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Non-thermal methods in characterization of anhydrous digoxin and two digoxin hydrates

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Summary

Anhydrous digoxin, digoxin · $\frac{1}{4}H_2O$ and digoxin · $\frac{1}{2}H_2O$ were characterized using X-ray powder diffractometry, diffuse reflectance Fourier transform infrared spectroscopy, intrinsic dissolution rates and scanning electron microscopy. X-ray powder diffraction patterns for the different forms showed small differences in the *D* values and intensities; differences were found in the ratio of the diffraction bands at $2\theta \approx 16.7$, 17.9 and 14.6°. FTIR positions were the same for the different forms. Initial dissolution rates were in the order of anhydrous > digoxin · $\frac{1}{4}H_2O$ > digoxin · $\frac{1}{2}H_2O$; both anhydrous digoxin and digoxin · $\frac{1}{4}H_2O$ converted to the hemihydrate over a period of 2 h. There is evidence that at least one higher hydrate of digoxin exists. Digoxin · $\frac{1}{4}H_2O$ consisted mainly of thin plates and digoxin · $\frac{1}{2}H_2O$ was relatively large crystals arranged in clusters as seen on SEM photomicrographs.

Introduction

Crystalline digoxin has been prepared by various researchers and investigated for the existence of either polymorphic forms and/or solvates (Chiou and Kyle, 1979; Draguet-Brughmans et al., 1985). However, these authors could not indicate the existence of polymorphism or pseudopolymorphism. Different investigators reported similar X-ray powder diffraction patterns and infrared spectra for crystalline samples prepared by various methods.

The preparation of anhydrous digoxin, digoxin · $\frac{1}{4}H_2O$ and digoxin · $\frac{1}{2}H_2O$ using different solvent systems and recrystallization temperatures has been reported in the preceding paper (Botha and Flanagan, 1992). Identification of these forms was achieved using a combination of thermogravimetric analyses (TGA) and coulometric Karl Fischer water determinations. In the present study, in order to characterize the prepared digoxin forms, X-ray powder diffraction patterns (XRD) and diffuse reflectance Fourier transform in-

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frared (FTIR) spectra were obtained. We were able to show differences in the ratios of the three main X-ray diffraction bands; FTIR spectra for the three forms differed only in peak intensities, the peak positions being essentially the same. Intrinsic dissolution rates for the different crystal forms were obtained; anhydrous digoxin had the fastest initial dissolution rate and digoxin · $\frac{1}{2}H_2O$ the slowest initial intrinsic dissolution rate. Both anhydrous digoxin and digoxin · $\frac{1}{4}H_2O$ converted to the hemihydrate over a period of time. Although the different forms were plates, as observed under scanning electron microscopy (SEM), the forms were different in size.

There are numerous reports of amorphous or crystalline digoxin being prepared by various methods. Nürnberg and Dölle (1980) prepared crystalline digoxin, as determined by X-ray diffraction studies, by spray drying a digoxin suspension in methanol:water. Amorphous digoxin, as determined by X-ray diffraction, has also been prepared by spray-drying (Nürnberg and Werthmann, 1978; Nürnberg and Dölle, 1980, 1983a), foam-drying (Nürnberg and Werthmann, 1978), rotary evaporation (Müller and Eckert, 1980) or rapid evaporation (Draguet-Brughmans et al., 1985). Amorphous spray-dried digoxin showed no X-ray diffraction peaks when heated to a temperature of 150°C; a further increase in temperature above 155°C showed the three major X-ray diffraction peaks of crystalline digoxin (Nürnberg and Dölle, 1983b).

Reported IR spectra of commercial samples in KBr pellets were similar, but not identical; peak intensities, peak widths and shoulders differed (Draguet-Brughmans et al., 1980). Crystalline material had absorption bands at 1800 and 3095 cm^{-1}, which were absent in amorphous digoxin (Black and Lovering, 1977; Nürnberg and Dölle, 1983b). After comminuting crystalline digoxin, some of the IR absorption bands in the 500–1500 cm^{-1} frequency range lost their definition and a shoulder appeared at 1775–1780 cm^{-1}, which grew with increased comminution to a distinct peak (Florence et al., 1974; Black and Lovering, 1977; Nürnberg and Dölle, 1983b). The degree of crystallinity can be estimated (Black and Lovering, 1977) from the ratios of peak height at 3095 cm^{-1} to that at 1618 cm^{-1} and that of the peak height at 1775–1780 cm^{-1} to that at 1618 cm^{-1}.

The aqueous solubilities of commercial digoxin samples have been reported to range from 24.3 to 60.2 μg/ml (Florence et al., 1974; Florence and Salole, 1976) at 25°C, and from 28.9 to 68.2 μg/ml (Chiou and Kyle, 1979) at 37°C. Increases of 7–118% in powder dissolution rates for milled samples were found and attributed to the formation of amorphous digoxin (Florence et al., 1974; Florence and Salole, 1976: Lovering et al., 1977). Trituration enhanced the solubility of one commercial sample by 44%, but had a negligible effect on the solubility of two other commercial samples (Chiou and Kyle, 1979). This failure of solubility enhancement by trituration was due to conversion of the amorphous form to a crystalline form during the solubility study period (Chiou and Kyle, 1979). A decrease in dissolution rate of triturated digoxin was ascribed to the dissolution of crystalline material (Florence et al., 1974; Nürnberg and Werthmann, 1978). The equilibrium solubilities were not identical (Florence et al., 1974) and these authors attributed the results to different digoxin forms. The amount dissolved from amorphous digoxin was about 2.5-times higher than that from crystalline material, yet the particle size distribution of the amorphous form showed a greater proportion of large particles than the crystalline material (Draguet-Brughmans et al., 1985). Digoxin recrystallized from chloroform or from ethanol had enhanced solubility; the solubility of the two recrystallized samples differed by 24% (Chiou and Kyle, 1979).

Depending upon the rotation speed during foam-drying, amorphous material had a dissolution rate 13–16-fold higher than that of crystalline digoxin, but the particle size of the foam-dried material was larger than that of the spray-dried material (Nürnberg and Werthmann, 1978). Spray-dried digoxin had an about 8-fold higher dissolution rate than crystalline material (Nürnberg and Werthmann, 1978; Nürnberg and Dölle, 1983b). After about 90 min the apparent solubilities for foam-dried and spray-dried material decreased to that for the crystalline material (Nürnberg and Werthmann, 1978). The intrinsic dissolution rates of milled digoxin standards were

about 2–3-fold higher than that of the crystalline standards; two samples showed a 4.5–10-fold increase in the intrinsic dissolution rate (Salole and Florence, 1975; Florence and Salole, 1976).

Materials and Methods

Digoxin samples were obtained from Sigma Chemical Co. (St. Louis, MO), Spectrum Chemical Mfg. Corp. (Gardena, CA) and as a gift from Burroughs-Wellcome Co. (Research Triangle Park, NC) (D_1, D_2 and D_3, respectively) and were used as received. Digoxin reference standards (D_4 and D_5) were obtained from Sigma Chemical Co. and USPC (Rockville, MD). All solvents used were of analytical grade. Digoxin obtained from Sigma was used in the preparation of the different forms from various solvent systems using recrystallization techniques at room temperature and 60°C (Botha and Flanagan, 1992).

X-ray powder diffraction

Powder diffractograms were recorded on an automated Philips PW1710 diffractometer using Philips Analytical PCAPD diffraction software. The hemihydrate was slightly ground in an agate mortar, the other forms were used unground. Samples were spread on double-sided tape on a glass plate placed into a standard aluminum sample holder. The powder patterns were recorded under the instrument conditions: 40 kV, 30 mA; scan rate, 3° 2θ/min; angular range, 2–50° 2θ; automatic divergence slit, receiving slit, 0.2°.

Infrared spectroscopy

Mixtures of 2% sample in powdered potassium chloride were packed into the sample cup of a diffuse reflectance cell and the spectra determined using a Nicolet 5DXB Fourier transform infrared spectrophotometer, connected to a Nicolet 5DX Data Processor. Additive inteferogram scans (300) at a gain of 4 were collected.

HPLC assay procedure

The USP XXII procedure for the assay of digoxin elixir was followed. A Shimadzu chro-

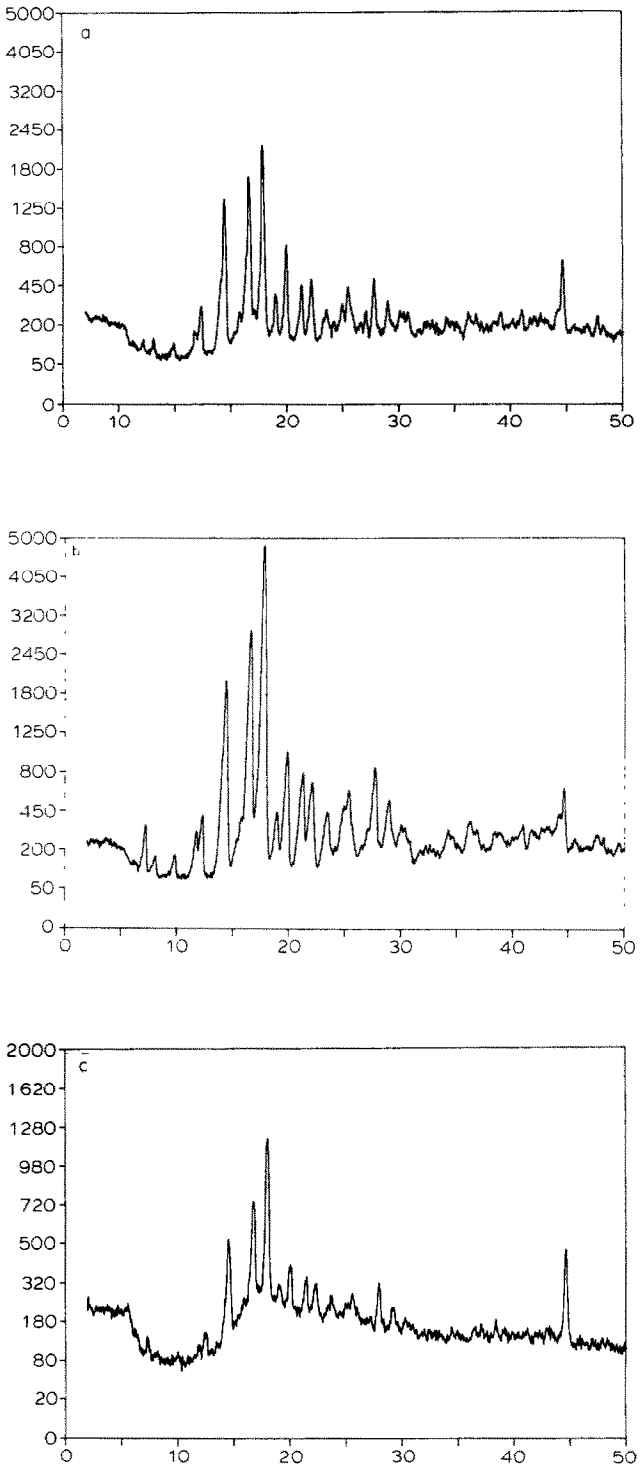

Fig. 1. X-ray diffractograms of commercial samples D_3 (a), D_4 (b) and D_5 (c).

matographic system equipped with liquid chromatographic pump (LC-600), UV detector (SPD-6A), Rheodyne six port manual injector system (Model 7125) and integrator (CR601 Chromatopac) was used, with the following column conditions: Waters μBondapak C_{18}, 3.9×100

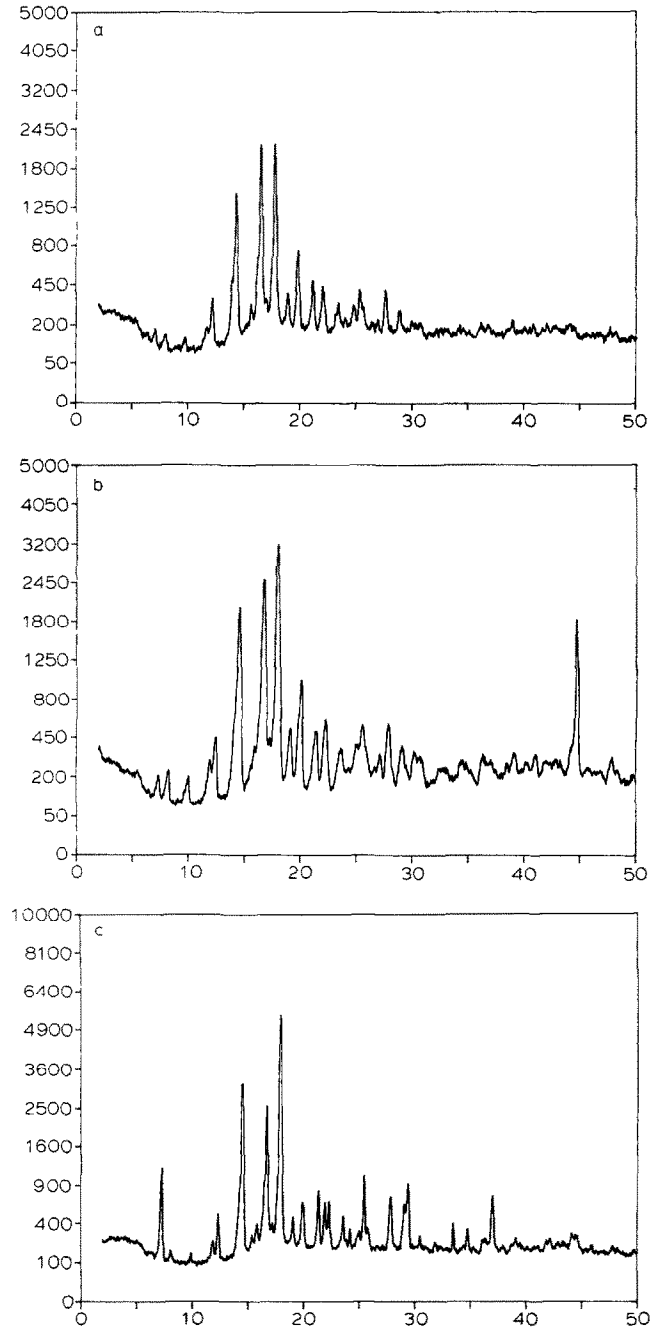

Fig. 2. X-ray diffractograms of anhydrous digoxin (a), digoxin · $\frac{1}{4}H_2O$ (b) and digoxin · $\frac{1}{2}H_2O$ (c).

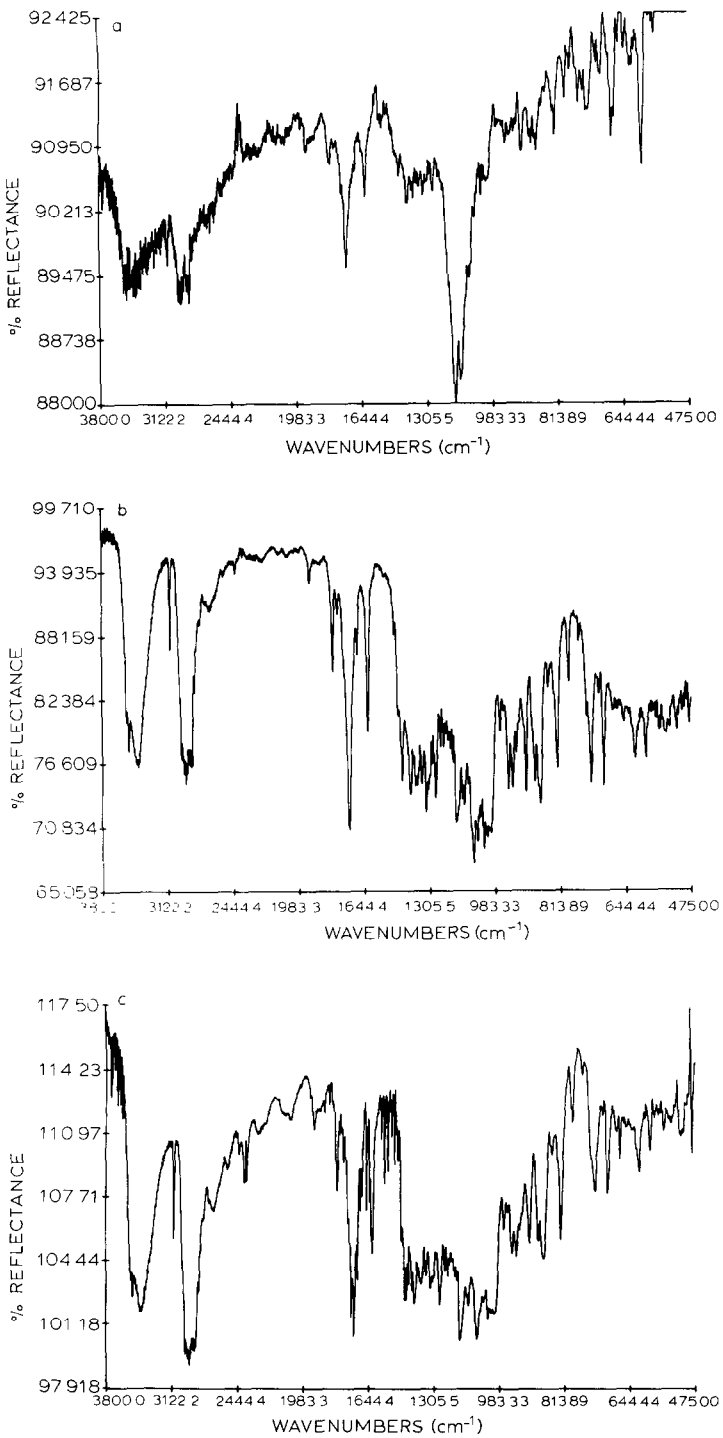

Fig. 3. FTIR spectra of anhydrous digoxin (a), digoxin · $\frac{1}{4}H_2O$ (b) and digoxin · $\frac{1}{2}H_2O$ (c).

mm, 10 μm particle size and a mobile phase flow rate of 0.8 ml/min. The retention time of digoxin was about 6 min.

Intrinsic dissolution rates

The intrinsic dissolution rates of the different crystal forms were determined in a propeller-stirred apparatus (Singh et al., 1968). The crystals were compressed into flat-faced 13-mm pellets at 10000 psi using a Perkin-Elmer potassium bromide pellet punch and die set on a hydraulic press (Pasadena Hydraulic Co.). The tablets were mounted in a Plexiglass holder so that the tablet surface was flush with the holder surface. The holder was mounted in a water-jacketed beaker containing 400 ml of 55.4% aqueous ethanol, maintained at 37 ± 0.1°C by means of a Haake E52 circulator. Stirring was achieved by a Teflon-coated stirrer attached to a Hurst Model CA synchronous motor (120 rpm). The position of the stirrer was such that the closest proximity of the blade to the tablet surface was 6 mm. Samples (2 ml) were withdrawn and replaced with 2 ml of dissolution medium. The amount of digoxin dissolved was determined by HPLC.

Scanning electron photomicrographs

Photomicrographs were obtained using the Hitachi S-570 scanning electron microscope. Samples were coated in vacuo with carbon (Emscope SC 500 Sputter-Coater) and finally with a gold-platinum film.

Results and Discussion

X-ray powder diffraction

X-ray diffractograms of commercial samples D_3–D_5 are shown in Fig. 1 with the five most intense peaks tabulated. The positions of the major peaks for the commercial samples were similar to those reported in previous observations (Black and Lovering, 1977; Draguet-Brughmans et al., 1980; Nürnberg and Dölle, 1983a). The position and intensities of the major peaks were identical for D_1 (not shown), D_2 (not shown) and D_4 (Fig. 1). The positions of the three most intense peaks of D_3 and D_5 were the same as for D_1, D_2 and D_4, but the intensities were different. X-ray diffraction analysis was used by Black and Lovering (1978) to calculate the degree of crystallinity during crystallization of moist amorphous digoxin; the height of the peak at $2\theta = 7.7°$ increased linearly with the degree of crystallinity. Digoxin samples D_1, D_2 and D_4 appeared to have

TABLE 1

FTIR peak ratios

Product	$Ratio_{3094}$	$Ratio_{1805}$
D_1	0.56	0.61
D_2	0.56	0.63
D_3	0.60	0.74
D_4	0.56	0.65
D_5	0.56	0.67
Anhydrous digoxin	0.60	0.62
Digoxin·$\frac{1}{4}H_2O$	0.60	0.67
Digoxin·$\frac{1}{2}H_2O$	0.74	0.67

TABLE 2

Intrinsic dissolution rates for anhydrous digoxin, digoxin·$\frac{1}{4}H_2O$, and digoxin·$\frac{1}{2}H_2O$

Form	Initial rate (mg ml^{-1} cm^{-2} min^{-1})	Intermediate rate (mg ml^{-1} cm^{-2} min^{-1})	Final rate (mg ml^{-1} cm^{-2} min^{-1})
Anhydrous digoxin	0–20 min (i) 7.14×10^{-4} (ii) 7.21×10^{-4}	30–90 min 5.85×10^{-4}	130–420 min 5.27×10^{-4}
Digoxin ·$\frac{1}{4}H_2O$	0–60 min (i) 6.71×10^{-4} (ii) 6.56×10^{-4}		180–360 min 6.21×10^{-4}
Digoxin ·$\frac{1}{2}H_2O$	0–45 min (i) 5.62×10^{-4} (ii) 5.76×10^{-4} (iii) 5.67×10^{-4}	60–120 min 5.53×10^{-4} 5.46×10^{-4}	120–360 min 5.20×10^{-4}
Digoxin ·$\frac{1}{4}H_2O$ Sigma (D_1)	0–30 min (i) 6.59×10^{-4} (ii) 6.37×10^{-4}		130–240 min 5.86×10^{-4}
Digoxin ·$\frac{1}{4}H_2O$ Burroughs Wellcome (D_3)	0–20 min (i) 6.84×10^{-4} (ii) 6.59×10^{-4} (iii) 6.67×10^{-4}	60–120 min 5.85×10^{-4}	180–360 min 5.53×10^{-4}

the same degree of crystallinity, using the peak height 7.3° 2θ as criterion. Samples D_3 and D_5 were about the same with the lowest crystallinity.

X-ray powder diffraction patterns for the different forms showed only small differences in the characteristic D values and intensities of the diffraction bands (Fig. 2). The band at $2\theta = 44.6°$ might be due to the aluminum sample holder. The main differences were in the ratio of the diffraction bands at $2\theta \approx 16.7$, 17.9 and 14.6°. The ratio $I_{16.7}/I_{17.9}$ was 0.9–1.0 for anhydrous digoxin; $I_{16.7}/I_{14.6}$ was 1.2–1.6 for digoxin · $\frac{1}{4}H_2O$ and 0.6–0.8 for digoxin · $\frac{1}{2}H_2O$. Anhydrous digoxin gave a diffraction pattern similar to that observed by Nürnberg and Dölle (1983b) for recrystallized amorphous digoxin.

FTIR

FTIR results obtained for the different crystal forms showed the absorption bands associated with crystalline digoxin (Florence et al., 1974; Black and Lovering, 1977) at 3093.7 and 1805.2 cm^{-1}, while the peak due to amorphous material was at 1624.7 cm^{-1} (Florence et al., 1974; Black and Lovering, 1977). Peak heights (PH) were measured from baselines drawn between the minima at about 3050 and 3150 cm^{-1} and 1600–1850 cm^{-1}. The ratios PH_{3094}/PH_{1625} ($ratio_{3094}$) were about the same for D_1–D_5 (Table 1). The peak height ratios, PH_{1805}/PH_{1625} ($ratio_{1805}$) varied from 0.61 to 0.74, with the higher values obtained for D_3 and D_5, which were the least crystalline on the basis of powder diffraction X-ray analysis. The shoulder at 1780.6 cm^{-1} was small and about the same size for the different samples. Digoxin · $\frac{1}{2}H_2O$ had the lowest value for PH_{3094}; XRD suggested that this form was the most crystalline. FTIR spectra for the three forms of digoxin differed only in peak intensities, the peak positions were essentially the same (Fig. 3).

Intrinsic dissolution rates

A number of aqueous dissolution media, with ethanol concentrations varying from 28.5 to 55.4%, were evaluated. An ethanol concentration of 55.4% gave adequate digoxin solubility and the dissolution disk retained its integrity over an exposure time of 6 h in the dissolution medium.

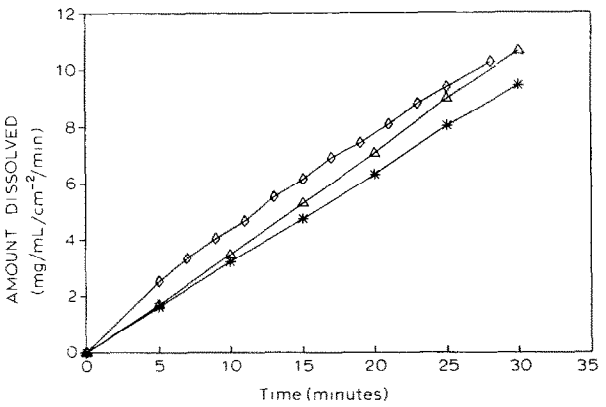

Fig. 4. Initial intrinsic dissolution rate profiles of anhydrous digoxin (◇——◇), digoxin · $\frac{1}{4}H_2O$ (△——△) and digoxin · $\frac{1}{2}H_2O$ (*——*).

Commercial samples D_1 and D_3 gave identical initial dissolution rates, which were also the same as that obtained for recrystallized digoxin · $\frac{1}{4}H_2O$ (Table 2). The initial dissolution rate of D_1 slowed to a final rate comparable to the initial dissolution rate observed for digoxin · $\frac{1}{2}H_2O$. The final dissolution rate of D_3 became slower than the initial rate observed for digoxin · $\frac{1}{2}H_2O$; it was comparable to the intermediate dissolution rate found for digoxin · $\frac{1}{2}H_2O$. Anhydrous digoxin had the fastest initial dissolution rate (Fig. 4) and

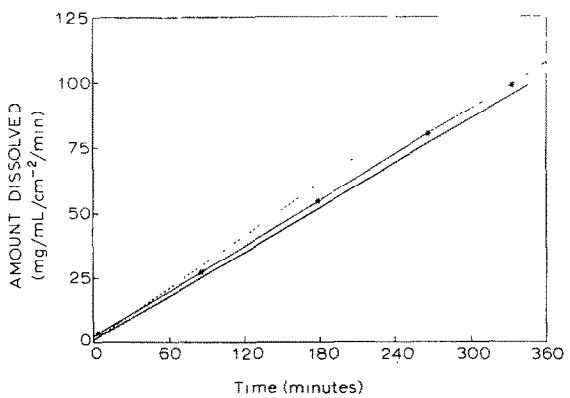

Fig. 5. Intrinsic dissolution rate profiles of anhydrous digoxin (*——*), digoxin · $\frac{1}{4}H_2O$ (- - - - -) and digoxin · $\frac{1}{2}H_2O$ (——).

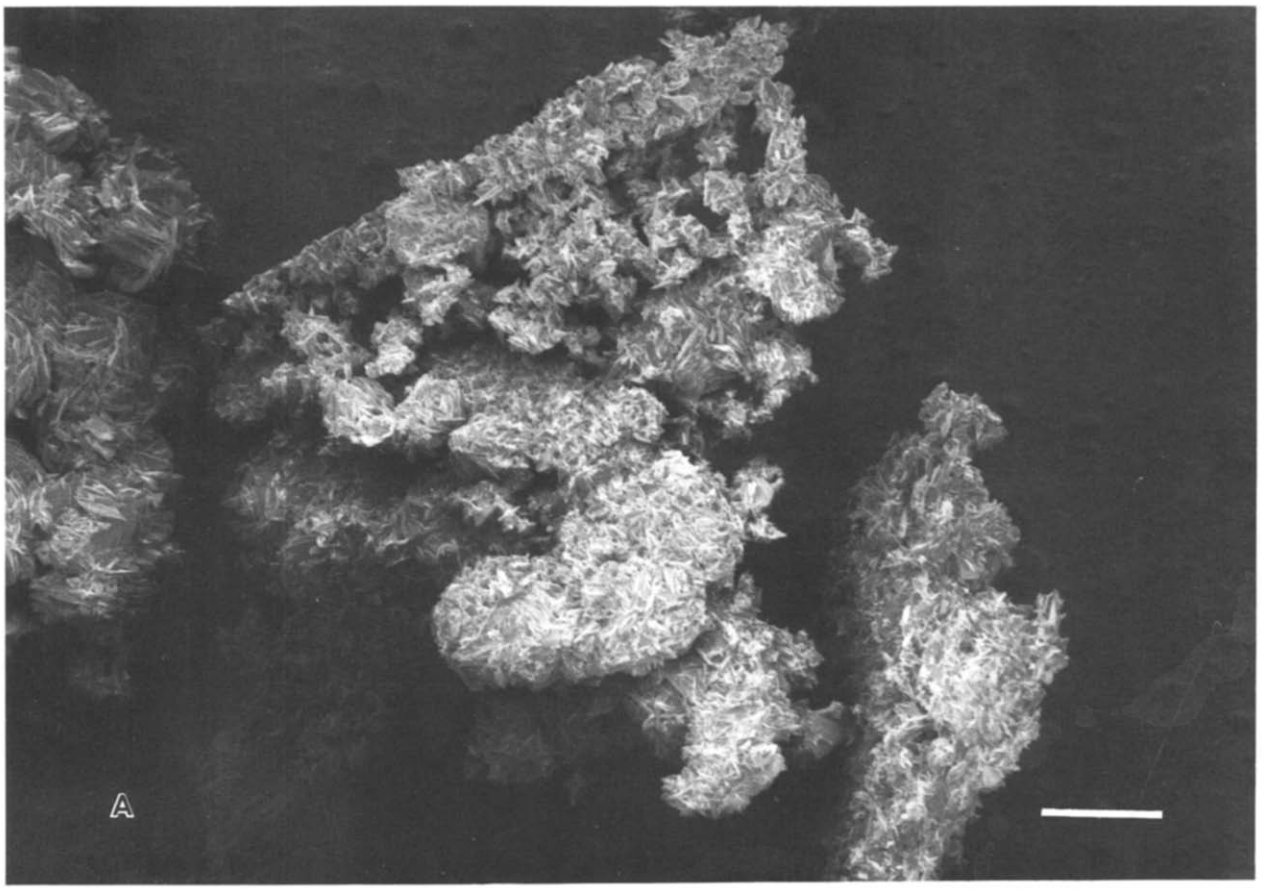

Fig. 6. SEM photomicrographs of anhydrous digoxin (A), digoxin · $\frac{1}{4}H_2O$ (B) and digoxin · $\frac{1}{2}H_2O$ (C).

slowed to a final rate approaching the initial dissolution rate observed for digoxin · $\frac{1}{2}H_2O$ (Table 2). The dissolution rate of digoxin · $\frac{1}{2}H_2O$, which was the lowest of the three forms, slowed as well (Fig. 5), which could indicate the existence of other hydrate forms of digoxin.

Dissolution rates indicated that the anhydrous and quarter-hydrate forms converted to the hemihydrate over a period of 2 h. There is also evidence that at least one higher hydrate of digoxin exists.

SEM

Anhydrous digoxin appeared as clusters of thin small plates; digoxin · $\frac{1}{4}H_2O$ consisted mainly of thin plates and appeared the same as previously reported (Florence and Salole, 1976; Nürnberg and Werthmann, 1978; Nürnberg and Dölle, 1980). Digoxin · $\frac{1}{2}H_2O$ was observed as relatively large plates arranged in clusters and was the same in appearance as that observed for the British Chemical Reference Substance by Florence and Salole (1976). SEM photomicrographs

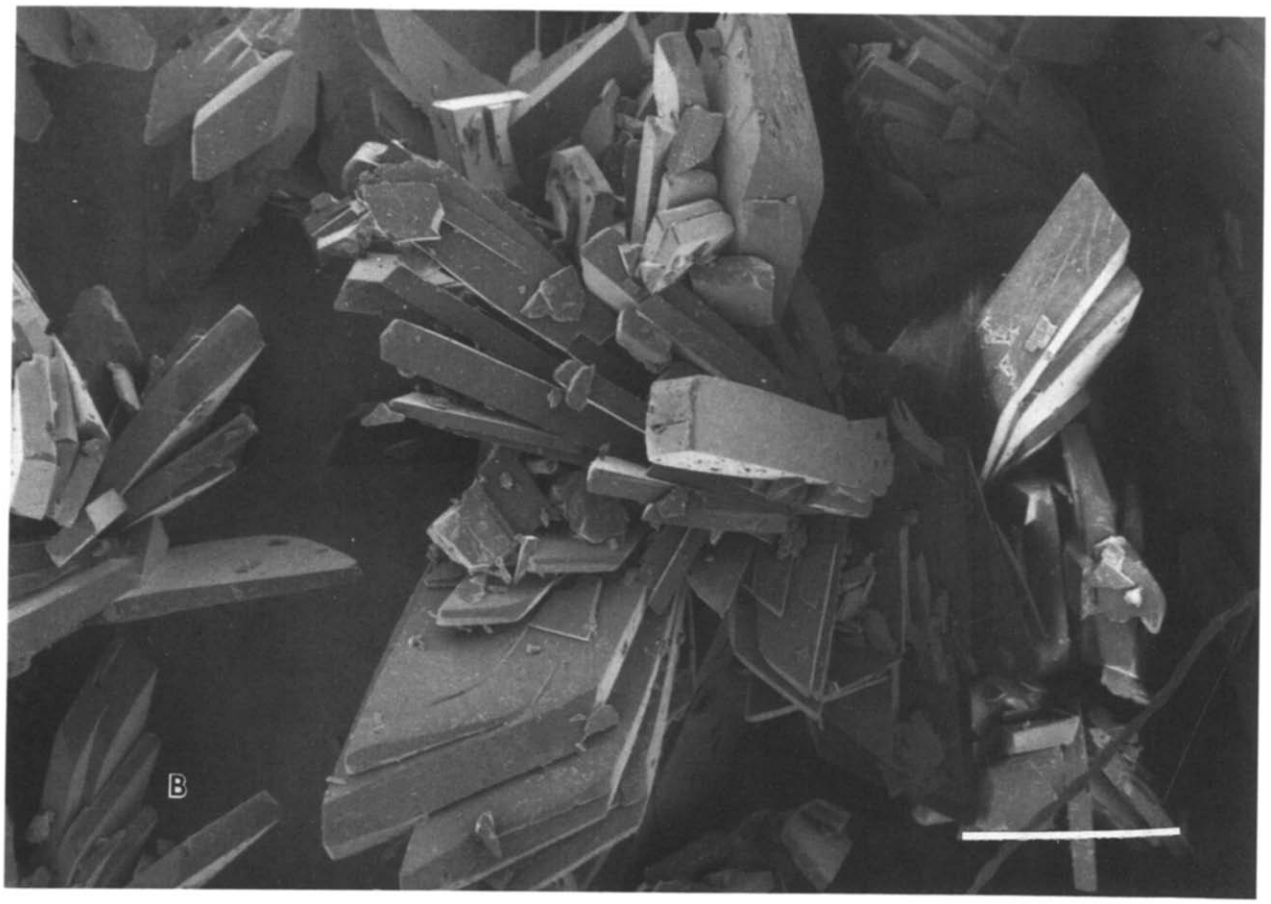

Fig. 6. (B).

of anhydrous digoxin, digoxin · $\frac{1}{4}H_2O$ and digoxin · $\frac{1}{2}H_2O$ are illustrated in Fig. 6.

Conclusions

Anhydrous digoxin, digoxin · $\frac{1}{4}H_2O$ and digoxin · $\frac{1}{2}H_2O$ were characterized using non-thermal methods. The ratio of XRD bands $I_{16.7}/I_{17.9}$ was 0.9–1.0 for anhydrous digoxin, $I_{16.7}/I_{14.6}$ was 1.2–1.6 for digoxin · $\frac{1}{4}H_2O$ and 0.6–0.8 for digoxin · $\frac{1}{2}H_2O$.

FTIR spectra for the three forms differed only in peak intensities and could not be used to characterize the forms.

Anhydrous digoxin had the fastest initial dissolution rate and digoxin · $\frac{1}{2}H_2O$ the slowest initial dissolution rate. Anhydrous digoxin and digoxin · $\frac{1}{4}H_2O$ converted to digoxin · $\frac{1}{2}H_2O$ over a period of 2 h; the dissolution rate of digoxin · $\frac{1}{2}H_2O$ slowed as well. There is evidence of at least one higher hydrate of digoxin.

SEM photomicrographs showed the different forms to consist mainly of plates; digoxin · $\frac{1}{4}H_2O$ was thin plates and digoxin · $\frac{1}{2}H_2O$ was relatively large crystals, arranged in clusters.

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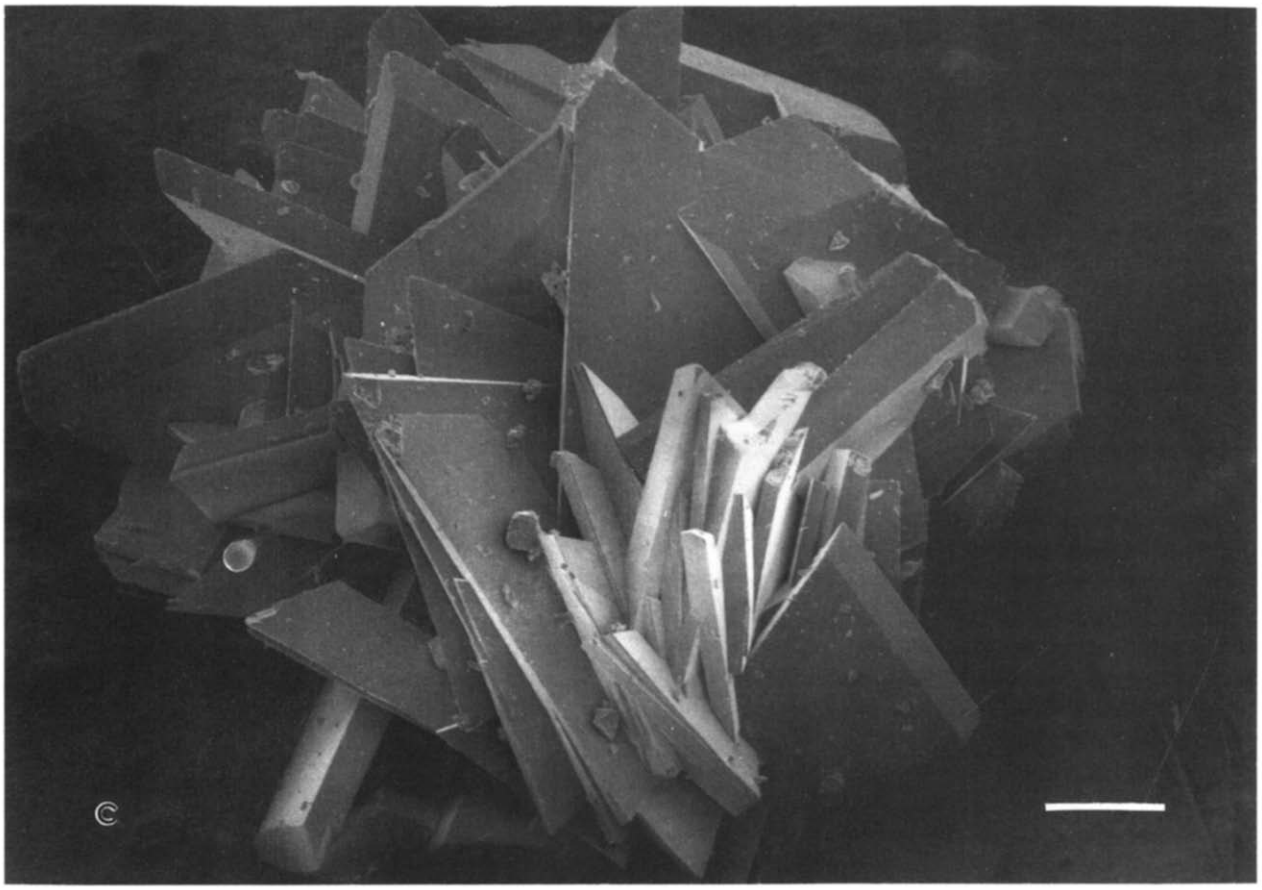

Fig. 6. (C).

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