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STUDIES OF METAL–SUGAR COMPLEXES IN THE SOLID STATE BY THE ^{13}C-N.M.R. C.P.–M.A.S. METHOD

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ABSTRACT

Several metal–sugar conjugates have been prepared. Chelate coordination complexes of copper(II) and zinc(II) were prepared from salicylaldimine ligands derived from combinations of methyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranoside or 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranose and salicylaldehyde. Similar Schiff's bases were prepared from chitosan and complexed to copper(II). Cross-polarisation–magic angle spinning ^{13}C-n.m.r. spectroscopy showed that paramagnetic ions have profound effects on the resolution obtainable and, at high concentration, result in broad, featureless spectra. In contrast, diamagnetic ions have little effect on the isotropic chemical shifts of the sugar chelates.

INTRODUCTION

The ability of sugars and their derivatives to sequester metals is of interest to the possible development of novel classes of metal-based affinity chromatography materials[1], of chiral homogeneous catalysts[2], of metal-chelators for clinical use[3], and of models for biologically important chelates[4]. Other ongoing interests include n.m.r. investigations of metal ion-binding to sugars. In this study, we examine, in the solid state, metal complexes which are diamagnetic and others which are paramagnetic.

Initially, specific, monomeric metal–sugar compounds were synthesised. Schiff's base ligands were obtained by combining amino sugars with aromatic aldehydes, followed by complexation with suitable metal ions. Subsequently, studies were made of the chelating polymers chitin and alginate, which can be obtained in enormous quantities from natural sources. Then, following from other recent attempts to improve the metal-chelating capability by chemical derivatisation of the native polymers[5], chitosan Schiff's bases were studied. This work also provided an opportunity to evaluate further the diagnostic potential of solid-state ^{13}C-n.m.r. methods[6–8] for examining carbohydrate structures.

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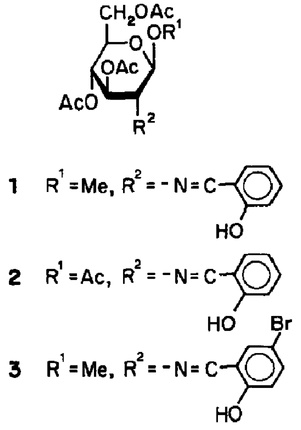
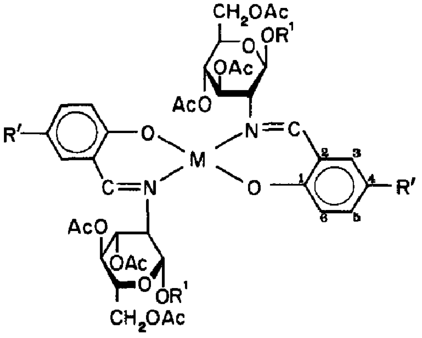
EXPERIMENTAL

Materials. — All compounds were either obtained commercially, or synthesised in this laboratory. The monosaccharide syntheses have either been documented in this laboratory or reported elsehwere[9]. Sugar salicylaldimines **1–3** were readily synthesised by adapting the method of Irvine and Earl[10]. Subsequently, the Schiff's base–metal complexes were prepared as follows. The ligands were first dissolved in hot ethanol, and an ethanolic solution of the appropriate metal acetate was added with stirring. On cooling, the metal complex separated as fine crystals that were then collected by filtration. Recrystallisation from either acetone (complex **4**), acetone–chloroform (complex **5**), ethanol (complex **6**), or acetone–ethanol (complex **7**) gave the pure compounds in good yields, except for the zinc(II)–sugar complex.

The methods of Hirano *et al.*[11] and Nud'ga *et al.*[12] were employed, with some modification, for the preparation of the chitosan Schiff's bases. However, "mixed" (*N*-benzylidene, *N*-salicylidene)chitosan was prepared by the following method. To chitosan (2 g, 12 mmol), dissolved in a mixture of methanol and aqueous 10% acetic acid, was added, with vigorous stirring, a methanolic solution (40 mL) of benzaldehyde (4 mL, 39 mmol). Saturated, aqueous sodium hydrogencarbonate (8 mL) was added and, within minutes, a very soft, colorless, semi-transparent gel was formed. Salicylaldehyde (1.4 mL, 14 mmol) in methanol (40 mL) was added immediately, resulting in a stiffening of the gel, to which was then added methanol (320 mL) and aqueous sodium hydrogencarbonate (4 mL). The "mixed" gel obtained was lighter in color compared with pure *N*-salicylidenechitosan. The product contained benzylidene and salicylidene moieties in the ratio 2:1 (estimated from ^{13}C-n.m.r. integration) [Found: C, 58.48; H, 6.10; N, 5.51. Calc. for





4 R^1 = Me, R' = H, M = Cu

5 R^1 = Me, R' = H, M = Zn

6 R^1 = Ac, R' = H, M = Cu

7 R^1 = Me, R' = Br, M = Cu

$(C_{13}H_{15}NO_5)_{0.33}(C_{13}H_{15}NO_4)_{0.67} \cdot 0.7\ H_2O$: C, 58.45; H, 6.19; N, 5.24%]. The wet gels (used without drying from these preparations) were complexed by vigorous stirring overnight in saturated, methanolic solutions of cupric acetate. The fragmented gels were filtered, washed with methanol (500 mL), and dried *in vacuo* at 56° for 1 day. Copper determinations were obtained by nitric acid digestion of the chitosan samples followed by atomic absorption measurements.

Methods. — All spectra were determined in the Fourier mode with a Bruker CXP-200 spectrometer, operating at the resonance frequency of 200 MHz for ^1H, and 50.3 MHz for ^{13}C. C.p.–m.a.s. ^{13}C-spectra were obtained at room temperature using a single-coil, doubly tuned probe. Spin-locked cross-polarisation was established by the single-contact Hartmann–Hahn procedure, using applied ^1H and ^{13}C r.f. fields of 15 and 60G, respectively. Delrin spinners were used, and the spinning frequency, estimated from the spinning side-bands, was 4.2 ±0.3 kHz. For diamagnetic organic solids and solid polymers, contact times varied from 0.5 to 5 ms, much longer than those used for the paramagnetic counterparts ($t_{cp} \leq 0.5$ ms). The repetition time between successive cross-polarisation times was 4–25 s, and between 1000 and 3000 scans were taken for each spectrum. The ^{13}C-n.m.r. signal of liquid benzene was used as the external reference to determine the chemical shifts, which were subsequently expressed as p.p.m. downfield from Me_4Si (the benzene signal is 128.5 p.p.m. downfield from this reference).

RESULTS AND DISCUSSION

The c.p.–m.a.s. ^{13}C-n.m.r. spectra of the zinc–sugar complex **5** [Zn(Sug I-sal)$_2$] along with the parent ligand **1** are shown in Fig. 1. Although the resonance line-widths are narrow and the peak is well resolved, there is still some ambiguity in the assignment of individual resonances in the solid state, even when these assignments are based on solution spectra of these and related molecules. The presence of the zinc atom disperses the aromatic region, but only the two non-protonated carbons can be differentiated from the rest by the dipolar dephasing technique[13]. The assignments for the protonated carbons must be considered tentative. Not surprisingly, small changes occur in the sugar region in comparison to that of the free ligand. The broad signal at 75.82 p.p.m. is assigned to C-2, and it is suggested that the broadening is caused by quadrupolar interaction with the ^{14}N nucleus, not averaged by magic angle spinning[14]. Surprisingly, metal ion chelation at O-1 and N-2′ has no pronounced effects on the chemical shifts of C-1 and C-2′. It may be that an up-field steric compression shift[15] counteracts the anticipated low-field shift. The methyl carbon atoms of the acetoxyl groups appear as a doublet of ~2:1 proportions, one of which is not affected by metal-induced shifts. Equivalent splittings of the carbonyl resonances may be obscured by the complexity of that spectral region.

It has been reported[9] that the ^1H-n.m.r. solution-state spectrum of this complex shows some free ligand which could not be removed by recrystallisation; if the

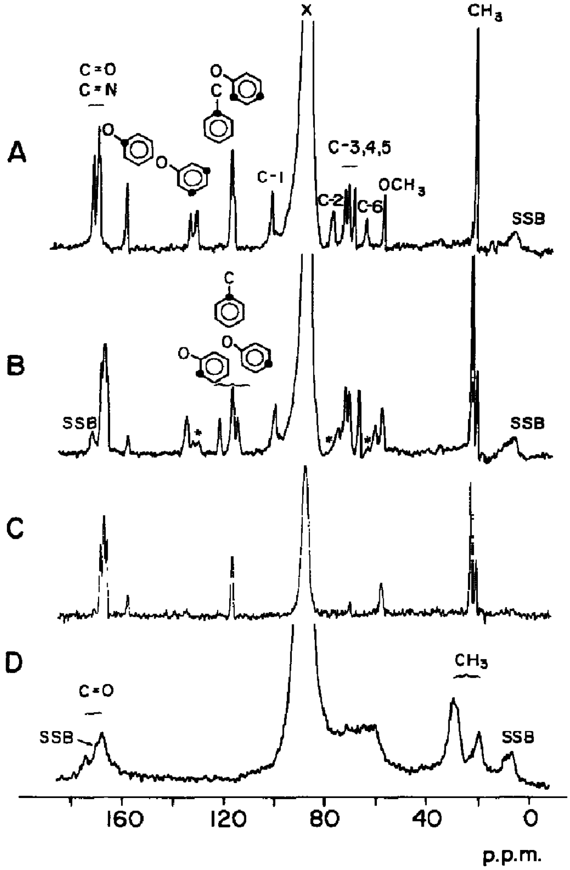


Fig. 1. C.p.–m.a.s. ^{13}C-n.m.r. spectra of methyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-salicylideneamino-β-D-glucopyranoside and its complex: A, free ligand **1** (2700 scans; contact time, 5 ms; repetition time, 10 s); B, diamagnetic zinc(II) complex **5**, same conditions as for A; C, dipolar dephasing spectrum of complex **5**, obtained by setting a 40-μs period without proton decoupling, prior to acquisition of ^{13}C data (530 scans; contact time, 5 ms; repetition time, 10 s); D, copper(II) complex **4** (1130 scans; contact time, 0.1 ms; repetition time, 5 s). Delrin signals and spinning side-bands are indicated as X and SSB, respectively.

complex decomposes in solution, the proportion of free ligand increases and can be monitored. Those signals from the residual, free ligand are marked by asterisks. The elemental analysis of the sample used here reveals a negligible amount of free ligand; thus, the splitting of the methyl resonances cannot be attributed to resonances from the free ligand. Possibly it is due to a crystallographic effect.

It is known[16] that the observed line-widths of carbon-13 resonances are dependent on the characteristic electron spin–lattice relaxation times of the copper ions, T_{1e}, which, in turn, are dependent on the coordination geometry about the paramagnetic ion. The shorter the T_{1e} the narrower the line-width; furthermore, it is known that copper(II) ions with a square planar geometry have a longer T_{1e} than in complexes with a more tetrahedral coordination. In general, the former geometry is preferred; in that geometry, the excited states are well separated as a result of the Jahn–Teller effect.

Although the geometry of the complex Cu(Sug II-sal)$_2$ (**6**) is closer to square planar geometry than the Cu(Sug I-sal)$_2$ complex (**4**), they are both essentially borderline cases in solution as evidenced from the i.r. studies reported earlier[9].

C.p.–m.a.s. ^{13}C-spectra of complexes **4** and **6** were obtained for different contact times. No signals whatsoever were observed for the square planar complex **6**, because the electron relaxation rate for copper(II) is so slow. On the other hand, use of a very short contact time (0.1 ms) in the c.p.–m.a.s. experiment revealed some broad ^{13}C resonances for the pseudotetrahedral complex **4** (Fig. 1D); only those sugar resonances which are relatively remote from the metal center were observed. The line-broadening effect of the copper(II) ion is a result of the spin–spin relaxation time, T_2 to which the line-width is inversely proportional. If the electron-to-carbon-13 dipolar interaction is dominant, T_2 varies directly with the sixth power of the metal-to-carbon distance; hence, resonances of carbon atoms closest to the metal binding sites are broadened most. It should be noted that the isotropic nuclear-electron spin exchange interaction need not be localised to the donor site but can also extend around the aromatic ring, which could further reduce the T_2 values. Thus, the disappearance of the aromatic resonances indicates extreme broadening and the binding of copper(II) ion to O-1 and N-2′. Only the signals of the acetyl groups of the sugar rings can be observed clearly, and the others appear as a single, broad peak that cannot be assigned. Although the line-widths are relatively broad in comparison to those for the diamagnetic zinc(II) complex **5**, the doublets for the carbonyl and methyl carbon atoms of the acetoxyl groups are well resolved; their separations are ~7.3 and 10.2 p.p.m., respectively. The effect of metal ions, paramagnetic or not, on the isotropic shifts is nicely illustrated by these complexes.

When the contact time is increased to 1 ms, all of the sugar signals disappeared; this suggests that the splittings of the acetoxyl resonances are not caused by the presence of free ligands, which should be quite remote from the nearest metal center. This finding also rules out the possibility that such splittings are due to paramagnetic anisotropic effects[17], because their separations are comparatively small; moreover, the integral ratio of the two signals is similar to that measured for the analogous, diamagnetic zinc–sugar complex.

Changing the substituent on the anomeric carbon of the sugar ring has a very substantial impact on the observed spectrum; compare, for example, complexes **4** and **6**. This is due to a geometric effect on the configuration of the complex. Similarly, broad signals were observed for Cu(Sug I-xsal)$_2$ (**7**) only for contact times shorter than those used for complex **4** (*i.e.*, $t_{cp} \leqslant 0.05$ ms); this suggests that its geometrical structure, and T_{1e}, are intermediate between those of complexes **4** and **6**.

It is appropriate now briefly to review the ways whereby the relaxation induced by the copper(II) ions results in the observed effects. This is best based on the expression derived for the matched Hartmann–Hahn condition[18], according to which, the carbon-13 magnetisation grows as

$$M(t_{cp}) = c\lambda^{-1}\{1 - \exp -(\lambda t_{cp}/T_{CH})\}\exp(-t_{cp}/T_{1\rho}{}^{H}), \qquad 1$$

where

$$\lambda = \left[1 + \frac{T_{CH}}{T_{i\rho}{}^{c}} - \frac{T_{CH}}{T_{1\rho}{}^{H}} \right],$$

$T_{i\rho}{}^{c}$ is the carbon rotating frame relaxation time, and "c" is a proportionality constant. Eq. *1* may be simplified to give

$$M(t_{cp}) = c\lambda^{-1} \left[\frac{\lambda t_{cp}}{T_{CH}} \right] \left[1 - \frac{t_{cp}}{T_{1\rho}{}^{H}} \right]. \qquad 2$$

This expression shows that the carbon magnetisation rises with a rate λ/T_{CH}, and falls off as $1/T_{1\rho}{}^{H}$. Since T_{CH} is of the order of the carbon–proton T_2 as a result of mutual carbon–proton spin flips under the Hartmann–Hahn condition, this expression is valid when $T_{1\rho}{}^{c} > T_{1\rho}{}^{H} \gg T_{CH}$.

For a sufficiently long value of T_1, two possible mechanisms may be involved in the course of the c.p.–m.a.s. ^{13}C-experiment. (*a*) The proton magnetisation decays so rapidly during the onset of spin locking that none remains for transfer to the carbon nuclei, whose signals are, therefore, eliminated from the obtainable spectrum. (*b*) The $T_{1\rho}{}^{H}$ is so shortened that cross-polarisation between spins occurs rapidly, decaying as a function of $1/T_{1\rho}{}^{H}$. A gradual decrease in $T_{1\rho}{}^{H}$ can result in the appearance of some reasonably broad ^{13}C resonances, observable for very short contact times. Presumably, this long (moderate) T_{1e} modulates the c.p. rate, which is subsequently decreased.

For situations where T_{1H} (and T_{1e}) is of intermediate range, conventional single-pulse ^{13}C-n.m.r. spectroscopy with high-power, gated decoupling of the proton spins can also be used to obtain the spectrum, for example, that of complex **4**. The T_{1c} and T_{2c} of **4** are reasonably short and the electron-to-carbon-13 dipolar interaction is not complicated by the spin diffusion effects discussed above. However, no signals were observed for complex **6** probably due to extremely short relaxation times.

Such polysaccharides as chitosan[19] and alginate show strong interactions with copper(II) ions, and it is appropriate to consider how their properties will influence the outcome of any measurements. First, we note that the metal binding sites can be either closely or distantly spaced, depending on the type of polysaccharide. Those carbons of the polymers which are located close to the metal-locus should behave similarly to those of the monomeric, metal–sugar complexes. Those of a distant sugar moiety can behave differently; it may remain far from the metal-bind-

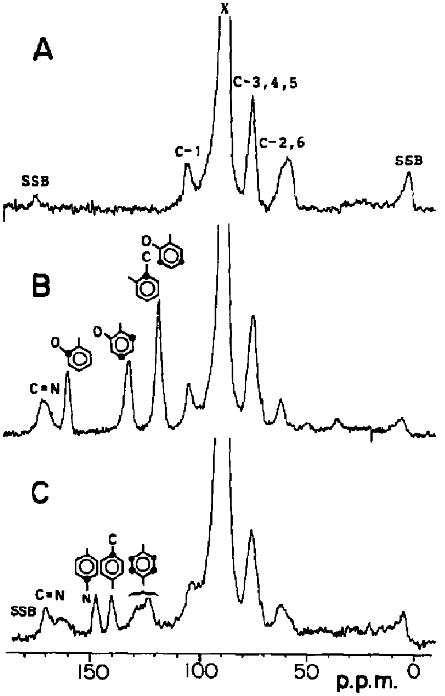


Fig. 2. ^{13}C-N.m.r. spectra of chitosan and its Schiff's base derivatives: A, chitosan (**8**); B, salicylidene-chitosan **9**; and C, 4-nitrobenzylidenechitosan **11** (1000 scans; contact time, 3 ms; repetition time, 10 s).

ing sites, but the crystalline or amorphous packing of the sample may bring the two zones into close proximity. Thus, solid-state n.m.r. techniques may be useful not only for studies of paramagnetically induced-shifts and -relaxation phenomena, but also for determination of the primary structure of some polysaccharides and their chemical derivatives.

The ^{13}C-n.m.r. spectra of chitosan (**8**) and its Schiff's base derivatives are shown in Fig. 2. Depending on the commercial batch of chitosan used, the residual amount of *N*-acetyl groups can vary up to 15% for incomplete hydrolysis. High acetyl-contents can easily be detected by the presence of the appropriate carbon resonances; their absence from the spectrum shown in Fig. 2A is indicative of almost complete deacetylation. The ^{13}C-n.m.r. spectrum of an amorphous sample of *N*-salicylidenechitosan (**9**) contains broad peaks reflecting a dispersion of chemical shifts, and the assignments of its resonances are made by comparison with the respective carbons of the starting materials. The copper(II)–salicylidenechitosan complex is analogous to CuII(Sug-sal)$_2$ in terms of its deep-green colour, and its characteristic e.s.r. spectrum which indicates a true chelate[5]. A low capacity for chelation was earlier reported for this polymer, in that only 20% of the binding sites were occupied by copper(II) ions after soaking for 12 h in the copper(II) solution. This effect is presumably due to its low porosity, and its inability to swell in

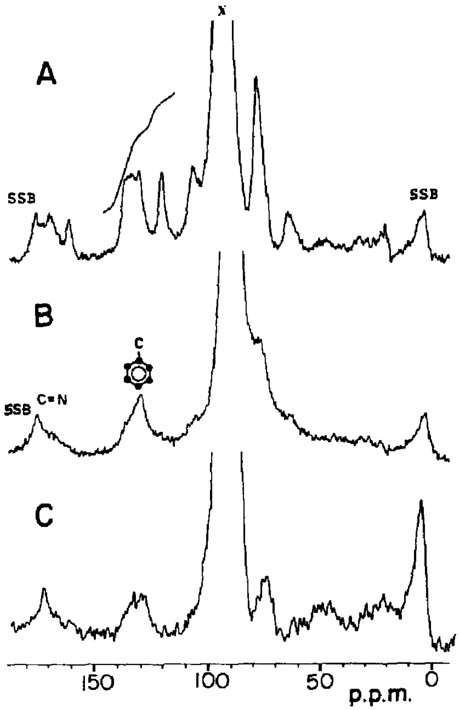
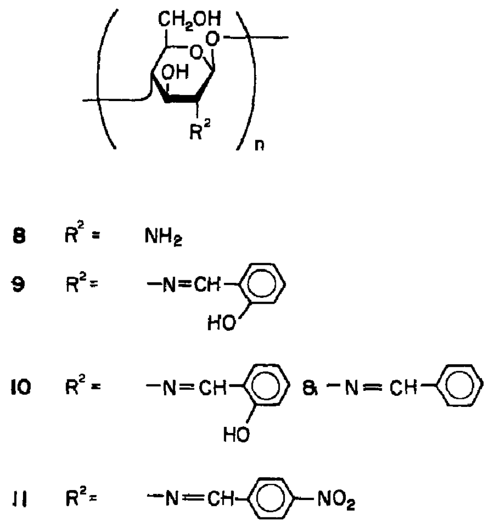


Fig. 3. ^{13}C-N.m.r. spectra of mixed Schiff's bases of benzylidenechitosan and salicylidenechitosan (**10**) with and without copper(II) ions: A, free ligand (1100 scans; contact time, 3 ms; repetition time, 10 s); B and C, copper complexed, with contact times set at 0.5 and 3 ms, respectively.



aqueous or alcoholic solution. In this work, an increase in the copper-chelating capacity [3.29 mmol of copper(II) per g; 87.4% of the theoretical value] was achieved by using the modified reaction procedure given in the Experimental Section. The absence of detectable carbon resonances can be interpreted as arising from the dense dispersion of copper(II) ions in the polymer "matrix"; this causes the resonances of non-metallated monomeric units to experience a T_{1e} comparable to that experienced by those which are metallated. This point is further illustrated by complex **10**, prepared by reacting chitosan with a mixture of benzaldehyde and salicylaldehyde. The colour of the mixed polymer is lighter, with or without copper(II) ions, when compared with that of salicylidenechitosan. The ratio of benzylidene to salicylidene groups is estimated to be 2:1 from Fig. 3. In the presence of copper(II) ions [2.09 mmol of copper(II) per g], the carbon signals of salicylidenechitosan disappeared and those of benzylidenechitosan were broadened significantly; the latter probably does not participate in metal chelation. By setting a longer contact time (3 ms), reasonably sharp signals were obtained, corresponding to "free" benzylidenechitosan unperturbed by the distant paramagnetic ions.

It is now appropriate to consider what effects copper(II) ions have on polymers which lack any major locus for strong metal chelation. For such compounds, it has been shown[20] in the solid state that

$$(1/T_1) \propto \mathrm{N.a.D}, \qquad (3)$$

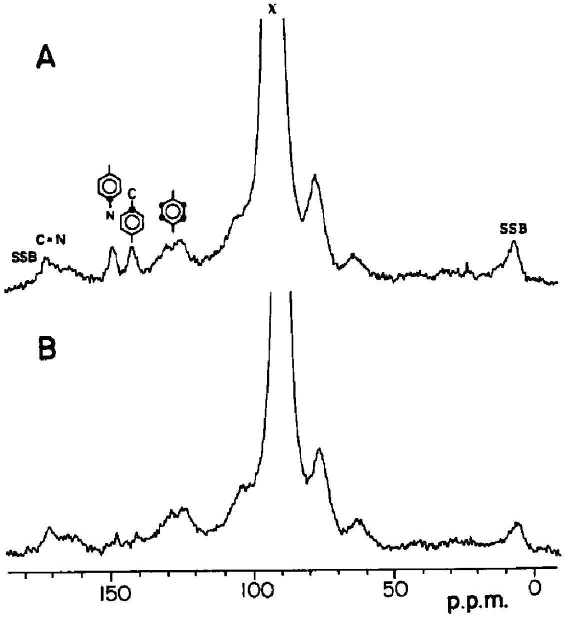


Fig. 4. ^{13}C-N.m.r. spectra of **11** in the presence of copper(II) ions: A, 3-ms, and B, 0.1-ms contact times (850 scans; repetition time, 10 s).

where N is the number of paramagnetic nuclei per unit volume, a is the distance between nuclei, and D is the spin-diffusion constant derived from the spin flip-flop between nuclei. At low concentrations of copper(II) ions in the polymer matrix, the carbon resonances are expected not to be shifted or broadened to any significant amount. *N*-(4-Nitrobenzylidene)chitosan (**11**) produces a copper(II) complex which is blue, and which binds 0.74 mmol of copper(II) per g of the polymer (21.8% of the theoretical value). The spectra in Fig. 4 indicate that the copper(II) ions decrease the relaxation times of the polymer, but to a lesser extent than for the salicylidene–copper(II) complexes discussed above. Slight line broadening was observed because of the high concentration of copper(II) ion, but no measurable shifts were detected for the carbon resonances. Reducing the contact time further, down to 0.1 ms, only decreased the intensity of the signals of non-protonated carbons of the aromatic ring; this observation is unlikely for a true metal chelate. Hence, it is concluded that the paramagnetic ions are diffused through the matrix, and weakly bound to the matrix by some long-range bonding to hydroxyl groups.

Alginate may be considered as a linear, block copolymer of β-D-mannuronic acid and α-L-guluronic acid residues, the relative amounts of which vary greatly for alginic acids from different species of algae[21]. The structure consists of three types of blocks, namely, homopolymeric blocks of mannuronic acid (MM) and of guluronic acid (GG), and blocks with alternating sequence (MG). It has been suggested that the metal ions are selectively bound in the long sequences of GG blocks and we hoped that chelation of copper(II) would eliminate the ^{13}C resonances of those blocks, leaving the others still detectable. The absence of any carbon signals in the spectrum of the copper–alginate complex [2.58 mmol of copper(II) per g] suggests that not only the copper(II)-complexed regions, but rather the whole polymeric unit experiences a long T_{1e}. This can be explained by two models: the polymer could be rich in L-guluronic acid residues, or the D-mannuronic acid residues could have some affinity for the metal ions and/or have been brought closer to the binding sites by molecular packing. We chose not to pursue this question further.

CONCLUSION

The metal–sugar complexes studied here often have intractable properties, and the solid-state ^{13}C-n.m.r. method has been used to help characterise them. The isotropic shifts of a diamagnetic sugar–metal complex are closely similar to those measured for the uncomplexed sugar-ligand. On the other hand, binding of paramagnetic ions has a profound effect on the obtainable resolution of the ^{13}C-n.m.r. spectrum. High concentrations of the paramagnetic metal result in broad, featureless spectra. Interpretation of the mechanism for those effects is complicated by spin diffusion of the protons; however, the major factors appear to be the T_{1e} and the inter-moment distances. For a sufficiently slow T_{1e}, all of the carbon resonances are broadened beyond detection; for moderate T_{1e} values, broad carbon resonances can be observed, with line-widths which depend on their proximity to the metal centers.

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