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Crystal modification of methisazone by grinding

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Methisazone (1-methylisatin 3-thiosemicarbazone) is an antiviral agent (Bauer, Dumbell & others, 1962). In the freshly prepared state it exists as long fibres, possessing low solubility, poor absorption, low bulk density and poor flow properties (Axon, 1972). It is used in various pharmaceutical formulations as freshly ground material, where there is a ten-fold increase in dissolution rate and accompanying increased bio-availability. The freshly ground material has a marked tendency to revert to the original fibrous crystals on storage; this crystal growth has been described as out-growths of 'whiskers' (Deavin & Mitchell, 1965).

The above results suggest a crystal modification of methisazone occurs on grinding to form an unstable polymorph (or amorphous form). Florence, Salole & Stenlake (1974) have reported that similar modifications occur during the grinding of digoxin.

We have examined infrared spectra and carried out X-ray diffraction and differential thermal analysis on three samples of methisazone—unground and micronized material as obtained from Burroughs Wellcome and freshly ground material, formed by ball-milling some of the unground material from the same source.

X-ray diffraction patterns were obtained using a Toshiba Model ADG-301 diffractometer with nickel filtered copper radiation at a scan speed of $0.5^\circ \text{ min}^{-1}$. All three materials gave distinctive diffraction patterns (Fig. 1), indicating the presence of different polymorphic forms (Pfeiffer, Yang & Tucker, 1970). Recrystallized material formed by either slow recrystallization from dimethyl sulphoxide or by seeding with unground material gave a different diffraction pattern from material formed by adding water to the solution in dimethylsulphoxide.

In the differential thermal analysis study, slight differences in the endothermic peak commencing about 239° were observed for the materials using a Mettler TA2000 (Fig. 2). The presence of a shoulder on these peaks indicates a phase change before melting, observed microscopically using a hot-stage. With different polymorphic forms of oxyclozanide, Pearson & Varney (1973) also found only slight differences in thermal properties.

Similar slight differences were obtained in infrared spectra using Nujol mulls in a Perkin Elmer, Model 337

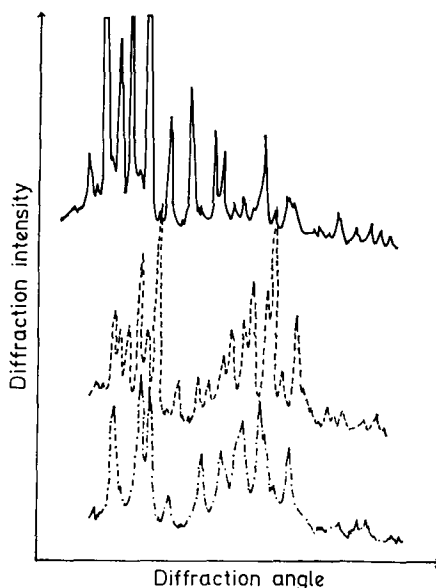


FIG. 1. X-Ray diffraction patterns of: unground methisazone —, micronized methisazone ---, milled/ground methisazone

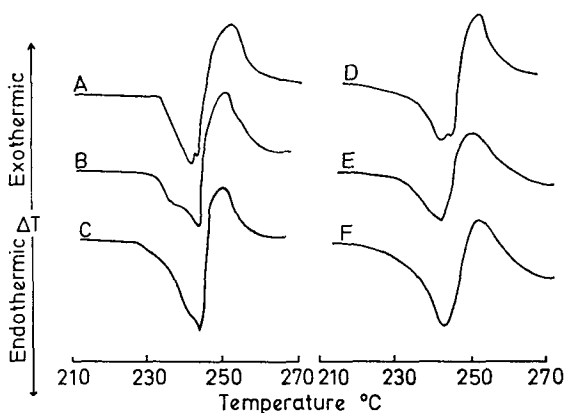


FIG. 2. D.T.A. traces of: A unground methisazone, B micronized methisazone, C milled/ground methisazone, D methisazone precipitated by water, E methisazone obtained by seeding, F methisazone obtained by slow recrystallization.

* Correspondence

grating spectrophotometer, i.e. at 610–620 cm^{-1} for each of the different forms. These differences have since been confirmed in a personal communication from Drs Axon & Mitchell.

Our results suggest that at least two polymorphic forms of methisazone exist. The unground and micronized materials both give evidence of a phase change in the shape of their endothermic peaks using differential analysis. The milled sample appears to give results somewhere between the unground and micronized samples for both X-ray and differential thermal analysis as might be expected from their previous history.

The unground material is probably prepared by precipitation as shown by the similarity of the two

thermograms (Figs 2A, D), whereas a single crystalline variety is produced by seeding or slow recrystallization (Figs 2E, F).

The unground methisazone may change crystal structure at elevated temperature as evidenced by its thermogram (Fig. 2A). Grinding of this material (Figs 2C, B) leads to the formation of a more active polymorph, which will revert more rapidly to a more stable polymorph as evidenced from the work of Deavin & Mitchell (1965). This would also account for the reported (Axon, 1972) increased solubility, biological activity and instability obtained with use of the micronized form in pharmaceutical formulations.

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Comparison of retention of activity of soluble and insolubilized forms of trypsin in a simulated gastric medium

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In pancreatic insufficiency, or after pancreatectomy, pancreatic extracts from animals (pancreatin) are administered orally to patients to supplement, or substitute, the secretion of proteolytic, lipolytic and amylolytic enzymes, normally secreted into the duodenum (Soergel, 1973; Warren & Jefferson, 1973; Anderson & Goodchild, 1976). Frequent incomplete response to even very high doses of pancreatin can be blamed on several factors:

- (i) gastric inactivation of trypsin and lipase have been well documented both *in vivo* and *in vitro* by Heizer, Cleaveland & Iber (1965); whereas trypsin is denatured by the combination of pepsin and low pH, lipase is inactivated by acidic pH alone;
- (ii) reduced pH in the duodenum due to poor bicarbonate secretion often accompanies pancreatic insufficiency (Hadorn, Zoppi & others, 1968); this is significant in view of the pH-dependence of enzymatic activity. pH optima for trypsin, lipase and amylase are 7 to 8, 7 to 9 and 6.9, respectively (Diem & Lentner, 1970);
- (iii) inadequate mixing of the enzymes with food;
- (iv) 'autodigestion' of trypsin, lipase and amylase by trypsin in pancreatin.

Attempts to overcome these problems by, e.g. sprinkling the enzymes directly on food, co-adminis-

tration of sodium bicarbonate, or enteric coating are either unsuitable for chronic treatment, or they do not always yield the expected results (Anderson & Goodchild, 1976).

It is now recognized that insolubilization of enzymes by physical or chemical attachment to macromolecular carriers is a versatile method for modification of the enzymic properties (Chang, 1975). Thus, it may be possible to formulate enzyme preparations with enhanced storage stability or pH-profiles optimized for a particular disease state. Our preliminary investigations (Wiseman & Gonda, 1976) indicated that trypsin immobilized on a variety of inert carriers exhibited a much greater resistance towards inactivation by simulated gastric fluid than the soluble form of trypsin. The experimental procedure reported in the present paper is a simplified version of the method described in the previous communication. The results demonstrate again the superior resistance of the insoluble forms of trypsin, although some quantitative differences from the results obtained in the previous tests can be observed.

Materials. A stock solution of trypsin (3150 NF, crystallized, Armour Pharmaceutical Company Ltd.) was prepared by dissolving approximately 25 mg of the enzyme in 10 ml 0.001 N HCl. This solution was