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Characterization of digoxin hydrates using thermal methods

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Summary

Anhydrous digoxin, digoxin · $\frac{1}{4}H_2O$ and digoxin · $\frac{1}{2}H_2O$ were prepared and characterized using a combination of TGA and coulometric Karl Fischer moisture determinations. Hot stage microscopy results correlated with TGA studies. DSC results were variable and inconclusive and could not be used as identification. Commercial digoxin samples were of the form digoxin · $\frac{1}{4}H_2O$.

Introduction

A number of cardiac glycosides were reported to exhibit polymorphism and/or pseudopolymorphism. Both gitoxigenin (Smith, 1931) (**I**) and digoxigenin (Smith1 1930; Rohrer and Fullerton, 1980) (**II**), the aglycones of gitoxin and digoxin, respectively, form hydrates when recrystallized from dilute alcohol. Digitoxin, which contains 1 mol of digitoxigenin (**III**) and 3 mol of digitoxose, may contain $\frac{1}{2}$ or 1 mol of water or ethanol (Merck Index, 1989). Two polymorphs each of digitoxin, gitoxin, digoxin (Oba and Koyama, 1968) and β-acetyldigoxin, as well as amorphous β-acetyldigoxin (Renz-Scharla et al., 1985), were described. Ouabagenin (**IV**), the aglycone of ouabain, has a monohydrate (Mannich and Siewert, 1942) and a methanol solvate (Go and Kartha, 1983), while ouabain (1 mol ouabagenin + 1 mol rhamnose) exists in six hydrate forms, an anhydrous form (Trivedi et al., 1959) and as ouabain diethanol (Go and Kartha, 1981).

Amorphous digoxin obtained by spray-drying (Nürnberg and Werthmann, 1978; Nürnberg and Dölle, 1980, 1983a) or rapid evaporation (Nürnberg and Werthmann, 1978; Müller and Eckert, 1980; Draguet-Brughmans et al., 1985) was extensively studied. Crystalline digoxin obtained by recrystallization from a number of solvent systems (Chiou and Kyle, 1979; Müller and Eckert, 1980; Nürnberg and Dölle, 1980; Draguet-Brughmans et al., 1985) was studied, but the existence of polymorphism or pseudopolymorphism could not clearly be demonstrated.

Conflicting and inconclusive results were reported by various authors. TGA of recrystallized digoxin (Draguet-Brughmans et al., 1985) showed

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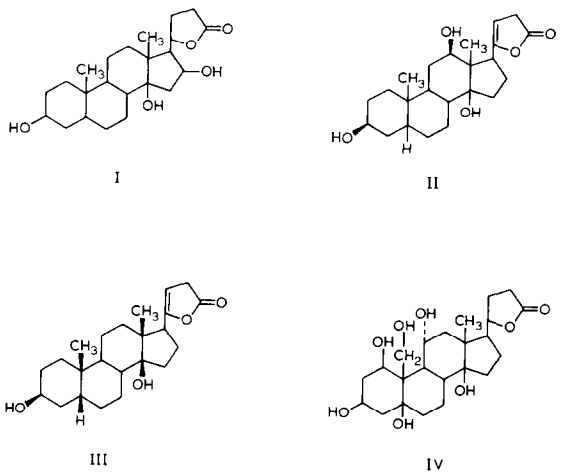

Scheme 1. Structures: **(I)** gitoxigenin, **(II)** digoxigenin, **(III)** digitoxigenin, **(IV)** ouabagenin.

weight loss of 7–25% above about 160°C, which varied in onset from sample to sample. The product obtained by rotary evaporation at 80°C of a digoxin solution in chloroform : methanol showed a weight loss of 2.5% at 80°C (Nürnberg and Werthmann, 1978), while digoxin spray-dried from the same solvent system contained 5% methanol (Nürnberg and Werthmann, 1978).

DSC studies on commercial samples (Florence et al., 1974; Florence and Salole, 1976; Nürnberg and Werthmann, 1978; Chiou and Kyle, 1979; Draguet-Brughmans et al., 1985) and digoxin recrystallized from various solvents (Nürnberg and Werthmann, 1978; Chiou and Kyle, 1979; Nürnberg and Dölle, 1983b; Draguet-Brughmans et al., 1985) showed wide melting ranges and large variations in melting temperatures (173–240°C). Micronized samples had sharper melting endotherms which were 50–60°C below those of the corresponding crystalline substance (Nürnberg and Werthmann, 1978; Chiou and Kyle, 1979; Nürnberg and Dölle, 1983b). Triturated digoxin showed a large endothermic peak at about 75°C (Chiou and Kyle, 1979), a relatively sharp exotherm at 175°C, followed immediately by a sharp endothermic peak. Chiou and Kyle (1979) attributed the first endotherm to a phase transition in the amorphous state and the exothermic peak to conversion of the amorphous form to the crystalline form, which subsequently melted with a sharp endothermic peak. Extensive DSC investigations of digoxin which was amorphous by X-ray diffraction (Nürnberg and Werthmann, 1978; Müller and Eckert, 1980; Nürnberg and Dölle, 1983b; Draguet-Brughmans et al., 1985) gave various results, depending upon the method of preparation. It was postulated that the first endotherm at 50°C found in some samples (Müller and Eckert, 1980; Nürnberg and Dölle, 1983b), was due to adsorbed water in amorphous digoxin since Karl Fischer water titrations after 20 days of storage at 55% relative humidity (RH) and room temperature showed a water content of about 14% (Müller and Eckert, 1980).

Using hot stage microscopy, evolution of volatile substances was detected in commercial samples by the appearance of gas bubbles above 200°C, a gradual darkening of the crystals simultaneously being observed (Draguet-Brughmans et al., 1985). Crystals obtained by the rapid evaporation of a saturated chloroform solution seemed to be amorphous at room temperature, with crystallization occurring at about 140°C (Draguet-Brughmans et al., 1985). An amorphous form, spray-dried from chloroform : methanol (7 : 3), changed to plate-like structures which did not have polarization colors at 150–170°C and became polarized at 180°C, when they were crystalline (Nürnberg and Dölle, 1983b). Digoxin recrystallized from a 96% ethanol solution, by both slow evaporation at room temperature and fast evaporation under vacuum, showed softening of the crystals at 150–175°C (Müller and Eckert, 1980).

Anhydrous digoxin was recrystallized by Go and Kartha (1980) and they reported the three-dimensional structure to be a triclinic system with P_1 space group.

In this study, anhydrous digoxin, digoxin $\cdot \frac{1}{4}H_2O$ and digoxin hemihydrate were prepared by recrystallization. Thermogravimetric analysis (TGA), coulometric Karl Fischer (CKF) water determinations, differential scanning calorimetry (DSC) and hot stage microscopy were used to characterize these forms.

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 crystal forms from various solvent systems using techniques of recrystallization at −18°C, room temperature and 60°C.

Anhydrous digoxin

Digoxin (500 mg), which had been dried for 12 h over P_2O_5 at 80°C, was added to 18 ml of a boiling chloroform/absolute ethanol solution (1:1) (Go and Kartha, 1980). The clear solution was filtered through Whatman No. 1 filter paper, transferred to a 60°C oven and allowed to evaporate to dryness. Anhydrous digoxin was stored at room temperature in a desiccator with desiccant.

Digoxin · $\frac{1}{4}H_2O$

A boiling solution of digoxin (0.1%) in ethanol:water (4:1) was reduced in volume (about 75%) with heat, filtered and transferred to a −4°C freezer. The container was closed once the first crystals appeared and allowed to crystallize for 24 h. The resulting crystals were collected by filtration through a fine sintered glass Buchner filter and air dried on the filter under suction for 15 min to remove residual solvent. The crystals were collected onto filter paper and exposed to room temperature and ambient humidity (≈ 55% RH) for 6 h; this method yielded crystals of small particle size. Larger crystals were obtained using the same solvent system at room temperature.

Digoxin · $\frac{1}{2}H_2O$

Boiling solutions of digoxin (0.1%) in ethanol:water (4:1 or 19:1) were reduced in volume (about 75%) with heat, filtered and transferred to an oven at 60°C. The container was closed once the first crystals appeared and allowed to crystallize for 24 h. The resulting crystals were collected by filtration and remained on the glass filter under a stream of air for 15 min to remove residual solvent.

Thermal analysis

DSC thermograms were recorded on a Perkin-Elmer differential scanning calorimeter (DSC-7) equipped with a Perkin-Elmer TAC 7/7 Instrument Controller and a Perkin-Elmer 7700 Professional Computer. The instrument was calibrated for temperature and energy with pure indium (99.999%; melting point 156.60°C, transition energy 28.45 J/g) and lead (purity 99.999%; melting point 327.27°C, transition energy 23.01 J/g) standards. Samples were weighed (Cahn C-31 microbalance) into aluminum pans for volatile substances and sealed with a Perkin-Elmer volatile sample pan crimper. An empty pan, sealed in the same way, was used as a reference. The thermal behavior of the different crystal forms was studied under a nitrogen purge at a heating rate of 10°C/min.

Thermogravimetric analyses (TGA) were performed using the Perkin-Elmer TGA-7 Thermogravimetric Analyzer, TAC 7/7 Instrument Controller and 7700 Professional Computer. The temperature axis was calibrated using the ferromagnetic materials furnished by Perkin-Elmer. Analyses were performed on samples in platinum pans at a heating rate of 10°C/ min.

Coulometric Karl Fischer moisture determinations

A coulometric Karl Fischer apparatus (Metrohm 684KF Coulometer) equipped with a variable-temperature controlled oven (Metrohm 688 KF oven) was used. Samples weighing 5–10 mg were introduced into the oven and the released moisture was transferred to the titration vessel with a stream of dried nitrogen. The oven temperature was 150°C and a titration time of 15 min was used. Coulomat A and C (Hydranal®, Riedel deHaën) solutions were used in the reaction vessel. The performance and accuracy of the instrument was checked against a sodium tartrate $\cdot 2H_2O$ standard (Hydranal®, Riedel deHaën; 15.67% moisture) at an oven temperature of 210°C.

Hot-stage microscopy

A Mettler FP82 Hot Stage, connected to a Mettler FP80 Central Processor and a Mettler GA44 Printer was used. The hot stage was set at a constant heating rate of 10°C/min; observations were conducted over 25–250°C under silicone oil to observe evolved gas.

Results and Discussion

TGA

Digoxin · $\frac{1}{4}H_2O$ should theoretically contain 0.57% water and a hemihydrate should contain 1.14% water. In contrast to Draguet-Brughmans et al. (1985), who reported a continuous weight loss from about 160°C, we found that TG analyses on both commercial samples and recrystallized forms showed three types of weight loss – an initial weight loss due to adsorbed water (≈ 30–125°C), a weight loss due to water of hydration, which was immediately followed by weight loss due to thermal degradation. A slight change in slope indicated the end of weight loss due to hydrate water (see Fig. 1). From these results, using the change in slope as an indication of hydrate water loss, it was concluded that TGA was of limited use for characterizing digoxin hydrates (Table 1). However, TGA was useful as a measure of adsorbed moisture. Digoxin · $\frac{1}{4}H_2O$ recrystallized at −4°C could have up to 16% of adsorbed water. Significant adsorbed moisture was always associated with this form. The adsorbed water was calculated from the TGA thermograms using weight loss up to a slight plateau, which was between 115 and 130°C. The water of hydration was calculated from the end of adsorbed water loss to the change in slope which varied from 195 to 220°C. The calculations for each crystal form are tabulated in Table 1. These

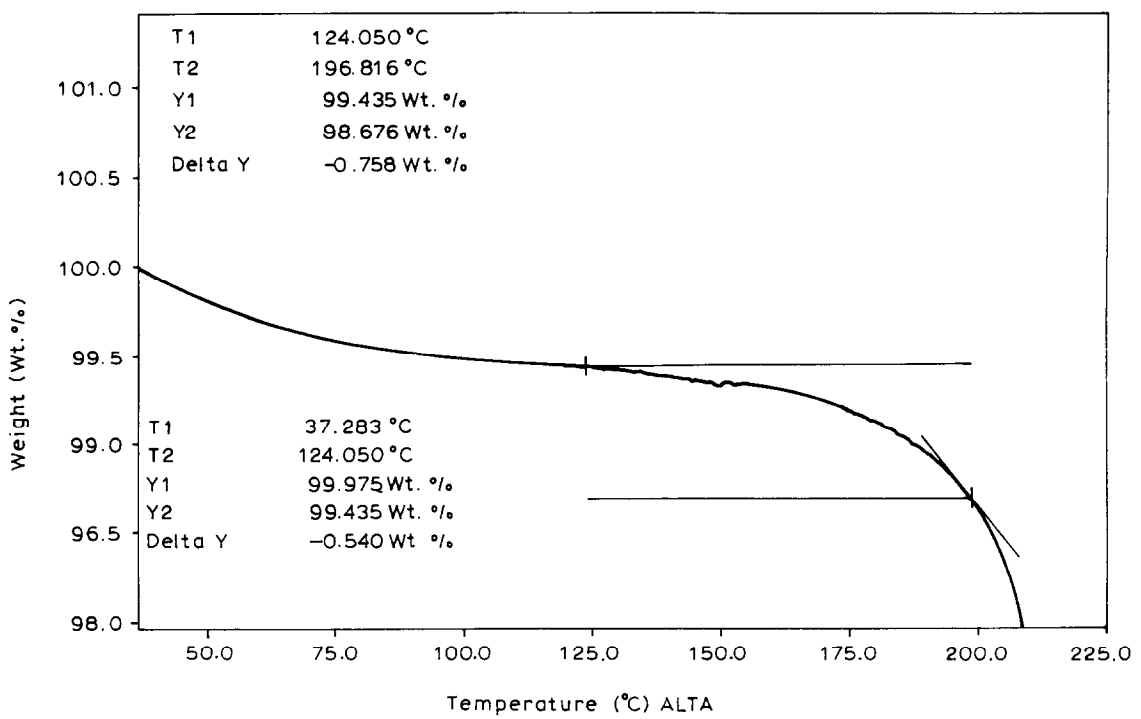

Fig. 1. TGA thermogram of digoxin · $\frac{1}{4}H_2O$ showing three stages of weight loss: (i) loss of adsorbed water 37–124°C; (ii) loss of hydrate water, 124–199°C; (iii) weight loss due to degradation – the change in slope at 199°C was used to indicate weight loss due to hydrate water.

TABLE 1

Coulometric Karl Fischer moisture determinations and TGA weight loss of commercial digoxin, anhydrous digoxin and digoxin hydrates

Sample	Coulometric KF: Total water (%)	Thermogravimetric analysis		Corrected water content (%)
		Temperature range (°C)	Weight loss (%)	
Digoxin (Sigma) (D_1)	0.78 ± 0.30 (n = 3)	33.1–116.5	0.14	0.64
		116.5–196.9	1.04	
Digoxin (Spectrum) (D_2)	1.02 ± 0.32 (n = 9)	35.5–127.0	0.25	0.77
		127.0–211.8	1.90	
Digoxin (B Wellcome) (D_3)	0.80 ± 0.22 (n = 6)	37.1–126.1	0.03	0.50
		126.1–191.8	0.44	
Digoxin (Ref. Std Sigma) (D_4)	0.99 ± 0.18 (n = 5)	34.2–127.4	0.07	0.92
		127.4–218.5	0.77	
Digoxin USP (D_5)	0.64 ± 0.14 (n = 4)	37.1–119.8	0.06	0.58
		119.8–210.2	0.50	
Anhydrous digoxin	0.28 ± 0.03 (n = 3)	37.3– 98.9	0.27	0.01
		98.9–159.4	0.12	
Digoxin · $\frac{1}{4}H_2O$	0.76 ± 0.09 (n = 2)	37.3–124.1	0.26	0.50
		124.1–198.9	0.85	
Digoxin · $\frac{1}{2}H_2O$	1.07 ± 0.02 (n = 7)	37.3–126.3	0.04	1.03
		126.3–218.0	0.70	

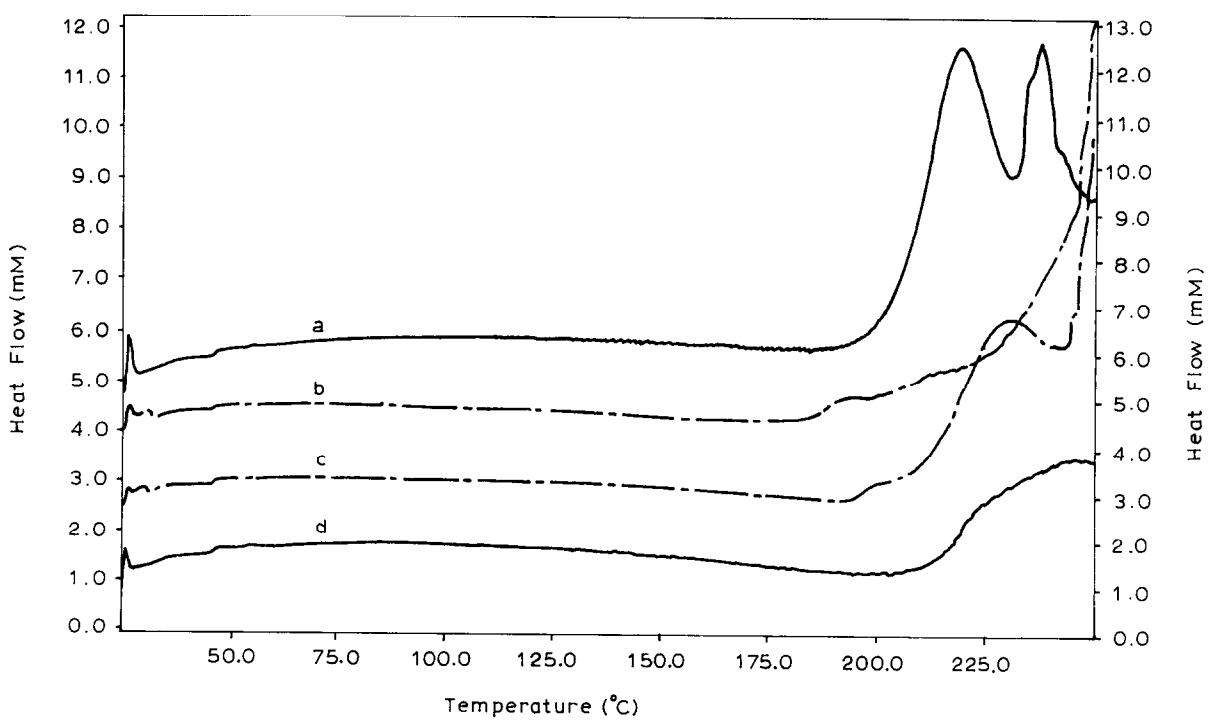

Fig. 2. DSC thermograms of commercial digoxin: D_4 (a), D_2 (b), D_1 (c) and D_5 (d).

results for water of hydration did not correlate well with the coulometric Karl Fischer data which were considered more reliable.

CKF determinations

A number of different oven exposure times and temperatures were evaluated before concluding on oven conditions of 150°C for 15 min. After an exposure time of 15 min at 150°C there was no change in the color of digoxin · $\frac{1}{4}H_2O$ and digoxin hemihydrate crystals, which is indicative of degradation (Go and Kartha, 1980). This temperature was also high enough to allow relatively fast water loss from the crystals. Due to apparent degradation of the anhydrous form at 150°C (sample discoloration), Hydranal®-Coulomat AK and CK, which are methanol-free titration solutions intended for the coulometric water determination in aldehydes, ketones and other substances which react with the standard titration solutions, were also used. The same results were obtained, regardless of the type of reaction solution used.

Water titration results from the recrystallized anhydrous, $\frac{1}{4}$- and $\frac{1}{2}$-hydrates are given in Table 1. The results were corrected for adsorbed water from the initial weight loss in TGA measurements. CKF results obtained from commercial samples, as well as USP and Sigma reference standards, are also included. From these results it was concluded that the commercial samples studied were of the form digoxin · $\frac{1}{4}H_2O$.

DSC

Although the commercial samples studied were the same hydrate form, large variations in melting temperature and behavior were found (Figs 2 and 3), which were consistent with other reported results. The D_1 thermogram (Fig. 2) showed a small endotherm (dehydration) with an onset of 196°C, a larger melting endotherm (onset 212°C) followed by a large endothermic degradation peak which began at 243°C. D_2 had two small endothermic peaks, the first (182°C) was at a lower temperature than the dehydration peak of D_1

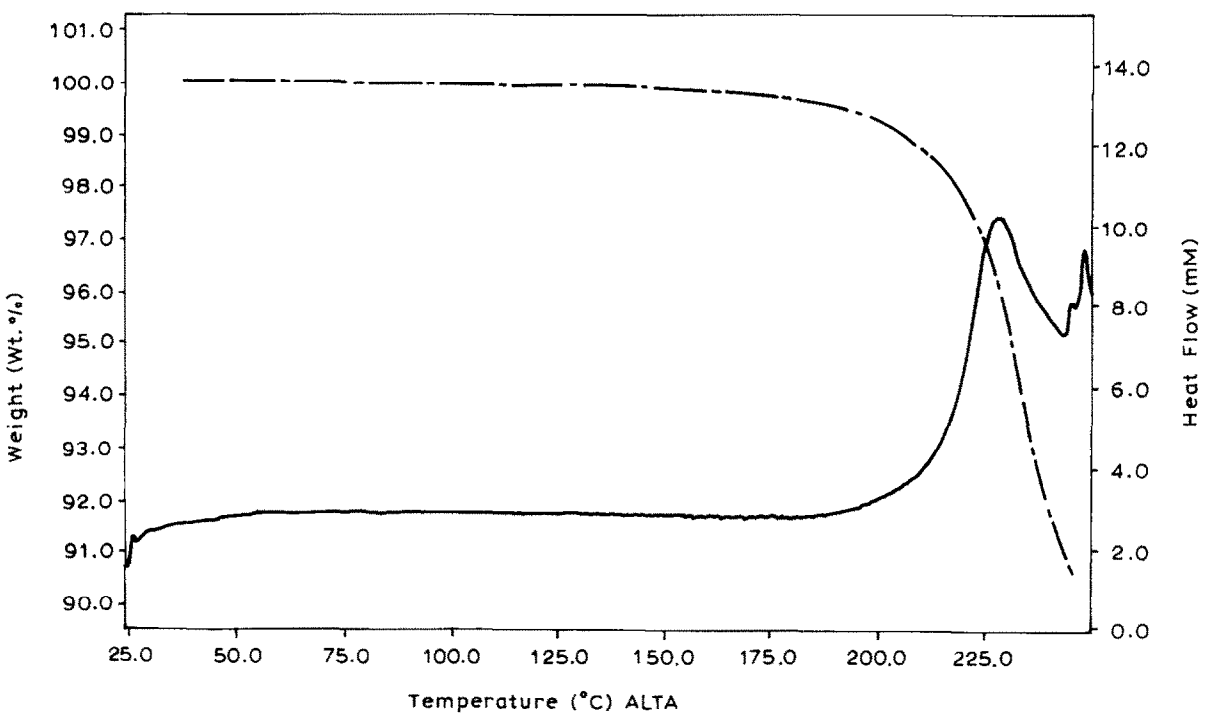

Fig. 3. TGA and DSC thermograms of commercial sample D_3.

and the melting peak started at a temperature of 224°C. Both D_3 (Fig. 3) and D_4 (Fig. 2) showed only one endotherm (216°C and 207°C, respectively) and D_5 (Fig. 2) had only one poorly formed endothermic peak with an onset of 205°C. Degradation melting started at 224, 243 and 233°C for D_2, D_3 and D_4, respectively. The first endotherms of D_1 and D_2 correlated with the secondary weight loss found by TGA (Table 1) and were due to loss of water of hydration. Samples D_3–D_5 did not show this endotherm, probably due to small particle sizes, even although these forms were the same hydrate forms as D_1 and D_2.

Anhydrous digoxin showed a change in baseline from 158–172°C (Fig. 4) that resembled that of a glass transition and melting or degradation started at about 225°C. The TGA thermogram of anhydrous digoxin showed rapid weight loss due to degradation starting at 172°C. A thermogram of digoxin · $\frac{1}{4}H_2O$ (Fig. 5) also showed a change in baseline, but at the higher temperature of 188–210°C. This baseline shift was interpreted as an endothermic dehydration peak since it correlated with TGA results. The onset of this peak was at 195°C and was followed by an endotherm (219°C), and a melting endotherm at 236°C. The dehydration endotherm (144–155°C) and degradation endotherm (201°C) of digoxin · $\frac{1}{2}H_2O$ (Fig. 6) were at lower temperatures as found for digoxin · $\frac{1}{4}H_2O$, and was followed by melting. Florence et al. (1974) reported endothermic transitions at around 170°C in milled samples, which they attributed to amorphous material. Amorphous digoxin showed glass transitions at 140–160°C with a T_G at 152°C (Müller and Eckert, 1980) and 142.5°C (Nürnberg and Dölle, 1983b). It was uncertain whether the baseline changes in anhydrous digoxin and digoxin · $\frac{1}{4}H_2O$ could be attributed to glass transitions.

Hot stage microscopy

Hot stage microscopy was carried out to temperatures above 215°C. The observations above

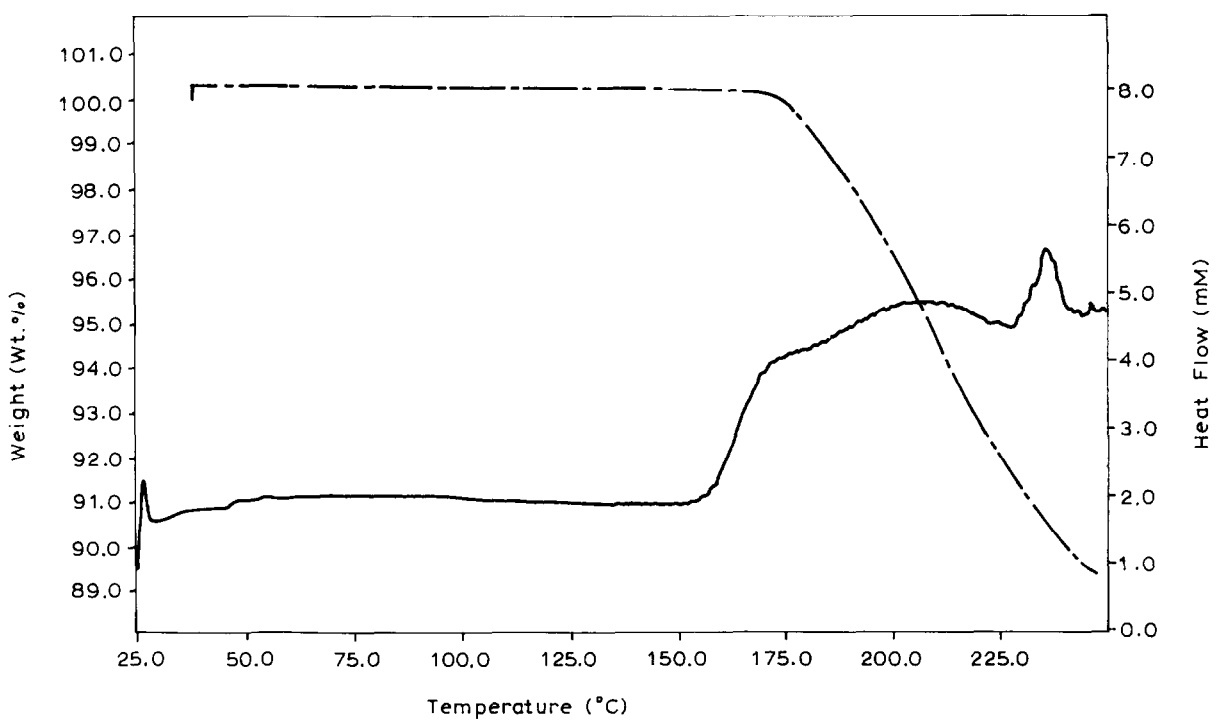

Fig. 4. TGA and DSC thermograms of anhydrous digoxin.

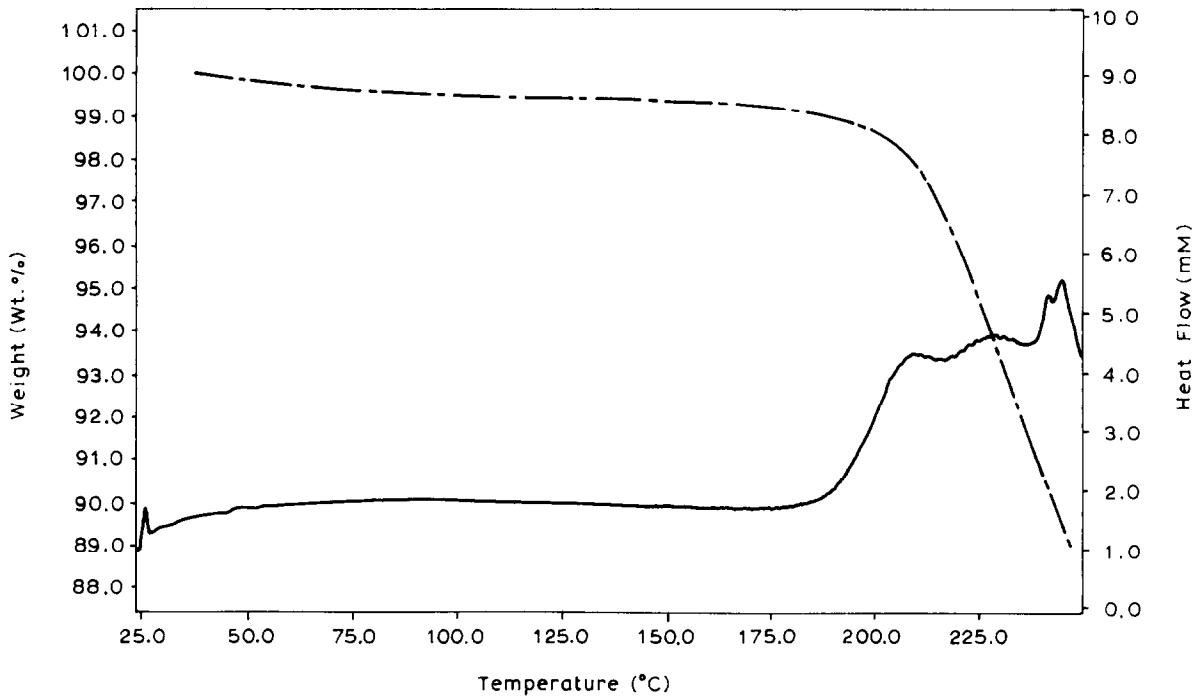

Fig. 5. TGA and DSC thermograms of digoxin · $\frac{1}{4}H_2O$.

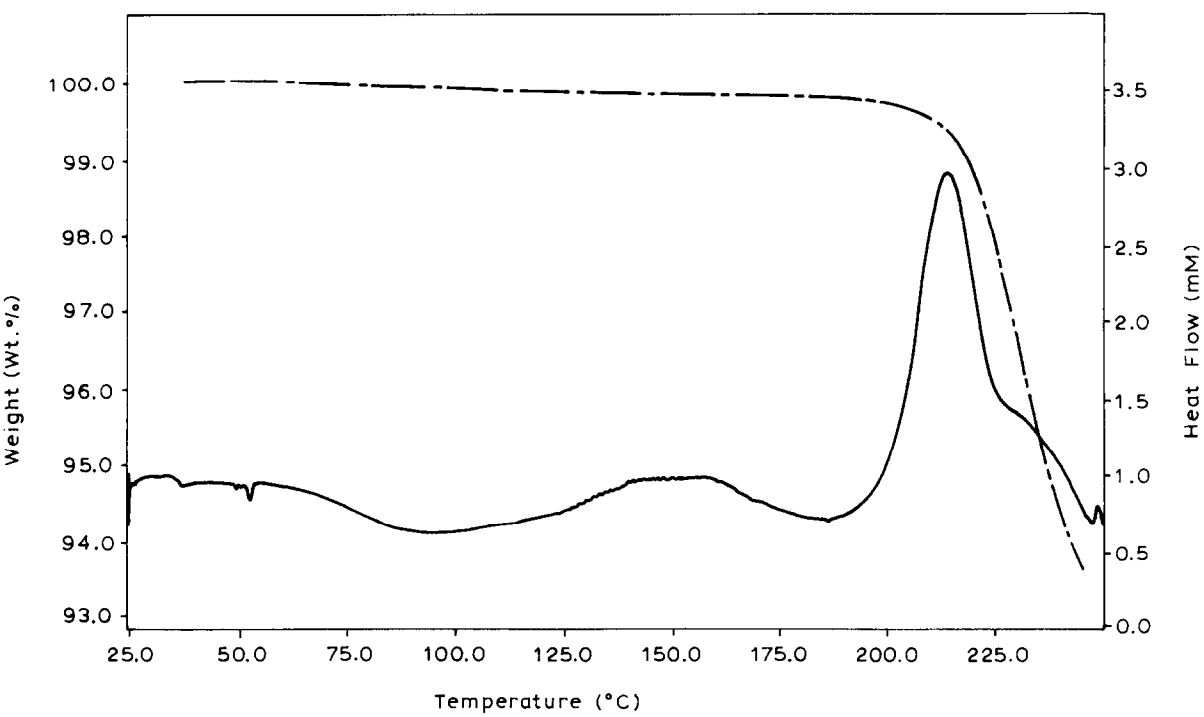

Fig. 6. TGA and DSC thermograms of digoxin · $\frac{1}{2}H_2O$.

215°C will not be presented since they are not relevant to the present discussion of digoxin hydrates.

Digoxin · $\frac{1}{4}H_2O$ appeared as polarized platelets. With heating, a few small bubbles appeared at 57.5°C, which grew slowly with further heating. At 118°C more bubbles appeared that grew faster; at 168°C bubbles appeared more rapidly. At 185.5°C the pattern of bubble appearance changed; small bubbles came in a rapid stream from channels in the crystals and simultaneously the crystals darkened. At 200°C the bubbles appeared very quickly, the crystals became dark brown and melting was complete at 263.5°C. These results were consistent with what was found with TGA studies, namely that adsorbed moisture was lost up to a temperature of 117°C, after which hydrate water was lost up to about 200°C. Above this temperature there was a slight change in slope in the TG thermogram, indicating a change in rate of weight loss, presumably due to thermal degradation.

Digoxin hemihydrate gave polarized plates. At 202°C, bubbles slowly started to form; at 215°C they formed more rapidly. At 218°C, a stream of small bubbles formed very rapidly and the sample became darker in color. The sample melted at 246°C. These results are consistent with TGA data, in that water of hydration was lost from 202–215°C.

A constant stream of small gas bubbles appeared at 198°C when anhydrous digoxin was heated, at 206°C the bubbles appeared even more rapidly and the crystals were darker in color. Melting of the darkened crystals was completed at 267°C.

Conclusions

The physical properties of amorphous and crystalline digoxin have been discussed by a number of authors, but the existence of polymorphism and/or pseudopolymorphism could not be demonstrated.

Anhydrous digoxin, digoxin · $\frac{1}{4}H_2O$ and digoxin · $\frac{1}{2}H_2O$ were recrystallized from different solvent systems and at different recrystallization temperatures. The different forms were identified using a combination of TGA and coulometric Karl Fischer moisture determinations. TGA studies showed weight loss to occur in three stages; weight loss due to adsorbed water and weight loss due to water of hydration which was immediately followed by weight loss due to thermal degradation. TGA was of limited use for characterizing digoxin hydrates, but was used as a measure of adsorbed water. DSC results were variable and inconclusive.

Hot stage microscopy correlated well with TGA results. Digoxin · $\frac{1}{4}H_2O$ lost water over the range approx. 30–125°C and water of hydration was lost from approx. 125–186°C; followed by degradation. Digoxin · $\frac{1}{2}H_2O$ lost water of hydration over the range approx. 120–220°C and anhydrous digoxin started to degrade at approx. 200°C.

Commercial digoxin samples studied were of the form digoxin · $\frac{1}{4}H_2O$.

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References

Chiou, W.L. and Kyle, L.E., Differential thermal solubility and aging studies on various sources of digoxin and digitoxin powder; biopharmaceutical implications. *J. Pharm. Sci.*, 68 (1979) 1224–1229.

Draguet-Brughmans, M., Bouche, R. and Levebvre, C., The physicochemical properties of digoxin. *J. Pharm. Biomed. Anal.*, 3 (1985) 227–234.

Florence, A.T. and Salole, E.G., Changes in crystallinity and solubility on comminution of digoxin and observations on spironolactone and oestradiol. *J. Pharm. Pharmacol.*, 28 (1976) 637–642.

Florence, A.T., Salole, E.G. and Stenlake, J.B., The effect of particle size reduction on digoxin crystal properties. *J. Pharm. Pharmacol.*, 26 (1974) 479–480.

Go, K. and Kartha, G., Structure of digoxin. *Acta Crystallogr.*, B36 (1980) 1811–1819.

Go, K. and Kartha, G., Ouabain diethanol·$2C_2H_5OH$. *Cryst. Struct. Commun.*, 10 (1981) 1329–1334.

Go, K. and Kartha, G., Structure of ouabagenin methanol solvate $C_{23}H_{34}O_8 \cdot CH_3OH$. *Acta Crystallogr.*, C39 (1983) 376–378.

Mannich, C. and Siewert, G., g-Strophantin (ouabain) and g-strophanthidin. *Chem. Ber.*, 75B (1942) 737–750. In *Chem. Abstr.*, 37 (1943) 3441[4].

The Merck Index, 11th Edn, Merck and Co., Inc. Rahway, NJ, 1989, p. 3144.

Müller, J. and Eckert, T., Thermoanalytische Untersuchungen am amorphem Digoxin. *Pharmazie*, 35 (1980) 471–473.

Nürnberg, E. and Dölle, B., Darstellung von Digoxin Sprüheinbettungen aus wässrigen Systemen: Eigenschaften dieser Produkte. *Pharm. Ind.*, 42 (1980) 1019–1026.

Nürnberg, E. and Dölle, B., Über das Auftreten parakristalliner Zustände, dargestellt am Beispiel des Digoxins. *Acta Pharm. Technol.*, 29 (1983a), 1–7.

Nürnberg, E. and Dölle, B., Physikalische Untersuchungen parakristalliner Zustände des Digoxins. *Acta Pharm. Technol.*, 29 (1983b), 75–83.

Nürnberg, E. and Werthmann, A., Zur Kenntnis biopharmazeutisch relevanter Eigenschaften von Digoxin. *Pharm. Ind.*, 40 (1978) 1061–1069.

Oba, T. and Koyama, R., Use of infrared absorption spectroscopy in the examination of drugs and their preparations. XVIII. Polymorphism of pharmaceuticals in Japanese Pharmacopeia. 4. Polymorphism of three cardiotonic digitalis glucosides. *Bunseki Kagaku*, 17 (1968) 53–56. In *Chem. Abstr.*, 69 (1968) 69678u.

Renz-Scharla, B.K.P., Canefe, K. and Speiser, P.P., Untersuchungen zur Polymorphie von β-Acetyldigoxin. *Pharm. Acta Helv.*, 60 (1985) 130–136.

Rohrer, D.C. and Fullerton, D.S., Structures of modified cardenolides. III. Digoxigenin dihydrate. *Acta Crystallogr.*, B36 (1980) 1565–1568.

Smith, S., *Digitalis* glucosides. II. Digoxigenin, the aglucone of digoxin. *J. Chem. Soc.*, (1930) 2478–2482.

Smith, S., *Digitalis* glucosides. III. Glucosides of *Digitalis lanata*. *J. Chem. Soc.*, (1931) 23–25.

Trivedi, J., Shell, J.W. and Biles, J.A., Some physical and crystallographic properties of the ouabain hydrates. *J. Am. Pharm. Assoc. Sci. Edn*, 48 (1959) 583–587.