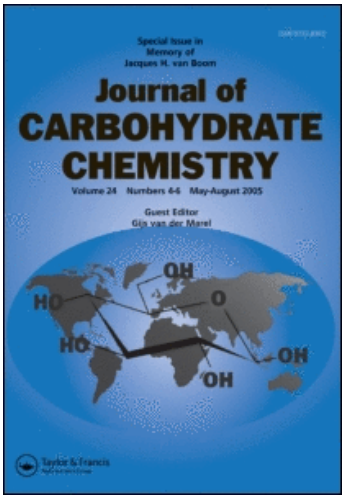

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High Resolution Solid State ^{13}C NMR and its Applications in Carbohydrate Chemistry

Philip E. Pfeffer[a]
[a] U.S. Department of Agriculture, Eastern Regional Research Center Philadelphia, Pennsylvania

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REVIEW ARTICLE

HIGH RESOLUTION SOLID STATE ^{13}C NMR AND ITS APPLICATIONS IN CARBOHYDRATE CHEMISTRY

Philip E. Pfeffer

U.S. Department of Agriculture
Eastern Regional Research Center
Philadelphia, Pennsylvania 19118

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ABSTRACT

The experimental concepts of high resolution solid state ^{13}C cross-polarization magic angle spinning (CP-MAS) NMR spectroscopy are examined and contrasted with those used for obtaining NMR spectra in the liquid state. The setting of the critical experimental parameters necessary for obtaining quantitative responses in the solid state experiment are discussed in terms of the relative magnitudes of relaxation processes that are essential for the successful implementation of the technique. Methods for evaluating morphological changes and identifying resonances of the spectra with regard to chemical and/or physical diversity are also described and illustrated with spectral studies of different carbohydrates.

INTRODUCTION

High resolution ^{13}C solution NMR spectroscopy has been used to solve analytical problems in carbohydrate chemistry for more than a decade. Yet, there are many situations in which comparable NMR information could be invaluable for studying the

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structure and chemistry of bulk solids, e.g., the elucidation of the structure of insoluble materials that do not form acceptable single crystals for X-ray crystallographic analysis, the characterization and quantitation of intractable polysaccharides and intact heterogeneous polysaccharide matrices, and determination of the dynamic properties of these substances. Fortunately, an NMR technique which can be applied to bulk solids is available. This paper presents a broad overview of the concepts and methodology associated with high resolution solid state ^{13}C cross polarization magic angle spinning (CP-MAS) NMR spectroscopy and provides illustrations of its application to problems in the area of structural carbohydrate chemistry.

SOLID STATE METHODOLOGY

High Power Proton Decoupling

Over 10 years ago three major innovations were developed which make it feasible to obtain high resolution NMR spectra of solids.[1,2] Of these, the removal of broadening due to strong dipolar interactions with neighboring protons was an important first step for obtaining high resolution ^{13}C spectra.[1] Typically, without the removal of these strong interactions, each ^{13}C resonance line could be 2-3 KHz in width and the spectra would show little or no definable response, as illustrated in the spectrum of powdered sucrose, Fig. 1a. This extreme broadening is primarily due to the lack of motion in the solid relative to the solution, i.e., an intimate dipolar interaction of the dilute spin ^{13}C (1.1% natural abundance) nuclei with an enormous population of neighboring abundant (nearly 100% natural abundance) protons that cannot be averaged out by fast tumbling as it is in solution. Consequently, in the solid, each ^{13}C nucleus is "surrounded" by many local proton magnetic fields which induce significant broadening. For ^{13}C solution spectra, scalar (through-bond) interactions can be easily removed with low power proton decoupling to give single line ^{13}C spectra. In the solid

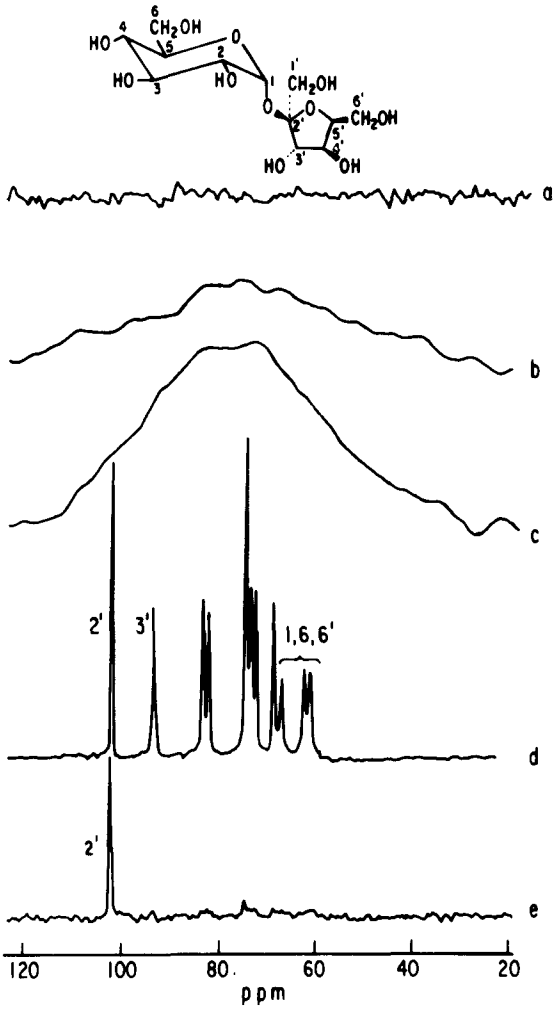


FIG. 1. 15 MHz ^{13}C solid state spectra of powered crystalline sucrose taken under the following experimental conditions: a) solution spectrum conditions, 50° pulse angle, 8 second pulse delay, and 5 KHz proton decoupling; b) same conditions as a except with high power decoupling of 45 KHz; c) same as b except with proton-carbon cross polarization, contact time of 2 msec; d) same as c except sample was spun at 2.0 KHz at the magic angle of 54.7°; e) same as d except a 40 μsec delay without decoupling was inserted into the pulse sequence following the contact time.

state, proton interactions are more severe and their modulation requires approximately 10 times the intensity of the decoupling power used for solution spectra to achieve acceptable responses. Figure 1b shows the effect of high power decoupling on the ^{13}C spectrum of powdered sucrose. Although no readily definable resonances are observed, a clear indication of an emerging spectrum is evident.

Sensitivity Enhancement of ^{13}C Signals

Additional problems in sensitivity associated with a dilute spin would normally limit the application of these experiments. Since ^{13}C nuclei do not "see" each other in the solid state as often as they do in the mobile liquid, they cannot readily undergo mutual spin flips which allow them to relax back to their equilibrium magnetization position following a 90° pulse. Consequently, ^{13}C T_1 (spin lattice relaxation times) are in the 10-100 second range in the solid, which makes the acquisition of data next to impossible under reasonable time constraints. To alleviate this problem, Pines et al. capitalized on the phenomenon of cross relaxation to allow the large proton population in the sample to control the rapid relaxation of the dilute spin ^{13}C nuclei. This methodology uses a pulse sequence that induces both protons and carbons to precess at the same frequency in their respective rotating frames. During this short period of time (milliseconds) called the cross-polarization time, ^{13}C and ^{1}H can exchange energy through mutual spin flips and thus the ^{13}C nuclei relax in a time period (fraction of a second) comparable to that of ^{13}C in solution. In addition to the rapid relaxation times obtained for the carbon nuclei, this process also produces a ^{13}C signal enhancement due to the polarization gained from the abundant proton population. The overall ^{13}C enhancement, due to time saving from the ability to recycle the experiment in the proton relaxation time domain and the gain in carbon magnetization from the energy transfer polarization process, results in an approximate fourfold increase in signal-to-noise over the conventional

non-cross polarization experiment. Figure 1c illustrates this point if we compare this spectrum with the signal-to-noise of spectrum 1b that was obtained with the same number of scans. Clearly, there is still a long way to go in terms of the significant broadening which continues to obscure the information contained in spectrum 1c. This is primarily due to the superposition of fixed orientations of the molecules in the solid matrix which is known as the chemical shift anisotropy (CSA).

High Speed Spinning at the Magic Angle

In the liquid state we generally observe an average isotropic value for the resonance or chemical shift due to the rapid averaging motion of the medium. In the solid state the rigid lattice does not permit such motional tumbling and consequently we observe broad lines arising from anisotropic powder patterns for each carbon atom in the spectrum.[3] These patterns represent the dispersion of chemical shifts in all possible orientations within the solid. In order to alleviate this broadening and average out the orientations within the material, the sample must be spun at a frequency equal to or greater than the observed linewidths[2,4] (at a ^{13}C frequency of 15 MHz, linewidths are approximately 2,000 Hz wide). If experiments are carried out at higher magnetic fields, linewidths will be inherently broader and consequently the spinning rate will have to be greater to achieve comparable narrowing. In order to achieve a high resolution narrow line spectrum, one must also orient the sample at the magic angle of 54.7° during the spinning process. This is necessary because the CSA line broadening is dependent on the angle θ which is the angle between the applied magnetic field and the axis of sample rotation according to the relationship $(3\cos^2\theta-1)$. When the sample is oriented at 54.7°, the value of $\cos^2\theta$ becomes 1/3 and the broadening factor vanishes.[5] Failure to orient the sample at the exact angle, i.e., a deviation as little as 0.7° from the magic angle will cause a coalescence of the resonances.[6] Likewise, lack of crystallinity can give rise to spectra much

like that seen in Fig. 1c. The final spectrum that results from the proper execution of all of the combined parameters of the CP-MAS experiment is seen in Fig. 1d.

Optimization of Experimental Parameters

In the cross-polarization process there are a number of relaxation phenomena that one must be aware of in order to avoid the problem of distorted spectral intensities. As depicted in the cross-polarization sequence (Fig. 2), the first process that must be considered is called the contact time. The contact time is a period (millisecond range) when both the proton and carbon pulses are on and thermal contact is made between the two spin reservoirs. During this time, magnetization flows from the proton spins to the carbons. In essence, the large proton population sacrifices a small loss in magnetization (e.g., becomes "hotter," dotted line) which ultimately corresponds to a large increase in the dilute carbon spin magnetization (becomes much "colder," dotted line). Since the entire proton population

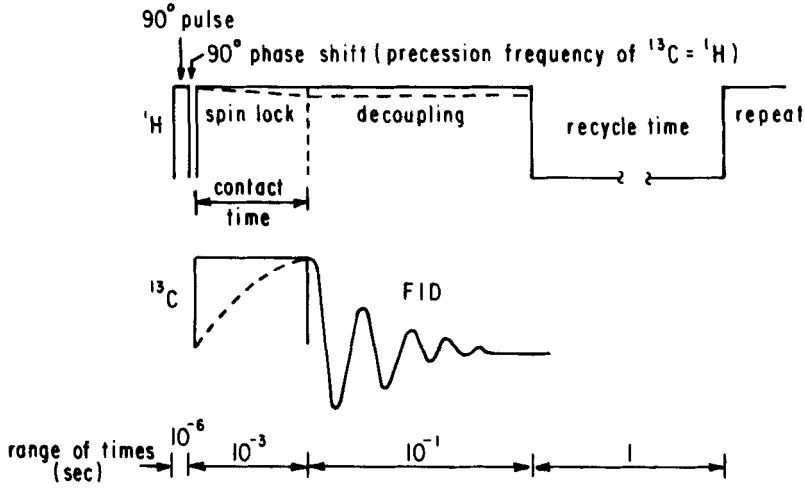


FIG. 2. ^{13}C solid state cross-polarization sequence.

undergoes rapid mutual spin flips (spin diffusion) all the protons in a homogeneous sample have the same characteristic spin lattice relaxation. During this condition when the protons are in the spin locked decoupled state, they begin to decay in a time $T_{1\rho}$ which is known as the rotating frame spin lattice relaxation time (usually in the order of milliseconds). This $T_{1\rho}$ is a most critical time constant since during this decay time a competitive transfer of the proton magnetization to the dilute ^{13}C population to induce its response is also occurring. The time constant for this competing process is characterized by the cross-relaxation time T_{C-H}. While proton $T_{1\rho}$ is uniform for all protons within the homogeneous sample, the value of the T_{C-H}, i.e., the time it takes for the proton magnetization to reach the carbon is a variable, dependent on the factor $1/r^6$ where r represents the distance to the closest proton. Typically, T_{C-H} values are in the range of 50 μsec to 1 ms. Because of this dependence on T_{C-H} not all carbons are equivalent, as is true for the proton population. Some carbons are isolated from neighboring protons (e.g., carbonyl carbons, tetra-substituted double bond carbons, and quaternary carbons) while others have directly bonded protons (e.g., methylene, methine, and methyl group carbons. For quantitative work the observed carbon resonance intensities must be proportional to the number of carbons of a given kind. This means that the carbons that cross polarize slowly must be given sufficient time to equilibrate with the spin locked protons which are concurrently decaying in the time $T_{1\rho}$. To satisfy this requirement it is essential that the value of $T_{1\rho}$ be greater (order of magnitude) than the corresponding T_{C-H} for each carbon.[7-11] Figure 3 illustrates this point. In the early part of the contact time when the proton population has had minimal decay (proton population has a long $T_{1\rho}$), optimum energy exchange is achieved and a large ^{13}C signal is generated. However, for those carbons having T_{C-H} values closer to the proton $T_{1\rho}$, a significant amount of proton magnetization is drained off before sufficient energy is interchanged. The result is a much diminished

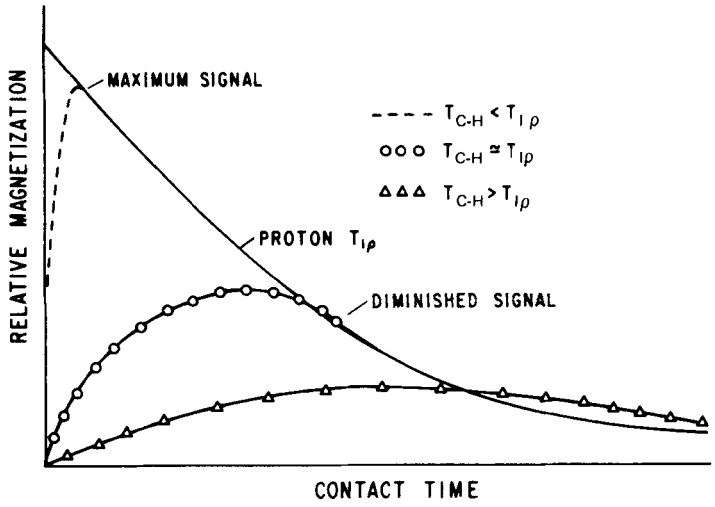


FIG. 3. Competing relaxation processes during cross-polarization.

^{13}C signal even when the experiment is optimized at a somewhat longer contact time. Finally, when the protons have decayed to the point where there is little or no magnetization left ($T_{CH} > T_{1\rho}$), virtually no ^{13}C signal is observed. In general this situation is not observed unless the carbons under consideration are significantly isolated from any protons, as has been found in some selected coal samples.[7]

Assuming the criteria of proton $T_{1\rho} >> T_{C-H}$ is met, one must also consider the relationship between the experimental parameter, contact time (CT), and these decay times. In general, the contact time should be much larger than the largest T_{C-H} value in the sample (approximately a factor of 5 to 10)[8,11] and much smaller than the proton $T_{1\rho}$ (approximately 1/5 the magnitude) to generate optimum responses. If we consider the range of contact time values for a ^{13}C spectrum of sucrose in the range of 0.05 ms to 2.0 ms, for which the criteria of $T_{C-H} >> T_{1\rho}$ is met, we see that loss of signal intensity for the nonprotonated C-2' carbon at 102.δ becomes significant for short contact time values

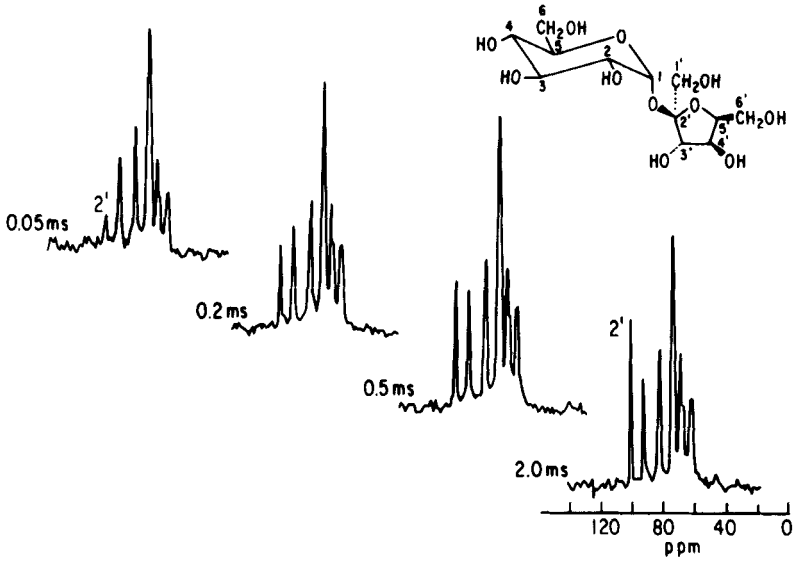
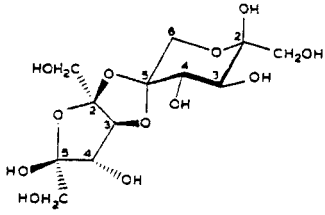


FIG. 4. Contact time study of powdered crystalline sucrose. Each spectrum was a product of 1000 scans, contact times of 0.05-2.0 msec, and recycle times of 8 seconds.

(Fig. 4). At a contact time of 2.0 ms the intensity of the C-2' resonances is maximized; however, all the other ring carbons and primary hydroxyl carbon resonances have begun to diminish in intensity. This phenomenon is due to the fact that the exponential decay representing the proton $T_{1\rho}$ process is constant for the entire proton population in sucrose, whereas the maximum intensity achieved for each carbon occurs at different times based on the individual T_{C-H} values. Consequently, a signal decay commences for the protonated carbons at approximately 1 ms while the non-protonated C-2' resonance decay is characterized by a parallel downward decay beginning after 4 ms of contact time. In many instances it is necessary to measure both T_{C-H} and proton $T_{1\rho}$ values from the slope of the early rise and final decay of the observed ^{13}C magnetization, respectively, as a function of contact time in the material under study.[9,10] From the magnitude and

ratio of these values one can usually optimize the contact time parameters to obtain quantitative responses. In some cases it is necessary to examine model systems with different ranges and ratios of T_{C-H} and $T_{1\rho}$ values to arrive at the optimum CT to be used in a specific experiment.[8-11] As we have observed, paramagnetic impurities can significantly shorten proton $T_{1\rho}$ values which ultimately results in the loss of carbon signal since the values of T_{C-H} are less unaffected.[10] This set of circumstances can give rise to serious signal intensity distortions in spectra of heterogeneous materials when specific components interact preferentially with such paramagnetic material.[8,10] While this process is detrimental to the quantitative aspects of the technique, it offers some interesting qualitative advantages, e.g., it can be useful for probing specific sites of preferential metal binding in multicomponent matrices.[10,12] In addition, purposely "too short" contact times can be used to advantage for identifying nonprotonated carbons in a homogeneous sample. A study of 5-keto-D-fructose revealed four nonprotonated carbons under short contact time conditions (preferential signal loss of four resonances). This finding leads to the identification of the structure as being a tricyclic spiran dimer (1).[13]



(1)

Following the contact period as illustrated in Fig. 2, the decay of the ^{13}C nuclei in the laboratory frame follows the proton spin lattice relaxation time T_1. As mentioned earlier, this relaxation time is significantly shorter than the corres-

ponding carbon T_1. Nevertheless, in some instances, as in the case with highly crystalline ordered materials such as glucose, this value can be abnormally large.[14] In any event, for homogeneous samples all proton T_1 values are the same, i.e., rapid spin diffusion maintains a uniform spin temperature among all the protons in the sample and represents the rate limiting relaxation process necessary for the recycling of the carbon nuclei. To determine the optimum recycling time it is necessary to measure the proton T_1 prior to the execution of these experiments. This is accomplished by the standard proton inversion recovery 180°-τ-90° method which has been previously described.[8,10] The recycle time should be at least 1.25 times the proton T_1 to maximize the total overall responses.[8] For nonhomogeneous materials the variation in proton T_1 values can be problematic since the recycling time used must be long enough to satisfy a range of relaxation requirements. If these requirements are not met, the intensity responses for some of the components could be distorted.[10]

One can also use the dipolar interactions of the protons to advantage for identifying specific types of resonances in the spectra. In the absence of proton decoupling, proton-carbon interactions destroy the carbon signal. Since the proton-carbon interaction has a $1/r^3$ dependence (where r is the distance between carbon and protons), the dipolar broadening induced on the carbon resonance is much more severe for carbon atoms that have directly bonded protons (~1A° separation) than for those that have no directly bonded protons (≥2A° separation). By inserting a 40 μsec delay with no proton decoupling between the end of the cross-polarization pulse (during which time the carbon magnetization is generated), and the start of the data acquisition (Fig. 2), the relaxation of the protonated carbon atoms (loss of phase coherence) is greatly enhanced over the nonprotonated ones.[15] The practical results of this interruption of decoupling in the pulse sequence is seen in Fig. 1e in which only the C-2' nonprotonated carbon resonance of sucrose persists at the expense of

all others. One slightly confusing aspect of this modified pulse sequence is that methyl carbon resonances also persist to some degree in the spectrum, as rotation of the methyl group modulates the carbon dipolar interactions with the directly bonded methyl protons.[15] In practice this is not a serious limitation since methyl carbon signals may be readily recognized by their characteristic shift positions.

Effects of Morphology and Structure on Spectra

When examining carbohydrates in the solid state, there are many structural parameters that must be considered in order to arrive at a correct interpretation of the observed spectral responses. Polysaccharides, for example, may exist in both crystalline as well as amorphous states simultaneously; likewise, pure crystalline sugars may crystallize from solution as mixtures of a) anomeric and/or tautomeric forms; b) conformational forms; c) inequivalent unit cell partners or molecules with different states of hydration. The problem we face in analyzing the ^{13}C CP-MAS spectra is to decipher which of the above phenomena are responsible for the observed resonance responses.

CP-MAS studies of cellulose from various sources suggest that both crystalline and amorphous domains can be observed simultaneously.[16,17] It has been suggested that the amorphous domains are located primarily at the surface.[18] Relaxation experiments clearly point to a distinct difference in mobility between these different regions.[16] Also, splittings of the C-1 and C-4 carbon resonances point to inequivalences of the anhydroglucose units in the unit cell of cellulose II.[16,19,20] A recent report on the spectra of model cellulose oligomers confirms this interpretation and indicates that there are two independent chains per unit cell of cellulose II.[21] A close look at wood pulp has uncovered homogeneous domains in which such polymers as cellulose are intimately associated with lignin.[12,22] In addition to quantifying the lignin in this material,[22] the criteria of homogeneous proton spin diffusion was used to characterize a

single-phase lignin-carbohydrate complex residue derived from cellulase-treated wood pulp.[12] Furthermore, doping of the intact wood pulp with paramagnetic Fe^{+3} gave a clear indication that rapid proton spin diffusion could be efficiently transferred from the Fe^{+3} bound to the carbohydrate to the intimate lignin matrix.[12]

Structural restriction imposed by cavities created by carbohydrates has been demonstrated in a study of substituted benzoic acid molecules that have been placed in the interior of cyclohexamylose. In these studies it was found that the molecular motion of the guest molecules (substituted benzoic acids) can be estimated by the change or loss of a specific carbon signal associated with the inside of the cavity.[23] Evidence for the homogeneous nature of such complexes, i.e., rapid proton spin diffusion from host cyclodextrin to guest benzoic acid has also been established via the measurement of proton T_1's.[24a] Another study indicates that guests such as benzene undergo rapid rotation about a sixfold axis with some additional angular fluctuations.[24b]

^{13}C CP-MAS spectra of polysaccharides often times do not give adequate information because of their diverse structural domains which generate somewhat broader lines. In most instances, as mentioned above, differential relaxation measurements have been resorted to in order to improve resolution as well as assess the dynamic behavior of these complex systems.[10,12,16] Mono- and oligosaccharides, on the other hand, exhibit excellent resolvable spectra. However, a problem that still remains is one of interpretation, i.e., what the observed spectral lines represent in terms of chemically or physically derived structural differences. Since all reducing sugars are capable of undergoing mutarotation, tautomerization, as well as conformational interconversions, their resulting ^{13}C solid state spectra are somewhat more complex than other classes of organic compounds. Add to this complexity the physical restrictions imposed by the crystal lattice, the ability to form hoichrometric hydrates, and the potential for existing in nonequivalent sites within the unit cell, and you have a formidable problem to solve.

A "simple" molecule such as glucose can provide an illustration of some of the problems that can beset the spectroscopist. The CP-MAS spectra of α-D-glucose as the monohydrate and anhydrous form are clearly different and so a mixture of glucose in these forms can be easily quantified. This kind of analysis cannot be done readily by any other means. Figure 5 compares the spectra of these two forms and shows a clear distinction in the ring carbon shift pattern and, in particular, the large difference in chemical shift between the two C-6 resonances. Strong lattice interactions are evidently reflected in glucoses' abnormally long proton T_1 values (monohydrate 40 second, anhydrous 77 second).[14] Clearly, such long T_1's present a problem for acquiring spectra, in that recycling times are inordinately long. In addition, we have reported that care should be taken when examining molecules that tend to form hydrates since they can

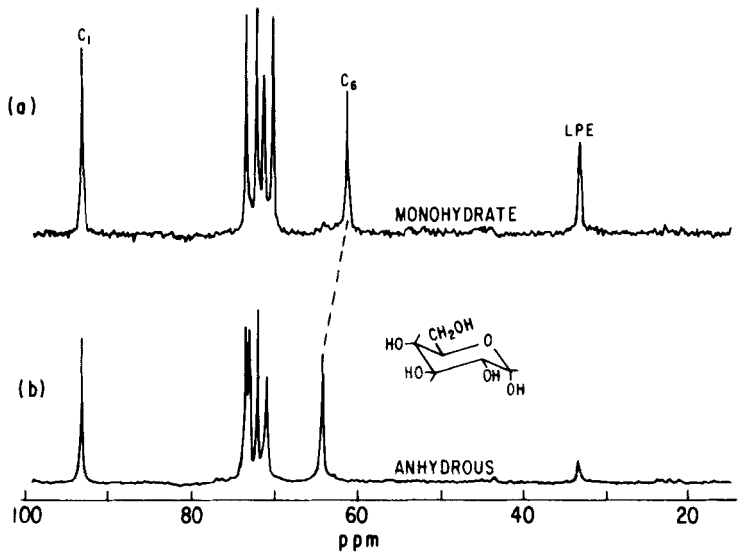


FIG. 5. 50 MHz ^{13}C CP-MAS spectra of a) α-D-glucose·H_2O and; b) α-D-glucose, LPE = linear polyethylene reference.

potentially undergo dehydration as a consequence of the CP-MAS experiment. The interconversion of α-D-glucose·H_2O to anhydrous α-D-glucose under the time course of spectral accumulations is shown in Fig. 6. This process is facilitated by radiated heat that enters the rotor at the time of strong proton decoupling. To eliminate this problem one should limit the length of the acquisition time following each pulse so little heat build-up can occur.

The diversity of interpretation of CP-MAS spectra is clearly evident in a study of two ketodisaccharides, lactulose and

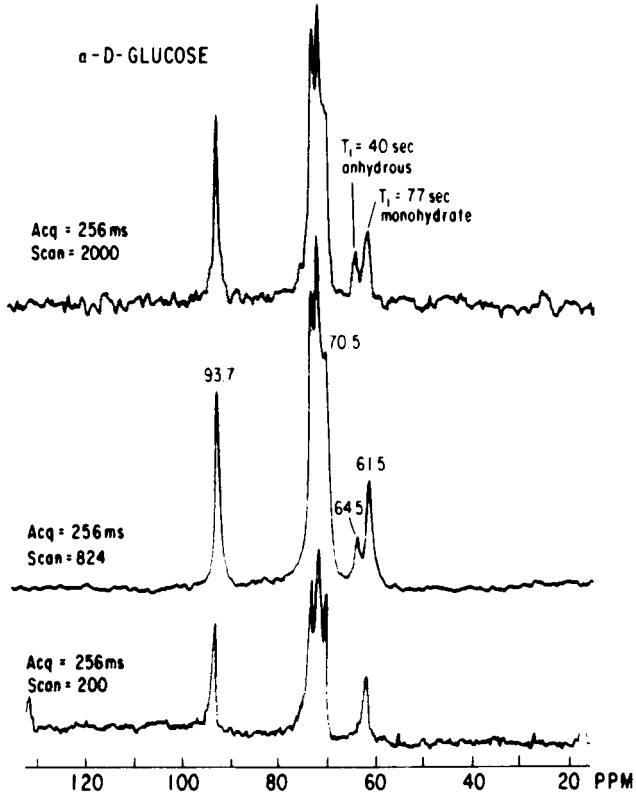


FIG. 6. 15 MHz ^{13}C CP-MAS spectra of α-D-glucose·H_2O, contact time = 1 msec, repetition rate = 280 seconds.

maltulose·H_2O.[25] Although both of these sugars exhibit spectra characteristic of mixtures, a careful examination of the samples has shown that the multiplicity of lines is attributable to different phenomena. A proton spectrum of lactulose in DMSO (a solvent that inhibits mutarotation) clearly demonstrated that when the restrictions of the crystal lattice were removed, three tautomeric forms of this sugar were present. The ratio of these three chemically nonequivalent species coincided with the ratio of the three nonprotonated C-2 carbon resonances observed in the ^{13}C spectrum using the interrupted decoupling procedure described above (see Fig. 7). This interpretation was also verified by a low temperature X-ray crystallographic study that showed the presence of a single-phase material containing three tautomeric forms of lactulose in the ratio given by the NMR spectrum.[26]

As mentioned earlier, the dynamic averaging of isotropic shifts that occurs in solution may not be present in the solid state. Hence, the freezing out of free rotation of certain conformations can be responsible for the observed multiplicity of certain resonances in the spectrum. The multiplets exhibited in the interrupted decoupling solid state spectrum of maltulose·H_2O (Fig. 8) are not characteristic of chemically different entities, i.e., the proton spectrum of maltulose in DMSO clearly demonstrated that only the β-pyranose isomer was present in the crystal. Thus, the only explanation that can accommodate these findings is that there are inequivalent sites in each unit cell.[27] Similar explanations have been suggested for the multiplicity observed in the solid state spectra of sodium potassium tartrate,[28] α-D-lactose,[29] and substituted benzoic acids.[30]

Lactose crystals have been examined as a single-phase mixture of discreet complexed anomeric forms in the ratios of α:β 5:3 and 4:1.[29] That these materials are true complexes has been demonstrated by the fact that simple mixtures of α and β-lactose corresponding to ratios found in these complexes give different spectra than the corresponding complexes. Evidently, perturbations of one molecular structure on the environment of the second

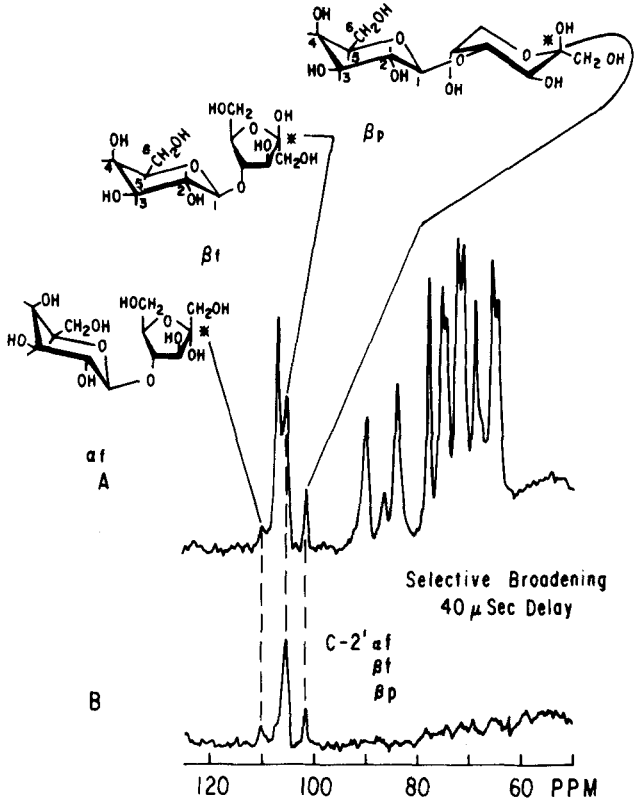


FIG. 7. 15 MHz ^{13}C CP-MAS spectra of lactulose taken with a) 500 transients, 10 second repetition times, 1 msec contact times, and 11G ^{1}H decoupling; b) same as a except for insertion of a 40 μsec delay with no proton decoupling prior to acquisition.

within a single phase yields a unique "fingerprint" pattern for the observed spectral responses of the complex.

Morphological changes in the structure of α,α-trehalose·$2H_2O$ have been investigated as a function of heat treatment. Figure 9 shows the dehydration of the dihydrate and the subsequent crystallization of the resulting anhydrous form in the solid state. Initial heating of the dihydrate[9a] (a molecule that shows

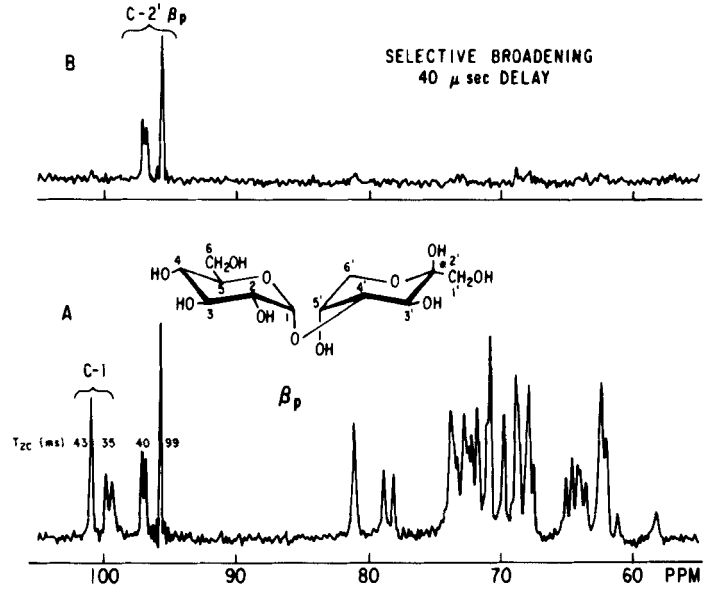


FIG. 8. 37.8 MHz ^{13}C CP-MAS spectra of maltulose·H_2O taken with a) 250 transients, 10 second repetition times, 1 msec contact time, and 25G ^{1}H decoupling and 3.2 KH_2 spinning; b) same as a except for insertion of a 40 μsec delay with no proton decoupling prior to acquisition.

evidence of asymmetry in carbon resonances 2 and 6) to 98° produces an amorphous material.[9b] The latter ultimately recrystallizes in the melt upon further heating to 130° to produce an anhydrous crystalline product.[9c] This final stage appears to contain inequivalent rings that could be a consequence of asymmetric crystal packing or different conformational ring preferences. The same material[9c] can be produced through azeotropic distillation of pyridine-water from a pyridine solution containing the dihydrate.[9a] Thus far, X-ray crystallographic studies have failed to define the nature of the dysymmetry displayed by this anhydrous crystalline form. In contrast, a collaborative X-ray crystallographic and solid state NMR study of an enopyranoside (ethyl 2,3-dideoxy-α-D-erythro-hex-2-enopyranoside) has been

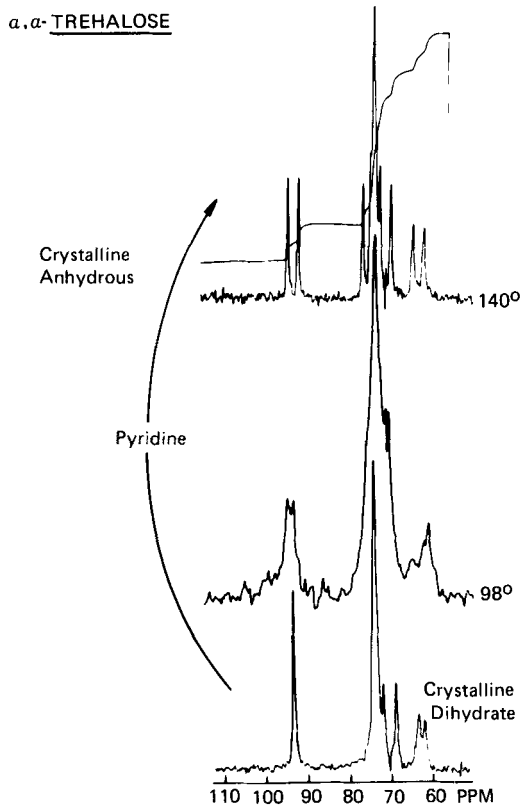


FIG. 9. ^{13}C CP-MAS spectra of a) α,α-trehalose·$2H_2O$; b) same sample after being heated to 98° for 4 h; c) after additional heating for 4 h at 140°. Spectra were obtained with 1K transients, 10G decoupling, 2K data points zero fitted to 8K, and a contact time of 3 msec.

successful in characterizing morphological changes that can come about through the interconversion of crystal forms within the solid state.[31] Crystallization of the enopyranoside from benzene yields a crystalline material of form I whose ^{13}C spectrum shows seven individual resonances (two overlapping) representing each of the eight carbon atoms (Fig. 10a). The X-ray crystallographic structure of form I shows that it is in space group $P2_1\ 2_1\ 2_1$,

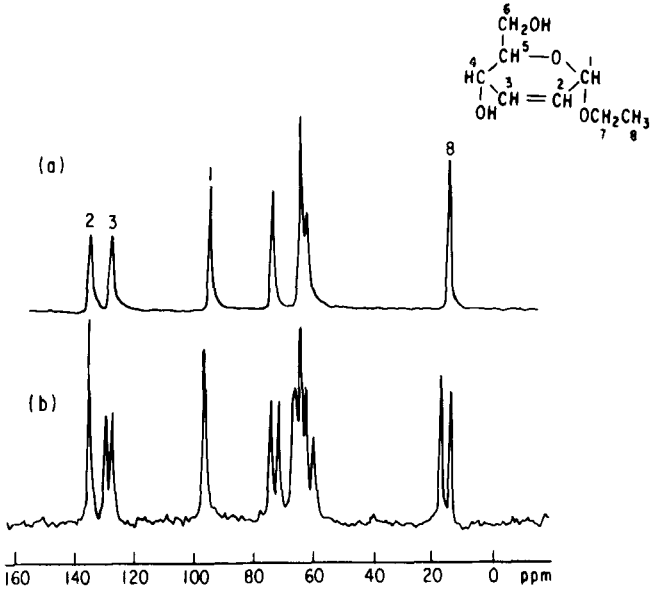


FIG. 10. 15 MHz ^{13}C CP-MAS spectra of ethyl 2,3-dideoxy-α-D-erythro-hex-2-enopyranoside a) form I - material crystallized from benzene; b) form II - material crystallized from benzene followed by sublimation.

and that it has one molecule per asymmetric unit. Upon standing for 2-3 weeks, or on following sublimation, this metastable form converts to a new structure, form II. The spectrum of form II (Fig. 10b) clearly shows that there are two peaks for all but one of the eight carbons displayed (the anomeric carbon resonance is seen as a split doublet at 50 MHz). In addition, the X-ray analysis of form II, whose space group assignment is identical with form I clearly verified that there are two molecules per asymmetric unit. Further NMR relaxation studies and comparisons of the thermal motion and hydrogen-bonding between molecules in the two crystal forms are presently being carried out to establish the identity of resonances of the two symmetry-independent molecules of form II and the nature of the transition between the two crystal forms.

Other workers have attempted to correlate ^{13}C spectral shifts of the C-6 (CH_2OH) resonance of various monosaccharides, oligosaccharides, and cellulose with the torsional angle χ about the exo-cyclic C-C bond.[32] From the analysis of the data they found that the chemical shift of this resonance falls into three groups, 60-62 ppm, 62.5-64.5 ppm, and 65.5-66.5 ppm corresponding to the gauche-gauche, gauche-trans, and trans-gauche conformations, respectively. For example, the high field shift range found for α-D-glucose·H_2O was referenced as the trans-gauche conformation and the lower field position of the anhydrous α-D-glucose as the gauche-gauche form.[32] Based on the assessment, the study concluded that the conformation of the CH_2OH group of regenerated crystalline cellulose II is gauche-trans. This finding is in conflict with the X-ray analysis that defines the geometry of the CH_2OH group as being a combination of both the gauche-trans and trans-gauche conformations.[32]

Specific Chemical Shift Assignments

Although many reports suggest that there is direct correspondence between the isotropic chemical shifts of ^{13}C solution and solid state spectra, recent reports have demonstrated that this is not necessarily true.[13,25,33-35] As mentioned above (see Fig. 1e), partial assignments can be made for nonprotonated and methyl carbons based on differences in C-H dipolar interactions, using the interrupted decoupling methodology.[15,25] Carbons directly bonded to nitrogen can also be identified by their characteristic residual dipolar splittings.[36-38] The magnitude of this coupling can also lead to the determination of the sign of the quadrupole coupling constant as well as its magnitude and orientation in the molecular frame.[38] A somewhat more complicated multipulse proton decoupling sequence has been devised to identify shifts through their respective proton coupled multiplicities, akin to the methodology used in solution ^{13}C NMR.[39] Unfortunately, this powerful technique cannot differentiate resonances that are derived from carbons having the same number of

protons attached. Crystalline sugars and polysaccharides are a class of compounds for which this situation is all too common, i.e., except for keto saccharides,[25] all ring carbons have the same doublet multiplicity due to a single bonded proton.

To circumvent this problem we have explored the use of ^{13}C enrichment at specific sites in model compounds such as glucose.[14,40] It is well known that in solution NMR, ^{13}C enrichment at specific positions is a convenient method for identifying the resonances associated with the site of enrichment as well as adjacent carbons.[41] The latter are generally observed as doublets due to the ^{13}C-^{13}C scalar coupling with the carbon at the enriched site. These couplings are approximately 40 Hz for one bond and 3-4 Hz for carbons two bonds away. To obtain similar information for solid samples we examined the ^{13}C solid state spectra of molecules specifically enriched with a single ^{13}C atom. In the usual high resolution ^{13}C NMR experiment of solids, the carbon-carbon dipolar interaction is very weak by virtue of the low natural abundance of ^{13}C. The other broadening mechanisms are removed by high power proton decoupling for the proton-carbon dipolar and scalar interactions and by magic angle spinning for the carbon chemical shift anisotropy as described above. In this exceptional case whereby a ^{13}C atom has been inserted in the structure, there is a significant probability of having two ^{13}C nuclei bonded to each other. Thus, carbon-carbon dipolar interactions become significant and have a pronounced effect on the spectrum obtained. In principle, this interaction produces broadening of the resonances corresponding to carbons directly bound to the enriched carbon even under fast spinning conditions. Figure 11 illustrates the effect of the homogeneous dipolar interaction for the example of ^{13}C-1 and ^{13}C-6-labeled α-D-glucose. If we compare the spectra of the natural abundance resonance of the enriched α-D-glucose molecules B and C (resonances of the enrichment sites are truncated for clarity) with natural abundance spectra of the unlabeled molecule A, it is apparent that the "missing" signals of the former correspond to the reso-

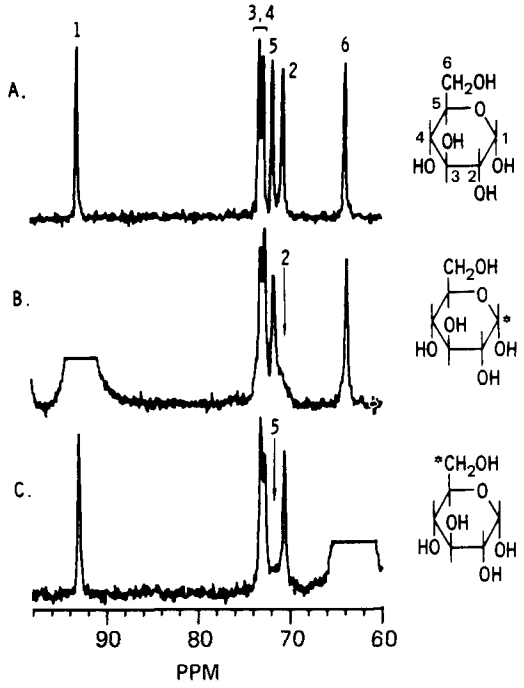


FIG. 11. 37.8 MHz ^{13}C CP-MAS spectra of a) unlabeled α-D-glucose after 64 transients, spinning rate of 2.5 KHz, 1.0 msec contact time, 25G decoupling, and 45 second recycling time; b) ^{13}C-1-labeled α-D-glucose, same as a except after 256 transients; c) ^{13}C-6-labeled α-D-glucose, same as b.

nances of each carbon adjacent to a label. A complete evaluation of α-D-glucose, α-D-glucose·H_2O, and β-D-glucose using this methodology has allowed us to make unambiguous spectral assignments of all the ring carbon resonances.[14] As anticipated, intermolecular forces play an important role in determining the isotropic chemical shift. Therefore, it is not surprising to see a poor correspondence in the relative order of chemical shift between the solution and solid state spectra as given in Table 1. Obviously caution should be used in extrapolating solution chemical shifts to those observed for solids.

TABLE 1

Relative Order of ^{13}C Shifts for 1, 2, and 3 in Solution[a] and Solid State[b]

	Chemical shift
α-D-Glucose·H_2O (1)	
Solution	C-1 > C-3 > C-2 > C-5 > C-4 > C-6
Solid	C-1 > C-3 > C-4 > C-5 > C-2 > C-6
α-D-Glucose (2)	
Solution	C-1 > C-3 > C-2 > C-5 > C-4 > C-6
Solid	C-1 > C-3 > C-5 > C-2 > C-4 > C-6
β-D-Glucose (3)	
Solution	C-1 > C-5 > C-3 > C-2 > C-4 > C-6
Solid	C-1 > C-2 > C-3 > C-5 > C-4 > C-6

a. In D_2O relative to internal p-dioxane 67.4 ppm.

b. Relative to p-dioxane 67.4 ppm.

PROGNOSIS

In spite of the ever increasing applications of high resolution solid state ^{13}C NMR, relatively little is known about its capabilities to give conformational information in carbohydrate molecules since relatively few spectra have been reported. As more and more ^{13}C solid state data become available, a coherent picture of the depth of structural information that is retrievable will emerge. Detailed studies of ^{13}C shift tensors in labeled compounds will ultimately yield more accurate data for comparison with X-ray crystallographic parameters. Motion dependent relaxation times, both proton and carbon T_1 and $T_{1\rho}$, need to be explored more fully so that they may be compared with

thermal motion as defined in the X-ray crystallographic experiments. Further work on the interaction of paramagnetic species has potential as a tool for defining carbohydrate binding sites.

Two-dimensional methods coupled with the solid state techniques promise the possibilities for measuring spin diffusion, microscopic heterogeneity,[42] and proton exchange rates for examining chemical exchange processes, i.e., hydrogen interchange, which can interconvert two equivalent structures.[43,44]

Although not mentioned, variable temperature CP-MAS is becoming an important method for studying the interconversion of molecules through chemical exchange processes, reactive intermediates trapped and stabilized at low temperatures, and molecular dynamics of solids and polymers.[45]

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