Pharmaceutical Research, Vol. 6, No. 12, 1989

Research Article

Determination 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 Powder X-ray Diffractometry

Raj Suryanarayanan[1]

Received January 18, 1989; accepted June 6, 1989

A powder x-ray diffraction technique has been developed to quantify the relative amounts of anhydrous carbamazepine ($C_{15}H_{12}N_2O$) (I) and carbamazepine dihydrate ($C_{15}H_{12}N_2O \cdot 2H_2O$) (II) when they occur as a mixture. The theoretical basis of this technique was developed by Alexander and Klug (*Anal. Chem.* 20:886–889, 1948). The powder x-ray diffraction patterns of I and II revealed that the lines with d-spacings of 6.78 and 9.93 Å were unique to I and II, respectively. The ratio of the integrated intensity of the 6.78 Å line in a mixture of I and II to the intensity of the 6.78 Å line in a sample consisting of only I was calculated as a function of weight fraction of I in the mixture. These ratios were also experimentally determined, and there was a good agreement between the theoretical and the experimental intensity ratios. Similarly good agreements between the theoretical and the experimental intensity ratios for the 9.93 Å line of II were obtained. The samples were compressed into tablets and subjected to x-ray analysis. When compressed to a certain pressure, the particles tended to orient the same way in replicate samples resulting in highly reproducible intensity values. Compression into tablets was necessary because the powder samples yielded unsatisfactory results.

KEY WORDS: quantitative powder x-ray diffractometry; carbamazepine; carbamazepine dihydrate; preferred orientation.

INTRODUCTION

Carbamazepine, 5H-dibenz[b,f]azepine-5-carboxamide, is used in the treatment of epilepsy and trigeminal neuralgia (1,2). It is practically insoluble in water (3). Anhydrous carbamazepine ($C_{15}H_{12}N_2O$) (I), when dispersed in water or in 0.1 *N* HCl, was observed to transform rapidly to carbamazepine dihydrate ($C_{15}H_{12}N_2O \cdot 2H_2O$) (II) (4,5). Several other pharmaceutical compounds have been reported to undergo a similar transformation in the presence of water (6,7). In order to study the kinetics of such transformations, the anhydrous material is dispersed in the appropriate liquid medium. An aliquot of the solid is removed at predetermined time intervals so as to determine its water content. The two common methods to determine water content in solids are (a) Karl Fischer titrimetry and (b) heating the sample to constant weight.

The water in solids is generally bound in two different ways. (a) Water held in a definite molar proportion within the solid is referred to as chemically bound water. Water of crystallization belongs to this category.[2] (b) Physically bound water wherein the amount held is variable. This depends on the method of solid preparation and/or the conditions under which the solid is stored. The adsorbed water belongs to this class (8).

The Karl Fischer method quantifies the total water in a sample and does not distinguish between physically and chemically bound water. Attempts to determine the water content in a solid by heating it to a constant weight can cause other undesirable events such as sample decomposition. Even if drying conditions are chosen to avoid sample decomposition, the weight loss is likely to reflect the total water in the sample. Therefore this method also possesses the same limitation as the Karl Fischer titrimetry.

Based on crystal lattice studies, three cases have been distinguished following the dehydration of hydrates: (a) the crystal lattice of the residue is nearly identical to that of the original hydrate, (b) the residue is poorly crystalline, and (c) the residue recrystallizes with a different crystal lattice (9). Many compounds of pharmaceutical interest belong to the last category. In these cases, the powder x-ray diffraction patterns of the hydrate and the anhydrous material will be different. If the differences in the powder pattern are pronounced, they can be exploited for quantification purposes. The aim of this study is the development of a quantitative x-ray technique to determine the relative amounts of I and II when they occur as a mixture.

[1] Department of Pharmaceutics, College of Pharmacy, 308 Harvard Street S.E., Minneapolis, Minnesota 55455.

[2] The existence of non-stoichiometric hydrates in pharmaceuticals is known. However, the formation of such hydrates has not been reported in case of carbamazepine.

0724-8741/89/1200-1017$06.00/0

Theory

The decrease in the intensity I of an x-ray beam as it passes through any homogeneous substance is given by the expression

$$-\frac{dI}{I} = \mu\, db \tag{1}$$

where b is the distance traversed by the x-rays and μ is the linear absorption coefficient. The value of μ depends on the substance, its density, and the wavelength of the x-rays (10). The mass absorption coefficient of a substance, μ^*, is obtained by dividing μ by the density of the substance.

The theoretical basis of the quantitative analysis of powder mixtures was developed by Alexander and Klug (11,12). Though a mixture may consist of N components, it was regarded to be composed of just two components: component J, which was the unknown, and the sum of the other components, which was designated the matrix. The intensity of line i of component J in a powder mixture was given as

$$I_{iJ} = \frac{K\, x_J}{\rho_J[x_J(\mu_J^* - \mu_M^*) + \mu_M^*]} \tag{2}$$

where

K = a constant
x_J = weight fraction of component J in the mixture
ρ_J = density of component J

μ_J^* and μ_M^* are, respectively, the mass absorption coefficients of component J and the matrix (for radiation of a particular wavelength).

In a mixture of I and II, I is first considered the unknown component and II is the matrix. The unknown component and the matrix are written with subscripts 1 and 2, respectively. Therefore, Eq. (2) can be written as

$$I_{i1} = \frac{K\, x_1}{\rho_1[x_1(\mu_1^* - \mu_2^*) + \mu_2^*]} \tag{3}$$

where x_1, μ_1^*, and μ_2^* are the weight fraction of I, mass absorption coefficient of I, and mass absorption coefficient of II, respectively. The line i of component 1 should be so chosen that in the 2θ range where this peak occurs, II should not exhibit any diffraction peaks. The intensity of peak i of a sample consisting of only I, $(I_{i1})_o$, is given as (12)

$$(I_{i1})_o = \frac{K}{\rho_1\, \mu_1^*} \tag{4}$$

Division of (3) by (4) gives

$$\frac{I_{i1}}{(I_{i1})_o} = \frac{x_1\, \mu_1^*}{x_1(\mu_1^* - \mu_2^*) + \mu_2^*} \tag{5}$$

Based on the chemical formulae of I and II, μ_1^* and μ_2^* can be calculated. It is then possible to calculate the intensity ratio, $I_{i1}/(I_{i1})_o$, as a function of x_1. This ratio can also be experimentally obtained. The intensity of the peak i of a sample consisting of only I is determined $[(I_{i1})_o]$. This is followed by the determination of the intensity of the same peak in mixtures containing different weight fractions of I and II. This enables the experimental intensity ratio, $I_{i1}/(I_{i1})_o$, to be obtained as a function of x_1.

Similarly, when II is considered as the unknown component, I forms the matrix. A peak of II should be selected such that in the angular range where this peak occurs, I does not exhibit any diffraction peaks. Both theoretical calculations and experimental determinations of intensity ratios can be made following the procedure outlined earlier.

MATERIALS AND METHODS

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 attainment of constant weight. The sample was then ground in a ball mill (Spex Mixer/Mill, Spex Industries, Metuchen, NJ) for 5 min using a sample holder and ball made of agate and stored at room temperature under 52% RH.

Thermal Analyses

A Du Pont 910 differential scanning calorimeter with a Du Pont 990 thermal analyzer was used. The calorimeter was calibrated with indium. About 1- to 3-mg samples were weighed into standard (open) aluminum sample pans and heated from 30 to 200°C at 10°C min^{-1} under a stream of nitrogen. The samples were weighed at the end of each run. Simultaneous differential thermal analysis and thermogravimetric analysis (TGA) were performed on a Stanton Redcroft STA780 thermal analyzer.

Powder X-Ray Diffractometry

Qualitative Studies. The samples were exposed to CuKα radiation (40 kV × 30 mA) at a scanning rate of 1° 2θ min^{-1}, in the step scan mode in a Siemens D500 wide-angle x-ray diffractometer. The Bragg–Brentano focusing geometry was used, with a 1° incident aperture slit, a 0.15° detector slit, and a scintillation counter as the detector. The signal-to-noise ratio was considerably enhanced by the use of a single crystal graphite monochromator (10). The sample holder, 2.0 × 1.5 × 0.2 cm, was packed from the side.

Quantitative Studies. For integrated intensity measurements, the diffractometer was operated in a step-scan mode with increments of 0.01° 2θ over two angular ranges: 8.20–9.19° 2θ for II (9.93-Å line) and 12.60–13.39° 2θ for I (6.78-Å line). Counts were accumulated for 3 sec at each step. The background was measured under similar conditions from 11.20 to 11.40° 2θ. The background counts had been obtained over an angular range of 0.2° 2θ (21 steps), while the intensity of the 9.93- and 6.78-Å lines had been obtained by integrating over 0.99° 2θ (100 steps) and 0.79° 2θ (80 steps), respectively. So the background counts were calculated for 100 and 80 steps and subtracted from the integrated counts of 9.93- and 6.78-Å lines, respectively. Such a correction is permissible because in the case of both I and II

the background counts did not undergo any perceptible change as a function of the scanning angle in the angular range of interest to us (8.20 to 13.39° 2θ). I, II, and mixtures with different proportions of I and II were prepared. Two hundred milligrams of the sample was accurately weighed and compressed in a hydraulic press (Fred S. Carver, Menomonee Falls, WI) to a pressure of 125 MPa and held for 1 min. The tablets obtained were 9.5 mm in diameter and 2 mm thick. A aluminum x-ray sample holder with a central cavity, 9.6 mm in diameter, was fabricated. The cavity had a depth of 2.2 mm. Two small pieces of molding clay were put at the bottom of the holder, the tablet was dropped into the cavity, and using a flat glass slide, the tablet was gently pressed down until the holder surface and tablet surface were coplanar. Tablets of I prepared as described above were periodically subjected to x-ray studies and the intensity of the 6.79-Å line was obtained by integrating from 12.60 to 13.39° 2θ and correcting for the background. The coefficient of variation (CV) of all such samples pooled together was less than 4%. Therefore long-term instrumental drift was assumed to be small enough not to require any correction. There was no measurable short term instrumental drift during the time of analysis of each sample. From the time of compression, until subjected to x-ray analysis, the tablets were stored at room temperature in a chamber maintained at 52% RH.

RESULTS AND DISCUSSION

The powder x-ray diffraction pattern of I (Fig. 1) was identical to that of β-carbamazepine reported both by the JCPDS (13) and by Lowes *et al.* (14). Carbamazepine has also been reported to exist in two other crystal modifications: the α form (14) and form III (15). The use of CuKα as the radiation source resulted in an intense peak around 5° 2θ for the former and several peaks between 5 and 10° 2θ for the latter. The absence of all of these peaks indicates that our sample consisted only of the β form and is not a mixture of polymorphs. The DSC curve obtained was similar to that reported earlier (14). There was no detectable weight loss on heating the samples suggesting the absence of both physically and chemically bound water.

The powder x-ray diffraction pattern of II (Fig. 2) was very similar to that of carbamazepine dihydrate reported (15). The existence of two crystal modifications of carbamazepine dihydrate has been suggested (16). Several batches of carbamazepine dihydrate were prepared and the x-ray powder diffraction patterns of all of the samples was identical to the pattern presented in Fig. 2. The relative peak intensities were very close to one another in all of these samples. Carbamazepine dihydrate was also prepared by storing I at room temperature under 92.9% RH and its powder x-ray pattern was superimposable on the Fig. 2 pattern. Therefore it was concluded that all batches of the carbamazepine dihydrate existed in one crystalline structure and were not a mixture of polymorphs. The DSC curve of II was similar to that of I but two additional endotherms at 88 and 100°C were observed. The first endotherm was attributed to the dehydration reaction and the second to vaporization of water of crystallization. In order to quantify the water loss, the DSC run was stopped after the appearance of the above two peaks and the samples were weighed. The weight loss of 13.0 ± 0.5% (w/w) agreed with the theoretical weight loss of 13.2% (w/w) for the complete transition of II to I. The weight loss obtained in the TGA was 13.5% (w/w). The amount of physically bound water, if any, in samples of II is likely to be very small. This conclusion is based on the fact that the observed

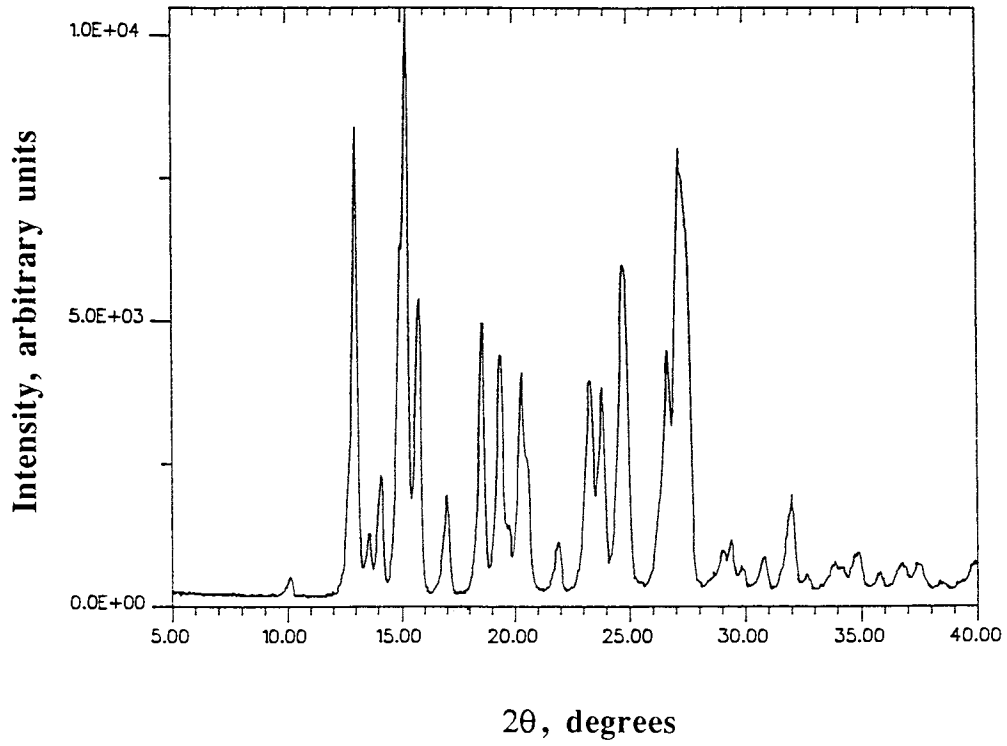

Fig. 1. The powder x-ray diffraction pattern of anhydrous carbamazepine (I).

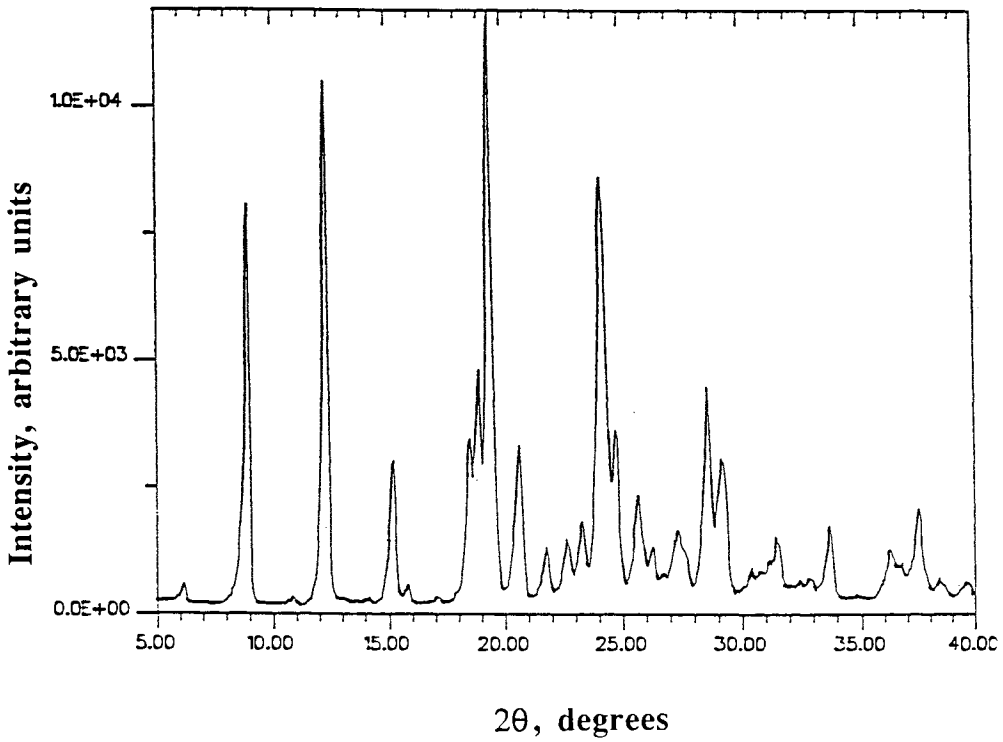

Fig. 2. The powder x-ray diffraction pattern of carbamazepine dihydrate (II).

weight loss and the theoretical weight loss (assuming two molecules of water of crystallization per molecule of $C_{15}H_{12}N_2O$) are close to each other. The presence of an appreciable amount of physically bound water will cause a weight loss substantially greater than that predicted theoretically.

When the powder x-ray patterns of I and II were compared (Figs. 1 and 2), it became evident that in the 2θ range where the 6.78-Å line in I occurred (peak at 13.05° 2θ), there were no peaks in the powder pattern of II. The 6.78-Å line is due to diffraction by the plane with Miller indices (TOI) (13). Diffraction by the (200) plane results in a peak at 12.75° 2θ (13). Therefore the integrated area is due to diffraction by both the planes (200) and (TOI), although the major contribution comes from the latter. Similarly in the 2θ range where the 9.93-Å line in II occurred (peak at 8.90° 2θ), there were no peaks in the powder pattern of I. The powder x-ray pattern of II is not listed in the powder diffraction files of the International Center for Diffraction Data (Swarthmore, PA) and the Miller indices of the diffracting planes are not known. The transformation of I to II will result in a progressive decrease in the intensity of the 6.78-Å line and will be accompanied by an increase in the intensity of the 9.93-Å line.

For the purposes of quantitative x-ray studies it is necessary to calculate the mass absorption coefficients of I and II. If $w_1, \ldots, w_n$ are the weight fractions of elements 1, $\ldots, n$ and if $\mu_1^* \ldots \mu_n^*$ are their respective mass absorption coefficients for radiation of a particular wavelength, then the mass absorption coefficient of the compound, μ^*, is (10)

$$\mu^* = \sum_{k=1}^{n} w_k \mu_k^* \qquad (6)$$

The mass absorption coefficients of I and II were calculated to be 5.21 and 5.87 $cm^2\ g^{-1}$ (CuKα radiation), respectively (17). The ratios, $I_{i1}/(I_{i1})_o$, Eq. (5), were calculated for different values of x_1 and x_2. If I is considered to be the unknown, then II formed the matrix, and vice versa. The calculated intensity ratios are presented in Table I.

To begin with, pure I and pure II were packed into 20 × 15 × 2-mm sample holders. Four hundred milligrams of powder was accurately weighed to ensure that the same amount of sample was used each time. In order to minimize preferred orientation, the specimen cavity was filled from the edge (18). Repacking of the same sample or packing of a

Table I. The Intensity Ratios $[I_{i1}/(I_{i1})_o]$ Calculated using Eq. (5)[a]

Wt fraction		Calculated intensity ratio $[I_{i1}/(I_{i1})_o]$	
I	II	6.78-Å line[b]	9.93-Å line[c]
0.00	1.00	0.00	1.00
0.10	0.90	0.09	0.91
0.20	0.80	0.18	0.82
0.40	0.60	0.37	0.63
0.60	0.40	0.57	0.43
0.80	0.20	0.78	0.22
0.90	0.10	0.89	0.11
1.00	0.00	1.00	0.00

[a] The 6.78-Å line is unique to I and the 9.93-Å is unique to II.
[b] In this series, $\mu_1^* = 5.21\ cm^2\ g^{-1}$ and $\mu_2^* = 5.87\ cm^2\ g^{-1}$. The theoretical line in Fig. 3 is based on these values.
[c] In this series $\mu_1^* = 5.87\ cm^2\ g^{-1}$ and $\mu_2^* = 5.21\ cm^2\ g^{-1}$. The theoretical line in Fig. 4 is based on these values.

fresh sample resulted in integrated area values that varied considerably (CV about 15%). When mixtures of I and II were prepared, the experimental intensity ratio $[I_{i1}/(I_{i1})_o]$ showed a similarly high variability and also did not agree well with the theoretical ratios predicted in Table I. Some of the possible sources of error were evaluated.

Preferred Orientation

Microscopic examination of I revealed irregularly shaped crystals. More than 95% of the particles were less than 3 μm (the longest dimension) in size. The remaining particles were 3–6 μm in size.

In case of II, particles either were irregularly shaped or were needle shaped and a sizable fraction was larger than 5 μm. Therefore the longest dimensions of 1000 particles were measured and 61.2% of these particles were in the size range of 1–5 μm, 19.5% of the particles in the 6- to 10-μm range, 18.3% of the particles ranged in size from 11 to 50 μm, and 1% of the particles were in the range 51 to 130 μm. Because of the large size of some of these particles, II was ground for 5 min in a ball mill. As a result, more than 95% of the particles were less than 3 μm and the remaining particles were in the size range of 3 to 6 μm. Particles above 6 μm in size were not found. The use of such fine particles should substantially aid in minimizing preferred orientation effects. In all further experiments, II ground for 5 min in a ball mill was used and the specimen cavity was filled from the edge.

It was also necessary to ensure that grinding for 5 min did not cause dehydration of II. The powder x-ray diffraction pattern of ground II was identical to that of unground II, with no trace of the 6.78-Å line (the line unique to I). Moreover, the weight loss on heating of both unground and ground II in the DSC was about 13.0% (w/w).

Extinction

In addition to the possible problems of preferred orientation outlined above, particle size can affect the diffracted intensity of x-ray in other ways. It has been observed that the diffraction intensity from substances crystallizing with a high degree of perfection decreases when the crystallites are larger than 10–15 μm (12). This is known as primary extinction. In particles smaller than this size, errors due to primary extinction are negligible. Particles of I and II were all less than 10 μm in size. Moreover, it is unlikely that these crystals have a very high degree of perfection. Therefore, primary extinction effects are unlikely in our samples.

Maximum Acceptable Particle Size

According to Brindley (19), the maximum acceptable particle size, t_{max}, in quantitative x-ray analysis is

$$t_{max} = \frac{1}{100\ \bar{\mu}} \tag{7}$$

where $\bar{\mu}$ is the average linear absorption coefficient of the material composing the powder. This formula assumes that absorption of x-rays within each particle $[-(dI/I)$ in Eq. (1)] is 1% and calculates the maximum particle size for satisfactory averaging of the absorption process. The density of I has been reported to be 1.3 g cm^{-3} (20). Its linear absorption coefficient was calculated (12) to be 6.8 cm^{-1} (CuKα radiation). The density of II was not available in the literature and was determined by the liquid floatation method to be 1.3 g cm^{-3} at room temperature (21). The linear absorption coefficient of II was calculated to be 7.6 cm^{-1}. Based on these linear absorption coefficient values, the t_{max} values of I and II were calculated to be 15.4 and 14.1 μm, respectively. Since the size of the particles was microscopically observed to be less than 10 μm problems due to particle size are unlikely.

Microabsorption

The accuracy of intensity measurements can also be affected by a phenomenon called microabsorption. Suppose a powder is a mixture of two phases, α and β. The problems of microabsorption are negligible if the following conditions are met: (i) $\mu_\alpha = \mu_\beta$, where μ is the linear absorption coefficient, and (ii) both phases have the same particle size or both phases consist of extremely fine particles (10). In our case, the linear absorption coefficient values of I and II are close to one another and both I and II occur as extremely fine particles. Therefore, microabsorption effects are likely to be negligible.

Statistical Accuracy of Counter Measurements

The magnitude of statistical errors depends on the total number of photons counted (12). The percentage standard deviation, 100 σ_ρ, in the net peak area is given by

$$100\ \sigma_\rho = \frac{100(N_T + N_B)^{1/2}}{N_T - N_B} \tag{8}$$

where N_T is the integrated counts for the peak (including the background) and N_B is the integrated counts for the background. The percentage standard deviation in the net peak area for one peak unique to I (12.60 to 13.39° 2θ) and one peak unique to II (8.20 to 9.19° 2θ) were calculated for various mixtures of I and II (Table II). The results indicate that at all the compositions studied, the statistical accuracy was acceptable.

Table II. The Standard Deviation in the Area of the Peaks (Corrected for Background) of I and II

Wt. fraction		Standard deviation in the net peak area (%)	
I	II	Peak unique to I (d-spacing 6.78 Å)	Peak unique to II (d-spacing 9.93 Å)
1.00	0.00	0.23	—
0.80	0.20	0.28	1.46
0.65	0.35	0.30	0.72
0.50	0.50	0.39	0.66
0.65	0.35	0.41	0.43
0.20	0.80	0.56	0.36
0.00	1.00	—	0.30

Sample Thickness

If the maximum diffracted intensity from a flat powder specimen is desired, the sample thickness t must satisfy the condition

$$t \geq \frac{3.2 \sin\theta}{\mu^* \rho'} \quad (9)$$

where θ is the incident angle of the x-ray and ρ' the density of the powder including interstices (12). The sample cell volume was 0.6 cm^3, into which 0.4 g of sample was loaded. The calculated value of ρ' was 0.67 g cm^{-3}. The sample thickness for I (peak at 13.05° 2θ) must be $\geq$1.1 mm, and for II (peak at 8.90° 2θ) t must be $\geq$0.7 mm. For mixtures of I and II, the minimum thickness required would range between 0.7 and 1.1 mm and would depend on the sample composition. Since all our samples were 2 mm thick, they satisfy Eq. (9) and there would be no loss in intensity due to inadequate powder thickness.

Phase Transition in Mixtures

Mixtures of I and II were stored at room temperature in a chamber maintained at 52% RH until subjected to x-ray analysis. It was necessary to ensure that transition of I to II or vice versa did not occur under these conditions. In a different study, I and II had been stored under various humidities at 25°C. The transition of I to II occurred at RH $\geq$ 92.9% while the transition of II to I occurred at RH $\leq$ 43.0%. Between 43 and 92.9% RH neither I nor II underwent a phase transition (22).

The results suggested that the particle size of the samples and the experimental conditions had been controlled so as to eliminate the major sources of error in quantitative powder x-ray diffractometry. Therefore we have no definitive explanation for the poor reproducibility of the results. It is possible that the sample does not pack uniformly in the sample holder. The differences in sample packing in replicate samples could explain the lack of reproducibility. We believed that compaction of the powder into a tablet may minimize the packing nonuniformity. Therefore the powder was compressed into a tablet in a hydraulic press, mounted in a holder, and subjected to powder x-ray diffraction studies.

Compression of a solid can cause it to undergo a polymorphic transformation (23). However, in our case, the x-ray patterns of the compressed samples and the uncompressed powder were identical over a compression pressure range of 62 to 250 MPa. Therefore it was concluded that when compressed to these pressures, I and II did not undergo any polymorphic transformation. Lefebvre *et al.* (23) had observed that compression did not cause any phase transformation of I.

Since compression is likely to cause preferred orientation, the next step was to determine the effect of compression pressure on the intensities of several peaks of I and II. The changes in the relative peak intensities as a function of compression pressure for the 10 most intense peaks of I and II are shown in Tables III and IV, respectively. In the case of I, the effect of compression pressure becomes clearly evident only at 125 MPa, while in the case of II, the effects are obviously right from the lowest compression pressure.

Table III. Effect of Compression Pressure on the Relative Intensities of the Ten Most Intense Lines of I

		Relative intensity (%)[a]				
			Compressed to			
2θ (deg)	d-spacing (Å)	Uncompressed	62 MPa	125 MPa	187 MPa	250 MPa
13.05	6.78	100	100	100	100	100
15.25	5.81	95.5	96.6	109	111	114
15.85	5.59	45.7	44.2	50.0	53.1	54.9
18.65	4.75	54.1	60.2	61.6	67.1	68.0
20.35	4.35	37.3	31.8	41.5	42.0	45.9
23.35	3.81	47.3	52.3	60.0	60.2	61.5
23.90	3.72	39.7	37.2	41.5	40.7	41.6
24.85	3.58	66.4	67.1	73.7	75.3	77.6
26.70	3.34	46.6	45.0	52.0	54.2	57.9
27.50	3.26	88.7	87.1	101	112	113

[a] For each of the 10 lines, the relative intensity was calculated as follows: (peak intensity of the line × 100)/peak intensity of 6.78-Å line.

It is not possible to prevent the preferred orientation of the particles during compression. When compressed to a certain pressure, if particles tend to orient in only one specific manner, then the variability in the area of a particular peak should be small in replicate samples. This must also be true for all of the diffraction peaks. In such an event, the problems due to preferred orientation are eliminated because our interest is not the absolute intensity of any peak but the intensity ratio $I_{i1}/(I_{i1})_o$ [Eq. (5)]. A compression pressure of 125 MPa was chosen. This was the lowest pressure at which satisfactory tablets were obtained. Table V shows that the CV values for the integrated intensities of the 10 most intense peaks of I are all below 4%. Occasionally it is possible to perform quantitative studies with the peak (maximum)

Table IV. Effect of Compression Pressure on the Relative Intensities of the Ten Most Intense Lines of II

		Relative intensity (%)[a]				
			Compressed to			
2θ (deg)	d-spacing (Å)	Uncompressed	62 MPa	125 MPa	187 MPa	250 MPa
8.90	9.93	45.5	76.3	86.8	102	106
12.30	7.19	64.4	130	165	195	197
18.90	4.69	31.4	49.7	64.3	72.4	71.8
19.45	4.56	100	100	100	100	100
24.20	3.68	81.0	103	124	131	129
24.70	3.60	30.0	53.8	70.2	81.3	80.7
28.55	3.12	47.8	70.7	86.5	95.9	96.4
29.20	3.06	36.4	53.4	67.1	72.2	71.8
37.50	2.40	22.2	40.2	149	152	154
38.45	2.34	31.3	46.6	61.8	70.2	69.4

[a] For each of the 10 lines, the relative intensity was calculated as follows: (peak intensity of the line × 100)/peak intensity of 4.56-Å line.

Table V. CV of the Areas and Intensities of the Ten Most Intense Lines of Compressed (to 125 MPa) I (n = 3)

Peak area		Peak intensity	
Integration angle (deg 2θ)	CV of integrated counts (%)	2θ (deg)	CV of peak counts (%)
11.55–13.40	3.8	13.05	4.0
14.45–15.55	1.9	15.25	1.5
15.55–16.35	0.7	15.85	2.1
17.95–19.00	1.1	18.65	2.0
19.95–21.25	2.2	20.40	4.2
22.35–23.65	1.6	23.35	3.4
23.65–24.20	1.1	23.90	1.8
24.20–25.60	1.3	24.85	1.1
25.85–26.85	0.6	26.65	0.4
26.85–28.25	0.6	27.35	1.2

intensity. This is possible only when it can be established that there is a constant proportionality between peak and integrated intensities (10). Interestingly, Table V also reveals that the CV values of the peak intensities are all less than 5% suggesting that these lines have the same shape in replicate samples. This was also confirmed by visual examination of the powder patterns. These observations lead to the conclusion that when compressed to a certain pressure, the particles orient in only one manner. The results of Table VI lead to a similar conclusion in the case of II. Finally, the effect of the presence of I on the intensity of peaks of II and vice versa were studied. It was not possible to determine the integrated intensities of the 10 most intense peaks because of the frequent overlap of peaks of I and II. Therefore the peak intensities of the five most intense peaks of I and II were determined in 50:50 (w/w) mixtures of I and II and the results are presented in Table VII. The low CV values suggest that in the presence of II, particles of I orient in only one manner and a similar conclusion can be drawn about the packing of particles of II in the presence of I.

Mixtures containing various weight fractions of I and II as well as pure samples of I and II were compressed and the integrated intensities of the 6.78-Å line (unique to I) and the

Table VI. CV of the Areas and Intensities of the Ten Most Intense Lines of Compressed (to 125 MPa) II (n = 3)

Peak area		Peak intensity	
Integration angle (deg 2θ)	CV of integrated counts (%)	2θ (deg)	CV of peak counts (%)
8.15–9.20	0.6	8.90	3.4
11.65–12.85	0.3	12.30	5.7
18.65–19.10	0.4	18.90	1.2
19.10–20.10	2.2	19.45	0.6
23.55–24.55	1.0	24.20	1.9
24.55–25.20	2.1	24.70	1.8
27.95–28.85	1.2	28.55	2.2
28.85–29.80	2.0	29.20	0.1
36.95–38.05	1.2	37.50	3.2
38.05–39.10	0.8	38.45	1.6

Table VII. CV of the Peak Intensities of the Five Most Intense Lines of I and II Following Compression to 125 MPa of 50:50 (w/w) Mixtures of I and II (n = 3)

Peaks unique to I			Peaks unique to II		
2θ (deg)	d-spacing (Å)	CV (%)	2θ (deg)	d-spacing (Å)	CV (%)
13.05	6.78	5.0	8.90	9.93	5.5
15.25	5.81	1.8	12.30	7.19	3.0
18.65	4.75	3.0	19.45	4.56	2.0
24.80	3.59	1.7	24.20	3.68	2.7
27.50	3.24	4.5	28.55	3.12	4.5

9.93-Å line (unique to II) were determined. The results are presented in Figs. 3 and 4. The line in Fig. 3 is based on the theoretical calculations given in Table I, while the points are experimental values. There is a good agreement between the theoretical and experimental intensity ratios. In the case of II we again see a good agreement between the theoretical line based on Table I and the experimental observations (Fig. 4).

The usefulness of powder x-ray diffractometry for the quantitative analysis of a mixture which consists of the anhydrous and hydrated forms of a compound has been demonstrated. The technique is capable of quantifying the relative amounts of the two phases without any interference

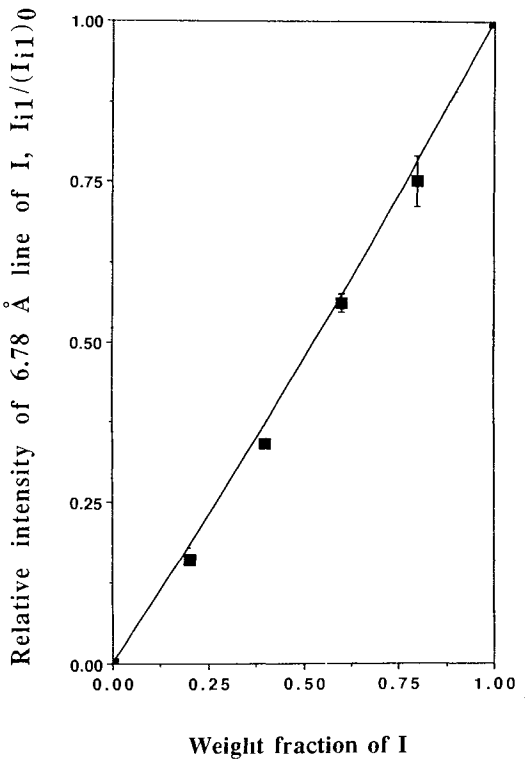

Fig. 3. The relative intensity of the 6.78-Å line of I as a function of the weight fraction of I in binary mixtures of I and II. The line is based on theoretical values (Table I), while the data points are experimental measurements. Each point is the mean of three determinations. Error bars represent standard deviations; in many cases they were smaller than the size of the symbol.

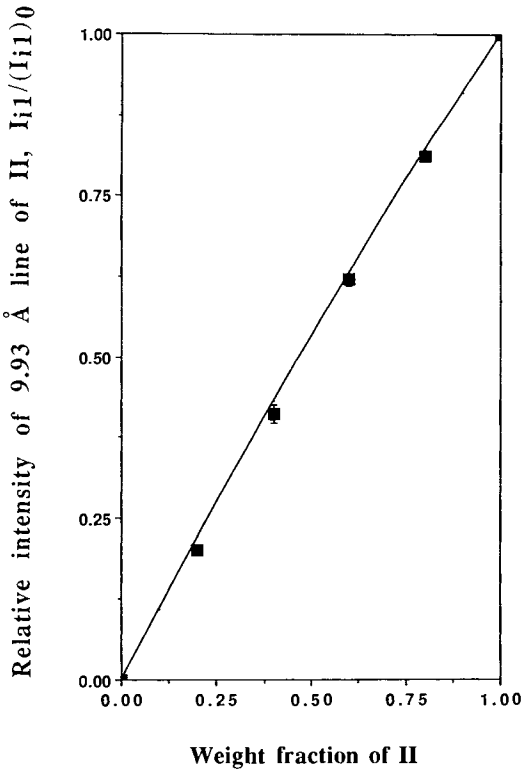

Fig. 4. The relative intensity of the 9.93-Å line of **II** as a function of the weight fraction of **II** in binary mixtures of **I** and **II**. The line is based on theoretical values (Table I), while the data points are experimental measurements.

from the physically bound water in the samples. Other possible applications of this technique are under investigation.

ACKNOWLEDGMENTS

The technical assistance of undergraduate students Ms. Mandana Asgharnejad and Ms. Winnie Young is gratefully acknowledged. Dr. Marlana Blackburn and Dr. Aharon Weissman provided many useful suggestions. Financial support was provided by the Graduate School, University of Minnesota, and by the American Association of Colleges of Pharmacy under the Grant Program for Young Investigators.

REFERENCES

1. J. W. M. Jongmans. *Epilepsia* 5:74–82 (1964).
2. S. Blom. *Lancet* 1:839–840 (1962).
3. M. Windholz (ed.). *The Merck Index,* 10th ed., Merck and Co., Rahway, N.J., 1983, p. 1758.
4. P. Kahela, R. Aaltonen, E. Lewing, M. Anttila, and E. Kristoffersson. *Int. J. Pharm.* 14:103–112 (1983).
5. E. Laine, V. Tuominen, P. Ilvessalo, and P. Kahela. *Int. J. Pharm.* 20:307–314 (1984).
6. E. Shefter and T. Higuchi. *J. Pharm. Sci.* 52:781–791 (1963).
7. S. Niazi. *J. Pharm. Sci.* 67:488–491 (1978).
8. W. J. Blaedel and V. W. Meloche, *Elementary Quantitative Analysis,* 2nd ed., Harper and Row, New York, 1963, pp. 150–156.
9. W. E. Garner. In W. E. Garner (ed.), *Chemistry of the Solid State,* Academic Press, New York, 1955, pp. 213–231.
10. B. D. Cullity. *Elements of X-Ray Diffraction,* 2nd ed., Addison-Wesley, Reading, Mass., 1978, pp. 13, 226, 417–418.
11. L. Alexander and H. P. Klug. *Anal. Chem.* 20:886–889 (1948).
12. H. P. Klug and L. E. Alexander. *X-Ray Diffraction Procedures for Polycrystalline and Amorphous Materials,* 2nd ed., Wiley, New York, 1974, pp. 95, 294, 362, 531–536.
13. Pattern No. 33-1565 (β-carbamazepine), Joint Committee on Powder Diffraction Standards, International Center for Diffraction Data, Swarthmore, PA.
14. M. M. J. Lowes, M. R. Caira, A. P. Lotter, and J. G. Van Der Watt. *J. Pharm. Sci.* 76:744–752 (1987).
15. N. Kaneniwa, T. Yamaguchi, N. Watari, and M. Otsuka. *Yakugaku Zasshi* 104:184–190 (1984).
16. G. Reck and G. Dietz. *Cryst. Res. Technol.* 21:1463–1468 (1986).
17. C. H. Macgillavry and G. D. Rieck (eds.). *International Tables for X-ray Crystallography, Vol. III,* 2nd ed., D. Reidel, Dordrecht, Holland, 1983, p. 162.
18. A. M. Bystrom-Asklund. *Am. Mineral.* 51:1233–1237 (1966).
19. G. W. Brindley. In G. Brown (ed.), *The X-Ray Identification and Crystal Structures of Clay Minerals,* 2nd ed., Mineralogical Society, London, 1961, p. 492.
20. V. L. Himes, A. D. Mighell, and W. H. De Camp. *Acta Crystal.* B37:2242–2245 (1981).
21. C. A. Hutchison and H. L. Johnston. *J. Am. Chem. Soc.* 62:3165–3168 (1940).
22. R. Suryanarayanan. AAPS Midwest Regional Meeting, Chicago, 1988, poster number 13.
23. C. Lefebvre, A. M. Guyot-Hermann, M. Draguet-Brughmans, R. Bouche, and J. C. Guyot. *Drug Dev. Ind. Pharm.* 12:1913–1927 (1986).