Study of polymer gels using paramagnetic probes: e.s.r. spectra of Cu^{2+} in reversible polyacrylamide gels

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The binding of the paramagnetic probe Cu^{2+} to the polymer network was studied by e.s.r. in reversible polyacrylamide gels containing various amounts of water. Analysis of the g values and the Cu ($I = 3/2$) hyperfine couplings indicates ligation of Cu^{2+} to water oxygens and to the pendant amide groups of the network. Four types of cation ligation were detected in the gels at 77 K. The number of N ligands increases from zero in the gels containing 95% water to three in gels containing 60% water. This effect can be correlated with the increase in the network density L, in cm fibre/cm^3. This approach can also explain the significant differences in the ligation of Cu^{2+} in the reversible gels, compared with crosslinked polyacrylamide gels containing the same amount of water. As the temperature increases, motional averaging of the e.s.r. parameters in the fully hydrated complex is observed; in the gels containing 60 and 70% water, anisotropic spectra from complexes with N ligation are observed even at 305 K. The changes with temperature indicate that the samples we measure at 77 K are representative of the binding of the paramagnetic probe in the gel phase in the temperature range studied.

(Keywords: electron spin resonance; polymer gels; polyacrylamide gels; paramagnetic probes; metal binding)

INTRODUCTION

The detailed binding of cations to polymeric networks is of considerable interest for the study of catalytic effects in biological systems[1] as well as in the applications of metal–polymer complexes to catalytic processes of technological importance[2,3].

In recent publications[4–6] we have suggested that specific solvation of cations in polymeric gels can be studied using the method of Electron Spin Resonance (e.s.r.) on networks doped with small amounts of a paramagnetic cation as a probe. These systems are of great fundamental interest because of recent detection of collapse in ionic gels on variations of the temperature, the swelling fluid and the nature of the ionic charges[7–9].

In polyacrylamide (PAA) gels prepared by simultaneous polymerization and crosslinking of acrylamide (AA) with the tetrafunctional monomer *N*,*N*′-methylene-bisacrylamide (BIS) we presented evidence that the binding of Cu^{2+} depends on the pore size of the gels[4]. In small pores ligation to the polymer backbone through a nitrogen ligand was observed while in pores larger than 7 Å the fully hydrated complex is detected exclusively. In the hydrated complexes we have analysed the lineshapes and the g and ^{63}Cu hyperfine tensors for pores in the range 7 to 60 Å in terms of a distribution of the $g_{\parallel}$ and parallel hyperfine splitting $A_{\parallel}$[5,6]. The widths of the $g_{\parallel}$ and $A_{\parallel}$ distributions were found to depend on the pore diameter.

In this paper we analyse the binding of Cu^{2+} in reversible gels formed from aqueous solutions of linear PAA. The water content of the reversible gels ranges from 60 to 95% and roughly parallels the water content of the crosslinked gels swollen to equilibrium[4–6]. The main objective of this study is to compare the binding and dynamics of Cu^{2+} in the reversible gels with the results obtained in crosslinked gels. We will present results which indicate significant differences in the ligation of Cu^{2+} in the two systems: reversible gels favour ligation by the nitrogen of the polymer network and complexes containing ligation to one, two and three N ligands were observed, in addition to ligation only by water oxygens. Higher N ligation is observed as the water content decreases. The difference between reversible and permanent gels can be related to differences in the network density L, which is the length of network (in cm) per unit volume of the solution (in cm^3). E.s.r. study of the gels in the temperature range 77 K to 320 K indicate that e.s.r. spectra measured at 77 K reflect the binding of the cation at higher temperatures, in the temperature range where the system is a gel.

EXPERIMENTAL

The linear PAA used for the preparation of the reversible gels was of high molecular mass, in order to reduce the effect of the chain ends in the network. It was prepared at 288 K from 100 g of a 10 wt% solution of AA in doubly distilled, deoxygenated water, to which sodium bromate (0.6 ml of a 1% w/v solution) and sodium bisulphate (0.01 ml of a 1% w/v solution) were added consecutively. The polymerization mixture was allowed to stand overnight, then transferred to a cellophane dialysis bag and washed three times with doubly distilled water during a three day period. The gel was cut into small sections and dried in a desiccator. A laboratory mill was used to

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pulverize the dry material into small granules. The molecular mass was determined by viscometry and the constants $k=6.8\times10^{-4}$ and $\alpha=0.66$ were used[10] to determine a molecular mass of 3.0×10^6.

The reversible gels were prepared by weighing the appropriate amounts of polymer and water and letting the mixtures stand for about two weeks to obtain clear gels. Smaller quantities of the more concentrated gels were prepared, to facilitate dissolution of the polymer. The gel to sol transitions were estimated by the tube tilting method[11] and by the steel and mercury ball drop methods[11-13]. All samples except the most dilute one were firm gels at 303 K. The sample containing 95% water was a sol at the ambient temperature of 295 K. The best method for the measurement of the gelation temperature was the mercury ball drop method[13], because of the high viscosity of the sample. The transition occurred between 278 and 288 K. It is interesting to note that on cooling the most dilute gel became opaque around 270 K, because of water freezing. The more concentrated gels became opaque at lower temperatures. For instance the gel containing 85% water becomes slightly opaque at 263 K while the gel containing 70% water is clear even at 263 K. These observations are in agreement with a comparative study of reversible and crosslinked PAA gels using proton n.m.r.[14] In this study it was found that the amount of bound or non-freezable water increases as the amount of polymer in the gel increases.

Magnetically dilute samples of Cu^{2+} in the gels were prepared by dissolving the dry polymer in a 0.01 M $CuSO_4$ solution, as described previously[4]. The Cu^{2+} concentration varied between 100 to 500 AA units for each ion, depending on the water content. Most experiments were carried out with normal Cu, which is a mixture of ^{63}Cu and ^{65}Cu isotopes. Some samples were prepared with ^{63}Cu (97% enrichment) but no significant effect on the lineshapes was detected.

E.s.r. spectra were measured at X-band with a Bruker 200D SRC spectrometer operating at 9.7 GHz, using 100 kHz modulation. Spectra at 77 K were taken in a liquid nitrogen dewar inserted in the e.s.r. cavity. In the region 100–320 K e.s.r. spectra were measured using the Bruker ER 4111 Variable Temperature unit with liquid nitrogen as coolant in a flow system. The temperature of the sample was measured by a digital chromel–constantan thermocouple with an accuracy better than ±2 K. The absolute value of the magnetic field was measured using the Bruker ER 035M NMR Gaussmeter. Calibration of g values was based on DPPH ($g=2.0036$) and $^{53}Cr^{3+}$-doped MgO single crystals ($g=1.9800$). Direct field readings were used for scan measurement.

Computer simulations of e.s.r. spectra were performed on a Burroughs 6800 mainframe computer and were plotted by using an IBM PC and a Hewlett–Packard 7470A digital plotter. E.s.r. spectra were calculated with the codes we developed and which included distribution widths $\delta g_\parallel$ and $\delta A_\parallel$ in the $g_\parallel$ and $A_\parallel$ parameters.

RESULTS

E.s.r. spectra of Cu^{2+} are easier to interpret, because of narrower lines, at low temperatures. We have measured the spectra at 77 K of gels quickly frozen by insertion in liquid nitrogen and assumed that the ligation shell is retained. This assumption seems justified by results obtained in a large number of experimental studies[15,16].

E.s.r. spectra at 77 K of Cu^{2+} in reversible gels containing 95, 85, 70 and 60% water are shown in *Figure 1*. All spectra are very similar in the perpendicular direction, with $g_\perp=2.078\pm0.005$ and no resolved hyperfine splitting. However, the spectra differ significantly in the parallel direction. Four sets of parameters for this orientation are indicated in the stick diagrams and tabulated in *Table 1*. The values of $g_\perp$, $g_\parallel$ and $A_\parallel$ in *Table 1* are typical of Cu^{2+} complexes with a distorted tetragonal symmetry. Of the four sets of parameters, complex **1** has values which are usually assigned to the completely hydrated ion and has been identified and simulated in detail in crosslinked PAA gels[4-6]. For tetragonal symmetry the values of $g_\parallel$ and $A_\parallel$ are very sensitive to the nature of the ligands. The expected change in the $g_\parallel$ and $A_\parallel$ values when one or more oxygen ligands are replaced by nitrogen ligands is described in the Peisach–Blumberg (PB) plots[17]. For $g_\parallel=2.3688$ and $A_\parallel=0.0152\,cm^{-1}$ as detected for **2**, the PB plots strongly suggest replacement of one oxygen ligand by a nitrogen ligand. In the gel N-ligation is from the pendant amide group $-CONH_2$. Inspection of the PB plots indicates that more than one N-ligand is very unlikely in **2**. The two species detected in gels containing 95% water are thus assigned to $Cu(H_2O)_6$ (**1**) and to $Cu(N_p)(H_2O)_5$ (**2**); N_p is a network ligand. Species **2** has been detected in PAA gels obtained by gamma irradiation and in chemically crosslinked gels containing only 73% water. In chemically crosslinked gels containing 95% water only species **1** has been detected[4].

In the reversible gels containing 85% water **2** is dominant but **1** is still present and its effect is evident in the asymmetry of the parallel absorptions. In gels containing 70% water **2** is present and, in addition, species **3** which is assigned to $Cu(N_p)_2(H_2O)_4$. In gels containing 60% water **2** is observed and also species **4** which is assigned to $Cu(N_p)_3(H_2O)_3$. The $g_\parallel$ and $A_\parallel$ values for **3** and **4** are in excellent agreement with the expected parameters for this type of ligation. A similar approach has been used recently for identification of Cu^{2+} ligation to poly(L-lysine) as a function of pH[18].

Additional support for the described assignments is presented in the simulation of the results for gels containing 85% water, *Figure 2*, by a superposition of simulated spectra from **1** and **2** in a 1:3 ratio. Both species are simulated by assuming a distribution of the $g_\parallel$ and $A_\parallel$ values[5,6,19]. The residual linewidth ΔH^R used for **2** is 23.9 G, much higher than the value of 12.0 G used for the simulation of **1**. The larger linewidth in **2** is most likely due to unresolved superhyperfine splittings from N ligands. The effect of N ligation is also detected in the larger width of the signal corresponding to the perpendicular orientation in *Figure 1*. The largest width for this signal is observed in gels containing 60% water, *Figure 1d*, where species **4**, containing the largest number of N ligands, is present.

E.s.r. spectra of the gels were also studied in the temperature range 100 K to 320 K. Typical results are shown for the 95% gels in *Figure 3a*. As the temperature increases **1** begins to disappear above 205 K and **2** becomes dominant. Around 270 K an isotropic signal centred on $g=2.19$ appears and is clearly due to the motionally averaged signal from **1**. At 270 K this isotropic signal and an anisotropic signal, mainly detected by the

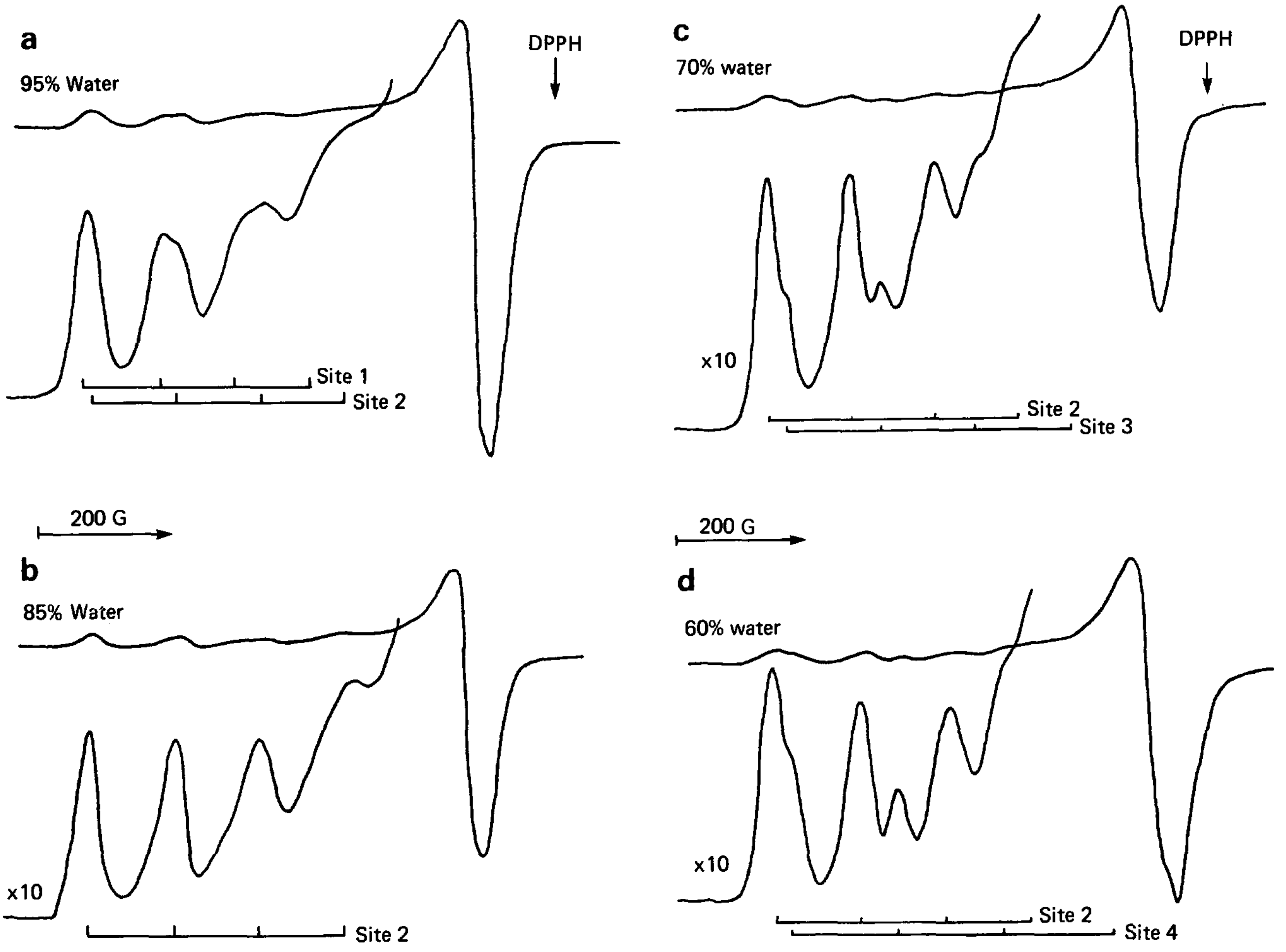


Figure 1 X-band e.s.r. spectra at 77 K of Cu^{2+} in reversible polyacrylamide gels containing the indicated amounts of water. The e.s.r. parameters corresponding to the 'stick' diagrams are given in *Table 1*

Table 1 E.s.r. parameters of Cu^{2+} complexes in reversible polyacrylamide gels

Species	$g_{\parallel}$	$g_{\perp}$	$A_{\parallel}$ (cm^{-1})	Assignment[a]
1	2.4047	2.083	0.0134	$Cu(H_2O)_6$
2	2.3688	2.078	0.0152	$Cu(N_p)(H_2O)_5$
3	2.3203	2.079	0.0168	$Cu(N_p)_2(H_2O)_4$
4	2.2891	2.073	0.0185	$Cu(N_p)_3(H_2O)_3$

[a] N_p is a nitrogen ligand from the network amide group $RCONH_2$

appearance of the high field $g_{\perp}$ signal, are observed. Above 270 K only the isotropic signal persists. The loss of the anisotropy is near the gel–sol transition temperature for this gel, observed around 280 K. In the reversible gels containing 70 and 60% water the anisotropy is retained at 305 K, again in agreement with our observation that these samples are gels at this temperature.

DISCUSSION

It is well known that gels are heterogeneous systems and are composed usually of phases with different network densities[20]. Two different binding sites for Cu^{2+} were obtained in each of the reversible gels studied, representing this heterogeneity. Complex **2**, in which the cation is ligated to one N ligand of the network, was detected in all the reversible gels studied and also in PAA gels obtained by gamma irradiation and chemical crosslinking and represents a major gel component.

The most striking result obtained in the reversible PAA gels is the appearance of complexes **3** and **4** which were not detected in chemically crosslinked gels with similar water contents. In addition species **1** and **2** were detected in reversible gels with 95% water in roughly the same proportion as in the crosslinked gels containing only 73% water. These differences must be assigned to fundamental variances in the structure and topology of networks in the two cases. We will attempt to rationalize these differences in terms of the network density L.

In solutions of linear chains L can be calculated from the water content, by assuming that the solution density is 1 $g\,cm^{-3}$ and the linear dimension of the monomer is 2.5 Å. The values of L in the reversible gels is given in *Table 2*. The calculation of L in crosslinked gels is more involved. The volume fraction of the gels available for a solute with a hydrodynamic radius R has been studied as a function of the network density L and the fibre radius r[21–23]. In PAA crosslinked by BIS L and r have been calculated from gel filtration of model proteins. It was found that r increased with increasing mole fraction of BIS while L decreased as the amount of water present during polymerization increased. The permeability of the gel increased as L decreased. The high permeability at high BIS concentrations was explained by assuming that chains bundle into thick fibres, leaving large voids in the network.

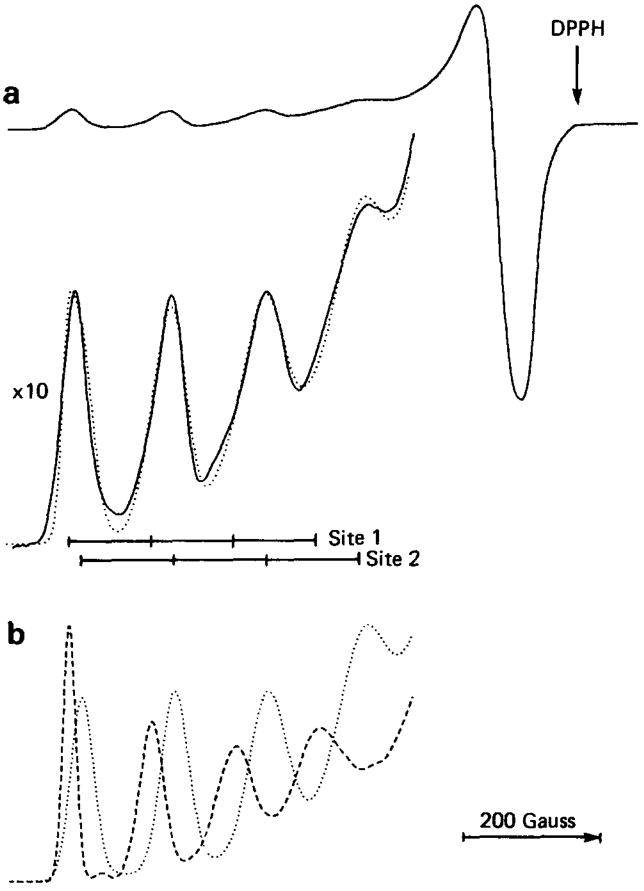


Figure 2 (a) X-band e.s.r. spectrum of Cu^{2+} in a reversible polyacrylamide gel containing 85% water, full line. The dotted line is a spectrum convoluted from simulated spectra of **1** and **2** in a 1:3 ratio. Stick diagrams for **1** and **2** are indicated. (b) Simulation of **1** (dashed line) and of **2** (dotted line) corresponding to the parallel component of the e.s.r. spectrum. The two simulated spectra are shown for the same integrated intensity. The parameters used for the simulated spectra are as follows. For **1** $g_\parallel=2.408$, $g_\perp=2.074$, $A_\parallel=120$ G, $A_\perp=8$ G, $\Delta H^R=12.0$ G, $\delta g_\parallel=0.0432$, $\delta A_\parallel=22.6$ G and $\varepsilon=0.98$; these parameters are similar to those used for simulations of the fully hydrated cation in crosslinked gels, and are based on e.s.r. spectra measured at X- and S-bands[5,6]. For **2** $g_\parallel=2.373$, $g_\perp=2.074$, $A_\parallel=136$ G, $A_\perp=8$ G, $\Delta H^R=23.9$ G, $\delta g_\parallel=0.0399$, $\delta A_\parallel=23.9$ G and $\varepsilon=0.739$

In order to test the effect of the network density on the cation ligation, we compare *L* values in gels containing 95% water and in crosslinked gels swollen to equilibrium by 73% water, because in these two systems we detected the same ligation, species **1** and **2**, with similar relative intensities. For the crosslinked gels we use the *L* values obtained previously[23] as a function of the total monomer concentration (AA and BIS), for 5% BIS, which is the monomer ratio used in our preparations of the crosslinked gels[4–6]. We then plot the literature values of *L* vs. the swelling ratios (SR) of the gels we prepared, as shown in *Figure 4*, and extrapolate to a swelling ratio of 73% water. We obtain $L=1.15\times10^{12}$ cm cm^{-3}. This value is very similar to the *L* value of 1.06×10^{12} obtained for the reversible gel containing 95% water.

In reversible gels the radius of the polymer chain is simply the radius of the monomeric unit, or 2.6 Å[7]. By contrast, the thickness of a typical crosslinked PAA gel is 8–10 Å[23]. These conclusions were recently quantified in a study of crosslinked PAA gels by static light scattering (SLS), small-angle neutron scattering (SANS) and small-angle X-ray scattering (SAXS)[20]. In this study it was found that in the dominant homogeneous gel phase the chains become both thicker and denser, and the separation between the chains increases when the amount of BIS increases.

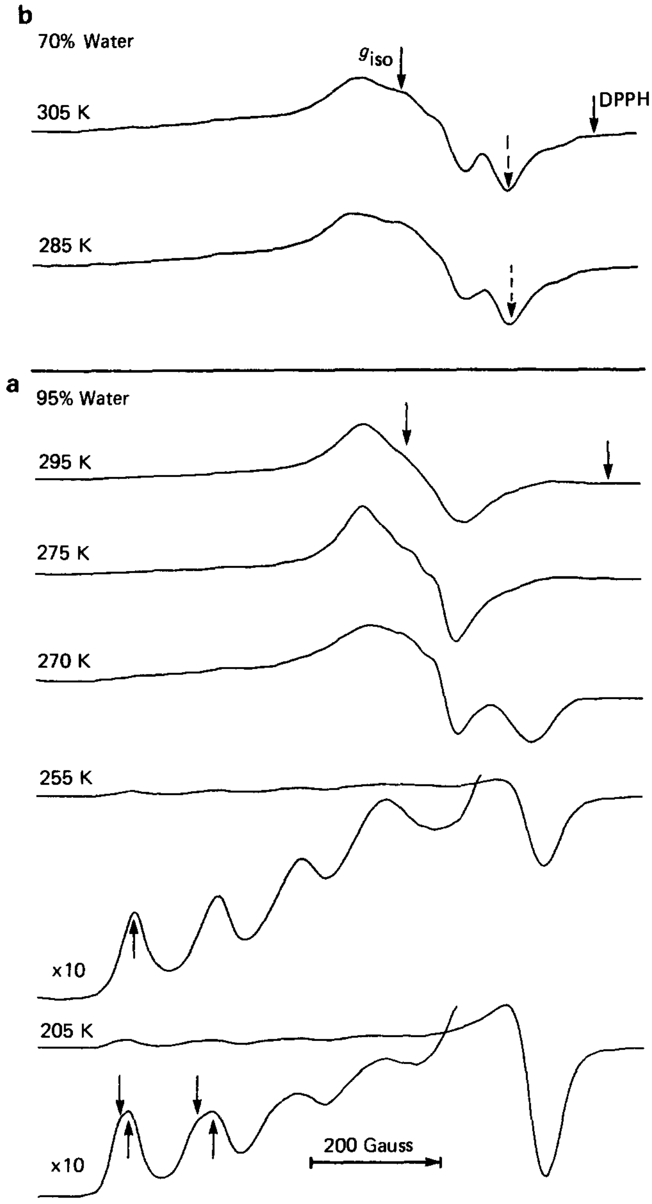


Figure 3 Variable temperature e.s.r. spectra. (a) Reversible polyacrylamide gels containing 95% water. At 205 K and 255 K the upward pointing arrows indicate complex **2** and the downward pointing arrows indicate complex **1**. (b) Reversible polyacrylamide gels containing 70% water. Dashed arrows indicate anisotropic signals

Table 2 Network density *L* in polyacrylamide gels

% Water	$L\times10^{-13}$ (cm cm^{-3})	ξ^a (Å)	Gel type	Species detected by e.s.r.
60	8.48	6.0 (6.9)	Reversible	**2, 4**
70	6.36	7.4 (8.6)	Reversible	**2, 3**
85	3.18	12.4 (14.4)	Reversible	**2**
92	–	19.9 (23.2)	Solution[b]	–
95	1.06	28.4 (33.0)	Reversible	**1, 2**
73[c]	1.15	7.0	Crosslinked	**1, 2**

[a] The two values of ξ are calculated values for $\xi=7.4$ Å or 8.6 Å in the gel containing 70% water, as explained in the text

[b] This water content was studied by scattering methods in ref. 20

[c] The value of ξ for this gel is calculated from gel titration of model proteins, ref. 23. See also ref. 4

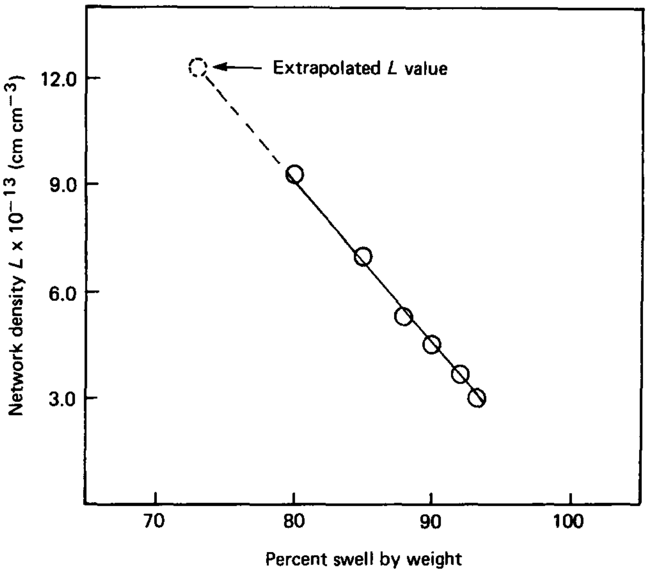
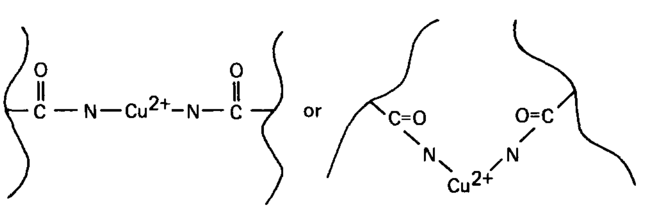


Figure 4 Plot of the fibre density L of polyacrylamide gels crosslinked from monomer mixtures containing 5% BIS (ref. 23) vs. the swelling ratio in wt% of water, as measured in ref. 4

In order to verify that the binding scheme is specific to the presence of the *polymer*, we prepared solutions of acetamide containing the same water content as the reversible gels; the e.s.r. spectra measured indicated the presence of the fully hydrated Cu^{2+} only. It seems that the increasing N ligation with increase in the network density is solely the effect of the polymer network.

The anisotropy which is evident in *Figure 3b* at 305 K might be a result of the smaller correlation length ξ, or distance between chains, in these samples. The fact that only one N ligand is observed in the most dilute gel indicates that it is unlikely to have Cu^{2+} ligated to two N ligands in the *same* chain. Therefore in the species with two N ligands, **3**, we must assume that two *chains* are linked by the cation. As seen below, the distance between these chains is ~7.4 Å if the N ligands are colinear with Cu^{2+} or ~8.6 Å if the N ligands are adjacent.



In gels containing 70% water species **3** was detected. If this distance between the chains is assigned to ξ, the correlation lengths for the other gels can be calculated using the scaling theories of de Gennes[24]. We use the scaling law[20,25] below, where w_1 is the weight fraction of the network in the gel.

$$\xi \sim w_1^{-3/4}$$

In *Table 2* we give the correlation lengths of the gels based on this assignment. These values must be considered with caution because they are based on ξ values derived from **3** which is only one component of the gels. The detection of **4**, which represents ligation of Cu^{2+} to three N ligands might indicate the presence of entangled or even 'strangulated' chains[26] in the reversible gels.

It is interesting to note that a correlation length of 22 Å has been deduced for PAA solutions containing 92% water from scattering experiments[20]. The value we obtain for this system is 19.9 Å or 23.2 Å and are also given in *Table 2*. The agreement is surprisingly good, considering the fact that we used an entirely different approach for the determination of ξ.

The e.s.r. spectra observed above 77 K, *Figure 3*, indicate a dynamic process in which the cation motion averages the g values and ^{63}Cu hyperfine interactions. Averaging processes for the hydrated cation and for cation ligated to the polymer network as well as transitions between N ligation and O ligation are possible. In many cases rates of exchange between two or more sites can be measured by analysis of the temperature variation of the linewidths. For Cu^{2+} the linewidths depend on the temperature even in the absence of any exchange processes, due to spin-lattice relaxation. Therefore it is not easy to isolate the effect of exchange. We are currently looking into other systems more amenable to this type of analysis.

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