × 2 cm) in isopropanol, with stirring, for about 24 h. After this stage the membranes were dry blotted with filter paper, dried in air for 2 h, and acidified in 9 M sulfuric acid at 350 K with stirring for 48 h. Acidified membranes were rinsed with deionized, doubly distilled water to pH = 6 and stored for further use. To prepare Nafion salts, the membranes were dried under vacuum to constant weight for 24 h at ambient temperature and for about 3 h at 353 K, to a final dynamic pressure of 2–3 × 10^{-5} Torr, and then equilibrated for 24 h with the stoichiometric amount of $CuSO_4$ containing ^{63}Cu (98 enrichment). The amount of Cu^{2+} retained by the membrane as a function of the amount of cations available in the neutralizing solution has been determined by atomic absorption and has been reported recently.[11] Additional experimental details have been published.[9-13]

Equilibrated membranes dried to constant weight were soaked with CH_3CN (Eastman Kodak, reagent grade) or with CD_3CN (Icon). Some samples were dried again for 24 h at ambient temperature to a final dynamic pressure of about 3 × 10^{-5} Torr and soaked again with acetonitrile. It is important to note that on heating under vacuum the membranes that have been soaked with CH_3CN turned brown. We decided not to use heating in the second drying process. The solvent uptake of the membranes was determined gravimetrically.

ESR spectra at X-band were measured with a Bruker 200D SRC spectrometer operating at 9.7 GHz (empty cavity at ambient temperature) and 100-kHz magnetic field modulation, interfaced with a data acquisition system based on an IBM PC/XT and the software EPRDAS (Mega Systems Solutions, Rochester, NY). The microwave frequency was measured with the HP 5342A frequency counter. The absolute value of the magnetic field was measured with a Micro-now gaussmeter Model 515B and with 2,2-diphenyl-1-picrylhydrazyl (DPPH, g = 2.0036) and Cr^{3+} in a single crystal of MgO (g = 1.9796) standards. All spectra were recorded at microwave power of 2 mW.

Results

All ESR spectra presented below are taken at 110 K; spectra at 77 K, taken in the finger Dewar, are identical. We prefer the higher temperature, because the noise is reduced for the spectra taken in the variable-temperature insert. The concentration of the cupric ions is given in percent of the total amount needed to fully neutralize the membranes, assuming two sulfonic groups per each cation. In previous studies we deduced that the amount of Cu^{2+} in fully neutralized membranes is 78.6% of the total amount calculated for full neutralization of the membranes.[11]

ESR spectra in Nafion that has been equilibrated with 4% Cu^{2+}, dried to constant weight as described above, and soaked with dried CD_3CN are shown in Figure 1. The spectrum shown in Figure

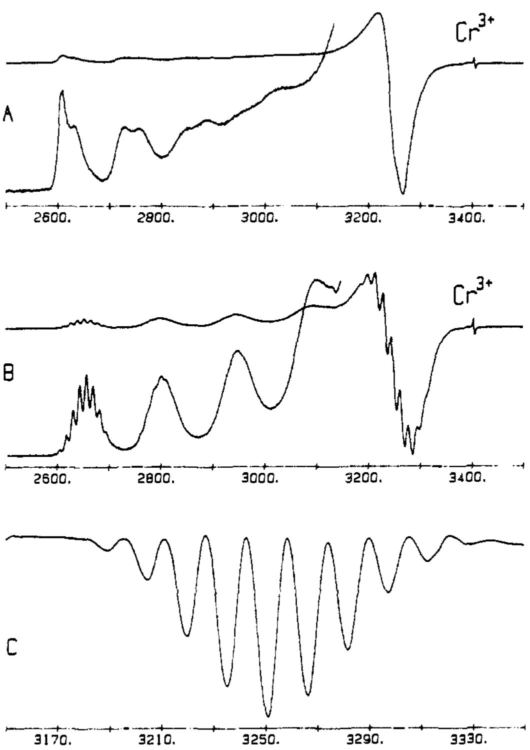


Figure 1. X-band ESR spectra at 110 K for Cu^{2+} in Nafion soaked by dried CD_3CN: (A) after one cycle of soaking; (B) after two cycles of soaking; (C) second derivative of the perpendicular signal in (B). The signal at high field is the standard, Cr^{3+} in a single crystal of MgO.

1A clearly represents two sites for the cupric ions, differing in their $g_\parallel$ and $A_\parallel$ (from ^{63}Cu). The ESR parameters for the two sites, site I and site II, are given in Table I. The values of $A_\perp(^{63}Cu)$ are not given, because of lack of resolution of the perpendicular component. The parameters given above are read directly from the spectra and are considered approximate; more accurate values will be deduced from spectra simulations.

Within experimental error the ESR parameters for sites I and II are identical with those detected for Cu^{2+} in dry Nafion that has been partially rehydrated with water (site I) and in Nafion fully equilibrated with water (site II), respectively.[16] Site I has been assigned to Cu^{2+} ligated to oxygens from four water molecules and from two sulfonic groups from the network, while site II has been assigned to fully hydrated Cu^{2+}. The spectra shown in Figure 1A indicate that, in spite of the large excess of CD_3CN available to the membrane, the cations are preferentially solvated by the small amount of water remaining in the membrane after drying under vacuum at 353 K and by the sulfonic groups.

When the sample whose spectrum is shown in Figure 1A is dried again overnight at ambient temperature to a final dynamic pressure of 2.5×10^{-5} Torr and resoaked with dried CD_3CN, the spectrum shown in Figure 1B is obtained. Clear superhyperfine splitting from ^{14}N is observed for the low-field parallel signal ($m_I = -^3/_2$) and for the perpendicular signal. The nine shf components for the parallel component correspond to *four* ^{14}N ligands in the equatorial position of an approximately tetragonally distorted octahedral Cu^{2+} complex. The axial ligands are too removed to significantly affect the ESR parameters. The line shapes from the four ^{14}N ligands are clearly seen in Figure 1C, where we present the computer-calculated second derivative of the perpendicular component. The resolution is signficantly improved compared with the first-derivative presentation and also indicates *nine* shf components with relative intensities close to the theoretically expected values of 1:4:10:16:19:16:10:4:1.[17] The ESR parameters for this site, site a, are given in Table I. Decrease of the $g_\parallel$ values and increase of the $A_\parallel$ values are expected when oxygen ligands are replaced by nitrogen ligands, in agreement with the results for site a. The magnitude of the $g_\parallel$ and $A_\parallel$ shifts, compared with the case of four oxygen ligands, is however smaller than expected for the four N ligands clearly indicated in the ESR spectra superhyperfine pattern.[14,15,17] This will be discussed in more detail below.

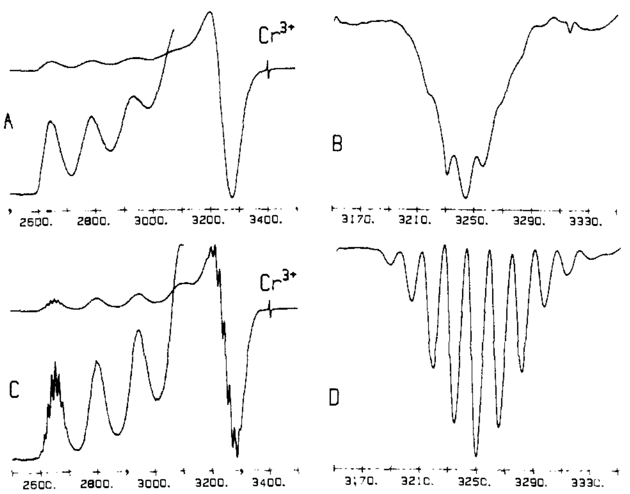


Figure 2. X-band ESR spectra at 110 K for Cu^{2+} in Nafion soaked by dried CH_3CN: (A) after one cycle of soaking; (B) second derivative of the perpendicular signal in (A); (C) after two cycles of soaking; (D) second derivative of the perpendicular signal in (C). The signal at high field is the standard, Cr^{3+} in a single crystal of MgO.

In Figure 2 we present corresponding results for Nafion swollen by CH_3CN. The ESR spectrum obtained after the first soaking of dry Nafion containing 4% Cu^{2+} with the dried solvent is shown in Figure 2A. The parallel signals are broad and most likely represent superimposed sites, with different $g_\parallel$ and $A_\parallel(^{63}Cu)$ values. The parameters, read directly from the center of the signals, are given in Table I and are assigned to site b. The resolution of the perpendicular component can be somewhat improved by taking the second derivative, as shown in Figure 3B; the five lines seem to indicate two ^{14}N ligands. The presence of nitrogen ligands is suggested also by the lower g and higher A for site b, compared to all-oxygen sites I and II. Less than four nitrogen ligands are also suggested by higher $g_\parallel$ and lower $A_\parallel$ values for site b, compared to site a.

In Figure 2C we present the ESR spectrum of the sample shown in Figure 2A, after an additional cycle of drying at ambient temperature to a final dynamic pressure of 2.5×10^{-5} Torr and soaking with dried CH_3CN. The ESR spectrum, site a′, is identical with that shown in Figure 1B (site a), indicating the presence of Cu^{2+} ligated to four equatorial nitrogen ligands. The second derivative of the perpendicular component shown in Figure 2D also indicates four nitrogen ligands.

If dried Nafion containing 4% Cu^{2+} is soaked with CH_3CN from a freshly opened container (not dried over molecular sieves), the ESR spectrum shown in Figure 3A is obtained; this is site c in Table I. The splittings observed for the perpendicular signal, shown more clearly in the second derivative in Figure 3B, correspond to ligation of the cupric ions with four nitrogen ligands. The additional lines detected in the second derivative at high fields, indicated by arrows in Figure 3B, and lack of resolution in the parallel signals, are most likely due to a superposition of sites with different $g_\parallel$ values.

In Nafion swollen by water we used the method of paramagnetic dilution to study ESR spectra of Cu^{2+} at low concentration, <10%, in Nafion neutralized by a Cu^{2+}/Zn^{2+} mixture.[16] The advantage of this method is the ability to obtain the maximum resolution of the spectra from paramagnetic cations, without contribution from dipolar interaction and broadening, in fully neutralized membranes. The ESR spectrum of Cu^{2+} in Nafion fully neutralized by a Cu^{2+}/Zn^{2+} mixture containing 4% Cu^{2+} and soaked with dried CH_3CN is shown in Figure 4A. The parameters for this site, site d, are given in Table I. The second derivative of

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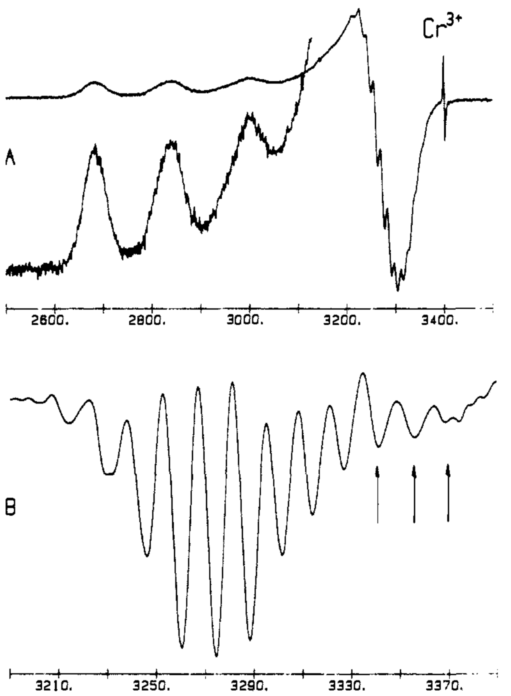

Figure 3. X-band ESR spectra at 110 K for Cu^{2+} in Nafion soaked by fresh (not dried) CH_3CN: (A) after one cycle of soaking; (B) second derivative of the perpendicular signal in (A). Arrows indicate additional lines due to a second site for the cupric ions. The signal at high field is the standard, Cr^{3+} in a single crystal of MgO.

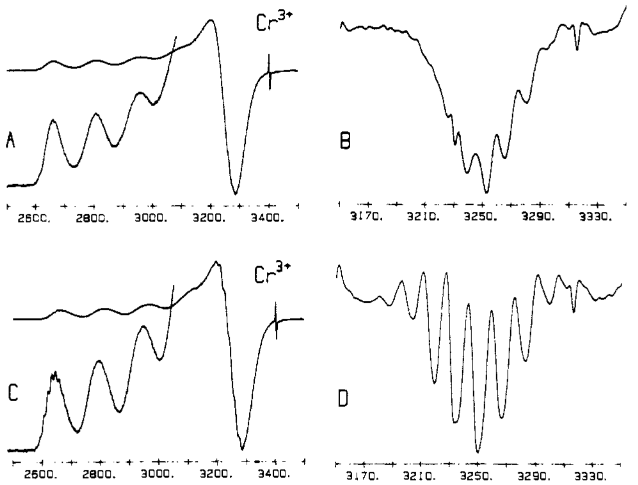

Figure 4. X-band ESR spectra at 110 K for Cu^{2+} in Nafion fully neutralized by a Cu^{2+}/Zn^{2+} mixture containing 4% Cu^{2+} and soaked by dried CH_3CN: (A) after one cycle of soaking; (B) second derivative of the perpendicular signal in (A); (C) after two cycles of soaking; (D) second derivative of the perpendicular signal in (C). The signal at high field is the standard, Cr^{3+} in a single crystal of MgO.

the perpendicular signal, Figure 4B, seems to suggest two or three nitrogen ligands. For site d the hyperfine splitting $A_{\parallel}$ is similar to, and $g_{\parallel}$ is less than, that for site b, where two ^{14}N ligands were suggested. On second drying and soaking with CH_3CN the spectrum shown in Figure 4C is obtained, which indicates shf from four ^{14}N ligands, clearly seen in the second derivative of the signal from the perpendicular direction, Figure 4D. The ESR parameters for this site, site a″, are given in Table I.

The ESR spectrum of Nafion fully neutralized with Cu^{2+}, dried at ambient temperature, and soaked with dried CH_3CN is similar to that shown in Figure 4A and is not reproduced here. The spectrum in the parallel orientation indicates more than one cation site. The major site, site b′ in Table I, has ESR parameters similar to those measured for site b; splittings measured in the second derivative of the perpendicular signal indicate not more than two nitrogen ligands.

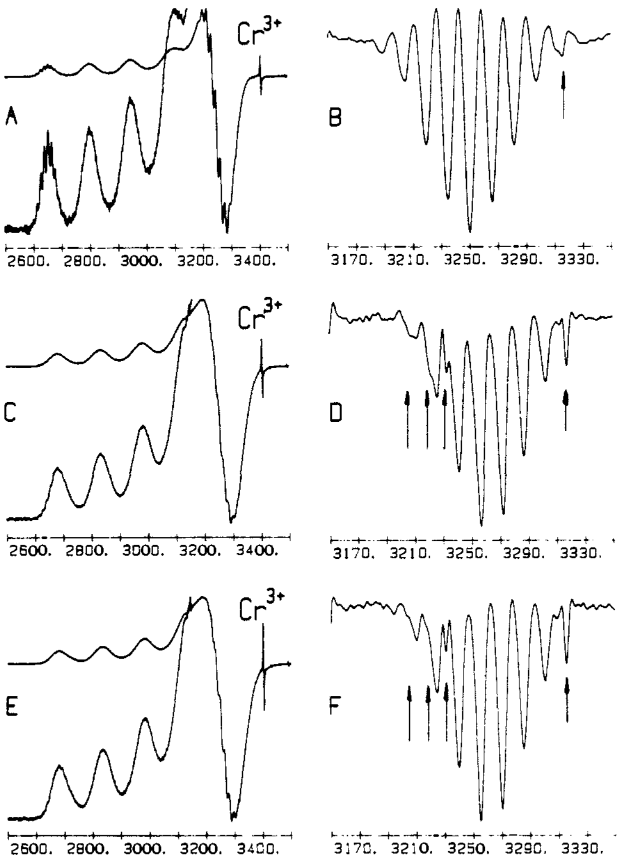

Figure 5. X-band ESR spectra at 110 K for Cu^{2+} in Nafion containing 4% Cu^{2+} and soaked by dried CH_3CN: (A) after two cycles of soaking; (B) second derivative of perpendicular signal in (A); (C) after 12 days; (D) second derivative of the perpendicular signal in (C); (E) as in (C), after 18 more days; (F) second derivative of the perpendicular signal in (E). Arrows indicate major changes in the spectra.

It is important to note that no indication of cation aggregation is detected in membranes fully neutralized with Cu^{2+}, in contrast to the results obtained for Nafion/Cu^{2+} swollen by water, but similar to those for Nafion/Cu^{2+} swollen by methanol, DMF, and THF.[13] In addition, the signal from Cu^{2+}–Cu^{2+} dimers[12] has not been detected in the half-field magnetic field region in Nafion/Cu^{2+} soaked by CH_3CN.

Over a period of weeks considerable changes occur in the spectra of the samples that have undergone two cycles of drying and soaking with CH_3CN and have been left in sealed tubes, so as to prevent loss of solvent. Figure 5 indicates the changes in the ESR spectrum given in Figure 5A (site a′) as a function of time, together with the corresponding second derivatives of the perpendicular signal. The progressive loss in the resolution of the spectra given in Figure 5, A, C, and E, most likely indicates changes in the ligation of the cation. The ESR parameters for the major sites e and e′ are given in Table I. Subtle changes can be detected in the second derivatives of the perpendicular components and are indicated by arrows in Figure 5, spectra D and F.

Discussion

The multiple sites detected for the cupric ions in Nafion progressively swollen by CH_3CN or CD_3CN seem to indicate a complex solvation process, important effects of the heat treatment on the ligation scheme of the cation, and slow changes in the membrane morphology occurring on a time scale of weeks at ambient temperature. These processes can be followed with great sensitivity by observing shf from nitrogen ligands, in addition to the ESR parameters normally measured, such as the principal values of the g tensor and the hyperfine tensor components from the central cation.

This discussion consists of three parts. In (a) we will select the most important classes of sites for the cupric ions and will discuss the process of solvation by acetonitrile. In (b) we will attempt to clarify the structure and symmetry of the sites where the cation is ligated to four ^{14}N ligands. In (c) we will suggest possible changes in the local environment of the cation as a function of time. We will discuss the significance of the results presented here to other methods for the study of the local environment of cations in ionomers.

(a) Major Sites and Solvation Process. From Figures 1 and 2 it is clear that the most stable ligation scheme for the cation, obtained after two cycles of drying the membranes to constant weight and solvation by dried CH_3CN or CD_3CN, consists of four nitrogen ligands, sites a, a′, and a″. The relative intensities of the shf signals that are clearly seen for the $m_I = -^3/_2$ parallel signal and in the second derivatives of the perpendicular signal, Figures 1C and 2D, are in agreement with this ligation scheme. The principal tensor components of the shf interaction are $A_\parallel(\mathrm{shf}) = 12.5$ G and $A_\perp(\mathrm{shf}) = 15.7$ G ± 0.2 G, and these indicate a predominantly isotropic shf interaction, in accord with results obtained in other complexes containing the CuN_4 group in an approximately tetragonal crystal field.[18] The appearance of shf lines in the parallel region only for $m_I = -^3/_2$ is due to the frequency and m_I dependence of the line widths in the presence of strain.[15]

ESR spectra obtained after the first cycle of solvation indicate strong preferential solvation of the cations by the small amount of water remaining in the membranes after this treatment. The CH_3CN content in the membrane, determined gravimetrically, is 17 wt % in Nafion-H and 14 wt % in Nafion fully neutralized by Zn^{2+}. Assuming that in membranes dried to constant weight there is one H_2O molecule per each sulfonic group,[19] we obtain in Nafion where 4% of the sulfonic groups have been neutralized by Cu^{2+} the following molar ratios: water/Cu^{2+} = 2.5, $CH_3CN/Cu^{2+} = 118$. Only partial ligation by nitrogen from CH_3CN and no ligation by nitrogen from CD_3CN have been detected. This is seen in the completely different spectra in Figure 1A (for CD_3CN), and in Figure 2, spectra A and B (for CH_3CN). Because the experimental procedures were identical, we must assign the different results to a marked isotope effect in the solvation process. In the experiments with CH_3CN, partial ligation of the cation by nitrogen is seen in the second derivative of the perpendicular signal, Figure 2B, which indicates five or seven shf lines, corresponding to *two* or *three* such ligands. In addition, the line width of the perpendicular component in Figure 2A is larger than that in Figure 1A, about 68 G compared to 47 G, due most likely to unresolved ^{14}N shf in Figure 2A and not in Figure 1A.

Explanation of observed isotope effect is not obvious. Although reliable numbers are not available, it is usually accepted that the $-CH_3$ group has larger volume than the $-CD_3$ group.[20] Thus CD_3CN should penetrate easier into the membrane, in contradiction to our observations. The experimental dipole moment of CD_3CN, 3.91D ± 0.0025 D, is larger than that of CH_3CN, for which values between 3.250 and 3.913 D are quoted.[21] Ligation of copper ions by the more polar, deuterated solvent should be easier, again in contradiction with experimental results. A larger dipole moment in CD_3CN could also result in a more structured solvent, and in stronger interactions between the solvent molecules compared to CH_3CN. This effect would lead to a less effective solvation of cupric ions in the deuterated solvent in accord with experimental observations.

Ligation to four N ligands is also detected in Nafion neutralized by a mixture of Cu^{2+} and Zn^{2+} containing 4 mol % Cu^{2+} and soaked with dried CH_3CN, Figure 4, C and D; partial ligation to two or three nitrogen ligands is suggested after the first cycle of soaking with dried solvent.

In our previous study of Nafion solvated by methanol, DMF, and THF, we exchanged the solvent in three cycles of drying to constant weight and soaking by the solvent. The process of solvation could not be followed in detail, because only oxygen ligands, and no shf splittings, were expected.[13] Use of acetonitrile enables monitoring of the solvation process in detail.

The exceptional sensitivity of the solvation scheme to traces of water is seen in the different results obtained for solvation of the membranes with fresh CH_3CN (not dried), as seen in Figure 3. The major site c seems to suggest ligation to four nitrogen ligands. Site c is, however, not the only site detected in the spectrum shown in Figure 3A; the lack of resolution in the parallel component and additional lines in the perpendicular component suggest an additional site. We must stress that the spectra shown in Figure 3 are hard to reproduce, most likely because of very small amounts of water in the solvent, which are impossible to control in our experiments.

(b) Cu^{2+} Ligation to Four Nitrogen Ligands. Complexes of cupric ions ligated to four N-ligands are numerous and have been extensively studied in biological and inorganic systems. Compared to oxygen ligands, the effect of the nitrogen ligands is to decrease the value of $g_\parallel$ significantly and to increase $A_\parallel(^{63}Cu)$. The specific effects of the presence of one to four such ligands have been extensively documented and plotted in the Peisach–Blumberg diagrams.[14] In most cases the shf splittings from the ligands are not completely resolved and the lines are broader, for both the parallel and the perpendicular orientations. As a result the strain effect, which manifests itself in a distribution of $g_\parallel$ and $A_\parallel$ (from copper nuclei), is less evident for nitrogen ligands than for oxygen ligands.[15]

For the case of *four* N ligands, typical values for the ESR parameters are approximately $g_\parallel = 2.25$ and $A_\parallel = 0.0190$ cm^{-1}. The corresponding values for Cu^{2+} in sites of type a (a and a′) are quite different, 2.349 and 0.0159 cm^{-1}. Yet the spectra indicate clearly *four N ligands*. We suggest that the reason for these ESR parameters is a tetrahedral distortion of the tetragonal symmetry. It is well-known that this type of distortion increases the value of $g_\parallel$ and decreases that of $A_\parallel$, compared to a tetragonal symmetry.[22,23] It is important to note that in the membranes neutralized with a mixture of Cu^{2+}/Zn^{2+} cations the parameters for this site, site a″ in Table I, are $g_\parallel = 2.3348$ (lower) and $A_\parallel = 0.01616$ (higher), indicating a slightly smaller tetrahedral distortion. The shf splittings, being dominated by an isotropic interaction, are not affected greatly by the tetrahedral distortion, as seen by comparing values of shf given in Table I with typical literature data.

(c) Long-Term Changes in Cation Ligation. The spectra shown in Figure 5 indicate that over a period of weeks the spectra from samples that contain the CuN_4 group change dramatically: The $g_\parallel$ value is reduced, suggesting less tetrahedral distortion, and the resolution of the shf lines is also reduced. In the second-derivative spectra (Figure 5, D and F), it appears that the complex pattern of lines that represent four N ligands remains, but some new lines appear and are indicated by arrows in these figures. We suggest that these changes are due to rearrangement of the ligands around the cation, while maintaining four N ligands. On the basis of these results we suggest that sites of type b (b and b′) and type c represent ligation to two, three, or four N ligands and, in addition, to two oxygen ligands from water or the sulfonic groups. After the second cycle of drying to constant weight the cation loses one axial ligand (water) to give $(CuN_4SO_3)^+$, which is a site of type a, and in which tetrahedral distortion is possible because of the absence of one axial ligand. Over a period of several weeks, the complex picks up a sixth, axial N ligand; the tetrahedral distortion is less and the $g_\parallel$ value is reduced. The number of N ligands close to the cation is the same, resulting in a major pattern of nine shf lines. The loss of resolution of the shf splittings is due to the slow

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process of change in the ligation and to superposition of different sites.

The effect of various solvents on the morphology of ionomers is a subject of great interest and has been investigated in many systems, including Nafion and sulfonated polystyrene (SPS). In the latter system the effect of thermal treatment has been detected in SAXS studies; heating to 165 °C seems to induce the formation of the ionic peak that did not appear at ambient temperatures.[24] In an extended X-ray absorption fine structure (EXAFS) study of SPS neutralized by Mn^{2+} it was not possible, however, to detect changes in the local structure of the cation due to heat treatment.[25]

To the best of our knowledge this paper contains the first study that gives a detailed molecular picture of the solvation process. Most importantly, it is necessary to keep in mind that simply adding a solvent to a dry ionomer will in general not replace all the ligands with the most abundant solvent.

Conclusions

1. Cupric ions in dry Nafion soaked with CD_3CN are preferentially solvated by the water remaining in the membranes, even though the following ratios have been measured: $CD_3CN/Cu^{2+} = 118$, water/$Cu^{2+} = 2.5$.

2. In membranes soaked with CH_3CN the water ligands around the cation are partially replaced after one cycle of drying of the membranes. The isotope effect in acetonitrile seems to be due to a higher polarity of CD_3CN, compared to CH_3CN.

3. The details on the solvation process can be followed by an analysis of the superhyperfine (shf) structure from ^{14}N ligands. The major site detected after *two* cycles of drying of the membranes and soaking with CD_3CN or CH_3CN clearly indicated four nitrogen ligands in a tetrahedrally distorted tetragonal symmetry. The shf is seen on the $m_I = -^3/_2$ component of the parallel signal and on the perpendicular signal. The resolution of the shf pattern in the perpendicular orientation can be significantly improved by computer-calculated second-derivative presentation of ESR spectra.

4. Changes in the local environment of the cation at ambient temperature on a time scale of several weeks are detected from changes in the shf pattern and are thought to reflect a decrease in the tetrahedral distortion.

5. No evidence for clustering of cations is detected in fully neutralized membranes that underwent one cycle of drying and soaking with CH_3CN.

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Homonuclear Three-Dimensional ^1H NMR Spectroscopy of Pike Parvalbumin. Comparison of Short- and Medium-Range NOEs from 2D and 3D NMR

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Abstract: The short- and medium-range NOEs that can be obtained from 2D NOE spectra and homonuclear 3D HOHAHA-NOE spectra of the protein parvalbumin III of pike have been compared. An extensive analysis has been made of a 3D HOHAHA-NOE spectrum in ^1H_2O of the cross sections perpendicular to the ω_3 axis at the amide resonance frequencies, where most of the sequential connectivities can be found. A single 3D HOHAHA-NOE spectrum resulted in 455 3D cross peaks involving short- and medium-range NOEs on which the assignment of 108 amino acids residues could be based. The 3D data set allowed definition of the secondary structure better than was previously possible, by the observation of a series of new medium-range NOEs. In addition, the 3D spectrum indicated that the amino acid sequence of pike parvalbumin III contained an error and that the protein actually consists of 109 amino acid residues rather than 108 as previously thought.

Structure determination of proteins in solution by high-resolution NMR spectroscopy relies on nuclear Overhauser effects (NOEs), from which interproton distance constraints can be derived.[1] For proteins, the NOEs are generally obtained from 2D NOE spectra and can be observed for interproton distances less than about 5 Å. For such an analysis it is essential to obtain first the sequence specific assignments of the proton resonances. This assignment is usually carried out with a combination of two types of 2D spectra, one involving *J* coupling and the other NOE transfer. However, due to overlap in 2D spectra of proteins the analysis remains a laborious and sometimes impossible task.

The general idea behind the development of 3D NMR spectroscopy is to increase the resolution by correlating the resonance frequencies of three individual spins in three independent dimensions. In this respect, 3D experiments turn out to be very promising for the NMR study of macromolecules.[2,3] In the last few years, various homonuclear 3D experiments such as NOESY-COSY[4] and 3D NOE-HOHAHA (or NOESY-TOCSY)[5,6]

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