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A ^{13}C solid-state NMR study of ion-exchange resins derived from natural polysaccharides

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Abstract

Several ion-exchange resins and resin precursor materials formed from natural polysaccharides have been examined by solid-state NMR spectroscopy. NMR spectra of the dry resin contained broad, featureless bands due to the polysaccharide backbone of the resin and additional broad bands due to attached groups. On addition of water to the resin, the resonances for the polysaccharide backbone remained broad, but those due to the attached groups were considerably sharpened. Many individual resonances of the attached groups could thus be assigned, so that changes brought about by chemical modification of the polysaccharide during the production of an ion-exchange resin could be followed.

Key words: NMR spectroscopy; CPMAS spectroscopy; Polysaccharide; Ion-exchange resin; Cellulose

1. Introduction

Many medium-performance ion-exchange resins are made by chemical derivatisation of natural polysaccharides such as agarose [1], dextran, and cellulose [2]. The resolution and retention properties of these ion-exchange resins depend on several factors, the most important being bead size, pore size, and the nature of the attached ligands. Bead and pore size are controlled by the physical method used to prepare the resin and by the conformational structure of the natural polysaccharide. Retention times of analytes are determined by their electrostatic and hydrophobic interactions with ion-exchange ligands bound to the resin.

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Formation of an ion-exchange resin from the natural polysaccharides will usually involve a number of chemical and physical modifications. These may include solution casting to form a porous beaded product, reaction with a cross-linking agent to strengthen and inhibit swelling of the bead, and covalent attachment of ligand and spacer groups. These cross-linking and derivatisation reactions are not specific for any particular hydroxy group, and they can give rise to more than one type of ligand, leading to a polymer with a nonregular repeating unit and reduced crystallinity.

Since the resin is an amorphous insoluble solid, only a limited number of spectroscopic techniques can be usefully applied to determine the structure of the resin and the resin precursor materials. Solid-state ^{13}C NMR spectroscopy is a particularly suitable technique, and solid-state ^{13}C NMR studies of a variety of polysaccharides and their derivatives have been reported. Cellulose has received the most attention, with studies of various structural forms of cellulose [3] and of a wide variety of cellulose derivatives, such as cellulose acetate [4], cellulose nitrate, and cellulose ethers [5], having been published. Reports of solid-state NMR studies of dextrans [6] and agaroses [7] are less frequent. In this paper we focus on the systematic interpretation of solid-state ^{13}C NMR spectra of commercially available ion-exchange resins and resin precursor materials formed from cellulose. Our results, however, are applicable to other similar polysaccharide-based materials.

Solid-state ^{13}C NMR spectra of resins in the dry state contained a series of broad, featureless peaks. However, spectra of moistened resins revealed several narrow resonances. These were assigned to noncarbohydrate mobile species attached to the carbohydrate substrate during modification of the polysaccharide. This allowed a significant part of the reactions that occur during modification of the polysaccharide to be followed.

2. Experimental

Resin precursor materials were obtained from Phoenix Chemicals (now Life Technologies Ltd, P.O. Box 3030, Richmond, Nelson, New Zealand). Whatman DE-52 was obtained from Aldrich Chemical Co.

Proton-decoupled ^{13}C CPMAS NMR spectra were recorded on a 4.7 T Varian XL200 spectrometer. Samples were packed into a sapphire rotor and spun at 3–5 kHz in a MAS probe from Doty Scientific. RF fields of 50 kHz were used for cross-polarisation and 60 kHz for broadband decoupling. Acquisition times were 0.04 s and recycle delays were 2 s. Samples that were to be examined wet were washed thoroughly in distilled water, air dried under ambient conditions, and then moistened with ca. 50% water by weight of dry resin. Optimum cross-polarisation times for the wet samples were about 2000 μs for the mobile groups and about 500 μs for the carbohydrate backbone. Adequate signal-to-noise (S/N) ratios were generally obtained after 5000 transients. For some resins examined in this study, especially those that tend to swell excessively on the addition of water, increasingly poor S/N ratios can occur in the CPMAS spectra with increasing water content. It is therefore preferable to keep water addition to the minimum required for obtaining reasonable resolution.

A 90° pulse width of 5 μs was used for the Bloch decay experiments, and spectra were acquired under MAS. Acquisition times were 0.04 s and recycle delay times were 0.2 s.

Washing the resin helped to remove salt contaminants that otherwise cause excessive dielectric loss within the probe. Even so the probe required retuning on addition of water to the resin, and dielectric loss resulted in decoupling fields of ca. 50 kHz. This required resetting of the Hartmann–Hahn match. The range of the proton RF field intensity over which a Hartmann–Hahn match occurred was found to be similiar to that of the dry samples. Conversion to the base form was accomplished by washing the resin with dilute sodium hydroxide, followed by repeated washings with distilled water until the washings were neutral. Unless stated otherwise all DEAE and DE resins were examined as the base form.

Chemical shifts are reported in ppm with respect to Me_4Si, with hexamethylbenzene used as an external secondary reference ($\delta = 17.5$ ppm for the methyl resonance). In acquiring ^{13}C CPMAS NMR spectra using the interrupted decoupling pulse sequence of Alemany et al. [8], interruption times of ca. 40 μs were used in order to achieve reasonable suppression of the signal of the nonmobile species. (Interrupted decoupling is also commonly called dipolar dephasing.) Proton rotating-frame relaxation times, $T_{1\rho}(H)$, and time constants for cross-polarisation, T_{CH}, were obtained by using a nonlinear least squares Maquardt method [9] to fit an exponential increasing function and an exponential decreasing function respectively, to the data. Errors were ca. ±10% for both of the resulting T_{CH} and $T_{1\rho}(H)$ values.

3. Results and discussion

Commercially available polysaccharide-based resins are usually supplied wet since drying can irreversibly collapse pores within the resin. Examination of samples as supplied, however, generally results in spectra with a poor S/N ratio due to probe detuning, especially if the sample contains ionic groups. Whatman DE-52, a weakly basic anion-exchange resin based on cellulose with diethylaminoethyl ligands was chosen for a study of the NMR dynamics and properties of the wet and the dry resins to see if methods could be developed that would allow resin samples to be characterised by NMR spectroscopy. The resin was examined in the base form since this allowed some of the resonances due to quaternary ammonium groups to be distinguished from those of the tertiary amine.

Solid-state NMR studies of Whatman DE-52 resin.—The CPMAS NMR spectrum of Whatman DE-52 that had been converted into the base form and dried, was shown to contain a number of broad peaks (Fig. 1a). Peaks from 60 to 110 ppm

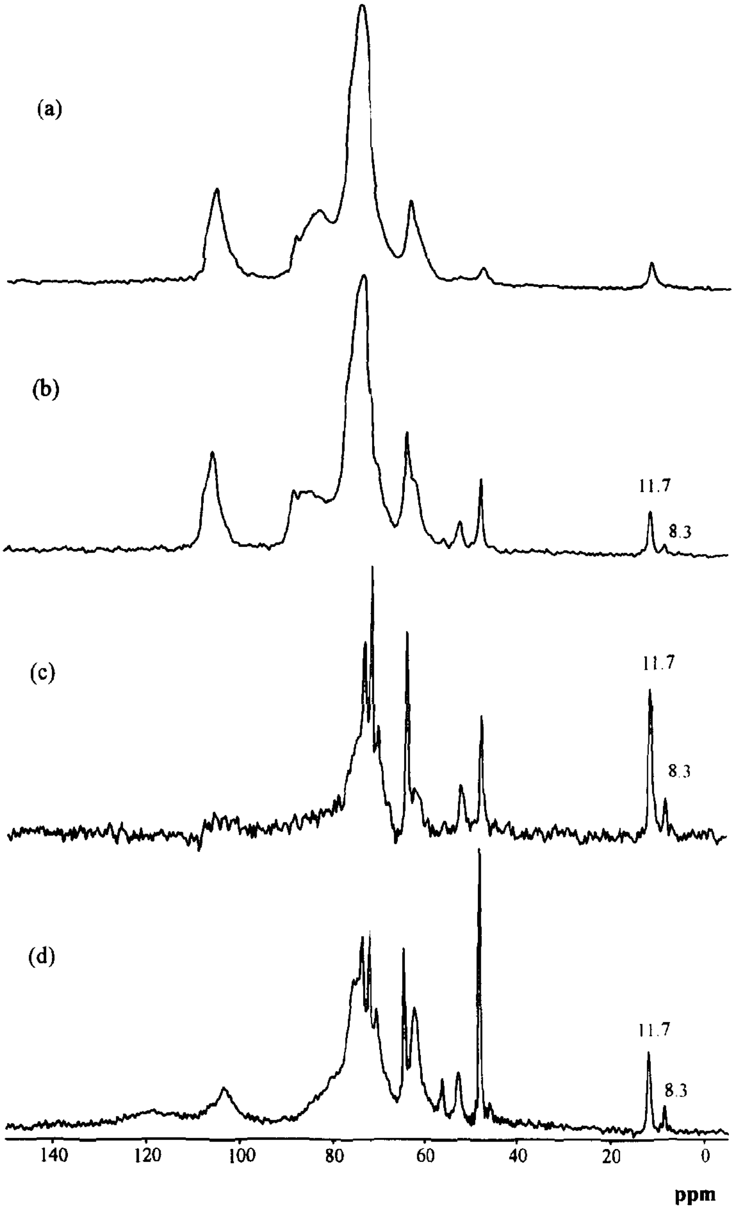


Fig. 1. Whatman DE-52: (a) ^{13}C CPMAS NMR spectrum of dry resin; (b) ^{13}C CPMAS NMR spectrum of dry resin moistened with water; (c) ^{13}C CPMAS NMR spectrum with interrupted decoupling of resin sample as in (b); and (d) ^{13}C NMR (Bloch decay) spectrum of moistened resin.

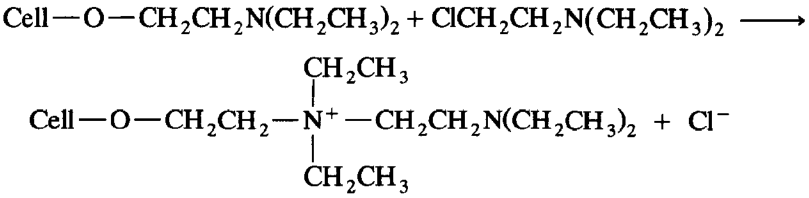
were assigned to cellulose, based on published data for crystalline and amorphous cellulose [3]. The distinct peaks at 11.7 and 47.8 ppm were assigned to methyl and methylene carbons, respectively, of the N-ethyl groups of the tertiary amine ligand.

All peaks assigned to the carbohydrate component of the resin had similar cross-polarisation time constants ($T_{CH} = 100 \pm 11$ μs) and rotating-frame relaxation times [$T_{1\rho}(H) = 2500 \pm 200$ μs]. T_{CH} values of ca. 100 μs are considered normal for methylene and methine groups. Cross-polarisation time constants were longer for the methyl carbons of the tertiary amine (330 ± 35 μs) due to partial averaging of the proton–carbon dipolar couplings by free rotation of the methyl group. In contrast the $T_{1\rho}(H)$ values of 3080 ± 260 μs were not greatly dissimilar to those of protons of the carbohydrate component, presumably because spin-diffusion between the protons is averaging the proton relaxation times.

Dried Whatman DE-52 readily reabsorbed water with some swelling. Although water addition does cause the probe to detune, it is possible to obtain good CPMAS NMR spectra of moist samples if they have first been thoroughly washed to remove salt contaminants and if care is taken in retuning the probe and resetting the Hartmann–Hahn condition for optimum signal intensity. The proton relaxation times [$T_1(H)$] of 0.6 s observed for dry and moistened resins were not favourable for rapid pulsing; nevertheless, spectra with reasonable S/N were obtained after a few hours of data accumulation, if the moisture contents were below 50% by weight of the dried material.

The ^{13}C CPMAS NMR spectrum of a moistened Whatman DE-52 resin sample is shown in Fig. 1b. Peaks of the noncarbohydrate substituents are now narrower ($\omega_{1/2}$ is 20–40 Hz). This is due to rapid rotational and translational motion of the substituents so that equivalent carbons experience a similar aqueous environment on the NMR time scale. Usually the nuclei associated with these narrower peaks have different relaxation behaviour, and specific pulse sequences may enhance these signals compared to the nonmobile species. Gidley [10] has called this "mobility resolved spectroscopy". As an increase in the time constant for cross-polarisation ($T_{CH} = 1300 \pm 140$ μs) for the methyl groups of the tertiary amines was observed, rapid motion of these substituents is also averaging dipolar couplings. The motion appears to be sufficiently rapid that the rate of proton–proton spin diffusion from the substituents to the carbohydrate backbone is now so slow that the protons on the substituents are effectively isolated from the protons of the carbohydrate backbone. This is discerned as an increase in the rotating-frame relaxation time [$T_{1\rho}(H) = 7300 \pm 600$ μs] of the methyl groups of the tertiary amines. Sufficiently rapid rotational and translational motion of a substituent can average proton–carbon and proton–proton dipolar couplings to zero so that cross-polarisation is no longer effective. However, the noncarbohydrate substituents do not have complete motional freedom as they are partially constrained by being tethered to the carbohydrate backbone.

The increased resolution in the spectrum of the moistened sample permits a small peak at 8.2 ppm to be identified and assigned to methyl carbons of quaternary tetraethylammonium groups. Quaternisation has occurred due to additional condensation reactions between the tertiary amine ligand and the diethylaminoethyl chloride reactant (Scheme 1). The cross-polarisation time constant $T_{CH} = 297 \pm 21$ μs and rotating-frame relaxation time $T_{1\rho}(H) = 5463 \pm 342$ μs for the methyl components of the quaternary ammonium groups are not as large as



$$\mathrm{Cell{-}O{-}CH_2CH_2N(CH_2CH_3)_2 + ClCH_2CH_2N(CH_2CH_3)_2 \longrightarrow}$$

$$\mathrm{Cell{-}O{-}CH_2CH_2{-}\overset{CH_2CH_3}{\underset{CH_2CH_3}{N^+}}{-}CH_2CH_2N(CH_2CH_3)_2 + Cl^-}$$

Scheme 1.

those determined for the methyl groups of the corresponding tertiary amines, suggesting that the quaternary ammonium groups have less motional freedom than the tertiary amine groups. This may be due to the larger size of, and the extra solvation about, the charged quaternary centre.

The T_{CH} values for nuclei in the carbohydrate backbone significantly shorten from 100 to 62 ± 5 μs on moistening the sample. Evidently inclusion of water somewhat stiffens the carbohydrate backbone, perhaps through additional hydrogen bonding. Rotating-frame relaxation times [$T_{1\rho}(H) = 2500 \pm 200$ and 2960 ± 140 μs for the dry and moist sample, respectively] did not significantly change. Taken together, these results suggest that moistening of the resin changes motional freedom of the cellulose backbone by at most a small amount. This is expected as cellulose chains are already involved in a network that should restrict conformational freedom, even in the presence of additional water.

In both the dry and moistened resins, resonances of the carbohydrate components were broad and ill defined. The carbohydrate backbone is held in a semifixed conformation, so that carbon atoms in otherwise equivalent monosaccharide units experience slightly different chemical environments and hence resonate at different chemical shift values. Some broadening also results from the variable chemical substitution pattern of each glucose monomer.

Since cross-polarisation times of the carbohydrate backbone and the substituents are very different, it is possible to acquire interrupted decoupling spectra of the moistened resin in which resonances of the carbons of the polysaccharide backbone are selectively suppressed. A spectrum of Whatman DE-52 using an interrupted decoupling pulse sequence [8] is shown in Fig. 1c. Peaks previously obscured by signals from the polysaccharide backbone are now clearly observed.

It is also possible to suppress signals due to the carbohydrate backbone by using a normal Bloch decay single-pulse sequence with MAS, and short delays (0.2 s) between pulses. This enhances the intensity of the signals of the faster relaxing carbons of the substituents compared to that of the slower relaxing carbons on the carbohydrate backbone (Fig. 1d). It is interesting to note that without MAS, peak widths of the mobile components are about 150 Hz, but with MAS they are 30 Hz. MAS is therefore required to average the chemical shift anisotropies. This again suggests that the mobile components do not have complete motional freedom, but are partially constrained by being tethered to the carbohydrate backbone. In general, the Bloch decay sequence gives better S/N ratio for the mobile components, over a fixed time period, than does the cross-polarisation sequence.

Resin and resin precursor materials.—Having defined appropriate experimental procedures for obtaining resolved spectra of the Whatman DE-52 resin, the techniques were applied to the analysis of resins manufactured in New Zealand by Phoenix Chemicals. A series of resin precursor materials were also examined to help with the assignment of specific peaks in the spectra of the ion-exchange resins. The primary precursor material was a cross-linked and expanded (EP) cellulose formed by reaction of regenerated cellulose particles with epichlorohy-

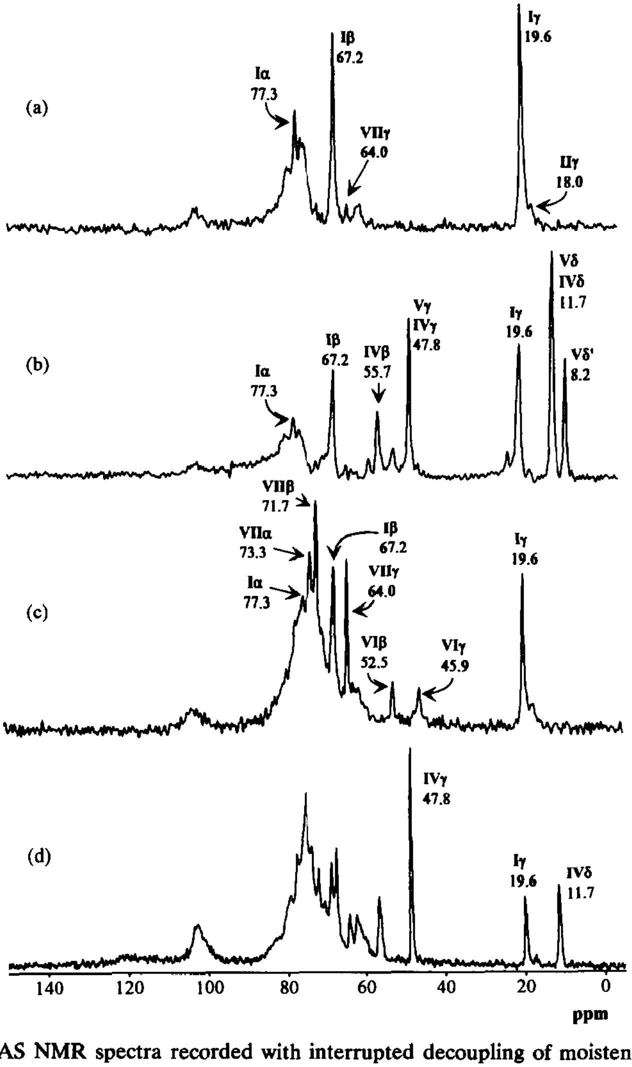
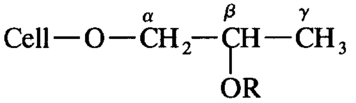


Fig. 2. ^{13}C CPMAS NMR spectra recorded with interrupted decoupling of moistened samples of (a) Phoenix EP cellulose resin and (b) Phoenix DEAE cellulose resin. Single pulse, Bloch decay spectra of moistened samples of (c) Phoenix AC cellulose resin and (d) Phoenix DE cellulose resin. (Note: the spectra acquired using either CPMAS or the Bloch decay sequences show similar features.)

drin and propylene oxide in sodium hydroxide. This material had then been treated with diethylaminoethyl chloride to produce the Phoenix DEAE resin.

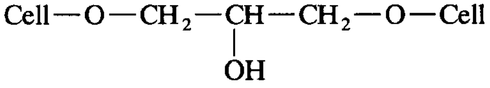
A diethylamine (DE) resin had also been prepared from the EP cellulose by reaction first with additional epichlorohydrin to introduce reactive glycidyl groups and form an activated (AC) cellulose, and then secondly with diethylamine to produce the Phoenix DE resin.

The ^{13}C CPMAS NMR spectra acquired with interrupted decoupling of moistened samples of resins of this series are shown in Fig. 2. The indicated assignments refer to the structural units shown in Fig. 3. Since the narrow peaks are due to



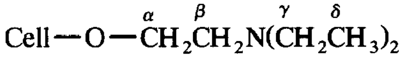
$$\text{Cell—O—}\overset{\alpha}{\text{CH}_2}\text{—}\underset{\text{OR}}{\overset{\beta}{\text{CH}}}\text{—}\overset{\gamma}{\text{CH}_3}$$

I R = H
II R = $CH_2CHOHCH_3$



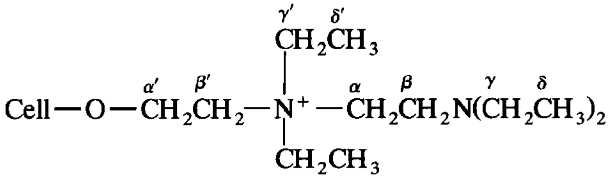
$$\text{Cell—O—CH}_2\text{—}\underset{\text{OH}}{\text{CH}}\text{—CH}_2\text{—O—Cell}$$

III



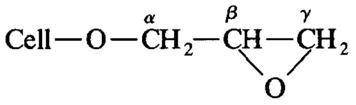
$$\text{Cell—O—}\overset{\alpha}{\text{CH}_2}\overset{\beta}{\text{CH}_2}\text{N(}\overset{\gamma}{\text{CH}_2}\overset{\delta}{\text{CH}_3}\text{)}_2$$

IV

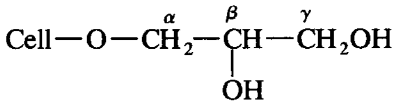


$$\text{Cell—O—}\overset{\alpha'}{\text{CH}_2}\overset{\beta'}{\text{CH}_2}\text{—}\underset{\text{CH}_2\text{CH}_3}{\overset{\overset{\gamma'}{\text{CH}_2}\overset{\delta'}{\text{CH}_3}}{\text{N}^+}}\text{—}\overset{\alpha}{\text{CH}_2}\overset{\beta}{\text{CH}_2}\text{N(}\overset{\gamma}{\text{CH}_2}\overset{\delta}{\text{CH}_3}\text{)}_2$$

V



VI



$$\text{Cell—O—}\overset{\alpha}{\text{CH}_2}\text{—}\underset{\text{OH}}{\overset{\beta}{\text{CH}}}\text{—}\overset{\gamma}{\text{CH}_2}\text{OH}$$

VII

Fig. 3. Structures of groups attached to the cellulose backbone where Cell-O represents a carbohydrate hydroxyl group.

groups in a pseudo aqueous environment, chemical shifts were expected to be similar to those of related small molecules dissolved in an aqueous solution [11].

The CPMAS spectrum of the EP cellulose is shown in Fig. 2a. The peaks at 19.6, 67.2, and 77.3 ppm were assigned to species **I**. A shoulder at 18 ppm was assigned to species **II** which is formed by reaction of a hydroxypropyl substituent with further propylene oxide. The intensity of this peak was observed to increase in a sample of EP cellulose subjected to reaction with additional propylene oxide. A weak peak at 64.0 ppm was assigned to glyceryl cellulose **VII**, the hydrolysis product of glycidyl cellulose **VI**, as discussed below. No peaks characteristic of the cross-linking groups in product **III** could be identified. These groups may not be observable in an interrupted decoupling experiment since they should have somewhat restricted mobility, both ends being connected to the rigid polysaccharide backbone of the resin. Additionally, the ratio of epichlorohydrin to propylene oxide used in the preparation of such resins is in the region of 1:10, so that peaks due to the cross-linking moiety would be small.

The CPMAS spectrum of the Phoenix DEAE resin in the free-base form is shown in Fig. 2b. In addition to the peaks observed in the spectrum of EP cellulose, there are peaks for the methyl groups of the tertiary amine [12] and the quaternary ammonium component [13] at 11.7 and 8.2 ppm. Other, small peaks have not been assigned. When the resin was examined in the hydrochloride salt form, the methyl peak of the now protonated tertiary amine (**IV**δ) shifted upfield to 9.5 ppm, while that for the quaternary ammonium group did not shift. In forming a DEAE resin, the desired product should primarily be the tertiary amine **IV**, since the presence of large amounts of quaternary ammonium groups **V** can cause irreversible binding of analytes to the resin, especially analytes with a high density of anionic groups, such as sulfated polysaccharides.

The Bloch decay spectrum of the AC resin is shown in Fig. 2c. It is apparent that hydrolysis of the epoxide groups of **VI** occurred prior to the acquisition of the spectrum, as a peak from the hydrolysis product **VII** is also observed. As noted above, a similar hydrolysis reaction can also occur during cross-linking.

The Bloch decay spectrum of the Phoenix DE resin formed by reaction of AC with diethylamine is shown in Fig. 2d. No peaks due to quaternary ammonium groups were observed in the spectrum. Thus the MAS NMR spectrum clearly shows that little or no quaternisation occurs during formation of a DE resin.

4. Summary

The results of this study demonstrate that DEAE resins can contain considerable amounts of quaternary ammonium groups, but that in general DE resins should be free of quaternary ammonium groups. A more extensive survey of ion-exchange resins, which included Pharmacia DEAE-Sephadex A-25, Chisso DEAE-Cellulofine, and Pharmacia DEAE-Sephacel, has shown that each of these resins contains significant amounts of quaternary ammonium groups. In addition, Whatman DE-52 contains quaternary ammonium groups even though the trade name suggests that it is nominally a DE resin. Since significant amounts of quaternary ammonium salts can change the ion-exchange properties of an ion-exchange resin, it would appear to be important that the ratio of quaternary ammonium groups to tertiary amine is monitored during manufacture in order to obtain a consistent product.

Acknowledgments

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