Solid-State Fluorescence of the Trihydrate Phases of Ampicillin and Amoxicillin

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ABSTRACT

The purpose of this work was to study the effects of crystal structure on the solid-state photoluminescence of the trihydrate phases of ampicillin and amoxicillin, and to contrast these spectra with analogous spectra obtained on the molecules dissolved in a solution phase. The polymorphic identity of the analytes was established using x-ray powder diffraction and Fourier transform infrared absorption spectroscopy, and the solid-state luminescence spectra obtained under ambient conditions. It was found that the solid-state excitation and emission spectra of ampicillin trihydrate and amoxicillin trihydrate were dominated by energy transfer and exciton effects, which were manifested as decreases in the energy of the excitation and emission bands of the solid-state systems relative to those of the free molecule in solution. The photoluminescence data revealed that in spite of the known structural similarity of ampicillin trihydrate and amoxicillin trihydrate, the magnitude of the Davydov splitting, and the degree of band energy shifting differed between the 2 systems. This finding indicates that the small differences in crystal structure existing between the 2 compounds leads to measurable differences in the patterns of energy transfer.

KEYWORDS: polymorphism, fluorescence spectroscopy, energy transfer, exciton splitting.

INTRODUCTION

The fluorescence spectroscopy of molecules dissolved in a fluid medium is usually readily understood in terms of the energy levels of the isolated molecule, and the excitation spectrum often completely parallels that of the absorption spectrum. However, owing to the effects of intermolecular energy transfer, the fluorescence spectra of the same compound in its crystalline solid state is often radically different relative to that obtained in a condensed phase.[1] Since the transfer of electronic energy between the excited molecules is rapid when compared with the time frame of fluorescence, excitation energy becomes delocalized, causing the excited state molecular orbital to extend over the ensemble of molecules involved in the energy transfer. The interaction leads to splitting of the single-molecule energy levels into a bundled set of levels, with the magnitude of the splitting being determined by the strength of the coupling. This type of splitting is commonly referred to as Davydov splitting and becomes manifest in the appearance of new bands in the excitation spectrum. In addition, the theory predicts that the mean frequency of the Davydov components would be displaced to lower energies relative to that of the free molecule value as a result of cooperative interactions in the crystalline state.

It is well established that solid-state spectroscopy can yield important information regarding the properties of polymorphic or solvatomorphic systems,[1-5] as long as some spectroscopic characteristic of the molecule is affected by differences in the crystal structures of the various forms. Less well recognized is the fact that when the differing crystal structures of polymorphs or solvatomorphs cause an alteration in molecular orbitals, then the analytical techniques that measure transitions among the electronic states derived from these orbitals can be used to obtain additional information on the systems. Since fluorescence spectroscopy is derived from transitions among molecular electronic states, it is to be anticipated that the excitation and emission spectra of luminescent molecules could be used to study the patterns of energy flow within the respective solids. Such methodology was recently used to deduce kinetic information associated with the phase transformation of carbamazepine anhydrate Form-III to its dihydrate phase[6] and to understand the solid-state fluorescence of 4 nonsolvated polymorphs of diflunisal.[7]

In the present work, solid-state fluorescence spectroscopy has been used to study the spectroscopic properties of ampicillin trihydrate and amoxicillin trihydrate, 2 compounds that crystallize in nearly isostructural crystal forms.

MATERIALS AND METHODS

Materials

Ampicillin trihydrate was obtained from Aldrich (St Louis, MO), while amoxicillin trihydrate was obtained from Sigma (St Louis, MO). Both compounds were recrystallized from water (isolation temperature of 5ºC-8ºC) prior to

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their solid-state characterization. For the solution phase studies, ampicillin and amoxicillin trihydrate were dissolved in 0.7 mM aqueous NaOH to obtain solutions having a concentration of ~0.7 mM (3 mg/mL).

Methods

X-ray powder diffraction patterns were obtained using a Rigaku MiniFlex powder diffraction system, equipped with a horizontal goniometer in the θ/2-θ mode (Tokyo, Japan). The X-ray source was nickel-filtered K-α emission of copper (1.54056 Å). Samples were packed into an aluminum holder using a back-fill procedure and were scanned over the range of 50 to 6 degrees 2-θ, at a scan rate of 0.5 degrees 2-θ/min. Using a data acquisition rate of 1 point per second, the scanning parameters equate to a step size of 0.0084 degrees 2-θ. Calibration of each powder pattern was effected using the characteristic scattering peaks of aluminum at 44.738 and 38.472 degrees 2-θ.

Infrared absorption spectra were obtained at a resolution of 2 cm^{-1} using a Shimadzu model 8400 Fourier-transform infrared spectrometer (FTIR) (Shimadzu, Tokyo, Japan), and represent the averaging of 25 interferograms. The data were acquired using the attenuated total reflectance (ATR) sampling mode, where the samples were clamped against the ZnSe crystal of a Pike MIRacle single reflection horizontal sampling accessory.

All solution-phase and solid-state fluorescence excitation and emission spectra were obtained on samples packed into 5-mm glass nuclear magnetic resonance (NMR) tubes. The spectra were obtained on a Perkin/Elmer LS 5B luminescence spectrometer (Fremont, CA), whose sample compartment was modified to enable measurements to be made on samples contained in the NMR tubes. The liquid cell holder was removed and replaced by an aluminum block that had a hole drilled through its length to permit kinematic placement of the sample tube. The block had an additional lateral removal of metal that permitted irradiation of the sample and fluorescence detection at right-angles.

Results and Discussion

Ampicillin and amoxicillin are structurally similar, and the 2 compounds differ only by the presence of an additional hydroxyl group in amoxicillin (Figure 1).

The properties of ampicillin have been summarized in a comprehensive profile,[8] as have the properties of amoxicillin.[9,10] As will be discussed shortly, the chemical similarity between the 2 compounds leads to a structural similarity between their trihydrate crystal phases.

Fluorescence Spectroscopy of Ampicillin and Amoxicillin in the Solution Phase

Since the photoluminescence of ampicillin and amoxicillin in the solution phase provides a basis for evaluation of the free-molecule properties, these data were obtained to provide a means to understand the spectroscopy of the respective molecules in their solid state trihydrate phases. The solution phase excitation and emission spectra of a 3 mg/mL solution of ampicillin dissolved in 0.7 mM aqueous NaOH are shown in Figure 2. The excitation spectrum consisted of a single peak having a maximum at 347 nm, while the single peak of the emission spectrum exhibited its peak maximum at 430 nm. Since the reported absorption spectrum of ampicillin[8] consists mainly of the intense $\pi \rightarrow \pi^*$ transition at 268 nm, it follows that the much weaker $n \rightarrow \pi^*$ transition is probably responsible for the fluorescence excitation.

The solution phase excitation and emission spectra of a 3 mg/mL solution of amoxicillin dissolved in 0.7 mM aqueous NaOH are also shown in Figure 2. The intensities of these spectra were found to be approximately half those relative to ampicillin in the same solution phase, and each amoxicillin spectrum was slightly red-shifted relative to its corresponding ampicillin spectrum. The excitation spectrum consisted of a single peak having a maximum at 357 nm, while the single peak of the emission spectrum exhibited its peak maximum at 437 nm. The reported absorption spectrum of amoxicillin[10] consists mainly of the intense $\pi \rightarrow \pi^*$ transition at 291 nm, but the presence of a shoulder at 325 nm was also noted. As with the case of ampicillin, it appears that the weaker $n \rightarrow \pi^*$ transition is responsible for the fluorescence excitation.

Spectroscopy of Ampicillin and Amoxicillin in the Solid State

The crystal structures of ampicillin trihydrate[11,12] and amoxicillin trihydrate[13] have both been reported, and the defining characteristics of the respective unit cells are found in Table 1. Both compounds crystallize in the orthorhombic system (space group of $P2_12_12_1$), with 4 molecules per unit cell. The molecular conformation of the drug substances in their respective structures is almost identical, and the crystal packing of each compound in its structure is very similar. The only difference between the 2 structures is the existence of an additional hydrogen bond between the aromatic hydroxyl group and an oxygen atom of a symmetry-related molecule.[13]

Nevertheless, as evident in Figure 2, the small differences in unit cell dimensions yield measurable differences between the x-ray powder diffraction patterns of ampicillin and amoxicillin trihydrate. These structural differences are

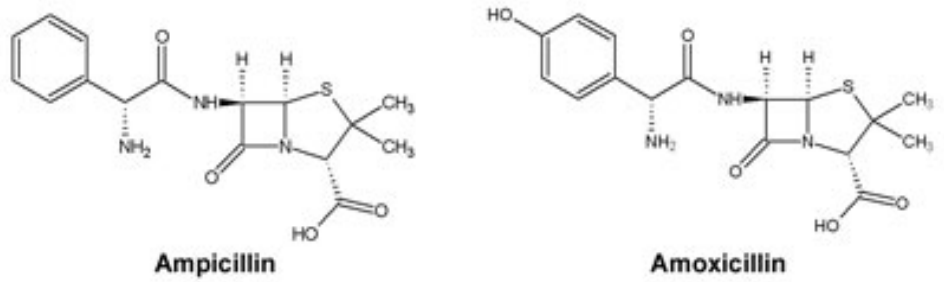

Figure 1. Structures of ampicillin and amoxicillin.

sufficient to alter the pattern of molecular vibrations of the compounds in their respective trihydrate crystal forms, so that the fingerprint regions of the respective infrared spectra contain several significant differences (see Figure 3). The fact that the structural differences between the 2 trihydrate crystal forms is sufficient to yield nonequivalent infrared absorption spectra suggests that one should be able to detect differences in other spectroscopies.

The solid-state excitation and emission spectra of ampicillin trihydrate are shown in Figure 4. The excitation spectrum was found to consist of 2 overlapping bands, with the main peak having a maximum at 391 nm, but coupled with a shoulder at 368 nm. Both of these excitation bands are seen to be fairly red-shifted relative to the solution phase excitation maximum that was observed at 347 nm. The emission spectrum consisted of a single peak having a maximum at 478 nm, which is also considerably shifted toward lower energies relative to the analogous peak observed for the compound in the solution phase (430 nm).

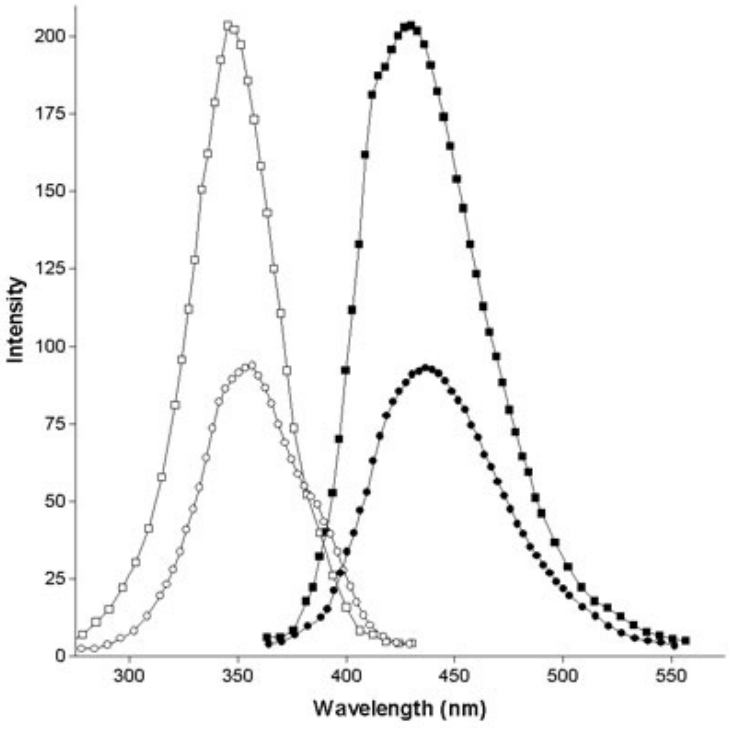

Figure 2. Solution phase excitation (○) and emission (●) spectra of a 3 mg/mL solution of ampicillin dissolved in 0.7 mM aqueous NaOH.

The solid-state excitation and emission spectra of amoxicillin trihydrate are also shown in Figure 5. As noted for ampicillin trihydrate, the excitation spectrum of amoxicillin trihydrate consisted of 2 overlapping bands, with the main peak having a maximum at 391 nm, and a shoulder at 367 nm. Both of these bands are seen to be shifted to lower energies relative to the solution phase excitation maximum of 357 nm. The emission spectrum consisted of a single peak having a maximum at 454 nm, which is only slightly red-shifted relative to the analogous peak observed for the solution phase compound (437 nm).

The origin of the band shifting observed in the excitation and emission spectra is known to originate from the cooperative effects of energy transfer existing in the solid state. Although the ground electronic state remains localized on individualized molecules in the crystalline state, the excited electronic states of these can be so strongly interactive that excitation energy becomes delocalized among the coupled molecules. Such a delocalized excited state is termed an exciton,[14] and a fairly complete exposition of the theory of excitons is available in the book by Davydov.[15]

Since the transfer of electronic energy between the excited molecules is rapid relative to the time frame of fluorescence,

Table 1. Crystallographic Data Reported for the Trihydrate Crystal Phases of Ampicillin and Amoxicillin*

	Ampicillin Trihydrate	Amoxicillin Trihydrate
Crystal class	Orthorhombic	Orthorhombic
Space group	$P2_12_12_1$	$P2_12_12_1$
Unit cell lengths	a = 15.490 Å	a = 15.622 Å
	b = 18.891 Å	b = 18.785 Å
	c = 6.662 Å	c = 6.645 Å
Unit cell angles	$\alpha = \beta = \gamma = 90°$	$\alpha = \beta = \gamma = 90°$
Molecules in unit cell	4	4
Cell volume	1949.4 Å^3	1429 Å^3
Density	1.37 g/mL	Not reported

*Data for ampicillin trihydrate are from James et al[11] and Boles and Gervin.[12] Data for amoxicillin trihydrate are from Boles et al.[13]

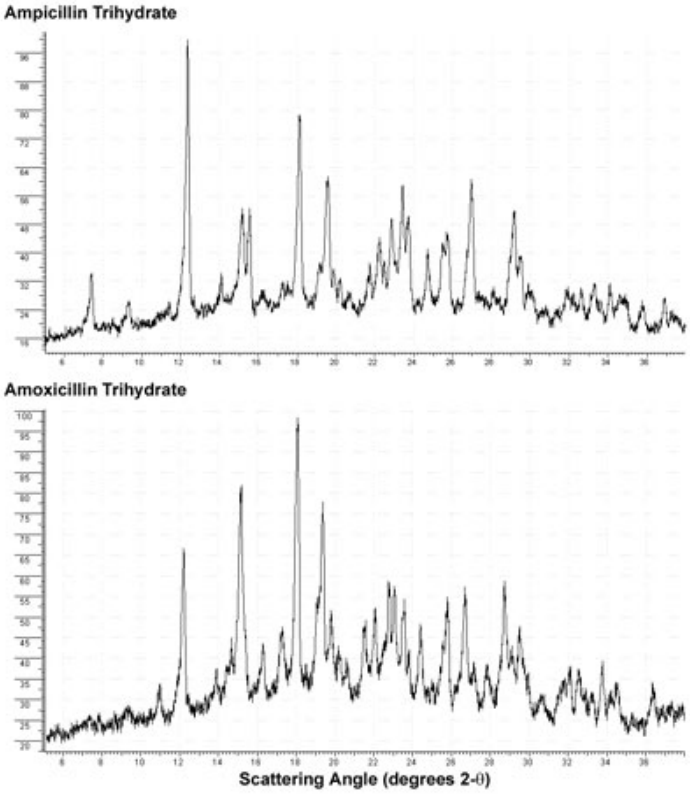

Figure 3. X-ray powder diffraction patterns of ampicillin and amoxicillin trihydrates.

the delocalization of excitation energy has the effect of extending the excited state molecular orbital over the molecules whose excited states are involved in the energy transfer.[16] For a pair of molecules, the interaction leads to a splitting of the single-molecule energy level into a pair of levels, with the magnitude of this splitting being determined by the strength of the coupling. This type of splitting is denoted as Davydov splitting, and its effects include the appearance of new bands in the excitation spectrum. In addition, the theory predicts that the mean frequency of the Davydov components would be displaced to lower energies from that of the free molecule value as a result of cooperative interactions in the crystalline state. The result of exciton coupling is to produce an excitation multiplet corresponding to a band of n levels, each differing in energy by small amounts, with the overall spread of the excitation band being determined by the strength of the coupling.

In crystals of organic molecules, the magnitude of the interaction energy and degree of exciton coupling is necessarily dependent on the relative orientation of the molecules in the crystal, as well as on their spatial arrangement. It follows that since polymorphic or solvatomorphic crystal forms are characterized by the existence of differing structural properties, the nature of the exciton coupling in the various forms would be influenced by the structural characteristics of each form. As a result, one would anticipate that the magnitude of the Davydov splitting, and the degree of shifting of the levels, would be dependent on the exact structural details existing in the different polymorphic forms.

The magnitude of the Davydov splitting can be calculated as the difference in energy between the 2 components of the solid state excitation bands. For ampicillin trihydrate, the energies of the 2 bands were 27 174 cm^{-1} (368 nm) and 25 575 cm^{-1} (391 nm), for an energy difference of 1599 cm^{-1}. In the case of amoxicillin trihydrate, the energies of the 2 bands were 27 248 cm^{-1} (367 nm) and 25 575 cm^{-1} (391 nm), for an energy difference of 1673 cm^{-1}. The near equivalence in Davydov splitting for the 2 trihydrate phases implies similar mechanisms of energy transfer in the 2 systems.

However, the 2 structures are not exactly the same, and the structural differences are reflected in the shifting of the excitation energies of the solid-state compounds relative to their solution phase analogs. The mean of the Davydov-split excitation bands observed for ampicillin trihydrate calculates as 26 375 cm^{-1}, which is red-shifted by 2443 cm^{-1} relative to the energy of the solution phase excitation peak (28 818 cm^{-1}, for the 347 nm band). On the other hand, the mean of the Davydov-split excitation bands of amoxicillin trihydrate was found to be 26 412 cm^{-1}, which is red-shifted by 1599 cm^{-1} with respect to the energy of the solution phase excitation peak (28 011 cm^{-1}, for the 357 nm band).

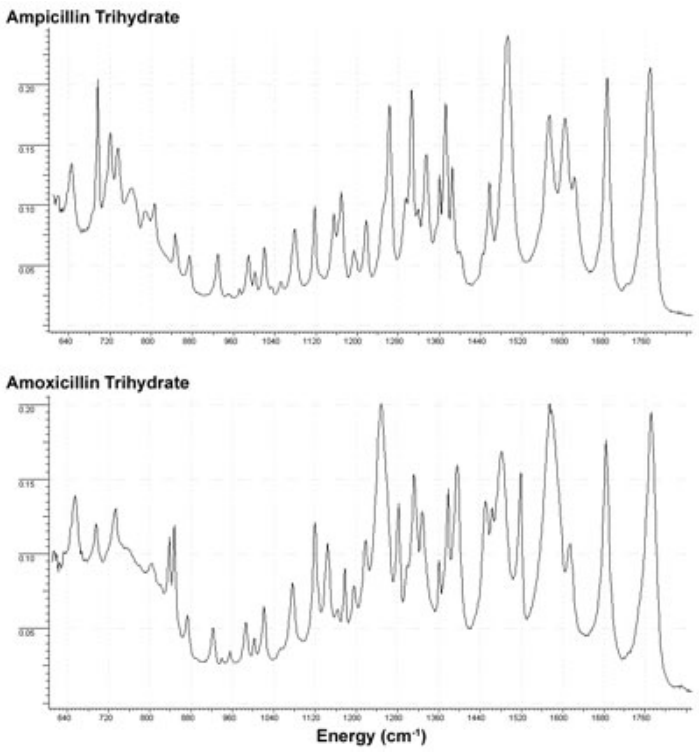

Figure 4. Fingerprint regions of the infrared absorption spectra of ampicillin and amoxicillin trihydrates.

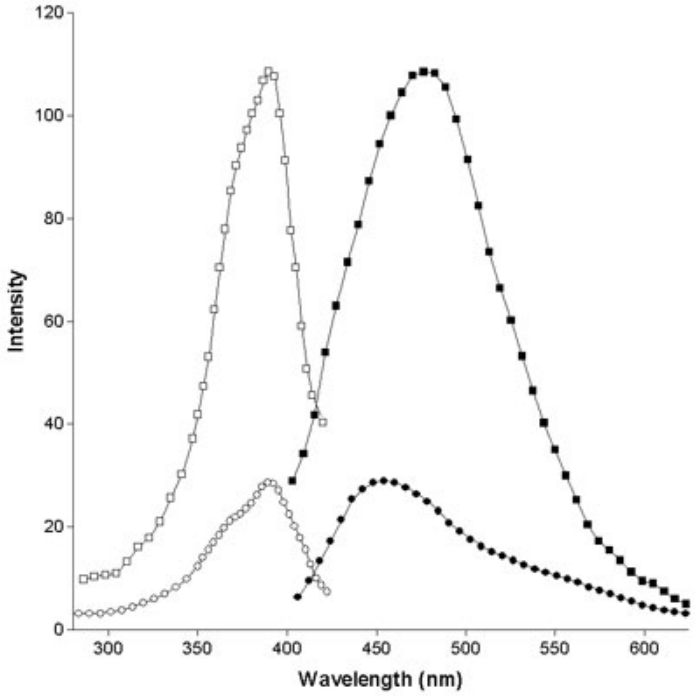

Figure 5. Solid-state excitation (□) and emission (■) spectra of ampicillin trihydrate, and solid-state excitation (○) and emission (●) spectra of ampicillin trihydrate.

Further spectroscopic realization of the structural differences between ampicillin and amoxicillin trihydrate are evidenced by the nonequivalence of energy displacements between the solution phase and solid-state emission bands. For ampicillin trihydrate, the energy of the solid-state emission peak was 20 921 cm^{-1} (478 nm), the energy of the analogous solution phase band was 23 256 cm^{-1} (430 nm), and so the solid-state peak was red-shifted by 2335 cm^{-1} relative to the solution phase peak. On the other hand, the energy of the solid-state emission peak of amoxicillin trihydrate was 22 026 cm^{-1} (445 nm), the energy of the analogous solution phase band was 22 883 cm^{-1} (437 nm), and so the solid-state peak was red-shifted only by 860 cm^{-1} relative to the solution phase peak.

CONCLUSION

It has been found that exciton effects dominate the excitation and emission spectra of ampicillin trihydrate and amoxicillin trihydrate, leading to a decrease in the energy of the excitation bands relative to that observed for the free molecule in fluid solution, and in a splitting of the excitation peak into 2 Davydov components. The magnitude of the Davydov splitting was comparable in the 2 crystal systems, indicating the existence of comparable delocalization of excitation energy. However, the observed shifting to lower energies observed for both the solid-state excitation and emission bands relative to the corresponding solution phase bands is reflective of perturbations in the spectroscopic states of the various systems induced by differences in the crystal structures.

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