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COMMUNICATIONS

High resolution electron microscopy study of sulphathiazole crystals

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Abstract—A high resolution electron microscopy study was undertaken on samples of sulphathiazole obtained by recrystallization at 0°C, 30°C and 70°C. Low magnification electron microscopy study of the crystals showed featureless morphology yet the resolved lattice images showed imperfections such as dislocations, lattice irregularities and regions of discontinuity.

This study forms part of a wider study of the effect of crystal defects on mechanical properties of pharmaceutical materials. The initial literature survey showed that there is no information available on the microstructure of sulphathiazole crystals.

A transmission electron microscopy study was therefore undertaken on sulphathiazole crystals in order to assess their morphology and structure. These features affect mechanical properties of crystals in general, because of structural defects and imperfections.

Previous work in this field is limited but results on large, well-formed crystals of potassium perchlorate grown in silica gel showed that these crystals could be of relatively low dislocation density (Burt & Mitchell 1981). It was also found that the number of dislocations incorporated into the crystal during growth affects its dissolution rate (Friesen et al 1981). Developments in high resolution electron microscopy with transmission microscopes being able to resolve 2 Å lattices, have permitted the successful investigations of the ultrastructure of various materials, including biologicals, polymers and metals.

Materials and methods

Three batches of sulphathiazole crystals were examined. They were prepared by crystallization of saturated solutions of sulphathiazole BP in distilled water.

Sulphathiazole BP, 20 g was dissolved in 500 mL of distilled water at 90°C. The solution was rapidly filtered into vessels maintained at 70°, 30° and 0°C for 30 min.

The resultant crystals for transmission electron microscopy were embedded in LR White Acrylic resin (hard grade) and cured at 60°C for 24 h under vacuum. The blocks were next sectioned with a Reichert (diamond knife) ultramicrotome and the thin sections picked up on 400 mesh copper grids. The crystal sizes ranged from tens of nm to about 1000 nm. Crystals from the smaller size range were used in transmission microscopy. The specimens were examined on a Jeol Jem-200CX electron microscope operating at 200 KeV and fitted with a high resolution pole piece. The images of specimens were improved in terms of contrast and stability by staining the crystals with 1–2 drops of 50:50 mixture of 0·25 M sodium sulphite and concentrated sulphuric acid and allowing it to react for about 1·5 min followed by washing in ether.

High resolution electron images can be obtained if diffracted beams including elastically and inelastically scattered electrons pass through the objective aperture.

Diffracted beams ought to correspond to distances within the point resolution of the microscope and the incident beam should lie precisely along the optical axis of the microscope (Loretto 1984). The imaging was carried out with a certain degree of defocus using many beam diffraction conditions.

The crystals were found to be electron beam sensitive and therefore it was necessary to maintain decondensed beam conditions to exclude damage to the crystal structure and morphology.

Results

A typical morphology of single crystal of sulphathiazole, 30°C sample, is presented in Fig. 1. The enclosed selected area diffraction pattern (SAD) shows [0001] orientation of a hexagonal close packed structure. All three types of crystals, 0°, 30° and 70°C, showed similar featureless morphology. High resolution electron micrographs of a 0°C specimen are presented in Figs 2 and 3.

Fig. 2 shows 7·60 Å lattice image and a corresponding selected area diffraction pattern, and Fig. 3 lattice planes of 5·97 Å spacing. The measurements quoted here were made from the negatives. The size of the objective aperture used was such that it allowed only the reflection corresponding to the largest lattice spacings to contribute to the image, as indicated by broken lines on Fig. 2, SAD. The images revealed areas of lattice discontinuity with the plane contrast becoming weaker or disappearing. It is also noticeable that the lattice pattern is distorted around such areas, being curved and slightly shifted.

Similar features were observed on lattices of 70°C samples shown in Fig. 4. In addition area A in Fig. 4 indicates the presence of a dislocation quite clearly, while area B shows curving and irregularity of the lattices near the dislocation. There are also other areas of lattice discontinuity present, C. The measured lattice spacing for this specimen is 9·0 Å which significantly differs from the value obtained from the corresponding diffraction pattern, 7·94 Å.

Figs 5 and 6 show lattice images of 5·2 Å and 2·75 Å spacing from a sample crystallized at 30°C. The resolved lattice areas are smaller in comparison to the other two specimens and seem to be more perfect. However, a reduced lattice contrast in certain areas was also observed.

Discussion

Differential Scanning Calorimetry, DSC, suggests that the incidence of crystal defects should increase as the temperature of crystallization decreases. Using the terms ΔS^P, where

$$\Delta S^P = \Delta S_{\text{sample}} + \Delta S_{\text{crystalline reference}},$$

Grant & York (1986) showed that ΔS^P increases as the disorder in a crystalline sample increases relative to the reference crystalline phase. Values of ΔS^P in this study were (approxima-

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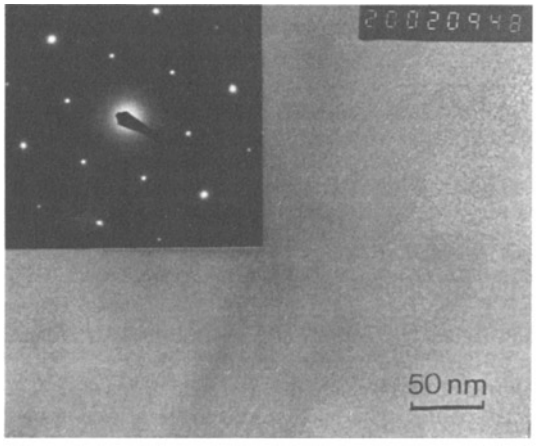

FIG. 1. Transmission electron micrograph of a single crystal of 30°C sulphathiazole and a corresponding selected area diffraction pattern showing the hexagonal close packed structure of [0001] orientation.

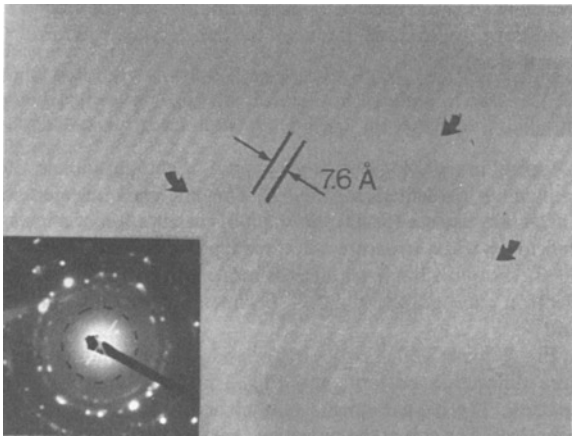

FIG. 2. High resolution electron micrograph of a 0°C specimen showing the 7·60 Å lattice image and a selected area diffraction pattern. The broken line indicates position of the objective aperture. Arrows indicate areas of discontinuity.

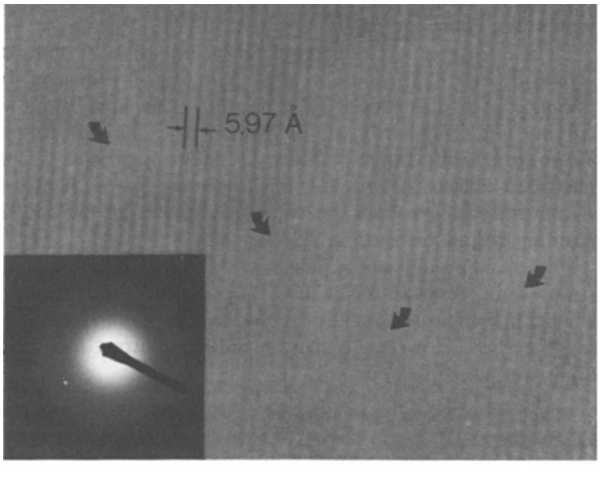

FIG. 3. High resolution electron micrograph of a 0°C specimen showing the 5·97 Å lattice image and selected area diffraction pattern. Arrows indicate areas of discontinuity.

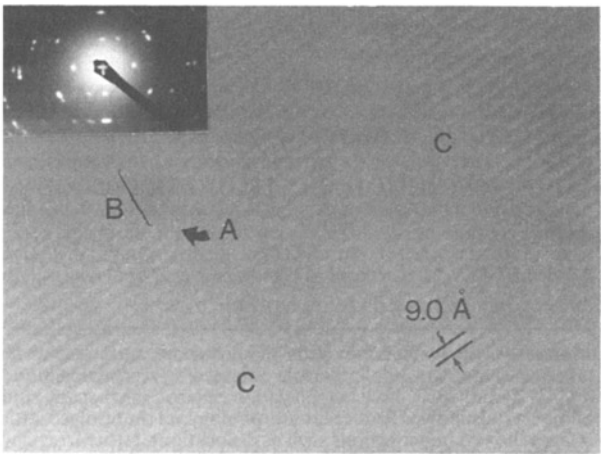

FIG. 4. High resolution electron micrograph of a 70°C specimen showing 9·0 Å spacing. Region A shows the presence of dislocation and region B lattice irregularity near the dislocation. Region C indicates other areas of lattice discontinuity.

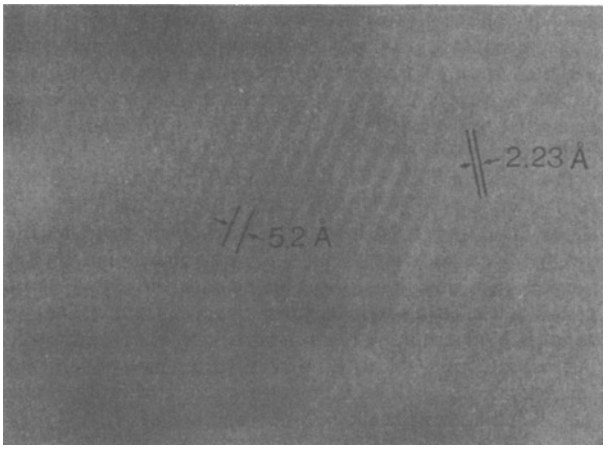

FIG. 5. High resolution lattice image of a 30°C specimen, 5·2 Å spacing and also 2·23 Å spacing in the background.

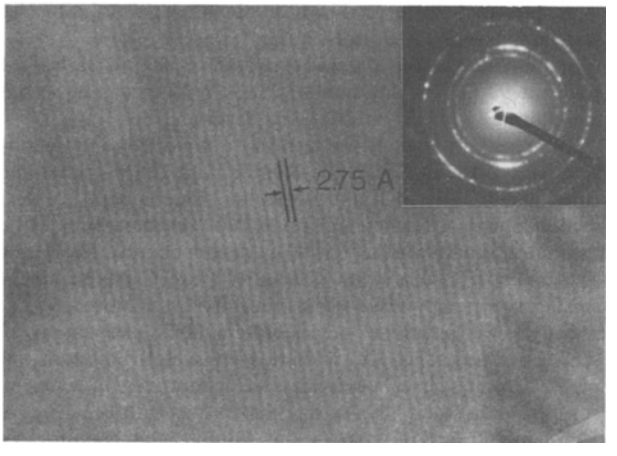

FIG. 6. High resolution lattice image of 2·75 Å spacing, 30°C specimen.

tely) 10 J K^{-1} mol^{-1} for samples crystallized at 70°C (using a commercial sample of sulphathiazole Form I as reference) 20 J K^{-1} mol^{-1} for samples crystallized at 30°C and 30 J K^{-1} mol^{-1} for samples recrystallized at 0°C. Those values are of the same order and therefore one would expect to find similarity in structure and morphology of the three types of crystals. The high resolution electron microscopy study revealed lattice imperfections in terms of either dislocations or lattice distortions and

Table 1. Lattice spacing (Å) (±0·05 Å) of sulphathiazole I, $C_9H_9N_3O_3S_2$.

Temperature of crystallization		
0°C	30°C	70°C
7·24	—	7·94
3·40	5·83	5·47
2·43	2·92	4·08
2·29	2·43	2·84
2·09	2·03	2·73
1·48	1·46	2·32
	1·26	1·96
	1·20	1·70
	1·03	1·46
	0·93	1·19
	0·86	1·05
	0·63	0·94

regions of discontinuity in every specimen examined. The regions of discontinuity showed lattice distortions near them and therefore it is thought that these regions are connected with the presence of dislocations. In particular, we can refer to Fig. 5, where a dislocation was seen due to favourable specimen orientations in relation to the electron beam.

Table 1 gives lattice spacings obtained in this study from selected area diffraction patterns. Reflections corresponding to the large lattice spacings could be missing due to strong (000) reflections.

There is considerable confusion in the literature concerning the number and status of the polymorphic forms of sulphathiazole and this is compounded by the use of different nomenclatures for the different forms (Moustaffa & Carless 1969; Kruger & Gafner 1972; ASTM Data). A mixture of forms II and III of Kruger & Gafner, on DSC, would resemble form I of this study and the presence of such mixtures may be difficult to confirm if one form is present in small quantities. The results given in Table 1 are of form I. The sulphathiazole crystals examined were very small, less than 1000 nm. It is probable that much larger crystals are required if the defects observed with potassium perchlorate (Burt & Mitchell 1981) are to be observed.

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Pharmacokinetic study of tempo carboxylic acid, a nitroxyl MRI contrast media, in control and streptozocin diabetic rats

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Abstract—The pharmacokinetics of tempo carboxylic acid (TCA), a nitroxyl contrast medium have been evaluated in control and streptozocin-diabetic rats. Previous magnetic resonance imaging (MRI) studies in diabetic rats showed prolongation of contrast visualization in the renal cavities after injection of TCA. Diabetes induced only slight alterations to the pharmacokinetic parameters. Rate constants and half lives were unchanged after four months of diabetes. A significant decrease of the apparent total body clearance and volume of distribution was observed while the area under the curve was increased. These alterations are not sufficient to explain MRI abnormalities which have to be elucidated.

Tempo carboxylic acid (TCA) is a piperidinic nitroxide spin label (nitroxide stable free radical) which has been successfully used as an experimental contrast medium for magnetic resonance imaging (MRI). TCA, as a meglumine salt, presented a renal elimination (Lamarque et al 1986) and has been used to assess renal function in normal rats and rats with experimentally induced ischaemia, hydronephrosis (Lamarque et al 1986) and diabetic nephropathy (Bonnet et al 1987; Chapat et al 1987). MRI studies in diabetic rats showed prolongation of contrast visualization in the renal cavities (45 min in diabetic rats versus 15 min in control rats) which was not clearly understood. The purpose of this study was to evaluate pharmacokinetics parameters of TCA after intravenous (i.v.) administration in control and diabetic rats to specify the causal factor responsible for MRI abnormalities.

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Material and methods

Induction of diabetes. Male Wistar rats, 210–230 g were randomly selected for this study and maintained on ordinary rat chow and water without restriction. Diabetes was induced, under light ether anaesthesia, by a single i.v. dose of streptozocin (STZ, Sigma Co., USA) 50 mg kg^{-1} administered in 1 mL kg^{-1} citrate buffer 0·05 M pH 4·5 in which it was dissolved just before injection. That dose of streptozocin is known to produce a mild diabetic state with hyperglycaemia but without ketosis (Tancrede et al 1983). Control rats received an equivalent volume of citrate buffer. Seven days after the onset of diabetes, plasma glucose was determined in STZ-treated rats (Destrostix-Ames Co.) and rats with glucose levels lower than 250 mg% were discarded. Diabetic state was controlled by taking into account the following parameters: water intake, food intake and body weight. Plasma glucose and urine volume were measured at the end of the study.