Differential Thermal, Solubility, and Aging Studies on Various Sources of Digoxin and Digitoxin Powder: Biopharmaceutical Implications

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Received June 2, 1978, from the *Clinical Pharmacokinetics Laboratory and Department of Pharmacy, College of Pharmacy, University of Illinois at the Medical Center, Chicago, IL 60612.* Accepted for publication April 4, 1979.

Abstract □ Unlike most organic compounds, both digoxin and digitoxin melted over a wide temperature range, with the widest range being 88 and 33° for both compounds, respectively. Furthermore, the melting ranges varied markedly among several untriturated powders obtained from commercial sources and after recrystallization. Trituration produced dramatically sharper and generally lower melting temperatures. Apparent equilibrium solubility also varied considerably among different untriturated compounds. Correlation between solubility and final melting temperature was found. Results from dynamic solubility studies were used to explain the failure of trituration to enhance the apparent equilibrium solubility in certain samples. Storage at room temperature increased the melting points and decreased aqueous solubilities. Several reasons such as the presence of polymorphic and amorphous forms, crystal defects, impurities, and solvate formation were postulated to explain the findings. In a preliminary study, the *in vitro* dissolution rates of two commercial tablet products stored at elevated temperatures for 4–8 weeks progressively decreased. Biopharmaceutical implications and areas for further studies are discussed.

Keyphrases □ Digoxin—stability studies, temperature, solubility, aging, various commercial powders □ Digitoxin—stability studies, temperature, solubility, aging, various commercial powders □ Cardiotonic agents—digoxin and digitoxin, stability studies, various commercial powders □ Stability—digoxin, digitoxin, various commercial powders

Oral bioavailability of digoxin, a potent water-insoluble cardiac glycoside, has been the subject of intensive studies (1, 2). Each solid dosage form product must pass the oral absorption test in humans (2), and the *in vitro* dissolution study must be performed as a routine quality control procedure by manufacturers (3). However, studies on the underlying physicochemical factors that might contribute to poor dissolution and hence to poor absorption of digoxin from the dosage forms have been quite limited.

The particle size of digoxin within a tablet was a major dissolution rate determinant: the smaller the particle size, the better the absorption (1, 4–6). Effects of milling or grinding of digoxin powder on the enhancement of dissolution and solubility (7, 8) and oral bioavailability in dogs (9) were recently reported. Different results from X-ray diffraction studies on the effects of milling or grinding of digoxin powder on the crystallinity were obtained (7, 8). A complete transformation to an amorphous form (6) and the absence of any crystal–crystal transition (8) after extensive grinding and trituration were both proposed, although the latter study (7) did not rule out the partial transformation to the amorphous form.

Relatively little attention has been directed to the physicochemical properties of digitoxin, another less widely used cardiac glycoside. Since digitoxin is much less soluble in water than digoxin (9), similar poor bioavailability problems from various solid dosage forms may exist. In some preliminary studies, however, complete absorption from intramuscular and oral administration was reported (10, 11).

The purposes of this paper are to report studies on some physicochemical properties of digoxin and digitoxin from various sources using differential thermal, equilibrium, and dynamic solubility methods and to point out their potential biopharmaceutical implications.

EXPERIMENTAL

Digoxin Samples from Commercial Sources and after Recrystallization or Trituration—Two lots of digoxin powder, designated as digoxin L1 and L2, were obtained from a commercial source[1]. Additional samples from two other commercial sources were designated digoxin Z[2] and digoxin S[3].

Digoxin samples also were obtained after recrystallization of digoxin L1 from chloroform[4] and 95% ethanol[5]. For recrystallization, 200 and 100 mg of digoxin powder were dissolved in 800 ml of boiling chloroform and 100 ml of ethanol, respectively. These solutions were reduced with heat to about 50 and 3 ml, respectively. After filtration, the filtrates were cooled in an ethanol and dry ice bath. Recrystallized particles were obtained after filtration and placed in a vacuum for 2 hr.

Three hundred milligrams of digoxin L1, L2, and Z samples were triturated in a porcelain mortar for 30 sec. The powder was loosened from the side of the mortar with a spatula. This process was repeated until a total trituration time of 15 min had been achieved.

Digitoxin Samples from Commercial Sources and after Trituration—Digitoxin powder samples were obtained from three commercial sources and were identified as digitoxin L[6], Z[2], and S[3].

Triturated samples were prepared from digitoxin L and Z as described for the preparation of the triturated digoxin.

Thermal Analysis Studies—Thermal studies on all digoxin and digitoxin samples were conducted in a thermal analyzer attached to a differential scanning calorimetry cell[7]. The temperature was usually scanned from 50 to 300° at 20°/min. One to 2 mg of sample was used for each study. The thermal analyzer was calibrated with the standard ammonium nitrate supplied by the manufacturer[7].

Equilibrium Solubility Studies—A preliminary study conducted over 7 days indicated that 24 hr was sufficient to achieve the equilibrium solubility. Therefore, all subsequent studies were conducted in 24 hr.

Ten-milligram digoxin or digitoxin samples were placed in 16 × 125-mm culture tubes fitted with screw caps, and 10 ml of water was

Table I—Literature Melting Points of Digoxin and Digitoxin

Compound	Source (Reference)	Melting Point
Digoxin	A (12)	270°
	B (13)	235°
	C (14)	240°
	D (15)	265°
	E (7)	234° [a], 236° [a], 240° [a]
Digitoxin	F (16)	240°
	G (17)	256–257°
	A (12)	265°

[a] Based on three different sources of digoxin powder.

[1] Digoxin USP, lots 0609-J9623 and 0609-K7193, Lederle Laboratory, Pearl River, N.Y.
[2] Digoxin USP and digitoxin USP, Zenith Labortory, Northvale, N.J.
[3] Digoxin USP and digitoxin USP, Sandoz Co., East Hanover, N.J.
[4] Certified ACS grade, Fisher Scientific Co., Fair Lawn, N.J.
[5] Alcohol USP, Commercial Solvent Co., Terre Haute, Ind.
[6] Digitoxin USP, lot W11097, Eli Lilly & Co., Indianapolis, Ind.
[7] DuPont 990 thermal analyzer, E.I. du Pont de Nemours & Co., Wilmington, Del.

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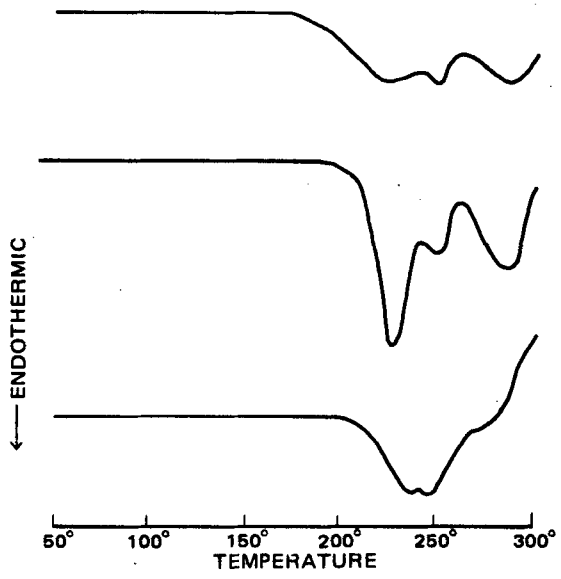

Figure 1—*Differential scanning calorimetric thermograms of three untriturated commercial digoxin samples. Key: top, S; middle, Z; and bottom, L1.*

added. The culture tubes were placed in an ultrasonic bath[8] for 30 min and then transferred to a shaker bath[9] maintained at 37° and shaking at 120 cpm. After 24 hr, the samples were filtered through 0.22-μm membrane filters[10], and the absorbance was read at 222 nm on a UV–visible spectrophotometer[11].

Concentrations were calculated according to a predetermined Beer's law plot. All studies were performed in triplicate. Excellent reproducibility was obtained; with some exceptions, only the average results are reported.

Dynamic Solubility Studies—Based on the results from the equilibrium solubility study, an excess amount (50 mg) of powder of digoxin L1, L2, and Z, digitoxin L and Z, and their triturated samples was added to 125 ml of water in a 250-ml beaker maintained at 37° in a water bath[12]. The medium was stirred at 200 rpm with a stirrer[13] centered in the beaker 2 cm from the bottom. The beaker was covered with parafilm to prevent water evaporation.

Three-milliliter samples were withdrawn at 5, 10, 20, 40, and 60 min and then periodically up to 24 hr. The samples were treated as in the equilibrium solubility studies, and the concentrations were determined. All studies were performed in duplicate, and only the average results are reported. The high agitation rate was necessary to overcome the effect of powder aggregation and agglomeration due to the hydrophobic nature of the cardiac glycosides and to enhance the dissolution rate.

Aging—Thermal studies were performed at various times on selected digoxin and digitoxin samples which were kept in sealed bottles at ambient temperature. The 24-hr equilibrium solubility study was conducted for digoxin L2 five times over a 1-year period.

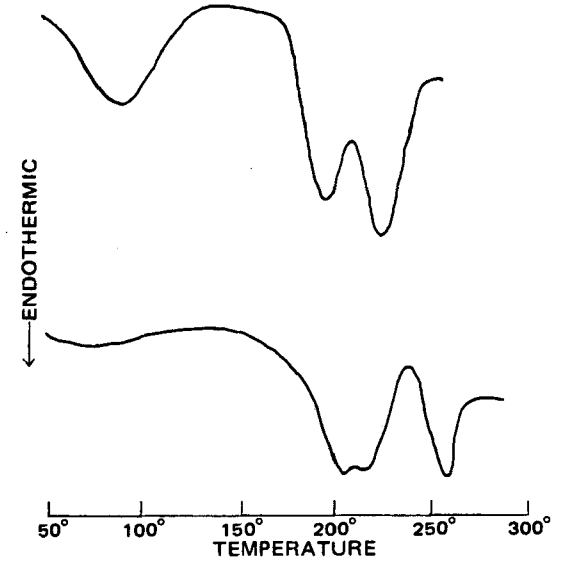

Figure 2—*Differential scanning calorimetric thermograms of digoxin recrystallized from chloroform (top) and 95% ethanol (bottom) from the L1 source.*

[8] Varian Aerograph, Walnut Creek, Calif.
[9] Eberbach Corp., Ann Arbor, Mich.
[10] Millipore filter (with Swinney filter device), Millipore Corp., Bedford, Mass.
[11] Beckman DBGT, Beckman Instruments, Fullerton, Calif.
[12] Fisher Versa-Bath, Fisher Scientific Co., Chicago, Ill.
[13] Nalgene stirrer, three blades, (1⅓ inch diameter), Fisher Scientific Co., Chicago, Ill.

Table II—Melting Ranges of Digoxin from Various Sources

Source	Date of Study	Beginning of Melting	End of Melting
L1	8/20/74	137°	225°
L1	10/22/74	146°	228°
L1	12/17/74	150°	230°
L1	10/14/75	182°	235°
L1 triturated	10/14/75	173°	176°
L2	10/28/74	150°	227°
L2	10/14/75	167°	233°
L2	3/5/76	167°	233°
L2 triturated	10/14/75	177°	184°
Z	10/28/74	192°	228°
S	10/22/74	180°	234°
L1 recrystallized from chloroform		175°	196°
L1 recrystallized from ethanol		150°	205°

RESULTS AND DISCUSSION

The melting test is often used for routine quality control of a drug or compound (3). The melting range is defined as those points of temperature within which the solid coalesces and is completely melted (3). Therefore, unless the melting of a compound takes place instantaneously, a melting temperature range should be reported. A literature survey on both glycosides found not only a large variation in the reported melting points but also an almost complete absence of any reported melting ranges. These literature data are summarized in Table I.

Differential thermal analyzers and differential scanning calorimeters are extremely sensitive instruments for studying the thermal behavior of compounds (18–22). They are particularly valuable in studying polymorphic transitions and the beginning of melting of a compound or a mixture. The temperature at which the suspected melting endothermic

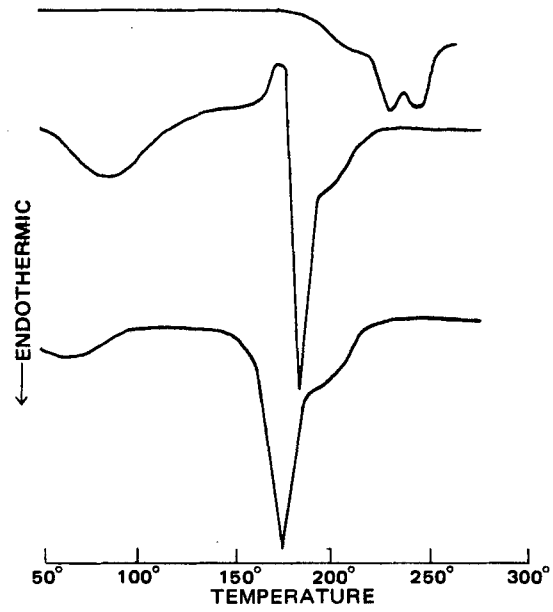

Figure 3—*Differential scanning calorimetric thermograms of digoxin from the L2 sample. Key: top, untreated; middle, triturated for 15 min; and bottom, triturated for 15 min, heated through exothermic transition, cooled, and reheated.*

Table III—Average Solubilities of Digoxin from Various Sources in Water at 37° after 24 hr of Equilibration

Source	Solubility, μg/ml
L1	41.8
L1 triturated	41.0
L2	68.2
L2 triturated	98.0
Z	40.1
Z triturated	39.5
S	28.9
L1 recrystallized from chloroform	64.7
L1 recrystallized from ethanol	52.1

peak begins (first deviation from the baseline in the thermogram) is considered to be the beginning of melting. When the conventional capillary tube method is used for the melting-point study, it is often difficult to determine the temperature at which the sample starts to melt, even when a small fraction of the sample has been melted already (20–22). On the other hand, when the sample melts slowly over a wide temperature range, the conventional capillary tube method is often superior in determining the final melting temperature.

In the present study, the differential scanning calorimeter was used to measure the beginning melting temperature, and both the differential scanning calorimetric and capillary tube methods were used to measure the final melting temperature. The final melting point of the sample generally corresponded to the first major endothermic peak in the differential scanning calorimetric study. Results on the melting ranges of digoxin are summarized in Table II, and some typical differential scanning calorimetric thermograms are shown in Figs. 1 and 2.

Consistent with the prior literature data, an extremely wide final melting-point range (176–235°) was found in the digoxin samples. The highest melting point reported previously was 270° (12). In contrast with previous reports, a wide melting-point range was found for each untreated digoxin sample obtained from the commercial sources or recrystallized from chloroform or ethanol. The largest range, 88°, was found in the L1 sample. Sample discoloration could be observed first at approximately the beginning of melting, and small droplets of melt could be observed with a hot-stage microscope well below the final melting temperature. Additional endothermic peaks above the melting point are believed to be due to decomposition.

Several possible reasons for such a marked variation in the melting range among all untreated digoxin samples studied can be postulated:

1. Polymorphic forms—The marked difference in melting ranges and thermograms might suggest that the phenomena are due to the existence of a different polymorphic (crystalline) form for each sample (19, 21). This possibility can be deemphasized, but not entirely ruled out, as a major cause because almost identical X-ray diffraction spectra were obtained previously (7, 8) from various digoxin sources, which also showed different dissolution, solubility, and melting properties.

The difference in the melting range possibly could result from various combinations of two or three polymorphic forms. Such a combination should produce the same X-ray diffraction peaks or lines with only different intensities from various samples. This hypothesis is not inconsistent with the reported X-ray diffraction data (7, 8). The possibility of varying fractions of an amorphous form with other crystalline digoxin seems unlikely because of the absence in the thermograms of an exothermic peak, which usually occurs when an amorphous form is converted to the crystalline form upon heating (21, 22). This point will be elaborated later.

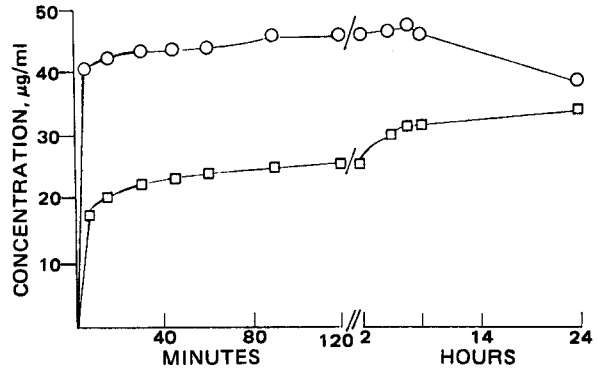

Figure 4—*Dynamic solubility study in water at 37° on the untriturated (bottom) and triturated (top) digoxin Z powder.*

Table IV—Effect of Aging on Solubility (Micrograms per Milliliter) of Digoxin in Water at 37°

Tube	Date of Study 3/16/75	7/11/75	9/15/75	12/3/75	3/2/76
1	67.5	63.7	61.0	55.2	53.5
2	68.5	62.2	61.0	56.2	53.8
3	68.5	62.7	61.5	56.7	52.5
Average	68.2	62.9	61.2	56.0	53.3

2. Crystal defects—Various degrees of extensive defects in the crystal lattice conceivably could produce various degrees of difference in the melting range, especially in light of the large molecular structure of digoxin (mol. wt. 780.92). Crystal defects might also be expected to alter the solubility and dissolution properties (19). Such a defect in the digoxin crystal might not be sufficient to produce any detectable shifts in diffraction peaks or lines.

3. Solvate formation—Various types of solvate possibly might be formed during the purification process with different solvents. The same digoxin sample (L1) recrystallized from chloroform and from ethanol yielded different thermal (Table II and Fig. 2) and solubility (discussed later) properties. Additional studies using the differential thermal gravimetric method should delineate this point.

4. Structurally related impurities—The possibility that the major cause of the wide melting range is the presence of impurities with chemical structures similar to digoxin is quite remote since the trituration of two digoxin samples resulted in sharper melting (Table II). This idea is an analogy to the common phenomenon of melting-point depression in the presence of impurities. The purity of digoxin samples seems to be supported by high-pressure liquid chromatographic (HPLC) analysis (23), which showed only one peak from both commercial and USP reference digoxin, and also by TLC analysis (24).

5. Any combination of the above four factors.

Digoxin samples recrystallized from chloroform and from ethanol had much lower final melting points than all of the untreated commercial samples in the present and previous studies (7). Their thermograms also were quite different from those obtained from the untreated commercial samples. A huge endothermic peak at about 80° was found in the sample recrystallized from chloroform, probably resulting from the chloroform desolvation. A quick and brief preheating of the sample to ~120° eliminated this endothermic peak upon rerun of the thermogram. This apparent desolvation endothermic peak was much weaker from the sample recrystallized from ethanol.

Trituration with a mortar and pestle dramatically affected the differential scanning calorimetric thermogram in both lots of digoxin powder (Fig. 3). A large endothermic peak at ~75° was followed by a relatively sharp exothermic peak at 175°, which was followed by a sharp endothermic peak. The reason for the first endothermic peak is unclear. It might be due to a phase transition in the amorphous or glassy state. The exothermic peak was probably due to the conversion of the amorphous form to the crystalline form, which subsequently melted with a sharp endothermic peak. The sharp melting of the triturated digoxin also was confirmed by the capillary tube method.

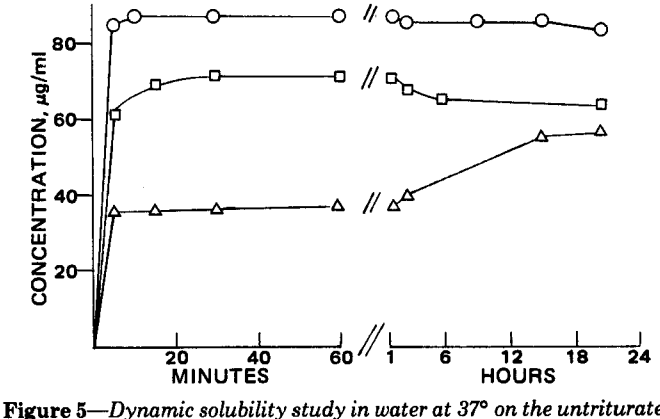

Figure 5—*Dynamic solubility study in water at 37° on the untriturated (bottom), freshly triturated (top), and triturated and stored (2–5 months; middle) digoxin L2 powder.*

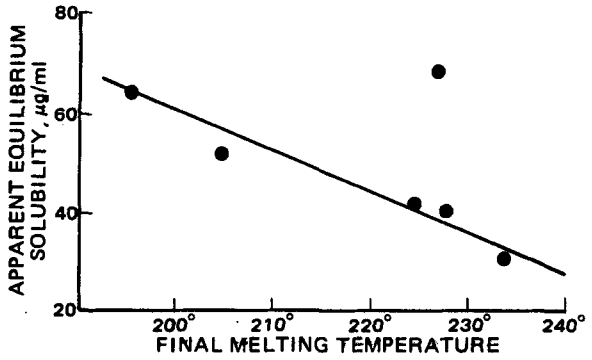

Figure 6—*Relationship between the apparent aqueous solubility and final melting temperature of various untriturated digoxin powders.*

This exothermic–endothermic thermogram was typical of the existence of an amorphous or glassy form, as found in melted and resolidified pure sulfisoxazole (21) and griseofulvin (22). Similar exothermic–endothermic thermograms obtained at temperatures above 140° also were reported for several ball-milled digoxin samples (7). As shown previously (7), the relative exothermic peak height varied with the source of the sample and the extent of trituration. This finding indicates that different degrees of conversion to the amorphous form might occur under various conditions. Furthermore, after rapidly heating the triturated sample in the differential scanning calorimetric cell through the exothermic transition, cooling to ambient temperature, and reheating, the exothermic peak disappeared and the final melting temperature decreased (Fig. 3).

Potential effects of storage on thermal, solubility, and dissolution properties of pure untreated digoxin powder apparently have not been reported. As shown in Table II, all three untreated commercial digoxin powders underwent some gradual changes in their thermal properties. An increase in the temperature for the beginning and/or final melting was generally observed. Two samples were studied for over 1 year, and these untreated samples were all in their metastable state during the study period. A very important question remaining to be answered is: "What is the final stable form?"

The results of the equilibrium solubility study conducted in water at 37° on digoxin powder from various sources are summarized in Table III. The solubilities ranged from 28.9 to 68.2 μg/ml among the untreated samples. A range of 24.3–63.6 μg/ml in water at 25° was reported from a study on four untreated commercial samples (7). Recrystallization from chloroform and from ethanol enhanced the solubility of the original L1 powder, and the two recrystallized samples differed in their solubility by 24.2%. Trituration significantly enhanced (43.7%) the solubility of the L2 sample and had a negligible effect on the L1 and Z samples. This finding is in contrast with the previous study (7) in which ball milling increased the solubilities of all four samples studied, with the highest increase being 118%.

The failure of trituration to enhance solubility can be explained by the results from the dynamic solubility study in which a large excess of digoxin powder was used and the concentrations were studied at very frequent intervals before the attainment of final equilibration. For digoxin Z, the triturated sample reached the highest concentration at 5 hr after study and started to decrease and approach the concentration obtained from the untreated sample (Fig. 4). A similar result was obtained with the L2 sample. Therefore, the failure of solubility enhancement by trituration clearly was due to the conversion of the higher energy amorphous form to the more stable, lower energy (crystalline) form during the 24-hr solubility study period. This effect, in turn, resulted in some precipitation of digoxin from the solution.

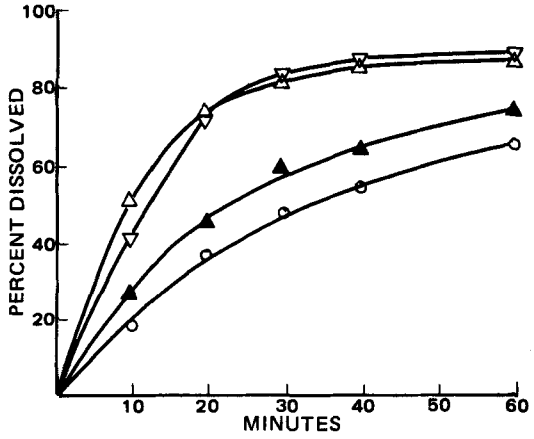

Figure 7—*Effect of temperature and length of storage time on the dissolution rate of digoxin from Tablet A. Key: △, fresh sample; ▽, sample stored at ambient temperature for 4 weeks; ▲, sample stored at 60–65° for 4 weeks; and ○, sample stored at 60–65° for 8 weeks.*

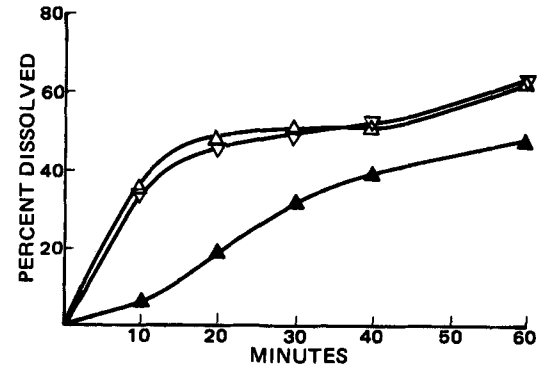

Figure 8—*Effect of storage on the dissolution rate of digoxin from Tablet B. Key: △, fresh sample; ▽, sample stored at ambient temperature for 4 weeks; and ▼, sample stored at 60–65° for 4 weeks.*

For digoxin L2, which had shown the solubility enhancement effect, the decrease in concentration during the dynamic solubility study was obvious also (Fig. 5). The same triturated sample after additional storage of about 2.5 months at ambient temperature showed a clear reduction in the digoxin concentration during the dynamic solubility study (Fig. 5). A further reduction in the dynamic solubility values will probably occur if the sample is studied again at a later date. Therefore, any gain in the apparent solubility and also the dissolution rate by the trituration or milling process appears to be temporary. These results demonstrate that the dynamic solubility method is superior to the equilibrium solubility method in detecting different thermodynamic states.

All solubility studies on the untreated digoxin samples reported in Table III were conducted on the same day. The different solubilities found in the two lots obtained from the same commercial source might be attributed to different aging or storage effects or to a different method of preparation.

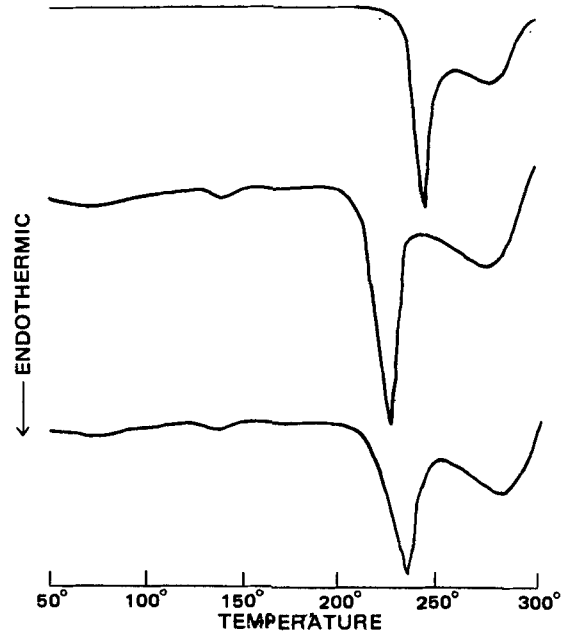

Figure 9—*Differential scanning calorimetric thermograms of three commercial untriturated sources of digitoxin. Key: top, Z; middle, L; and bottom, S.*

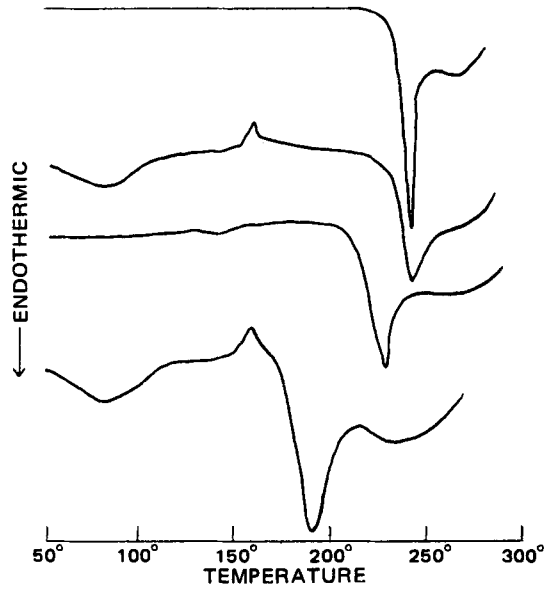

Figure 10—*Differential scanning calorimetric thermograms of digitoxin. Key: top, untriturated Z; upper middle, triturated Z; lower middle, untriturated L; and bottom, triturated L.*

The fact that essentially the same "apparent" equilibrium solubility was found over several days during the study for each untreated digoxin sample indicates a very slow phase transition in water. This transition is different from many drugs exhibiting polymorphism (19, 21).

Table IV summarizes the effects of aging at ambient temperature on the apparent equilibrium solubility of the untreated digoxin L2 sample. The decline in the solubility over the 1-year period was linearly related to time (coefficient of variation = 0.9913). This type of finding has not been reported to date. A slight increase in the melting range of this sample was found over the 1-year period (Table II).

The temperature at which the final melting of the sample takes place could be indicative of crystal intermolecular forces or thermodynamic stability, which both could be related to the dissolution rate or apparent equilibrium solubility. For all six untriturated digoxin samples studied, except L2, the apparent solubility, as expected, decreased almost linearly with the increase of the final melting temperature (Fig. 6). This result shows that the simple and fast differential thermal analysis method might be useful for routine quality control of digoxin powder.

In light of these findings on the effects of aging on the thermal and solubility properties of the untriturated digoxin powder, one could speculate that storage of commercial digoxin solid dosage forms at room temperature might also reduce the dissolution rate and oral absorption of digoxin from the products. A preliminary study on the effect of aging or storage at room temperature (22–26°) and at an elevated temperature on *in vitro* dissolution was conducted on two widely distributed 0.25-mg tablet products obtained from a local pharmacy. Three tablets were dropped gently to the bottom of a 600-ml beaker containing 500 ml of water maintained at 37 ± 0.5°. The stirrer[13], with a predetermined stirring rate of 60 rpm, was quickly lowered to the center of the dissolution medium. The dissolved digoxin concentration was measured by the official compendial method (11) at 10, 20, 30, 40, and 60 min.

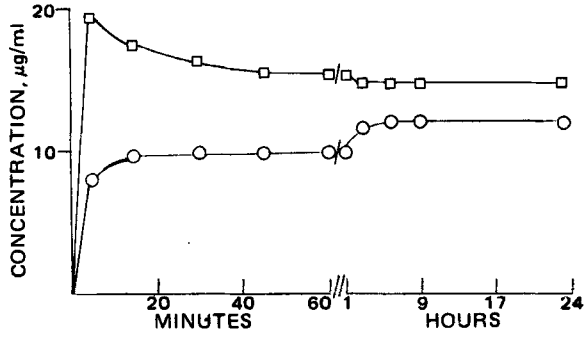

Figure 11—*Dynamic solubility study in water at 37° on the untriturated (bottom) and triturated (top) digitoxin Z powder.*

Table V—Melting Ranges of Digitoxin from Various Sources

Source	Date of Study	Beginning of Melting	End of Melting
L	11/11/74	195°	225°
L	9/20/75	195°	227°
L	10/15/75	201°	228°
L triturated	10/15/75	170°	192°
Z	10/15/75	230°	244°
Z triturated	10/15/75	220°	244°
S	11/11/74	200°	233°

Four duplicate studies were performed on each type of sample. For each tablet product, an initial control and samples stored at room temperature and at 60–65° in an oven for 4–8 (only one product) weeks were studied. All tablets were stored in air-tight glass vials. Very reproducible results were obtained from the duplicate studies. The average data on the percent dissolved as a function of time are shown in Figs. 7 and 8. Differences in dissolution rate from samples stored at room and elevated temperatures were highly statistically significant at all of the times ($p < 0.01$) for both products. Storage at room temperature for 4–8 weeks had a negligible effect on the dissolution rate. The reduction in the dissolution rate in the elevated temperature studies could not be attributed to digoxin chemical decomposition, as shown by the assay of both products. For the product studied for 8 weeks, the aging effect continued throughout this period (Fig. 7).

Based on the results from powder and tablet studies, it is obvious that serious consideration should be given to the possible aging effect of all solid digoxin products when evaluated by either the dissolution or the oral absorption method. A freshly manufactured product that has passed the dissolution and absorption tests might not pass these tests after storage. The potential clinical implications are obvious. These results should be regarded as preliminary since the experimental conditions were not very realistic.

The thermal analysis studies on digitoxin generally paralleled those of digoxin since digitoxin has only one hydroxyl group less than digoxin. Most of the discussion on digoxin can also be applied to digitoxin.

As shown in Fig. 9 and Table V, the melting ranges from the three untreated sources varied less than for digoxin. The widest range was 33°, found in digitoxin S. The final melting points for both digoxin and digitoxin in the present study were all much less than those reported in most of the literature (Table I). Although a very narrow melting range (256–257°) was reported for digitoxin from once source (17), it probably represents the final melting temperatures at which most of the sample was "observed" to melt.

The reason for the rough baseline below the beginning melting temperatures of digitoxins L and S is unknown. This phenomenon was not found in digitoxin Z or in the untreated commercial digoxin samples. The apparent endothermic peaks at ~135–140° found in the digitoxin L and S samples might be due to the eutectic melting caused by impurities (20, 22). This contention is supported by the fact that the official pharmacopeia only requires that digitoxin (powder) contain ≥90.0 and ≤101.0% (mean 95.5%) of pure digitoxin calculated on the dried basis (11), thereby allowing the presence of a significant amount of impurity. However, for official digoxin powder, a concentration range of 97–103% (mean 100%) is required (11).

The differential scanning calorimetric thermograms from two triturated digitoxin samples are shown in Fig. 10. Significant endothermic and exothermic properties prior to melting were observed in both samples, indicating the presence of an amorphous form after trituration. No reduction in the final melting temperature was found in the triturated Z sample.

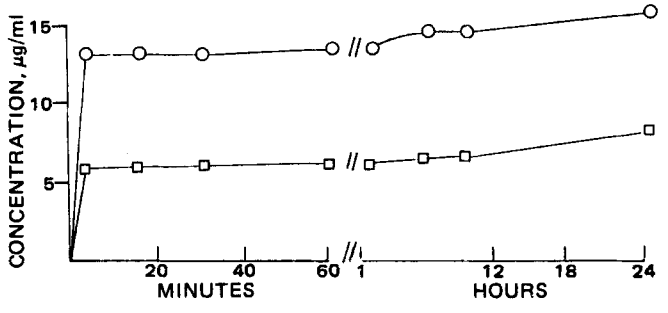

Figure 12—*Dynamic solubility study in water at 37° on the untriturated (bottom) and triturated (top) digitoxin L powder.*

Table VI—Average Solubilities of Digitoxin from Various Sources in Water at 37° after 24 hr of Equilibration

Source	Solubility, μg/ml
Z	15.2
Z triturated	15.8
L	8.0
L triturated	15.8
S	10.0

The results of the digitoxin solubility studies are summarized in Table VI. An almost twofold difference in the apparent equilibrium solubility was found between the Z and S samples. The highest solubility found in the Z sample (15.2 μg/ml) is 3.2 times that (4.8 μg/ml) reported in a previous study (25), indicating marked variations with the sources of material.

Trituration had a significant enhancement effect on the L sample but not on the Z sample. Such a discrepancy could be rationalized by the results from the dynamic solubility studies (Figs. 11 and 12).

Similar aging effects were also observed for the untriturated digitoxin stored at room temperature (for example, the thermal study of digitoxin L, Table IV). For the digitoxin Z sample, the solubility also decreased with time (Table VI and Fig. 11). The dynamic solubility study was conducted several months after the equilibrium solubility study.

The results of this preliminary study on digoxin and digitoxin raise many questions regarding the physicochemical properties and quality control of both cardiac glycosides. A more detailed and systematic investigation on the mechanisms behind the wide range and variation in melting, dissolution, and solubility is needed. The effect of particle size on these properties should be evaluated. All samples used in this study appeared to be very fine. For example, the particle size of digitoxin L1 ranged from 3.1 to 12.4 μm from the microscopic study and that of digitoxin L ranged from 12.4 to 86 μm.

Other questions remaining to be answered include:

1. Could a stable form with a sharp melting point be found for both drugs, and what is the most thermodynamically stable form?
2. How pure is the powder that meets all pharmacopeial requirements?
3. What are the effects of aging on dissolution and bioavailability, and are current regulatory requirements in these aspects adequate?
4. Could more water-soluble hydrates or solvates of both drugs be prepared?
5. Could a quantitative differential scanning calorimetric method be established for powder quality control? In this study, no attempts were made to evaluate thermal energy either quantitatively or qualitatively. This energy possibly might be directly related to dissolution and solubility.

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ACKNOWLEDGMENTS

Presented in part at the Basic Pharmaceutics Section, APhA Academy of Pharmaceutical Sciences, New Orleans meeting, 1976.

Abstracted from a thesis submitted by L. E. Kyle to the Graduate College, University of Illinois at the Medical Center, in partial fulfillment of the Master of Science degree requirements.