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# Changes in crystallinity and solubility on comminution of digoxin and observations on spironolactone and oestradiol*

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Using infrared spectroscopy, X-ray diffractometry, differential thermal analysis, scanning electron microscopy, solubility and dissolution rate measurements, it was demonstrated that the comminution of digoxin results in the appearance of an amorphous phase. The examination of spironolactone and 17β-oestradiol by infrared spectroscopy and differential thermal analysis showed that these compounds also undergo changes in their crystallinity on grinding. Since the dissolution characteristics of poorly soluble drugs may be complex functions of surface area and crystallinity, it is concluded that the most pertinent method for standardizing a sample of a polymorphic drug of low solubility is by means of a powder dissolution test, as the results embrace the influences of particle size, aggregation and polymorphism.

It has been shown that polymorphic transitions may be induced in certain organic compounds, particularly those with a steroidal structure, by means of comminution (Baker, 1957; Roberts, 1957; Moustafa, Ebian & others, 1971). Fluprednisolone has been reported to form the amorphous phase on lyophilization (Haleblian, Koda & Biles, 1971), as does methylprednisolone (Moran, Gillard & Roland, 1971). Digitoxin is thought to be present as the amorphous phase when in solid solution in polyethylene glycol 6000 (Chiou & Riegelman, 1971), or coprecipitated with polyvinylpyrrolidone 40 000 (Stupak & Bates, 1973). The poorly soluble crystalline saponins aescin and digitonin were both found to be converted to a much more soluble amorphous phase on grinding (Rosoff, Schulman & others, 1967).

Since there was evidence to suggest that reducing the particle size of digoxin improved its dissolution and bioavailability (Jounela & Sothmann, 1973; Shaw, Carless & others, 1973), the similarity of the molecule to the above mentioned compounds led us to investigate the effects of comminution on its crystal properties (Florence, Salole & Stenlake, 1974). This paper discusses the changes that occur on the grinding of digoxin in particular, with reference to studies on structurally similar spironolactone, 17β-oestradiol and digoxigenin. The problem of standardizing poorly soluble drugs which are prone to alterations in their physical characteristics during pharmaceutical manipulations is examined.

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## MATERIALS AND METHODS

*Digoxin samples:* 'British Standard', 'Swiss Standard' and 'Swiss Micronized (Sandoz)' were obtained through Courtin-Warner Ltd., Lewes, Sussex, and 'British Chemical Reference Substance' digoxin was obtained from the British Pharmacopoeia Commission, London.

Digoxigenin was obtained from the Aldrich Chemical Co., and spironolactone and 17β-oestradiol from the Sigma Chemical Co., London.

*Comminution* of crystalline samples (500 mg) was effected either by manual grinding in an agate mortar, or by milling in a Glen Creston Model M270 ball mill (1·3 cm i.d., × 3·8 cm Nylon 66 chamber, with a single 1·03 g steel ball of 0·65 cm diameter) for between 3½ and 8 h.

*Powder dissolution tests* were performed as follows: 225 ml of an aqueous 0·005% solution of polysorbate 80 (Tween 80, Koch Light Labs) were equilibrated at 37·0 ± 0·2° in a 250 ml Quickfit conical flask fitted with a magnetic stirrer (2·5 cm × 0·7 cm) and a filter-stick (6 cm long, with a 1 cm diameter sintered plate of 5–10 μm porosity, Sinta Glass Ltd.), suspended through the rubber bung. At zero time, 25 mg of digoxin was washed into the flask with 25 ml of pre-warmed dissolution medium. At intervals, 5 ml samples were removed through the filter-stick assembly and immediately filtered (Millipore MF membrane, 0·1 μm mean pore diameter). The samples were assayed by ultraviolet spectrophotometry at 221 nm.

*Equilibrium solubilities* were determined by measuring the concentration of filtrates of suspensions held in a water-bath at 25·00 ± 0·05°, using both ultraviolet spectrophotometry and the B.P. fluorimetric assay method (British Pharmacopoeia 1973, Addendum 1975). The values were obtained after equilibration of solid and solvent for as long as 8 weeks, during which time no breakdown of the digoxin was detected by t.l.c. in several systems.

*Infrared spectra* were obtained, using both the KCl disc and Nujol mull techniques, with Perkin Elmer Model 157 and 237 spectrophotometers.

*Differential thermal analysis* (*DTA*) was carried out on a Stanton Redcroft Model 671 analyzer. Samples (10 mg) were heated in nickel alloy ('Inconel', Stanton Redcroft Ltd) cups at the rate of 10° $min^{-1}$, under static normal atmospheric conditions, with sintered alumina (10 mg) as reference.

*X-ray diffraction* patterns of powders were obtained with a Guinier camera, using the $K\alpha_1$ line of a copper target. The samples were attached to 'Sellotape' strip.

*Electron micrographs* were obtained with a Cambridge Stereoscan Mk IIA scanning electron microscope, after the samples had been coated with palladium-gold 40 : 60 alloy to a thickness of 5–20 nm.

*Specific surface areas* of powders were determined by the BET method, using a Perkin Elmer Model 212 C sorptometer and nitrogen gas. The precision of the technique was ±4%, the accuracy ±10%.

Organic solvents used were analytical reagent grade (Analar, BDH Ltd).

## RESULTS AND DISCUSSION

The four types of commercial untreated crystalline digoxin were found to have the same infrared absorption spectrum (Hayden, Sammul & others, 1962) and X-ray diffraction pattern (Fig. 1a), and therefore by definition, are of identical crystal structure. However, under the scanning electron microscope, the samples revealed different characteristics. Swiss Standard digoxin was found to consist mainly of thin plates, British Standard and Swiss Micronized samples of a mixture of fines and thick angular crystals, and the British Chemical Reference Substance material of relatively large crystals arranged in clusters (Fig. 2). The samples surprisingly differed in their equilibrium solubilities at 25° and

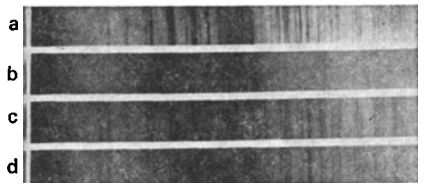


FIG. 1. Guinier X-ray diffraction photographs of crystalline and comminuted digoxin: (a) Swiss Standard digoxin, (b) Swiss Standard ball milled, (c) and (d) Swiss Micronized mortar ground.

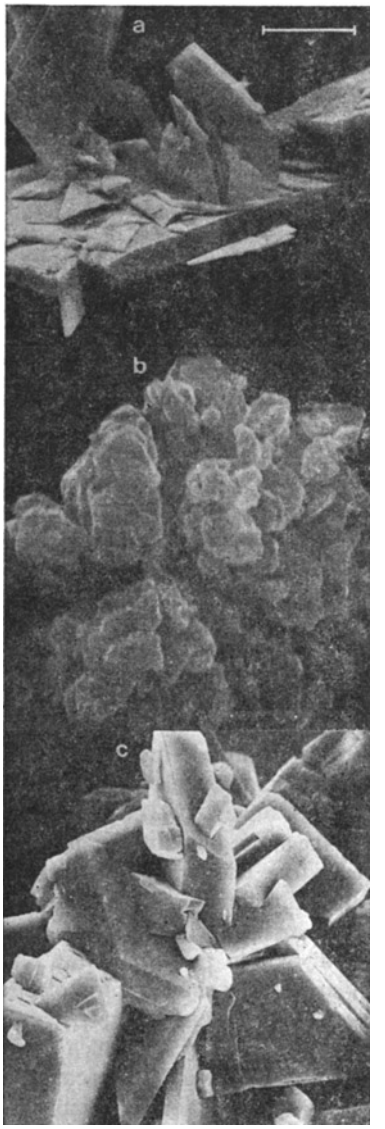


FIG. 2. Scanning electron micrographs of crystalline and comminuted digoxin samples: (a) Swiss Standard, (b) Swiss Standard ball milled, (c) British Chemical Reference Substance. The scale marker is equivalent to (a) 17·0 μm, (b) 3·1 μm, (c) 22·7 μm.

melting behaviour, as determined by DTA (Table 1 and Fig. 4), and powder dissolution rates (Fig. 3; Florence & others, 1974). The DTA thermograms suggest that the commercial samples differ in thermodynamic properties from at least the reference substance: this would seem to be reinforced by the disparity in solubilities. Nevertheless, as far as dissolution is concerned, the overriding factor appears to be surface area, since when plotted against initial dissolution rate (Table 1) it was found to correlate well, although the dissolution rate of these commercial samples when plotted against the apparent solubility of each sample is also linearly related.

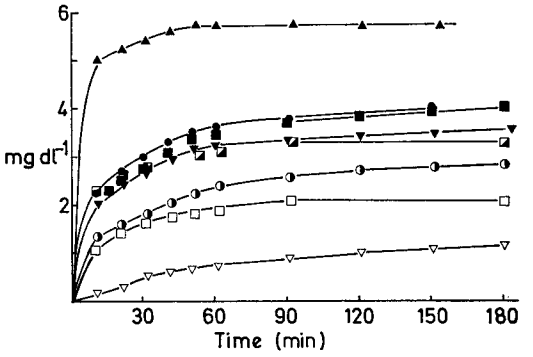


FIG. 3. Dissolution of original and comminuted digoxin samples (mg dl$^{-1}$) in 0·005% polysorbate 80 at 37°: ▽, ▼ British Chemical Reference Substance; ◑, ● Swiss Standard; ▲ Swiss Micronized, □, ◨, ■ British Standard. Open symbols represent the original unground samples, partly closed symbols the mortar ground samples and closed symbols the milled samples.

On comminuting crystalline digoxin, several physical characteristics were altered to an extent partly dependent on the degree of comminution. Some of the infrared absorption bands in the 700–1500 cm$^{-1}$ frequency range lost their definition, and, characteristically, a shoulder appeared at 1780 cm$^{-1}$ and grew with increased comminution to a distinct peak (Florence & others, 1974). Spectral changes of this kind are generally indicative of alterations in the intermolecular forces within crystals, and in this instance the extraneous peak at 1780 cm$^{-1}$ was found to be associated with the aglycone part of the molecule, since digoxigenin displayed the same changes on grinding.

X-ray diffraction patterns for digoxin became more diffuse with increased comminution, the lines finally broadening to an extent such that individual detail disappeared and the picture was essentially that of an amorphous sample (Fig. 1, c, d and b). Since X-ray diffraction patterns depend on the existence of long-range crystalline order, a blank picture is indicative of either a genuine phase transition to an amorphous phase or the presence of a substantial proportion of particles below 10 nm (which is effectively equivalent). Scanning electron micrographs show that even at high magnification the aggregated particles of milled digoxin do not have a distinctly crystalline appearance (Fig. 2b).

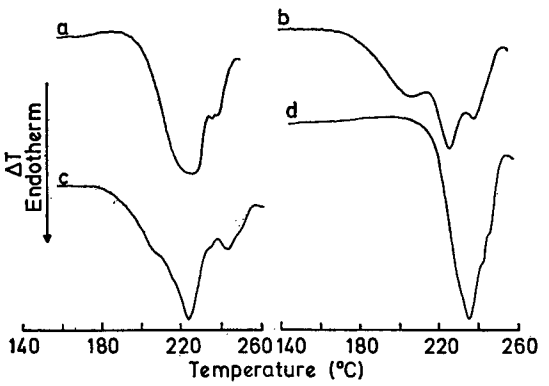


FIG. 4. Differential thermal analysis thermograms of crystalline digoxin samples: (a) Swiss Standard, (b) Swiss Micronized, (c) British Standard, (d) British Chemical Reference Substance.

The DTA thermograms revealed an altered melting behaviour, with milled samples having much sharper melting endotherms than untreated samples, the peaks occurring some 50–60° below those of the corresponding crystalline substance (Fig. 5). This alteration is undoubtedly due in part to the much

Table 1. *Solubility (Sol), specific surface area (SSA), initial dissolution rate (Init. diss. rate) and melting points of crystalline and comminuted digoxin samples.*

| Digoxin | Sol.[1] mg dl$^{-1}$ | S.S.A. m$^2$ g$^{-1}$ | Init. diss. rate[2] mg dl$^{-1}$ min$^{-1}$ | M.P.[3] °C |
|---|---|---|---|---|
| British Standard | 6·02 | 0·63 | 0·11 | 234 |
| Mortar ground | | 4·15 | 0·23 | |
| Ball milled | 6·48 | 4·00 | 0·20 | 178 |
| Swiss Standard | 4·06 | 0·43 | 0·08 | 234 |
| Mortar ground | | 6·63 | 0·13 | |
| Ball milled | 5·76 | 4·54 | 0·23 | 179 |
| Swiss Micronized | 6·36 | 0·76 | 0·18 | 236 |
| Mortar ground | | 1·83 | 0·31 | |
| Ball milled | 9·79 | 3·04 | 0·50 | 184 |
| British Chemical Reference Substance | 2·43 | 0·30 | 0·02 | 240 |
| Mortar ground | | 2·33 | 0·09 | |
| Ball milled | 5·30 | 5·22 | 0·20 | 179 |

1. The solubility figures are equilibrium solubility values in water at 25·00 ± 0·05°. 2. Dissolution rate between t = 0 and t = 10 min, extrapolated from the powder dissolution rate curves (Fig. 3; Florence & others, 1974). 3. Melting points were determined from DTA thermograms.

reduced particle size, but considering the dissimilarity between the thermograms of crystalline samples it would seem reasonable to suggest that milling reduces the samples to a common form, which is essentially amorphous.

The apparent equilibrium solubilities at 25° of milled samples showed increases of between 7 and 118% over those of the starting materials (Table 1), supporting the suggestion that a transition to the amorphous phase occurs on comminution. The specific surface areas of comminuted samples also increased, as one would expect, and this was reflected by an increase in sample dissolution rate (Table 1);

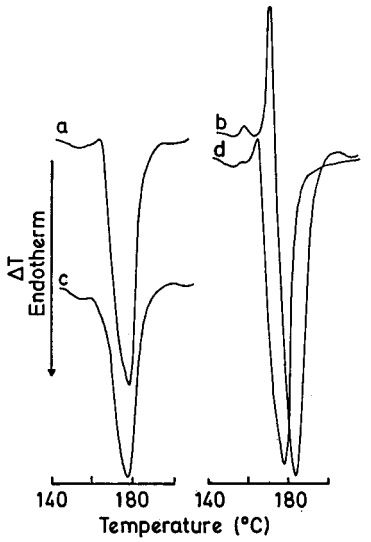


FIG. 5. Differential analysis thermograms of ball milled digoxin samples: (a) Swiss Standard, (b) Swiss Micronized, (c) British Standard, (d) British Chemical Reference Substance.

however, the positive correlation between dissolution rate and specific surface area or solubility no longer obtained. This may be partly due to the strong aggregation of particles in suspension despite the presence of surfactant, but may also be a reflection of the varying degrees of crystallinity resulting from different comminution processes.

That reducing the particle size of digoxin causes it to adopt a more amorphous character was shown in other ways. Digoxin was precipitated out of a hot concentrated pyridine solution by the rapid addition of a large excess of cold water: the dried fine precipitate exhibited the infrared band at 1780 $cm^{-1}$ characteristic of 'amorphous' digoxin. Similarly, digoxin evaporated onto powdered KCl from ethanolic solution produced the partially amorphous form. The coprecipitation of digoxin with high molecular weight polyvinylpyrrolidone to produce a fine dispersion in a soluble matrix resulted in its being present as the amorphous form, as detected again by infrared spectroscopy.

The diuretic spironolactone is known to crystallize out in different polymorphic forms and to undergo structural rearrangements on heating (Mesley & Johnson, 1965: Sutter & Lau, 1975). Its structural similarity to digoxigenin, in that both molecules possess a steroidal nucleus substituted at C17 with a lactone ring, and its poor solubility, prompted us to undertake a brief investigation into the effects of grinding on its crystal properties, particularly in view of reports that reducing its particle size considerably improved its dissolution characteristics and bioavailability (Bauer, Rieckmann & Schaumann, 1962; Levy, 1962; Shaldon, Ryder & Garsenstein, 1963). On grinding our sample of spironolactone no major changes in the infrared spectrum were produced (reference spectrum Sadtler Reference Spectra), apart from a decrease in resolution of the absorption peaks. The melting-point, as determined by DTA, remained the same, but the pre-melting behaviour was found to be radically altered (Fig. 6). This would indicate that grinding significantly affected the sample's crystallinity.

It had been reported that the amorphous modification and three crystalline forms of the steroid 17$\beta$-oestradiol were all converted to a fourth crystalline form, form A, on grinding (Smakula, Gori & Wotiz,

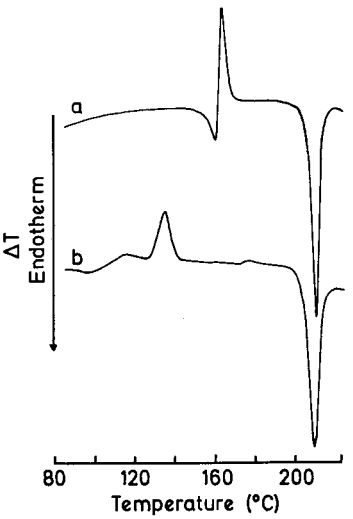


FIG. 6. Differential thermal analysis thermograms of (a) crystalline and (b) mortar ground spironolactone samples.

1957). It was shown by DTA that grinding this A form of oestradiol altered its crystal properties, as reflected by changes in its pre-melting behaviour, the melting-point and infrared spectrum remaining the same (Fig. 7).

Tablets made from triturations of digoxin and lactose prepared by ball-milling were reported to have a better dissolution rate than tablets manufactured from triturations prepared by what was considered the equivalent technique of depositing the drug onto lactose from ethanolic solution (Shah,

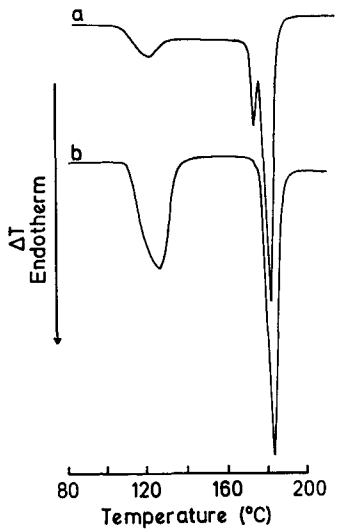


FIG. 7. Differential thermal analysis thermograms of (a) crystalline and (b) mortar ground 17$\beta$-oestradiol samples.

Pytelewski & others, 1974). This illustrates the necessity for a method of standardizing poorly soluble drugs which, like digoxin, spironolactone and oestradiol may be susceptible to variations in their crystal properties in the course of pharmaceutical manipulations. As described above, a certain amount of quantitative information may be obtained by a variety of methods. However, as also shown, the information most of these provide is not necessarily directly related to the dissolution characteristics of the material, and in any case many of the techniques are experimentally inconvenient.

The measurement of dissolution rates from the single exposed face of a non-disintegrating disc has been extensively used to characterize solid drug samples (Wadke & Reier, 1972; Sekiguchi, Kanke & others, 1973), and has been found in this work to be useful in distinguishing between crystalline and amorphous samples of digoxin. Dissolution rates from non-disintegrating discs, in spite of complications which do not allow calculation of precise intrinsic dissolution rates, show that British Standard "milled" has a rate of solution of $16 \times 10^{-4}$ mg dl$^{-1}$ min$^{-1}$ and that of the unmilled sample $6 \times 10^{-4}$ mg dl$^{-1}$ min$^{-1}$, i.e. the milled sample shows a rate of solution 2·6 $\times$ that of the unmilled sample and a rate of solution 5 $\times$ that of the British Chemical Reference material. But data from these intrinsic dissolution rate experiments must be carefully considered because scanning electron micrographs of both digoxin and sodium chloride discs revealed that the surfaces had sizeable fissures and pits in them, as well as the exposed edges of small collections of crystals, all of which could serve as sites for preferential dissolution.

Therefore, since the dissolution characteristics of poorly soluble drugs may be complex functions of surface area and degree of crystallinity, it is concluded that as the rate of solution is such an important pharmaceutical parameter the most pertinent and convenient method for standardizing a sample of a polymorphic drug of low solubility is by means of a powder dissolution test, because the results embrace the influences of particle size, aggregation and polymorphism.

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