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Estimation of the degree of crystallinity in digoxin by X-ray and infrared methods

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The X-ray procedure for estimation of the degree of crystallinity in digoxin is based upon measurement of the total X-ray scattering and the scattering from crystalline regions of the drug. The infrared procedures are based upon measurement of the peak height ratios, 1775/1618 and 3095/1618 cm^{-1}. Correlation between results obtained by the two methods is good. These methods are of value in the physico-chemical characterization of digoxin, particularly as the properties may be altered by comminution.

Studies by Florence, Salole & Stenlake (1974) and Florence & Salole (1975) showed that ball milling may lead to a transformation of digoxin from the crystalline to the amorphous state, with a consequent increase in the dissolution rate and the apparent solubility. Other workers (Jounella & Sothmann, 1973; Shaw, Carless & others, 1973) demonstrated that the bioavailability of digoxin depends upon particle size and solubility and/or dissolution rate of the drug administered. Thus, the bioavailability of digoxin may be dependent in part upon whether the drug administered is amorphous or crystalline, or is a mixture of these states. Dosage forms manufactured from drug in the amorphous state may be of questionable stability because of the tendency of the metastable amorphous form to revert to the stable crystalline form (Szinai, Harder & Tulley, 1975). These considerations indicate a need for analytical methods to measure the degree of crystallinity in digoxin. The degree of crystallinity is defined as the percentage of crystalline drug in a sample containing drug in both the amorphous and crystalline states.

X-Ray procedures for estimation of the degree of crystallinity in organic materials have been described by Klug & Alexander (1974). These procedures require measurement of the X-ray scattering from the entire sample and from the crystalline regions of the samples.

The degree or per cent crystallinity, C, can be calculated from

$$C = \frac{I_c}{I_c + I_a} \times 100 \quad .. \quad .. \quad (1)$$

where I_c and I_a are the intensities of X-rays scattered from the crystalline and amorphous regions respectively. The intensity of X-rays scattered from the crystalline regions of a specimen is proportional to the area under the sharp peaks of the diffraction spectrum, while the intensity of radiation scattered from the entire sample ($I_c + I_a$) is proportional to the total area under the curve. The intensity of radiation scattered from the amorphous regions is proportional to the area under the broad, diffuse peak which underlies the crystalline peaks.

Infrared procedures for measuring the degree of crystallinity are based upon estimation of the height of peaks characteristic of the crystalline state with reference to peaks which are independent of the state of the drug. This procedure has been used, for example, to calculate the relative amounts of inactive polymorph in chloramphenicol palmitate (Borka & Backe-Hansen, 1968).

MATERIALS AND METHODS

Chemicals. Digoxin U.S.P. was obtained from five commercial manufacturers and coded from A to E inclusive. Digoxigenin, digoxigenin monodigitoxoside and digoxigenin bisdigitoxoside were obtained from the Wellcome Foundation Limited, Dartford, Kent.

Amorphous digoxin was prepared by comminution of 1 g of product A for 15 min at −196° in a freezer mill (Spex Industries Inc., Metuchen, N.J., U.S A.).

Samples of differing crystallinity were prepared by shaking mixtures of crystalline (product A) and amorphous digoxin in glass vials. The mixtures contained 0, 20, 40, 60, 80 and 100% amorphous digoxin.

X-Ray spectra (Philips X-Ray Diffractometer Model No. P.W. 1130/00/60). Drug samples, 28 ± 0·5 mg,

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were pressed into a sample holder consisting of a brass plate with a concave cavity, 10 mm diameter by 1·5 mm in maximum depth. Samples were irradiated with MoK_α X-rays over a range of 2θ from 30 to 3°. Recorder sensitivity and time constant were 4×10^3 counts s^{-1} full scale and 1 s respectively. Total areas and areas under the peaks were estimated by making photocopies of the original traces, cutting the appropriate areas from the photocopies, and weighing.

Infrared spectra. Samples were prepared by mixing 4 mg of drug with 196 ± 5 mg of potassium bromide for 2 s in a WIG-L-BUG (Cresent Dental Mfg. Co., Chicago, U.S.A.). The P.E. 621 spectrophotometer was calibrated to the peak at 1603 cm^{-1} in a polystyrene film. Although spectra in Fluorolub or Nujol may be preferable for studying changes in physical state (Borka & Backe-Hansen, 1968), in our experience digoxin spectra in these media were poorly resolved.

Thin-layer chromatography. The procedure was that of White & Oeth (1966). Precoated Silica Gel G plates, 0·25 mm, and a solvent system of chloroform–methylethylketone in a ratio of 1:4 were used. Plates were developed in equilibrated, filter paper lined tanks for 30 min, blown dry, and sprayed consecutively with 5% *m*-dinitrobenzene and 16% sodium hydroxide solution. The purple spots were marked at once as rapid fading occurred. By this method, no evidence of chemical degradation after the freezer mill treatment was found.

Table 1. *Ratios of X-ray scattering: crystalline to total peak areas.*

Product	Area ratio
A	0·53[a]
B	0·52
C	0·56
D	0·54
E	0·55

[a] Mean of three determinations.

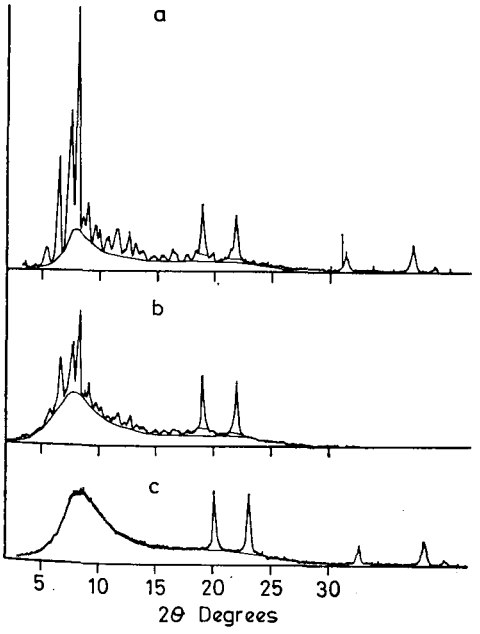

FIG. 1. X-Ray spectra of digoxin; (a) crystalline (product A), (b) 40% crystalline, (c) amorphous. The peaks at 2θ values of 19 and 22° are due to the β-brass sample holder and serve as reference peaks.

RESULTS AND DISCUSSION

The X-ray diffraction spectra of crystalline (product A) and amorphous digoxin and a mixture of the two are presented in Fig. 1. That portion of the spectrum which would appear to be due to scattering from the amorphous region of the curve has been drawn in. According to equation 1 product A (Fig. 1 (a)) would be about 50% crystalline. However, examination of product A under a polarizing microscope suggests it to be crystalline. The broad peak in the X-ray spectrum may be due to other factors such as overlap of the crystalline peaks. There is little difference in the ratios of the area

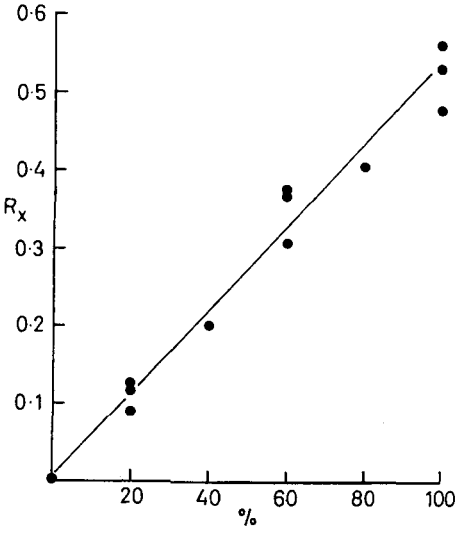

FIG. 2. The degree of crystallinity (%) *vs* R_x, the ratio of the area under the diffraction peaks to the total area.

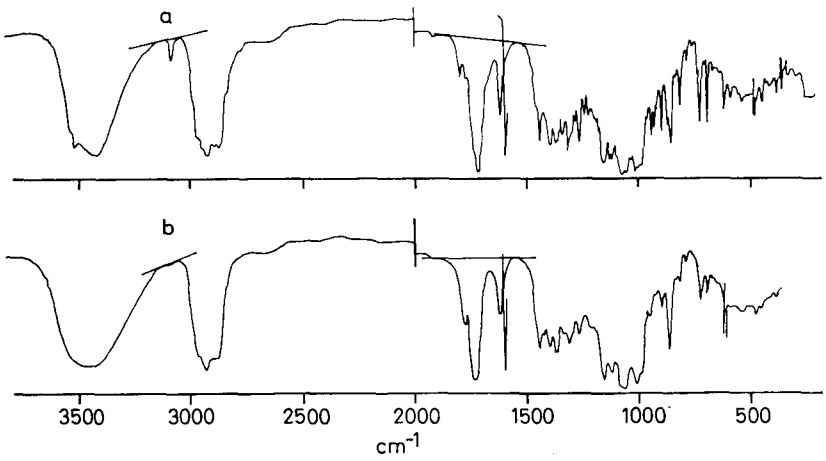

FIG. 3. Infrared spectra of digoxin; (a) crystalline (product A), (b) amorphous.

under the crystalline peaks to total area, R_x, of the five commercial products (Table 1). This is indicative that all are 100% crystalline, otherwise various degrees of crystallinity would be expected in products from different sources.

Equation 1 predicts a linear relation between the degree of crystallinity and R_x. This was tested by plotting R_x *vs* the degree of crystallinity in mixtures made up of product A and amorphous digoxin (Fig. 2). Repeat measurement of samples which are 20, 60 and 100% crystalline suggests a reproducibility in the measurement of about ±10%. The line of best fit is given in Fig. 2.

Amongst the differences in the infrared spectra of crystalline (product A) and amorphous digoxin are peaks at 1800 and 3095 cm^{-1} in crystalline digoxin which are not present in the amorphous material, and a peak at 1775 cm^{-1} which increased in the amorphous specimen (Fig. 3). There are also a number of differences in the region between 500 and 1500 cm^{-1}.

To utilize these differences to calculate the percent crystallinity, peak heights were measured from base lines drawn between the minima at about 3050 and 3150 cm^{-1} and between 1550 and 1900 cm^{-1}, as shown in Fig. 3.

The ratios, R_{3095}, the height of the peak at 3095 cm^{-1} to the height of the peak at 1618 cm^{-1} and R_{1775}, the height of the peak at 1775 cm^{-1} to the height of the peak at 1618 cm^{-1}, of the five

Table 2. *Ratios of infrared peaks.*

Product	R_{1775}	R_{3095}
A	0·43[a]	0·229[a]
B	0·41	0·219
C	0·43	0·219
D	0·46	0·194
E	0·42	0·243

[a] Mean of three determinations.

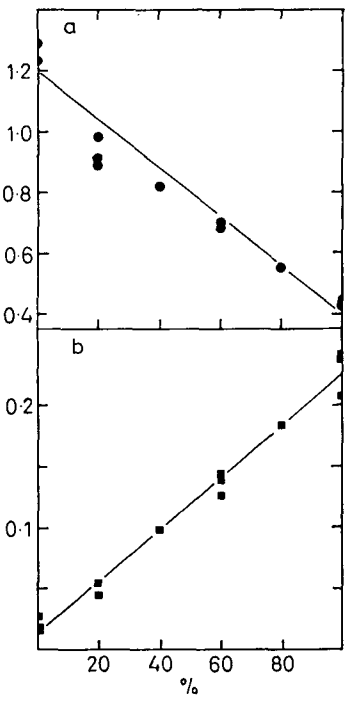

FIG. 4. Ratio of peak heights, (a) 1775/1618 and (b) 3095/1618 *vs* the degree of crystallinity (%).

Table 3. *Comparison of X-ray and infrared methods.*

Commin. time (s)	Degree of crystallinity (%) X-ray	R_{1775}	R_{3095}
5	58	53	58
15	48	45	52
30	39	36	40
60	3	6	8

commercial products are given in Table 2. Again the ratios for all five products are similar.

The ratios R_{1775} and R_{3095} were determined for mixtures of crystalline (product A) and amorphous digoxin. The ratios were plotted against the degree of crystallinity, taking the crystallinity of product A to be 100% (Fig. 4). In both cases the relation can be represented as linear but a better fit is obtained from R_{3095}. This may be due to overlap of the peak at 1775 cm^{-1} by the intense peak at 1730 cm^{-1}. The reproducibility on repeat measurements is about $\pm 10\%$.

Samples of product A were comminuted in the freezer mill for various periods of time, and the degree of crystallinity determined by the X-ray and infrared methods. The results (Table 3) show good agreement between the X-ray and infrared procedures.

The degree of crystallinity of a solid substance is an important physico-chemical property. Changes in the degree of crystallinity may affect the processing characteristics, dissolution rate, bioavailability and stability of drugs. The procedures and concepts described should be applicable to other drugs.

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