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SPECTRAL METHODS FOR DETERMINATION OF WATER

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ABSTRACT

Techniques based on the interaction of electromagnetic radiation with materials can be extremely useful for the characterization of water in solids of pharmaceutical interest. When combined with other physical methods, such as calorimetry, thermogravimetry, or titration, spectral techniques can be used to deduce information regarding the nature of water contained within a solid. The spectral methods are particularly useful in the study of hydrate species, and in the differentiation of hydrate water from entrapped or adventitious water. The most useful methods are powder x-ray diffraction, fourier transform infrared spectroscopy, and solid state nuclear magnetic resonance spectroscopy. These particular methods have been used in the study of ampicillin hydrate species.

INTRODUCTION

The characterization of water in a solid material is of extreme importance to the pharmaceutical scientist for a variety of reasons. In solids, water may be bound as part of the crystal lattice (crystalline water), be contained in interstitial voids (entrapped water), or be loosely bound on the surface of particles (adventitious

water). The crystal structure of a compound will generally differ depending on the nature of its water, yielding pseudopolymorphs which have differing bulk properties and chemical stabilities. The characterization of water in a solid becomes increasingly more critical when the contained water is involved in a decomposition pathway of a substance, where the more labile the water the more rapid will be the rate of degradation. These factors all require the full characterization of water in pharmaceutical substances.

A variety of physical techniques are suitable for the determination of water in solid materials. These may be divided into two main classes, based on how the information is acquired. Thermal methods involve the observation of a bulk material property (sample wieght or heat capacity) as the temperature of the sample is uniformly increased. The most widely used thermal methods include differential scanning calorimetry (DSC) and thermogravimetry (TG). The determination of total moisture in a solid is usually obtained by thermally dehydrating a subatance, and then titrating the evolved water (Karl Fischer method). Spectral methods involve the measurement of how various types of electromagnetic radiation interact with the sample, and these techniques are more indicative of the microscopic properties of the material. The most useful methods for the study of water in solids are infrared spectroscopy (IR), nuclear magnetic resonance (NMR), and powder x-ray diffraction (XRD). The combination of spectral methods with thermal and titrimetric methods yields a unified and powerful approach toward the characterization of water in solids. In the present work, the use of various spectral methods to the characterization of water in pharmaceutical solids will be outlined, and each application will be illustrated using ampicillin hydrate species as a particular example.

MATERIALS AND METHODS

Ampicillin trihydrate was obtained from Bristol-Myers, and was used as received. This material can be thermally dehydrated to an

amorphous phase by simple heating [1], or can be transformed into an anhydrous crystalline phase [2]. The anhydrous crystalline phase was obtained by suspending the trihydrate phase in water, and heating at 90°C for two hours. At the end of this time period, a crystalline material separated out of the aqueous phase. This was filtered and dried.

Measurements of differential scanning calorimetry and thermogravimetry were obtained on a DuPont 9900 thermal analyzer system. Samples were heated at a rate of 10°C/min, from ambient room temperature up to 225°C. The morphology of each crystal form was first studied using scanning electron microscopy, with all data being obtained on an Amray model 1820T system. Powder x-ray diffraction patterns were obtained on a Philips APD-3720 XRD system, and were scanned between 2 and 32 degrees 2-theta. The infrared spectrum of each sample was obtained on a Nicolet model 740 FTIR spectrometerm, and were scanned between 400 and 4000 cm^{-1}. ^{13}C Solid state nuclear magnetic resonance spectra were obtained on a Bruker AM-250 spectrometer, using a combination of magic angle spinning (MAS) and cross polarization (CP) to obtain optimal signal-to-noise ratios.

RESULTS AND DISCUSSION

Differential scanning calorimetric and thermogravimetric profiles of ampicillin trihydrate are shown in Figure 1, while analogous data obtained on the crystalline amorphous phase are located in Figure 2. The theoretical TG weight loss for the trihydrate has been calculated as 13.4% [1], in exact agreement with the value obtained for the trihydrate material used in the present work. The strong DSC endotherm observed at 120°C is clearly associated with the dehydration, and other work has shown that an amorphous phase results from this type of dehydration [1]. The width of the DSC dehydration endotherm indicates that the water is fairly crystalline. The anhydrous nature of the material obtained through heating of trihydrate in an inert solvent [2] is evident in its TG weight loss of

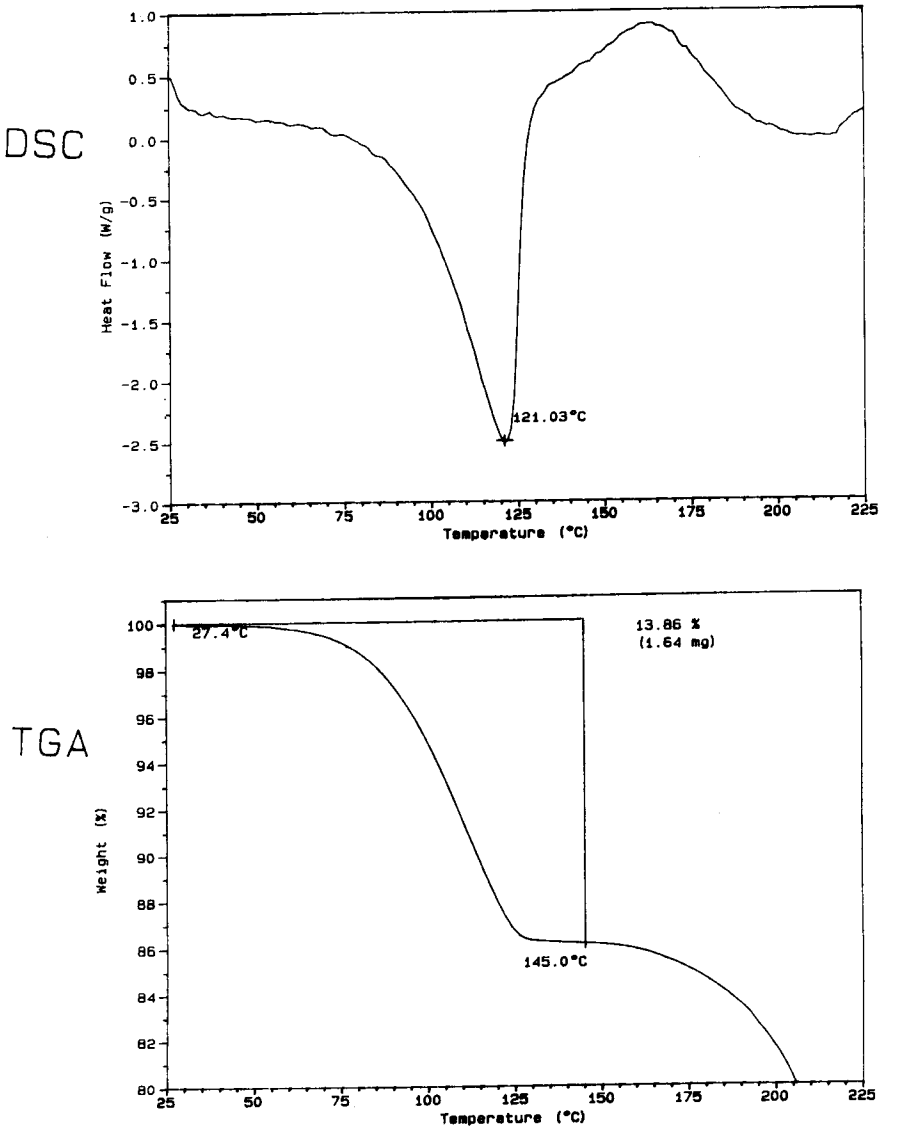

Figure 1. Differential scanning calorimetric and thermogravimetric profiles obtained for the trihydrate phase of ampicillin.

0.6%, and in the absence of any DSC feature below 175°C. Comparison of the DSC trace with the TG thermogram reveals that all of the weak DSC features observed above 175°C are associated with the thermal decomposition of the compound.

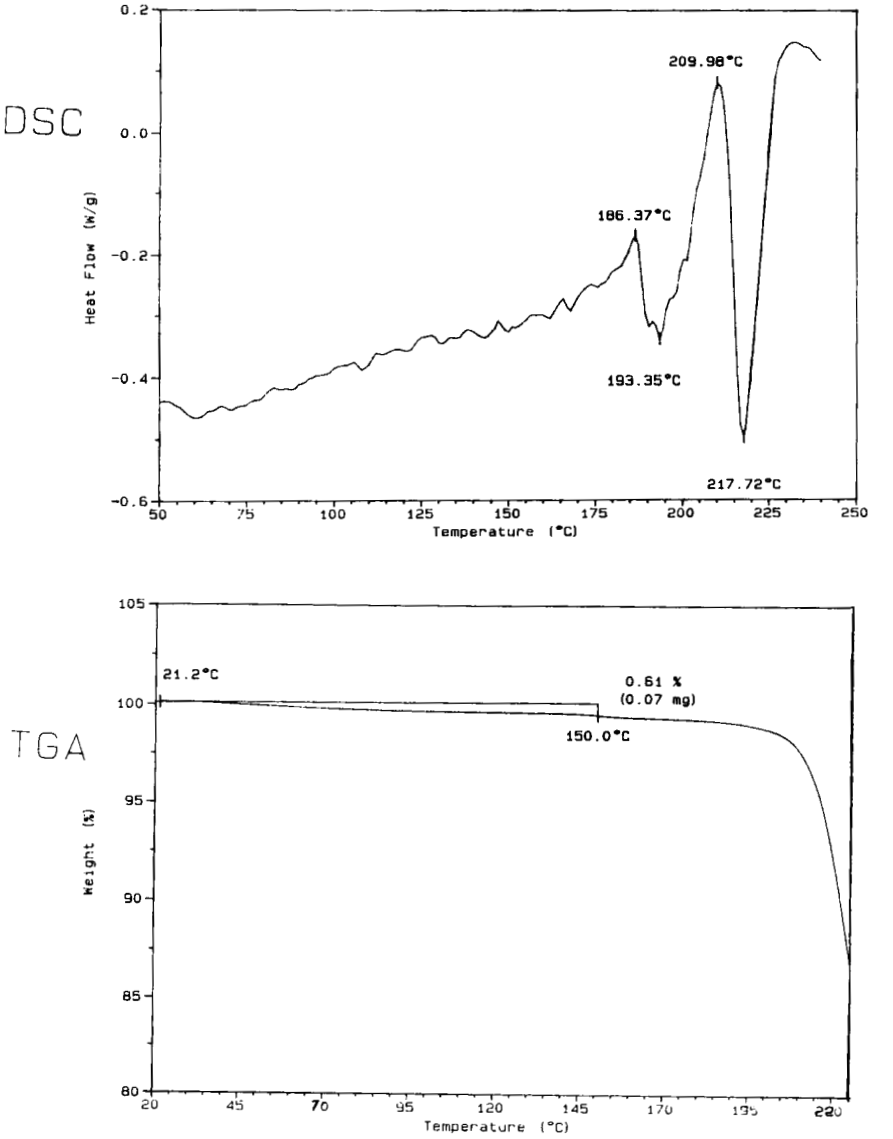

Figure 2. Differential scanning calorimetric and thermogravimetric profiles obtained for the crystalline anhydrous phase of ampicillin.

The overall morphological properties of the trihydrate and anhydrous phased materials were established through the use of scanning electron microscopy (SEM). Representative SEM pictures of the two ampicillin materials are shown in Figure 3. The trihydrate crystals were would to be essentially orthorhombic in their appearance, while the crystalline anhydrous phase was essentially triclinic in its appearance.

The thermal and microscopic studies of materials provide the basis for further study of the nature of water contained in a solid. The existence of various hydration states is always evident in the DSC and TG studies, and the microscopic examination will usually indicate the possible existence of pseudopolymorphism. Upon completion of the thermal and microscopic examinations, the next stage in material characterization involves a characterization of the crystal structures. The existence of different crystal structures resulting from differences in hydration (pseudopolymorphism) is established by means of x-ray diffraction. Since different hydrates normally form crystals of differing structures, it follows that powder x-ray diffraction is the primary screening method to differentiate the pseudopolymorphs [3].

The diffraction of x-rays by crystalline substances is of great analytical interest, since no two compounds would be expected to form crystals in which the spacing of planes is totally identical in all analogous directions. In most instances, a powdered sample will present all possible crystal faces at a given interface, and the diffraction off this surface will therefore provide information on all possible atomic spacings.

Modern instrumentation uses a direct recording x-ray diffraction spectrometer for measurements of powder patterns. A x-ray detector (usually a scintillation counter) is mounted on a goniometer, and slowly rotated across the face of a powdered sample. The intensity of diffracted radiation is recorded as a function of the angle of

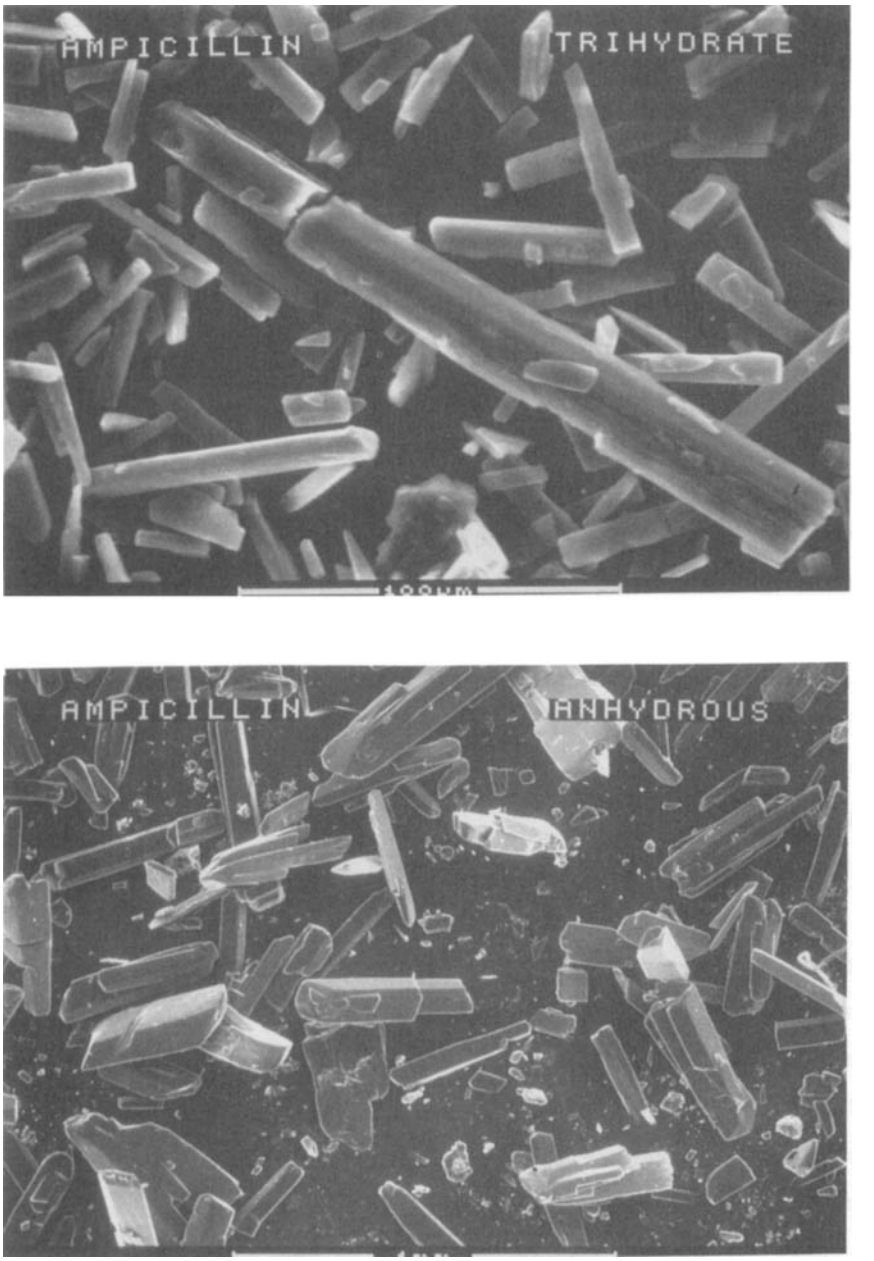

Figure 3. Scanning electron microscopic images obtained for the pseudopolymorphs of ampicillin. The trihydrate phase (upper trace) was photographed at a magnification of 500x, and the anhydrous phase (lower trace) was photographed at a magnification of 50x.

rotation, thus providing a powder pattern consisting of a series of peaks detected at various angles. These angles, and their relative intensities, are correlated with computed d-spacings to provide a full crystallographic characterization of the powdered sample. Upon indexing all the scattered lines it is possible to derive unit cell dimensions from the powder pattern of a substance.

The full XRD powder patterns obtained for the trihydrate and anhydrous forms of ampicillin are shown in Figure 4. The two structures are evidently extremely dissimilar, with the most notable differences being observed below 13 degrees 2-theta. It has been noted that compaction of ampicillin trihydrate contaminated by the anhydrous phase results in expulsion of lattice water. It is relatively trivial to then develop a XRD method for the determination of anhydrous phase levels in trihydrate phased material. As shown in Figure 5, the relative areas of the trihydrate peak (7.3 degrees 2-theta) and the anhydrous peak (8.1 degrees 2-theta) can be used to deduce the relative amounts of each phase present in a given ampicillin lot.

Once the morphology and crystallinity of a sample is established, the chemical nature of the water can be studied by fourier transform infrared spectroscopy (FTIR). The FTIR method is far more useful in this regard then are conventional dispersive IR methods, since transmission and beam attenuation problems are miminized in FTIR. In addition, the FTIR method makes use of all frequencies from the source simultaneously (rather than sequentially as in a scanning instrument), thus providing an immediate increase in the signal-to-noise ratio. The infrared spectra can be obtained in the solid state by means of diffuse reflectance, and extensive compilations of group vibrational frequencies exist which allow the ready assignment of observed bands [4].

Characterization of water in a solid involves measurement of the stretching frequency associated with the -OH group, and how this

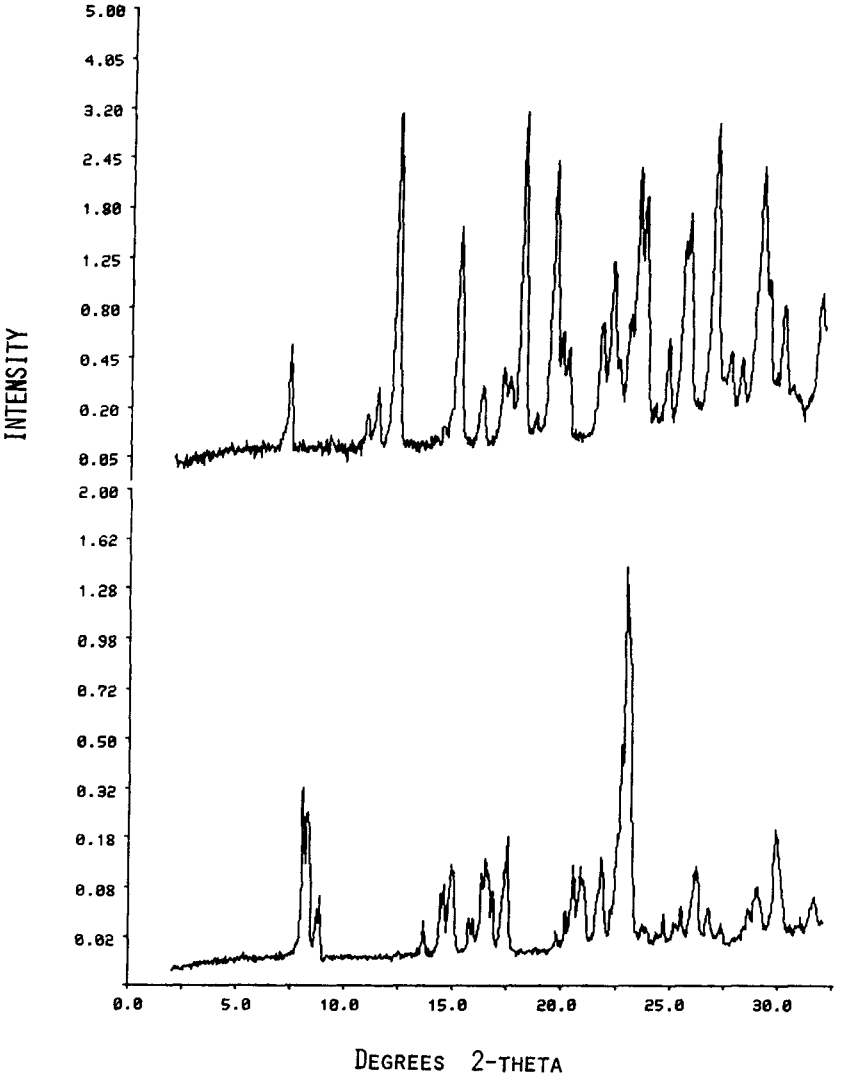

Figure 4. Complete powder x-ray diffraction patterns obtained for the trihydrate (upper trace) and anhydrous (lower trace) phases of ampicillin.

frequency is perturbed by the environment. For this purpose, the critical spectral regions are located in the mid-infrared (3000-4000 cm^{-1}, used to study fundamental vibrational bands), and in the near-infrared (1200-2400 nm, used to study overtone and combination vibrational bands). The fundamental energies associated with the -OH

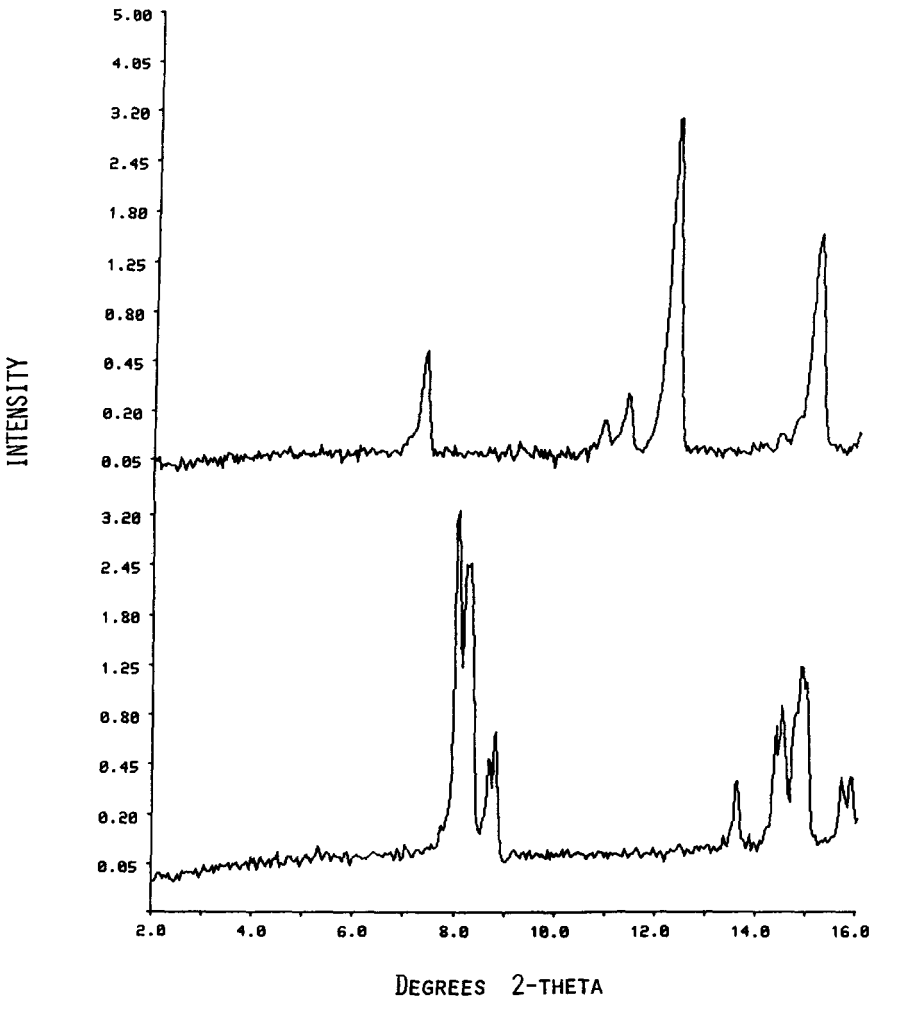

Figure 5. Partial powder x-ray diffraction patterns obtained for the trihydrate (upper trace) and anhydrous (lower trace) phases of ampicillin. The diagnostic peak region for the trihydrate phase is at 7.3 degrees 2-theta, while the diagnostic region for the anhydrous phase is at 8.1 degrees 2-theta.

vibrational stretching mode are quite high (3275 to 3650 cm^{-1}), and distinctive. Little interference from -CH (2850 to 3100 cm^{-1}) or -NH stretching modes (3075 to 3500 cm^{-1}) is normally encountered. Other high frequency vibrations (-FH, -SiH, -PH, -SH, or ClH) are totally resolved from the -OH mode, as are all skeletonal vibrational modes.

The -OH stretching mode associated with free water (gaseous state) exhibits a characteristic energy (3655 cm^{-1}), the frequency of which is decreased upon binding in a solid. For instance, in solid water, the -OH stretching mode decreases in energy to 3400 cm^{-1}. The -OH band position associated with water can therefore be used to evaluate whether the water is bound, entrapped, or merely adventitious. This approach requires that all non-aqueous -OH stretching modes be identified, and this information can usually be obtained from a study of the anhydrous material. Comparison of spectra obtained from the hydrated and anhydrous materials serves to identify the -OH bands associated with the compound, and permits identification of the -OH bands associated with the contained water. Crystalline water will generally yield sharper absorption bands than trapped or adventitious water, and is normally quite distinctive. In the situation where water is present in multiple forms, it is quite possible to observe several bands assignable to contained water.

The FTIR spectra obtained for trihydrate and anhydrous phased ampicillin are shown in Figure 6. Of particular importance is the 2200-3500 cm^{-1} spectral region, which contains the -OH modes of interest, and this region is shown in greater detail in Figure 7. Most of the absorption in this region is broad and featureless, but one sharp resolved band was observed in the FTIR spectrum of the trihydrate material. The extremely sharp absorption noted 3334 cm^{-1} for the trihydrate material (and absent in the anhydrous phase) is therefore assigned to the water of hydration. The sharpness of this band confirms that the water is indeed fairly crystalline, as had been deduced from consideration of the DSC data.

The ultimate characterization of a pharmaceutical material concerns the chemical environment of each atom in the compound, and this information is best obtained through the use of nuclear magnetic resonance (NMR) spectroscopy. With recent advances in instrumental technique, these studies can now be carried out in the solid state.

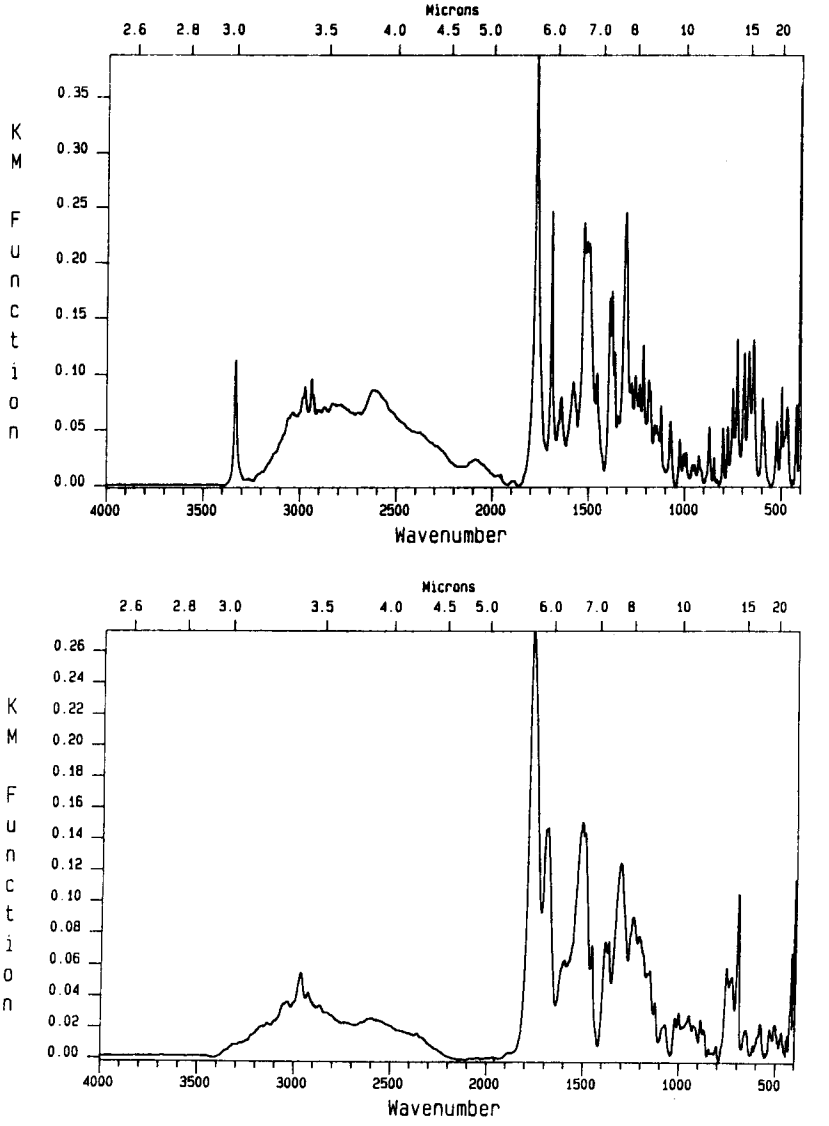

Figure 6. Complete fourier transform infrared spectra obtained for the trihydrate (upper trace) and anhydrous (lower trace) phases of ampicillin.

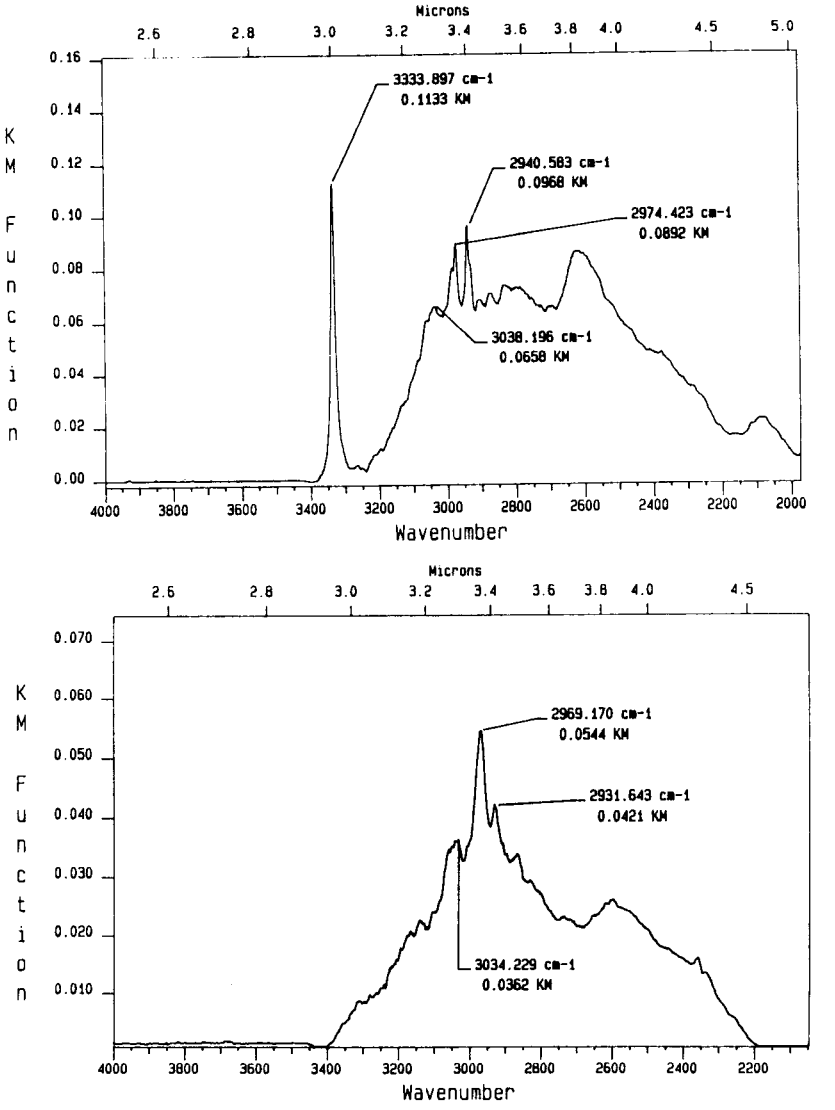

Figure 7. Partial fourier transform infrared spectra obtained for the trihydrate (upper trace) and anhydrous (lower trace) phases of ampicillin. The unique absorption assignable to lattice water in the trihydrate phase is located at 3334 cm^{-1}.

Although any nucleus which can be studied in the solution phase can also be studied in the solid state, most of the work has focussed on ^{13}C studies. As in the case for FTIR, extensive compilations of ^{13}C resonances for various functional groups are available in the literature [5]. ^{1}H NMR which remains an extremely difficult measurement in the solid state, and the data obtained from such work could be considered as only medium resolution at best. Since protons are abundantly present in organic compounds, the removal of hetereonuclear dipolar interactions is necessary to obtain high-resolution ^{1}H data in solids. Although it is possible to do so, the resulting ^{1}H NMR spectra are still inferior to those obtainable in solution phase. Other nucleii yield far better data, and it is anticipated that solid state NMR studies will be of great importance to the physical characterization of all pharmaceutical solids.

The random molecular motions which take place in the liquid phase result in an averaging of the effects which would broaden NMR resonance lines. It was recognized that spinning the sample at an angle of $54^{o}44'$ (the so-called "magic angle") with respect to direction of the applied magnetic field would result in an averaging of the chemical shift anisotropy. Since it is this anisotropy that is primarily responsible for the spectral broadening associated with solid samples, this advance made it possible to obtain high-resolution NMR spectra of solid materials. In a solid sample, the anisotropy reflects the chemical shift dependence of chemically identical nuclei on their spatial arrangement with respect to the applied field. High-power proton decoupling is also used simultaneously to eliminate the additional line broadening effects due to ^{13}C-^{1}H dipolar interactions.

Even though high-resolution spectra can be obtained on solids using the MAS technique, the data acquisition time is lengthy due to the long relaxation times exhibited by the nuclei in the solid state. This problem has been circumvented through the use of cross polarization (CP), a process which involves transfer of spin

polarization from the high-abundance, high-frequency nucleus (^{1}H) to the rare, low-frequency nucleus (^{13}C). This results in a more efficient buildup of ^{13}C magnetization, and thus shorter waiting periods between pulses are required. The CP experiment also allows the measurement of several relaxation parameters that can be used to study the dynamic properties of the solid under investigation. A complete description of the special techniques required for NMR work in solids is available [6].

If the pseudopolymorphism associated with the existence of various hydrate forms perturbs the chemical environment of the nucleus under investigation, then the resonances detected in solid state NMR experiments will be a function of the crystal form. For ^{13}C spectra, the carbonyl region normally contains the resonance lines which are most sensitive to bulk structural changes, since absorbed water would tend to hydrogen-bond to the carbonyl oxygens. Methods suitable for the quantitative determination of hydrate phases can be developed by integrating the resonance lines characteristic of each phase.

Solid state ^{13}C spectra obtained on the trihydrate and anhydrous phases of ampicillin are shown in Figure 8, and the effects of pseudopolymorphism (as caused by the presence or absence of water of hydration) are evident. The solid state spectra strongly resemble the solution phase spectrum, but do contain significant differences related to the differences in crystal structure. The higher degree of crystallinity noted earlier for the trihydrate phase is also observed in the NMR spectra, where peaks of smaller bandwidth are observed in the trihydrate phase when compared to the anhydrous phase. The three carbonyl resonances (located at 169.7, 172.7, and 174.6 ppm) are fully resolved in the anhydrous material, but only two peaks (170.4 and 172.6 ppm) are observed in the trihydrate phase. Since three carbonyl groups are found in ampicillin, it is concluded that two of these are structurally equivalent in the trihydrate phase and are not in the anhydrous phase. Differences related to crystal phase were also

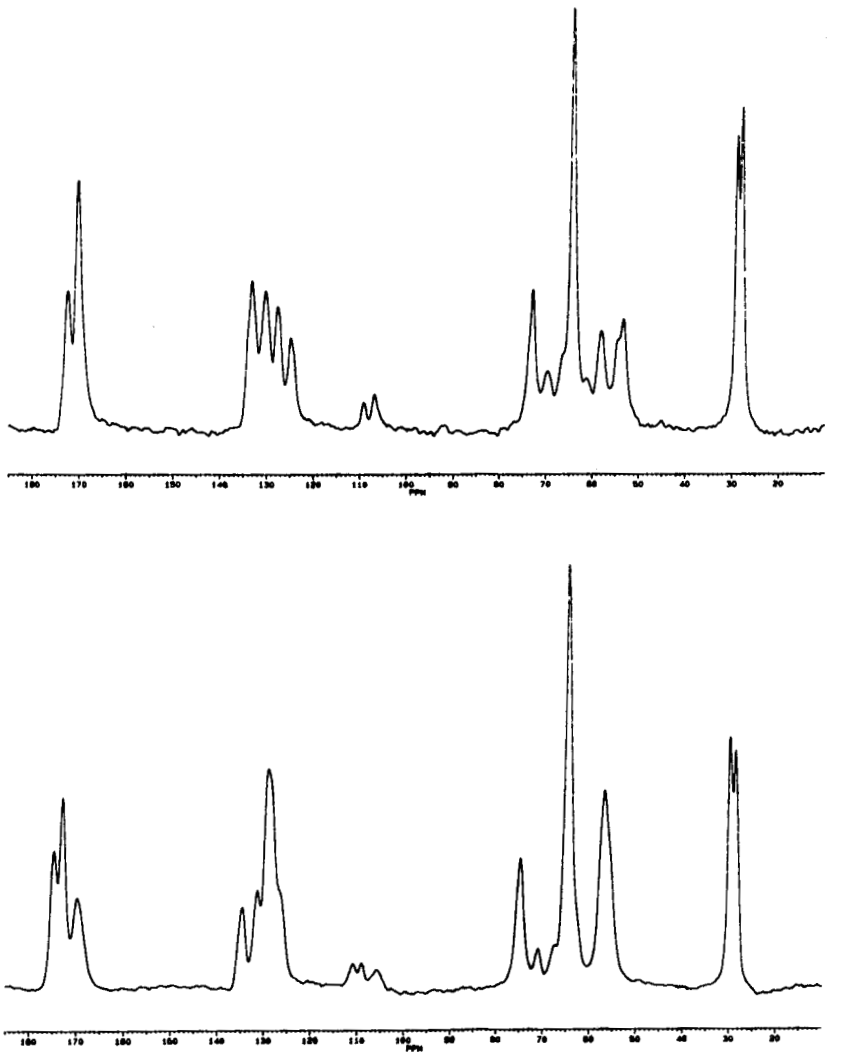

Figure 8. Solid state ^{13}C nuclear magnetic resonance spectra obtained for the trihydrate (upper trace) and anhydrous (lower trace) phases of ampicillin.

observed for the carbons contained in the aromatic ring. In the trihydrate phase a well-resolved pattern of four peaks (resonance positions 125.0, 127.8, 130.3, and 133.3 ppm) was observed, while imperfect resolution (peaks at 128.9, 131.3, and 134.6 ppm, together with a shoulder near 127 ppm) was noted for the anhydrous phase. These observations are two examples of structural variations which originated in the differing hydration states of the two materials.

SUMMARY

The study of pharmaceutical solids is often research in solid state chemistry. While in the past the physical methods consisted simply of thermal or x-ray characterization, the studies of the future will make increasingly more use of sophisticated spectral techniques. These methods are ideally suited to determine the accessibility and mobility of water contained in a pharmaceutical solid, and such information would be more useful than a mere determination of the total amount of water present. The goal of the physical scientist will be to develop suitable methods, and to implement these as part of a comprehensive approach to materials science.

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