Effect of Humidity-Dependent Changes in Crystal Structure on the Solid-State Fluorescence Properties of a New HMG-CoA Reductase Inhibitor

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It has been shown previously that the disodium salt of a new HMG-CoA reductase inhibitor (SQ-33600) is capable of existing as a number of hydrate species [1]. Three crystalline solid hydrates and one liquid crystalline phase have been identified, each having a definite stability over a defined range of humidity. These forms have been found to exhibit varying fluorescence properties in their respective solid states, with differences in bandshapes and intensities being noted for each. These spectral variations have been correlated with the known pseudopolymorphism of the compound.

KEY WORDS: HMG-CoA reductase inhibitor; SQ-33600; crystal structure; hydrates; solid-state fluorescence.

INTRODUCTION

The ability of pharmaceutical solids to form hydrate species is well known, and it is possible that more than one crystal form can be obtained for a given substance depending on environmental conditions [2]. It is also established that the various hydrate species of a compound can exhibit widely differing physical properties, and that these variations must be considered during drug development [3]. Outside of nuclear magnetic resonance and infrared spectral properties, it is not generally recognized that other spectroscopic characteristics of the compound can also be profoundly affected by alterations in crystal structure.

In a previous work, the effect of relative humidity on the crystal form of SQ-33600 (the disodium salt of a novel HMG-CoA reductase inhibitor) was characterized using a wide variety of experimental techniques [1]. Evaluation of the data permitted a classification of the hydrate species, which is summarized as follows:

Type I. This form is characterized by solids containing up to 1.5 moles of water per mole of SQ-33600. The water is most likely contained within "channels" in the solid. Evidence exists that even though the water was not an integral part of the crystal lattice, it was bound at some type of localized site (nonspecific hydration).

Type II. This crystalline phase is formed upon sorption of up to 2 additional moles of water per mole of drug, with the maximum drug-to-water ratio being 1:3.5. All data indicated that the crystal lattice could expand reversibly over the water content range while still maintaining its basic crystal structure.

Type III. This hydrate phase appears to contain an average of 5.5 water molecules per molecule of SQ-33600, and is a well-defined hydrate species.

Liquid Crystalline Phase. At the highest levels of water content a liquid crystalline phase is formed by the sorption of at least 9 water molecules per molecule of SQ-33600.

It has been found that SQ-33600 exhibits fluorescence when in the solid state, and that the details of its fluorescence are strongly dependent on the crystalline phase of the material. In this report, we summarize the results of solid-state fluorescence investigations conducted on the various pseudopolymorphic forms of SQ-33600.

MATERIALS AND METHODS

Materials

SQ-33600 was manufactured by the Chemical Process Development division of Bristol-Myers Squibb Pharmaceutical Research Institute, New Brunswick, New Jersey. Samples were equilibrated to varying moisture levels by storing samples in closed desiccators over saturated aqueous solutions of LiCl, CH_3COOK, $MgCl_2$, K_2CO_3, $Mg(NO_3)_2$, NH_4NO_3, NaCl, and KCl. These chambers effectively maintained relative humidities (RH) of 11%, 22%, 33%, 43%, 52%, 60%, 75%, and 84%, respectively. Anhydrous $CaSO_4$ (Drierite®, Hammond, OH) provided the 6% RH condition.

Fluorescence Studies

Fluorescence spectra were obtained on a Spex 2:1:2 fluorolog system, which featured double monochromators on the excitation and emission sides. Samples were filled into 2 mm glass tubes, with the spectral data being obtained through the use of front-face excitation. Fluorescence spectra were obtained on all samples using excitation wavelengths of 315, 340 and 400 nm, while excitation spectra were obtained at fluorescence observing wavelengths of 370 and 485 nm.

RESULTS AND DISCUSSION

The photophysical processes associated with fluorescence spectroscopy have been amply reviewed [4,5], so only a brief synopsis will be given here. Having undergone an electronic transition to an excited state, most molecules rapidly lose excess energy and fall to the lowest vibrational level of the excited state. From this level, the molecule can return to any of the vibrational levels of the ground state either through a radiationless deactivation of the excited state, or through the emission of radiant energy. This process is known as fluorescence, and a plot of fluorescence intensity (obtained at a given wavelength of excitation) against wavelength is known as the *fluorescence spectrum*. Since the emission of fluorescence always takes place from the lowest vibrational level of the lowest excited state, the shape of the fluorescence spectrum is not dependent on the wavelength

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used for excitation. The dependence of emission intensity (measured at a specific value) upon the wavelength of exciting light is termed the *excitation spectrum.*

The principles just described hold as long as the observed fluorescence originates from the same excited state. Should the structural details of the fluorescent state be altered, then an entirely new pattern of excitation and fluorescence spectra would be obtained. The transformation of a solid from one crystal phase to another would effect the necessary perturbation in molecular energy levels, and thus each structural modification of SQ-33600 should exhibit a characteristic pattern in photophysical properties.

The fluorescence spectrum obtained for SQ-33600, Type I, was obtained through the characterization of material equilibrated at relative humidities of 6% and 11%. As shown in Figure 1, the spectrum consisted of two bands located at 371 and 418 nm. An identical spectral pattern was obtained for Type II materials (equilibrated at relative humidities of 22%, 33%, and 43%), although the overall intensities were substantially reduced relative to the Type I samples.

The excitation spectra obtained for activation of either Type I or Type II material (also shown in Fig. 1) was independent of the fluorescence band chosen for monitoring, and consisted solely of a band at 345 nm. This finding would indicate that both fluorescence bands originated from the same excited state. The spacing between these two fluorescence bands (calculated to be 3030 cm^{-1}) coincides with a well-developed feature noted in the infrared spectrum at the same energy [1], suggesting that the two bands represent a vibronic sequence.

In the previous work [1], it had been noted that the break between Type II and Type III crystal characteristics took place after exposure of SQ-33600 to 52% relative humidity, and that this environmental condition represented a definite cross-over situation. Perhaps not surprisingly, samples of SQ-33600 equilibrated at 52% relative humidity were found to exhibit complicated photophysical characteristics. In addition to the two fluorescence peaks at 371 and 418 nm, a new fluorescence feature was also noted at 485 nm. The relative intensity of the various fluorescence peaks was found to depend critically on the wavelength used for excitation. As shown in Figure 2, the intensity ratio of the 371/418 nm pair to the 485 nm band was completely inverted by shifting the excitation wavelength from 340 to 315 nm.

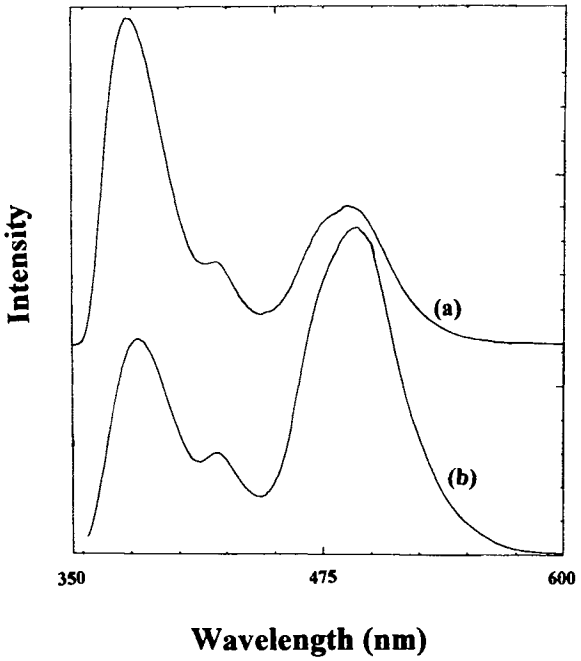

Fig. 2. Fluorescence spectra of the cross-over SQ-33600 material obtained after equilibration at 52% RH. The spectra were obtained at excitation wavelengths of (a) 315 nm and (b) 340 nm.

The excitation spectrum of the 485 nm fluorescence band is shown in Figure 3. Although activation of this band can be accomplished using 340 nm irradiation, use of the band at 400 nm is considerably more efficient. Of course, 400 nm activation of a SQ-33600 sample equilibrated at 52% RH yielded only the fluorescence band at 485 nm.

Samples of SQ-33600 equilibrated at either 60% or 75% RH had been shown to consist solely of Type III, and equivalent fluorescence was obtained from such materials. As shown in Figure 4, the fluorescence spectrum consisted solely of the 485 nm emission and the excitation spectrum consisted solely of the 400 nm band.

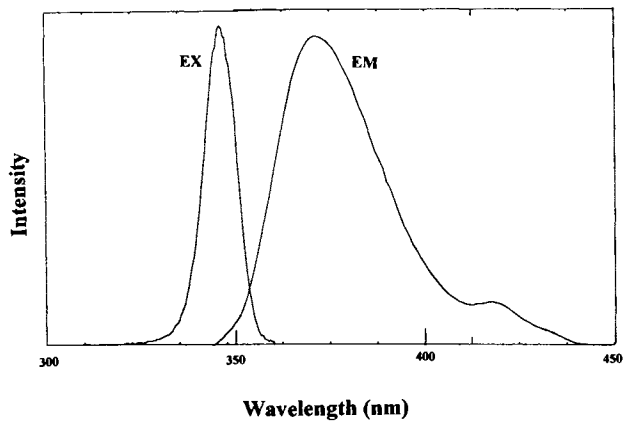

Fig. 1. Excitation (EX) and fluorescence (EM) spectra obtained for SQ-33600, Type I. Equivalent spectral lineshapes exist for Type II material.

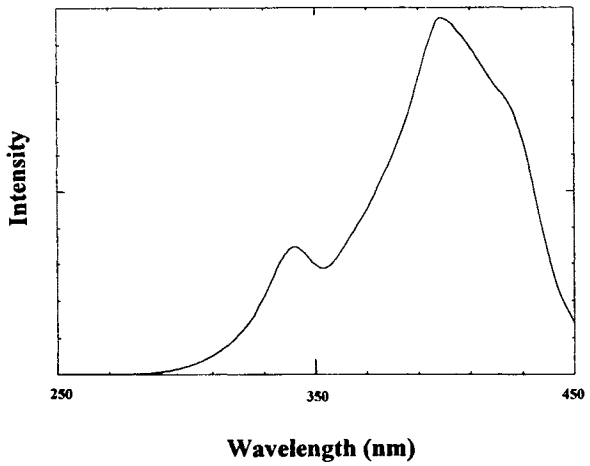

Fig. 3. Excitation spectrum of the 485 nm fluorescence band, measured for the crossover SQ-33600 material obtained after equilibration at 52% RH.

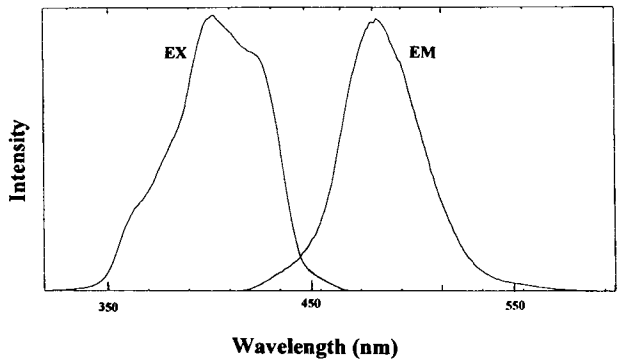

Fig. 4. Excitation (EX) and fluorescence (EM) spectra obtained for SQ-33600, Type III.

When equilibrated at a relative humidity of 84%, SQ-33600 was previously found to convert into a liquid crystalline form [1]. The fluorescence and excitation spectra of this species were found to be equivalent to those obtained for Type III, but were significantly reduced in intensity.

The effect of equilibration conditions on the intensity of observed fluorescence is shown in Figure 5 for the various excitation/emission possibilities. The transition between Type I and Type II is clearly evident in the reduction of fluorescence intensity of the 371 nm band between samples equilibrated at 6% or 11% and those equilibrated at 22%, 33%, or 43%. That material equilibrated at 52% relative humidity can be activated through irradiation at either 340 or 400 nm supports the earlier conclusion that this particular relative humidity represents a transition point. The completely different photophysical features observed for samples equilibrated at either 60% or 75% are intrinsic to the Type III material, and the intensity reduction noted for samples exposed to 84% RH must accompany the transformation to the liquid crystalline phase.

An alternate way to view the fluorescence intensity trends is to plot the data in terms of the number of coordinated water molecules, using the previously reported data [1] to make the connection. This has been illustrated in Figure 6, and the data permit additional conclusions to be drawn regarding the plausible structures for each pseudopolymorph. Type I is essentially a monohydrate species, and addition of a second water of hydration to this structure yields Type II. The lack of fluorescence bandshape changes on passing from Type I to Type II suggests that the structural modification is relatively minor. Since it is certain that the observed fluorescence is associated with the indole functionality, the data suggest little perturbation of this system on passing from Type I to Type II.

It is interesting to note that the species formed after equilibration at 52% RH corresponds roughly to a trihydrate, but the luminescence data indicate that the solid is actually a mixture of types II and III. This particular solid represents the only situation where excitation at either 340 or 400 nm yields fluorescence at 485 nm, while 340 nm excitation is still able to yield the 371/418 nm system. This situation could only arise if the solid isolated upon exposure to 52% RH was obtained as a mixture of phases.

Type III is formed at a 5.5 mole ratio of water to SQ-33600, and is stable up to a 6.5 mole ratio. The structural change induced by this transformation must be substantial since the nature of the photophysics undergoes such a dramatic change. The red-shifting of the fluorescence excitation and emission are consistent with the existence of indole excimers, which would arise from a stacking of the ring systems within the solid [6].

Finally, the liquid crystalline phase corresponds to a

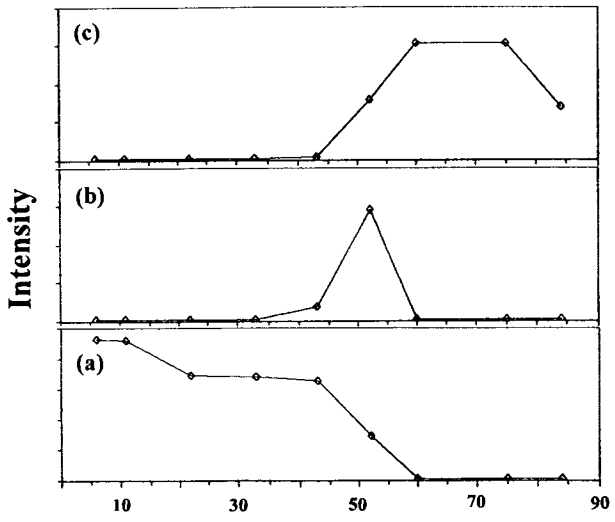

Fig. 5. Relative fluorescence intensities of SQ-33600 materials equilibrated at different relative humidities, obtained at various excitation wavelengths. Data are illustrated for (a) fluorescence intensity at 371 nm when excited at 340 nm, (b) fluorescence intensity at 490 nm when excited at 340 nm, and fluorescence intensity at 490 nm when excited at 395 nm.

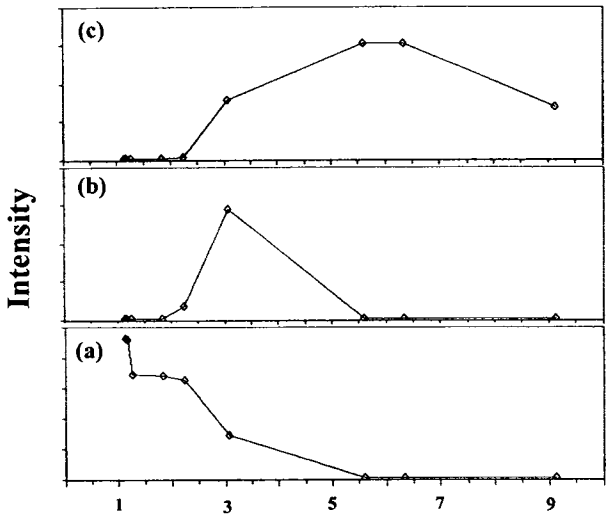

Fig. 6. Relative fluorescence intensities of SQ-33600 materials as a function of the number of coordinated water molecules, obtained at various excitation wavelengths. Data are illustrated for (a) fluorescence intensity at 371 nm when excited at 340 nm, (b) fluorescence intensity at 490 nm when excited at 340 nm, and fluorescence intensity at 490 nm when excited at 395 nm.

species containing nine waters of hydration. The similarity of the fluorescence spectrum obtained for this phase to that of Type III suggests the existence of ring stacking, while the reduction in fluorescence intensity indicates a loss of long-range order in the solid.

SUMMARY

The excitation and fluorescence data obtained on SQ-33600 materials equilibrated at a series of relative humidities have been found to be sensitive toward details of the phase composition. Type I was known to be a monohydrate species, and was characterized by an excitation maximum at 345 nm and a main fluorescence maximum at 371 nm. Type II was a dihydrate, whose photophysical parameters were equivalent to those of Type I, but for which the overall levels of fluorescence were greatly reduced. Addition of additional waters of hydration produced Type III material, which exhibited an excitation maximum at 400 nm and a fluorescence maximum at 485 nm. Finally, upon binding of nine water molecules, the structure relaxed into a liquid crystalline form which exhibited photophysical properties equivalent to those of type III, but which were considerably reduced in intensity.

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