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Report

Quantitation of the Relative Amounts of Anhydrous Carbamazepine ($C_{15}H_{12}N_2O$) and Carbamazepine Dihydrate ($C_{15}H_{12}N_2O \cdot 2H_2O$) in a Mixture by Solid-State Nuclear Magnetic Resonance (NMR)

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The application of solid state nuclear magnetic resonance (NMR) for the quantitation of the relative amounts of carbamazepine anhydrate (I) and carbamazepine dihydrate (II) in a mixture is presented. The techniques of cross polarization, dipolar decoupling, and magic angle spinning have been used to obtain high-resolution NMR spectra of the samples in the solid state. Although the chemical shifts of I and II were similar, the proton spin lattice relaxation time of II was much shorter than that of I. A delay time of 10 sec between pulses resulted in saturation of the signal from I and in a spectrum arising solely from II. The dependence of the observed signal intensity on the contact time was evaluated for II and glycine, the internal standard, to allow theoretical estimation of the peak area ratios. Various molar ratios of I and II were then mixed with glycine, and the resulting peak area ratios of II to the area of the alpha and the carbonyl carbons of glycine was linearly related to the relative proportion of II in the mixture.

KEY WORDS: anhydrous carbamazepine; carbamazepine dihydrate; solid-state nuclear magnetic resonance (NMR).

INTRODUCTION

Carbamazepine, 5H-dibenz[b,f]azepine-5-carboxamide is used to treat epilepsy and trigeminal neuralgia (1,2). It is commercially available only as a solid dosage form (3), but methods for extemporaneous preparation of suspensions are available (4).

Anhydrous carbamazepine, $C_{15}H_{12}N_2O$ (I), is practically insoluble in water (5). When suspended in water it is rapidly converted to carbamazepine dihydrate, $C_{15}H_{12}N_2O \cdot 2H_2O$ (II) (6,7). I is used for the preparation of tablets, while in aqueous suspensions, carbamazepine exists as II. When adult volunteers received 847 µmol of carbamazepine as both I and II, the administration of II resulted in a significantly earlier peak time and also a higher peak level in serum (6). The results have been explained by postulating that the administration of I (in tablets) results in its rapid conversion to II and the particle size of the crystals so formed is much larger than the crystals of II administered as a suspension (6).

The transition of I when dispersed in water to II is not a phenomenon unique to carbamazepine since several other pharmaceutical compounds undergo a similar transformation (8,9). The kinetics of such transitions can be studied by dispersing the anhydrous substance in water, taking an aliquot of the solid at predetermined time intervals and quantifying the amount of hydrate formed. The two common methods of water content determination in solids are (a) Karl Fischer titrimetry and (b) heating the solid to a constant weight.

In solids, water is generally bound in two different ways. (a) Water held in a definite molar proportion with the solid is referred to as chemically bound water. Water of crystallization belongs to this class.[3] (b) Water held in a variable amount is called physically bound water. This depends on the method of solid preparation and/or the conditions of storage. The surface-adsorbed water belongs to this category (12). Both methods of water content determination do not readily distinguish between physically and chemically bound water. In order to use either of these methods in the kinetic studies it is necessary first to dry the solid in such a manner that all of the physically bound water but none of the chemically bound water is removed. This can be difficult to achieve.

Recently, a powder X-ray diffraction technique was developed to quantify the relative amounts of I and II when they occur as a mixture (13). Solid-state ^{13}C NMR has already found application in studying drug polymorphism (14,15). The object of this investigation is the development

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[3] The existence of nonstoichiometric hydrates in pharmaceuticals is known (10). However, the formation of such hydrates has not been reported in the case of carbamazepine (6,7,11).

of a solid-state NMR technique to quantify the relative amounts of I and II in a mixture. The advantage of such a method is that it will unambiguously distinguish between physically and chemically bound water.

Materials. Anhydrous carbamazepine (I) was obtained from Sigma Chemical Company (St. Louis, MO). It was stored at room temperature (≅25°C) under 0% relative humidity (RH). Carbamazepine dihydrate (II) was prepared by dispersing I in water at room temperature for 24 hr. The slurry was filtered and stored at room temperature under 52% RH until the attainment of constant weight. The details of the characterization of the two solids has been presented elsewhere (13).

NMR Measurements. Spectra were obtained with a 100-MHz (for proton) superconducting magnet and IBM NR/100 AF console. Spin-locked cross-polarization was used to establish the single-contact Hartmann-Hahn condition. The Hartmann-Hahn condition was optimized with the use of 1,4-di-*t*-butylbenzene as an external standard. Phase cycling was used throughout signal acquisition to minimize baseline and intensity artifacts. The samples were spun at the magic angle, which was adjusted by the KBr method, at a frequency of 3.9 ± 0.2 kHz (16). The chemical shifts were measured relative to tetramethylsilane using glycine as the internal standard. Integrated peak areas from spectra in the absolute intensity mode were used to reflect the carbon magnetization. The optimum time for transfer of polarization was determined by varying the contact times over the range of 0.02 to 20 msec. The number of scans accumulated and the delay time between successive sampling pulses depended on the experiment. The specific experimental conditions are listed under the figures. Estimates of the cross-polarization time and the spin lattice relaxation time were obtained by fitting the integrated peak areas as a function of mixing time using a nonlinear curve fitting routine (17).

It was necessary to confirm that spinning did not cause dehydration of II. Therefore samples of II were subjected to powder X-ray diffraction studies after the NMR experiment. The 6.78-Å line unique to I was absent (13).

RESULTS AND DISCUSSION

The solid-state spectrum of II is given in Fig. 1. The carbons directly attached to nitrogen atoms appear as broad peaks due to the ^{14}N quadrupolar interaction (18). Furthermore, at a spinning rate above 3.7 kHz, the spectrum is unaffected by rotational speed, which indicates the absence of spinning side hands. The spectrum obtained with I did not appear different, although good signal-to-noise requires extremely long accumulation times due to the slow relaxation rate. This apparent difference in the proton spin lattice relaxation time was exploited for quantitative purposes. Thus the relaxation time of II was determined by acquiring the signal at several interpulse delay times. Essentially full intensity of II was obtained with a delay time of 10 sec (Fig. 2), while at this delay time no signal is seen for I (see below). Thus in mixtures of I and II, a short delay time of 10 sec resulted in signals only due to II. Alternatively if experiments were conducted at both a short and a long delay time (in excess of 2000 sec), two spectra would be obtained: one due solely to II and the other due to both I and II. This was deemed undesirable since the experiment would require an unduly long time. Instead, the use of an internal standard permitted the completion of the experiment in a short time.

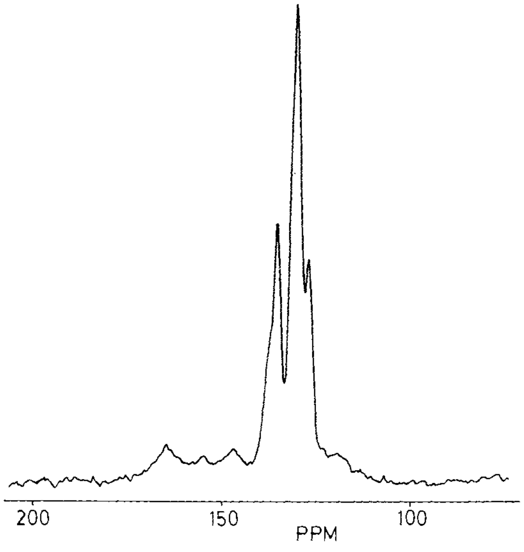

Fig. 1. ^{13}C spectrum of carbamazepine dihydrate (II). Number of scans = 279; contact time = 1.5 ms; delay time = 3 sec.

To this end, carbon magnetization was measured as a function of contact or mixing time to determine the optimum time for quantifying the relative amount of II in a mixture of I and II. This also has the advantage of providing cross relaxation and proton spin lattice relaxation time in the rotating frame of II and glycine. This information permits prediction of the area ratio at any contact time. The peak time for the carbons in II and for the α-carbon in glycine was 0.4

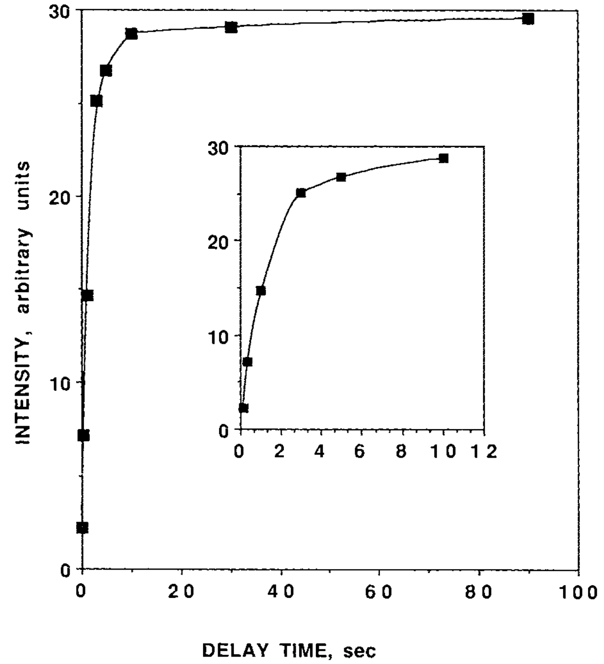

Fig. 2. The intensity of the carbamazepine dihydrate (II) peak as a function of the delay time. The inset is an expanded view of the intensity at short delay times. Number of scans = 24; contact time = 1 msec.

msec (Fig. 3). On the other hand, the carbonyl carbon of glycine with no directly attached protons had a peak time of 1.5 msec.

The observed magnetization depends on the carbon-proton contact due to the finite time required for polarization transfer and also on the loss of magnetization of the protons to the lattice through spin lattice relaxation processes (16). The results obtained are consistent with theory in that the transfer rate is proportional to the number and proximity of protons to the carbon (19). The observed carbon magnetization should follow a biexponential function, with the rate constant for the transfer process inversely related to the cross-polarization time and the terminal decay rate constant related to the reciprocal of the proton spin lattice relaxation time in the rotating frame (19). In Fig. 3, the fitted curve is provided along with the observed carbon magnetization. The importance of these results lies in the ability to relate quantitatively the observed magnetization to the number of carbons as is routinely done in high-resolution, solution NMR. Thus, at a contact time of 1 msec, the percentage maximum signals for II, the α-carbon of glycine, and the carbonyl carbon of glycine were found to be 87.0, 97.1, and 91.3, respectively. Furthermore, this allowed sample preparation of the mixtures of I, II, and glycine, at a proper weight ratio to yield a standard curve with a slope of two. The peak area ratio is related to the weight ratio by the following formula.

$$\frac{\text{area of II}}{\text{area of glycine}} = \frac{N_{\text{II}}}{N_{\text{G}}} \cdot \frac{E_{\text{G}}}{E_{\text{II}}} \cdot \frac{M_{\text{II}}}{M_{\text{G}}} \cdot \frac{W_{\text{II}}}{W_{\text{G}}} \quad (1)$$

where N is the number of carbons in the molecule, M is the molecular weight, W is the mass in the sample, and E corrects for the effects of cross relaxation and proton spin lattice relaxation time in the rotating frame. Subscripts G and II refer to glycine and carbamazepine dihydrate, respectively. The value of E_{II} was 0.870, while the value of E_{G}, calculated as the average of the two carbons, was 0.942.

The spectra obtained for mixtures of glycine with dif-

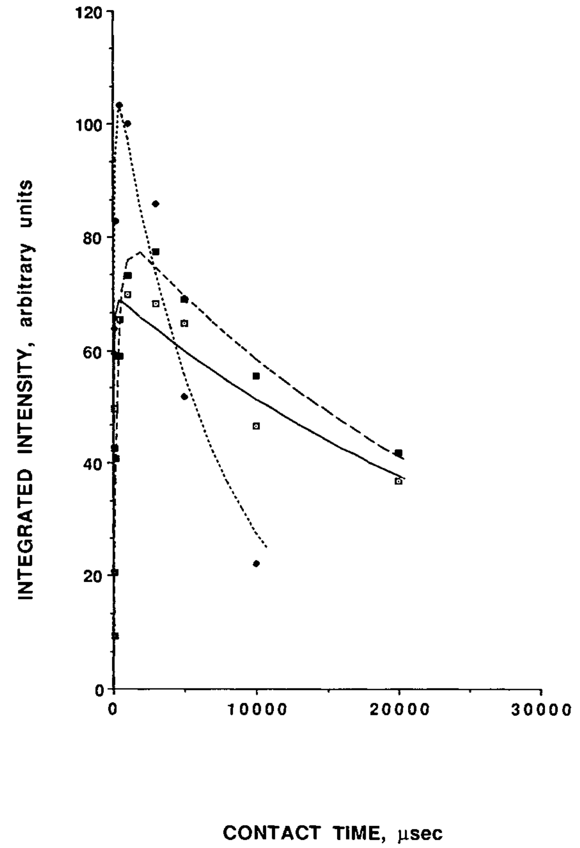

Fig. 3. Effect of contact time on the integrated intensities of II (——♦————), carbonyl carbon in glycine (——■——), and α-carbon in glycine (———□———). The data points as well as the lines based on fitting of the experimental points are shown. Number of scans = 24; delay time = 5 sec.

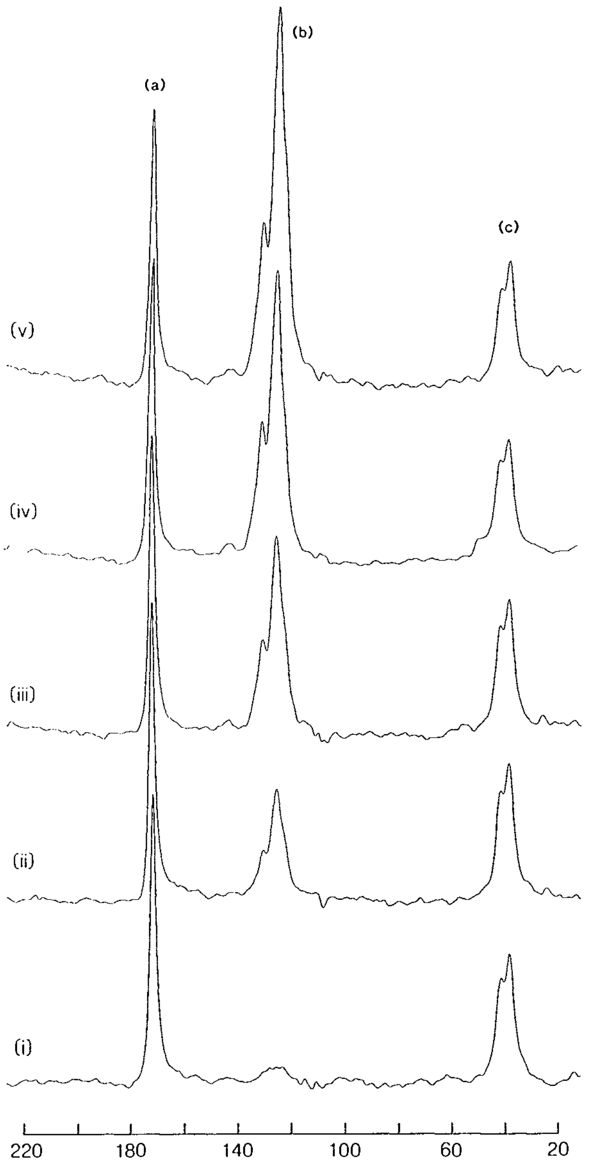

Fig. 4. ^{13}C spectra of mixtures of I, II, and glycine. The peak assignments are (a) the carbonyl carbon of glycine, (b) all carbons of II, and (c) the α-carbon of glycine. The mole fraction of II in the mixtures was as follows: (i) 0.00, (ii) 0.25, (iii) 0.50, (iv) 0.75, and (v) 1.00. Although the samples contain I, II, and glycine, the mole fraction calculation is based only on the amounts of I and II in the mixture. Number of scans = 200; contact time = 1 msec; delay time = 10 sec.

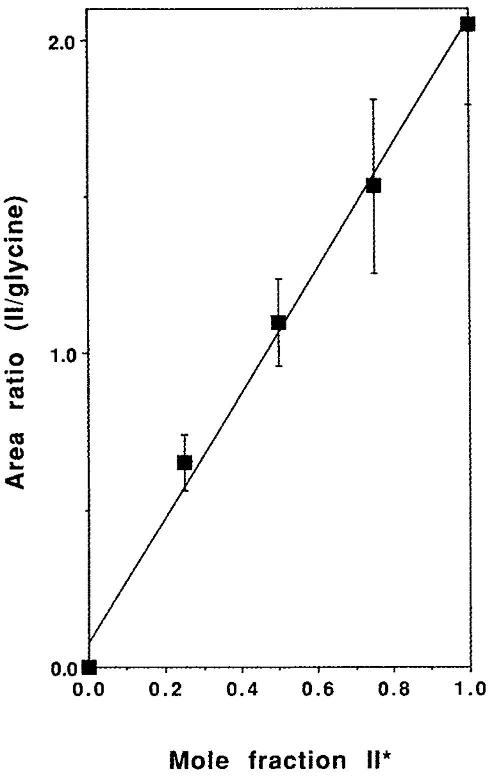

Fig. 5. The peak area ratio of II to glycine (mean ±S.D., n = 3) as a function of the mole fraction of II. *The samples contain I, II, and glycine. The mole fraction calculation is, however, based only on the amounts of I and II in the mixtures.

ferent ratios of I and II are given in Fig. 4. The carbonyl carbon and the α-carbon of glycine give rise to the peaks at 175 and about 40 ppm, respectively. The latter is split because of the presence of the quadrupolar ^{14}N (19). Due to the overlap of the chemical shifts of the carbons of carbamazepine, the spectrum appears as a featureless, broad peak. No peaks were observed between 100 and 150 ppm as indicated in spectrum i in Fig. 3 for a mixture of I and glycine. The other spectra reveal that the peaks of II and the internal standard do not interfere with one another. With increasing amounts of II, there is a progressive increase in the peak areas in the region between 100 and 150 ppm.

In Fig. 5, the integrated peak area ratios of II to the two peaks of glycine are given as a function of the mole fraction of II in the mixture. As can be seen in Fig. 5, a linear response was obtained. The slope was found to be 1.99, which is in good agreement with the theoretical slope of 2.00 based on the results of the contact time study.

The linear relationship in Fig. 5 permits the quantitation of II in a mixture of I and II. Although initially time-consuming, the standard curve need not be replicated each time for the analysis of an unknown sample. Thus, solid-state NMR spectroscopy provides a rather rapid means of sample analysis, since actual measurement time is only about 30 min. It does not require the use of solvents or other chemicals as in Karl Fischer titrimetry and, as such, represents an extremely safe method of analysis. Finally, once the instrumental parameters are optimized, the methodology is relatively straightforward.

However, the greatest advantage of the technique lies in the unambiguous quantitation of the solid phase of interest. Like the X-ray method published recently (13), the technique is capable of quantifying the amount of II in a mixture of I and II without any interference from the physically bound water in the samples.

CONCLUSION

A quick, safe, and unambiguous technique using solid-state NMR has been developed to quantify the relative amounts of anhydrous carbamazepine and carbamazepine dihydrate in a mixture.

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