Polymer Bulletin 41, 199–206 (1998)

Polymer Bulletin

An EPR study of polysaccharide copper(II) complexes in composite dextran/epichlorohydrin gels

Artur Bartkowiak[1], **Julia Jezierska**[2], **Tadeusz Spychaj**[1]

[1] Polymer Institute, Technical University of Szczecin, Pulaskiego 10, PL-70 322 Szczecin, Poland
[2] Faculty of Chemistry, University of Wroclaw, F. Joliot-Curie 14, PL-50 383 Wroclaw, Poland

Received: 30 January 1998/Revised version: 13 May 1998/Accepted: 10 June 1998

Summary

The EPR spectra of composite dextran and carboxymethyldextran gels were studied in order to identify the functional groups involved in copper(II) complexation depending on pH. The porous structure resulting from crosslinking of polymer chains of dextran, *via* epichlorohydrin, forces the immobilization of Cu^{2+} ions surrounded by different oxygen groups of the polymer gel matrix. The different polynuclear structures are postulated to be formed depending on the type of polysaccharide derivatives. The results indicate that carboxyl and deprotonated hydroxyl groups participate in the complex formation.

Introduction

The crosslinking of polysaccharides leads to porous hydrogels widely applied in medicine and used as packings for various kinds of chromatography [1]. It has been stated recently that the participation of the metal ions in the crosslinking of polysaccharides, such as dextran, by an organic agent epichlorohydrin affects distinctly the structure and properties of the final gels [2-5]. Polyvalent metal cation modified dextran hydrogels combine properties characteristic for both known classes of dextran gels i.e. dextran/epichlorohydrin cross-linked materials as well as dextran/metal cation complexes. Their properties depend on a type and content of the metal cation modifier as has been shown by atomic adsorption spectroscopy, scanning electron microscopy, infrared spectroscopy and derivatography [3]. Some composite dextran gels can also be applied as stationary phases for size exclusion/liquid chromatography [4]. Furthermore some applications covering catalytic activities in dry and swollen aqueous gel state as well as slow cation delivery characteristics have been tested [3,5].

Electron paramagnetic resonance (EPR) spectroscopy can be used to characterize the micro-environment of paramagnetic spices (for example metal cations like Cu^{2+}, Mn^{2+} or Fe^{3+}) in complex systems, and to obtain information on the extent of binding, the chemical nature and the spatial distribution of the specific binding sites. This has been identified during the structural investigation of the copper complexes with functional groups of various polymers which possess donor atoms like oxygen or nitrogen [6-8].

For biopolymers, exceeding majority of studies have been carried out on proteins, primarily enzymes carrying copper as an essential component of the active site [9]. EPR has also been applied to investigate metal complexes of saccharides (cyclodextrins and piranoses [10]) and carboxysaccharides [11]. However, there is a rather limited number of publications related to EPR spectroscopy of Cu^{2+}/polysaccharide complexes.

In this investigation, EPR characterization of various of the different composite dextran gels were carried out in order to i) confirm the creation of stable Cu(II)/ polysaccharide ligands complexes ii) identify the structures of different complexes and iii) estimate the variation of complexes structure as a function of pH and chemical composition of the polysaccharide.

Experimental

Materials

Dextran (D) with average molecular weight of ca. 15,000 was obtained from “Polfa” Pharmaceutical Works S.A. (Kutno, Poland).

The carboxymethyldextran (CD) was synthesized in alkali aqueous/ethanol dextran solution in the presence of α-chloroacetic acid (Koch Light Laboratories Ltd., Great Britain) (Table 1) [12]. The product obtained in the reaction for 1.5 hour at 70°C at the lowest α-chloroacetic acid and alkali concentrations was selected as the substrate for gel preparation [5]. The degree of substitution of synthesized CD with $-OCH_2COOH$ groups was determined by titration with 0.1 m aq. NaOH solution in the presence of phenolphthalein to be 10% relative to dextran hydroxyl groups.

Table 1. Reaction Parameters for the Carboxymethyldextran Synthesis

Reaction Parameters	**Range**
α-chloroacetic acid concentration	0.275-0.55 g/g of dextran
NaOH concentration	0.25-0.5 g/g of dextran
Temperature	60-80 °C
Time	1.5-4.5 hours

Gel Preparation

Two series of the gels based on dextran and carboxymethyldextran were synthesized. The first series consists of unmodified gels (DG and CDG, respectively) while the second composite dextran gels were obtained in the presence of $CuSO_4 \cdot 5H_2O$, with a molar concentration of the copper cation - 0.2 mmol/Cu^{2+}/g of polysaccharide (DG/Cu^{2+} or CDG/Cu^{2+} gels, respectively). All syntheses were performed at 50°C using toluene as dispersing medium and the constant ratio of epichlorohydrin to polysaccharide 1.5/1 mol/mol. A detail procedure of gels preparation has been described in the literature [2-5].

Cu^{2+} /Polymer Complex Preparation

The copper(II) complexes with dextran (Cu^{2+}-D), carboxymethyldextran (Cu^{2+}-CD) and poly(acrylic acid) (Cu^{2+}-PAA) were precipitated from aqueous solution by adding the methanol in order to render the final concentration of alcohol 95% vol./vol.. The pH of initial aqueous solution was adjusted, as appropriate, with HCl and NaOH. The precipitate formed was collected by centrifugation (2000 rpm, 10 min.) and was dissolved in small volume of water. The pH of this solution was adjusted again to the previous value, and methanol was added until the complex was reprecipitated. The final precipitate was collected by centrifugation, washed with acetone and dried. The same procedure was applied in preparation of the synthesized composite gels with adjusted pH. Independently, one gel sample was prepared by postpriori introduction of copper ions to the dextran gel crosslinked with epichlorohydrin in the absence of Cu(II) (Cu^{2+}-DG).

EPR Measurements

The EPR spectra were recorded on a Bruker ESP 300 (Bruker, Germany) spectrometer operating at X-band and equipped with the Bruker NMR gaussmeter ER 035M and the Hewlett-Packard microwave frequency counter HP 5350B at room temperature and at 77 K. The comparison of the Cu complexes of epichlorohydrin crosslinked and uncrosslinked dextran complexes was possible only in the dry state due to the solubility of covalently uncrosslinked copper dextran complex in water. Moreover it is evident from literature that even long-term air drying does not change usually the coordination properties of polymer functional groups like amino, carboxyl or phosphate, some modificaton is observed upon complete dehydratation of the samples at higher temperature [6-8]. To avoid any effect provided by the change of solvent contribution the measurements of all samples were performed at similar conditions.

A powder (polycrystalline) pattern spectra of all solid samples were simulated using program SimFonia Software Version 1.2 provided and copyrighted by Bruker Instruments, Inc. The algorithm used in the SimFonia is based on a second order perturbation theory [9].

The spectra were simulated without consideration of different contribution of Cu-63 and Cu-65 isotopes, what is a usual practice for polycrystalline type spectra with relative broad lines due to copper hyperfine interaction of parallel orientation (in comparison to those observed for the lines due to particular orientation of mono crystals).

Results and Discussion

Typical EPR spectra of the investigated dextran or carboxymethyldextran gels with Cu(II) as well as of the complexes formed between copper cations and functional groups of the linear polymers (dextran, carboxymethyldextran and polyacrylic acid) are illustrated in Figures 1 and 2, while Table 2 lists the values of the most informative parameters $A_{||}$ and $g_{||}$ [9] of all samples investigated. In all cases the spectra correspond to $g_{zz}=g_{||}>>g_{xx}=g_{yy}=g_{\perp}>2.0023$, $A_{zz}=A_{||}>>A_{xx}=A_{yy}=A_{\perp}$, which is typical of tetragonal Cu(II) compounds having $d_{x2\text{-}y2}$ orbital as ground state. The anisotropic character of the spectra even at ambient temperatures indicates the immobilization of Cu^{2+} cations which can be caused only by interaction of metal cation with hydroxyl or carboxyl oxygen atoms of glucosidic units in polymer chain. Furthermore, all spectra exhibit a very well resolved anisotropic copper(II) hyperfine structure what proves a good dispersion of the Cu^{2+} within the polymer matrix. However, the close neighborhood of the copper ions usually result in the spectra broading and loss of the copper hyperfine resolution. All EPR spectra of investigated systems indicate that the metal cations are uniform bound into the polymer matrix and cause the changes of synthesized material properties.

Specific values of $g_{||}$ and $A_{||}$ parameters for $Cu(H_2O)6^{+2}$ ($g_{||}$=2.410 and $A_{||}$=137·10^{-4} cm^{-1}) allow us to discriminate between Cu^{2+} aquaions and the complexes with oxygen donors provided by functional groups of the polymer chain. A general tendency in these parameters can be observe, where decrease of $g_{???}$ value occurs with increasing negative charge and/or increasing stability of the complex [9].

It follows from the EPR ($g_{||}$ and $A_{||}$) parameters in Table 2 that, for all investigated dextran and carboxymethyldextran systems, with an exception of complex with pure dextran, complexes of the copper with ligands others than water are formed. The comparison of the EPR parameters of DG/Cu^{2+} with those of carboxymetylodextran (Cu^{2+}-CD, CDG/Cu^{2+}) and Cu^{2+}-PAA provides evidence for the coordination of copper cations in composite gels also by carboxyl groups (Table 2, Figs 1 and 2).

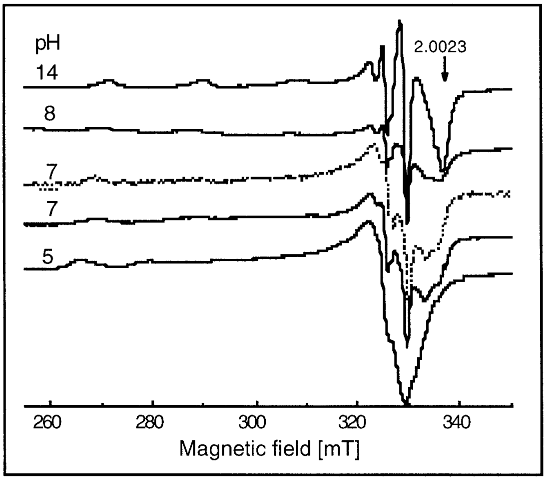

Figure 1. The spectra of composite dextran gels with copper(II) (DG/Cu^{2+}) at various pH; temp. 77 K (solid lines) and 295 K (dotted line).

Only in the case of Cu^{2+}-D (the complex of Cu^{2+} with uncrosslinked dextran) the parameters of the spectra at lower pH than 5 are close to those for an aqua complex, whereas at pH>5 the complete disappearance of the spectra is observed. It seems to be probable, that spin-spin interactions between Cu^{2+} ions in the formed polynuclear structure are responsible for the complete lost of EPR spectra after introducing of copper ions into uncrosslinked dextran above pH 5. Furthermore, apparent decrease of EPR signal intensity in pH range 8-10 (Fig. 1) determined for DG/Cu^{2+}, can be assigned to the same spin-spin interactions, weaker for this covalently crosslinked system.

Table 2. EPR Parameters for the Copper(II) Complexes

System	pH	$g_{\|\|}$	$A_{\|\|} \cdot 10^{-4}$ cm^{-1}
DG/Cu^{2+}	2	2.356	141
	5	2.351	153
	7	2.310	170
	8-14	2.257	185
CDG/Cu^{2+}	2	2.356	141
	5-7	2.370	146
	12	2.257	185
Cu^{2+}-DG	2	2.350	150
	5	2.340	146
Cu^{2+}-D	2	2.410	137
	5	2.410	137
Cu^{2+}-CD	2	2.410	137
	5-6	2.368	145
	8-10	2.337	155
	13	2.250	180
Cu^{2+}-PAA	2	2.410	137
	3-4	2.368	145
	5-8	2.330	160
	12	2.265	180
Cu^{2+}-piranose [10]	14	2.232-2.256	~190
$[Cu(OH)_4]^{2-}$			
[15]	14	2.273	194
[10]	14	2.271	190

As was mentioned above the EPR parameters $g_{||}$ and $A_{||}$ of DG/Cu^{2+} measured at pH range 2-6 are similar to these observed for Cu(II) complexed by carboxylic groups in Cu^{2+}-PAA system (Table 1, Figs 1 and 2). This suggests the existence of some number of carboxyl groups in the initial dextran or formation of these groups during the crosslinking with epichlorohydrin in the presence of Cu^{2+} cations. The existence of carboxylic groups was also identified in the case of sample (Cu^{2+}-DG) when dextran was crosslinked by epichlorohydrin in the absence of Cu(II) and copper ions were introduced into DG after the covalent crosslinking of dextran. On the other hand, EPR spectra for uncrosslinked dextran - copper complexes reveal the lack of carboxyl groups. This confirms our assumption of carboxylic group formation during the reaction of dextran with epichlorohydrin in aqueous alkali solution. Another confirmation of this thesis was provided by FTIR spectral data [5].

The change in spectral characteristic of DG/Cu^{2+} in the pH range 7-8 point to the stepwise inclusion of other oxygen donors in the complex formation. Furthermore, the EPR parameters are close to these of polysaccharide derivatives with carboxyl groups, where mixed coordination of carboxyl and deprotonated hydroxyl groups was postulated [11]. The involvement of oxygen atoms from polysaccharide hydroxyl groups in coordination to Cu(II) at higher pH appears to be possible in view of recently published structural data [13,14]. These provide evidence of growing participation of deprotonated hydroxyl groups in copper ions complexation with increasing pH.

All EPR parameters observed from pH 8 to 14 differ specially in $g_{||}$ from the parameters of $Cu(OH)_4^{2-}$ (Table 2) [10,15]. This indicates that -O$^-$ donors from deprotonated hydroxyl groups may participate in Cu(II) coordination. The anisotropy of EPR spectra observed at ambient temperature is strong evidence of the immobilization of Cu^{2+} by interaction with functional groups occurring in covalently crosslinked dextran gel.

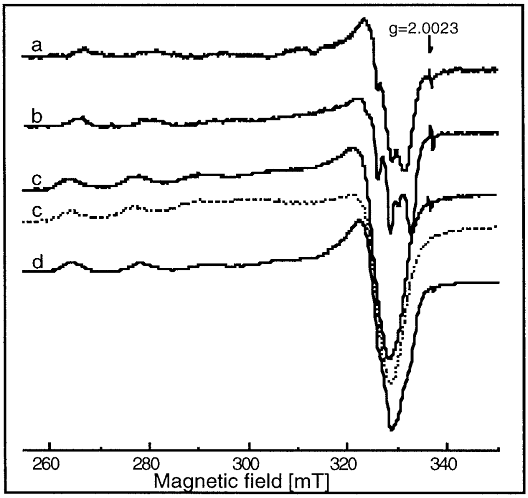

Figure 2. The EPR spectra of Cu^{2+} complexes with carboxymethyldextran (CD) and poly(acrylic acid) (PAA): a) Cu^{2+}-CD at pH 10; b) Cu^{2+}-PAA at pH 8; c) CDG/Cu^{2+} at pH 7 ; d) Cu^{2+}-PAA at pH 4; temp. 77 K (solid lines) and 295 K (dotted line).

Distinctly smaller values of the $g_{???}$ parameter for the sample of DG/Cu^{2+} in relation to CDG/Cu^{2+} in the pH range 5-7 (Table 2) indicate that other oxygens than those provided by carboxylic groups in a greater extent are bound to Cu^{2+} in the case of DG. The comparison of the CDG/Cu^{2+} and Cu^{2+}-PAA spectroscopic parameters reveals the quantitative domination of complexes with two carboxylic groups of carboxymetylodextran chain (Fig.2 c,d) at lower pH. On the other hand, in the case of uncrosslinked carboxymethyldextran complexed with copper ions (Cu^{2+}-CD) the $g_{||}$ and $A_{||}$ values in the pH range 8-10 agree with those found for Cu^{2+}-PAA (Fig.2 a,b) suggesting the presence of four carboxyl groups in the coordination sphere around Cu(II) in the *xy* plane [6]. This clearly indicates that covalent crosslinking of dextran limits the coordination of Cu^{2+} with free -COOH groups. It is noteworthy, that the spectral parameters at pH>10 of all above mentioned systems are in good agreement with those [Table 2] found [10] for piranoses and cyclodextrins [10]. Moreover, the significant decrease of $g_{||}$ values in respect to these found for $[Cu(OH)4]^{2-}$ (Table 2) should be related to the system stabilization caused by immobilization of the copper ions through the deprotonated oxygen atoms of dextran hydroxyl groups.

Conclusion

It follows from the experiments that the porous structure resulting from crosslinking of polymer chains of dextran or carboxymethyldextran with epichlorohydrin forces the immoblization of Cu^{2+} ions surrounded by various oxygen groups of the polymer gel matrix. On the other hand, the different type of coordination between the polymeric carrier and copper ions is responsible for significant changes in matrix structure of the synthetic product. The results obtained for materials based on carboxymetylodextran indicate that covalentaly crosslinking of dextran via epichloerohydrin limits the coordination of Cu^{2+} with free -COOH groups.

References

1. Finch CA (1981) Chemistry and Technology of Water Soluble Polymers, Plenum Press, New York
2. Spychaj T, Bartkowiak A, Hunkeler D (1995) Int J Polymer Anal. & Characterization **2**: 1
3. Spychaj T, Bartkowiak A (1997) Polymers for Advanced Technologies **8**: 1
4. Spychaj T, Bartkowiak A (1996) Macromol. Symp. **110**: 191
5. Bartkowiak A (1996) Ph.D. Thesis, Technical University of Szczecin
6. Trochimczuk A, Jezierska J (1997) Polymer **38**: 2431
7. Jezierska J, Kolarz BN, Pawlow B, Trochimczuk A (1997) React Polym **33**: 127
8. Jezierska J, Kozlowski H, Kolarz BN, Trochimczuk A (1991) Polym Bull **26**: 231
9. Pilbrow JR (1990) Transition Ion Electron Paramagnetic Resonance, Claredron Press, Oxford
10. Ohara M, Kobayashi Y, Sasaki H (1984) 12th Int Carbohydr Symp, Abstr Utrecht 478

11. Micera G, Dessi A, Kozlowski H, Radomska B, Urbanska J, Decock P, Dubois B, Oliver I (1989) Carbohydrate Research **188**: 25
12. Pat Jap (1986) 61,152,702, Chem Abstr (1986) **105**: 210 711
13. Fuchs R, Habermann N, Kluefers P (1993) Angew Chem Int Ed Eng **32**: 852
14. Kluefers P, Schuhmacher J (1994) Angew Chem Int Ed Eng **33**: 1742
15. Falk KE, Ivanova E, Ross B, Vanngard T (1970) Inorg Chem **9**: 556