counterion site in the micellar systems, the substrate, itself, could compete at the binding site. To examine this possibility, the effect of initial concentration of cephalexin was investigated in the presence of 20 mM CTAB at pH 6·5. The observed rate constant, k_ψ, was found to be decreased as the initial concentration of cephalexin increased. At its initial concentrations of 0·1, 1·0, 2·0, 5·0 and 10·0 mM, k_ψ were 0·6482, 0·6254, 0·5620, 0·4951 and 0·3905 h^{-1}, respectively. Using equation 7, giving the initial concentration of cephalexin as [I], a good straight line was obtained ($r = 0·9973$). This indicates that the substrate molecules are competitively occupying the binding site of CTAB micelles.

These observations demonstrate that the degradation of cephalexin is specifically accelerated by cationic micelles and the catalysis is explicable in terms of the interactions between cephalexin and the micelles. Many factors undoubtedly contribute to the catalysis. The dependence of the extent of micellar catalysis on pH reflects the importance of electrostatic interactions.

May 6, 1977

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The polymorphism of acetohexamide

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Analytical studies made in recent years on the oral hypoglycaemic compound acetohexamide do not report any incidence of polymorphism, although this phenomenon is known to occur with other sulphonylureas, and both tolbutamide (Kuhnert-Brandstätter & Wunsch, 1969; Kuhnert-Brandstätter & Bachleitner-Hofmann, 1971; Simmons, Ranz & others, 1972; Burger, 1975b) and chlorpropamide (Burger, 1975a) have been shown to exist in different polymorphic forms. No significant differences have been reported in the biological activities of these forms, but the *in vitro* availability of chlorpropamide from tablets has been shown to be affected by the type of polymorph used in the tablet preparation (Burger, 1976a, b). This paper presents evidence to show that acetohexamide can also exist in at least two polymorphic forms that can easily be detected by infrared spectroscopy.

Acetohexamide was kindly supplied by Eli Lilly and Company Ltd. A sample of acetohexamide U.S.P. reference standard was also obtained. Infrared spectra were recorded from potassium bromide discs using a Perkin-Elmer Model 357 grating spectrometer. Nmr spectra were recorded with a Perkin-Elmer R32 (90 MHz) spectrometer. Differential scanning calorimetry was carried out with a Perkin-Elmer DSC-1B apparatus using pans with crimped aluminium covers and a heating rate of 4° min^{-1}. For the determination of melting points, samples were inserted into an Electrothermal melting point apparatus at a temperature of 170°.

Preparation of polymorph A. Acetohexamide (1 g polymorph A or B) was dissolved in a minimum volume of glacial acetic acid (about 30 ml) by heating on a boiling water bath, and the solution allowed to crystallize at room temperature. The crystals were washed well with cold water, and dried at 105°, m.p. 180–183° dec.

Preparation of polymorph B. Acetohexamide (1 g polymorph A or B) was dissolved in chloroform (30 ml) by heating on a boiling water bath, and the solution left to crystallize at room temperature. The crystals obtained were dried at 105°, m.p. 183–185° dec.

The nmr spectra of the two polymorphic forms of acetohexamide were determined in trifluoroacetic acid and found to be identical showing therefore that the two species were chemically the same.

Infrared spectroscopy revealed a number of differences by which the polymorphs could easily be identified. The spectra recorded were unchanged when the re-crystallized forms A or B were dried *in vacuo* at 60° instead of at 105° at atmospheric pressure. The main distinctions between the forms were as follows:

1. The infrared spectrum of polymorph A (Fig. 1) shows a strong well-resolved doublet with maxima at 3310 and 3240 cm^{-1}, and a second medium intensity doublet at 2940 (with a shoulder at 2920) and 2850 cm^{-1}.
2. In the infrared spectrum of polymorph B (Fig. 2), the first doublet is replaced by a single strong peak at 3360 cm^{-1}, and the second appears as a medium intensity band at 2940 cm^{-1} with shoulders at 2890 and 2850 cm^{-1}.
3. Between these two bands, polymorph B shows a broad medium intensity band at 3095 cm^{-1} with a shoulder at 3150 cm^{-1}. This band is almost absent from the spectrum of polymorph A.
4. Form A produces a single strong band at 1685 cm^{-1};

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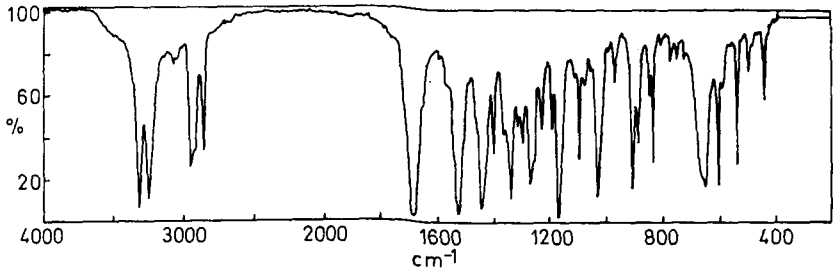

FIG. 1. Infrared spectrum of acetohexamide polymorph A.

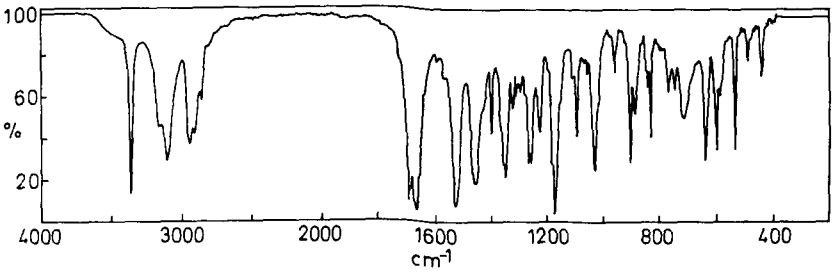

FIG. 2. Infrared spectrum of acetohexamide polymorph B.

in form B this is resolved at its apex giving a doublet with peaks at 1690 and 1665 cm^{-1}.

5. Form A gives rise to a shoulder peak at 1365 cm^{-1} which is not given by form B.

6. Two weak but clearly resolved peaks are shown by form A at 1315 and 1295 cm^{-1}; form B correspondingly shows three peaks at 1320, 1305 and 1290 cm^{-1}.

7. Form A produces a narrow medium intensity band at 1190 cm^{-1} which is not seen in the infrared spectrum of form B.

8. The spectrum of polymorph A has a narrow weak band at 725 cm^{-1}, and a fairly intense broad band with maximum at 650 cm^{-1}. With form B there is a shift in the positions of these bands, so that the broad medium intensity band appears at 715 cm^{-1} and reveals a fairly intense well-resolved peak at 640 cm^{-1}.

The infrared spectrum of polymorph A was found to correspond with that of the commercial sample of acetohexamide, and also with published spectra for this compound (Baltazar & Ferreira Braga, 1966; Salim & Hilty, 1967; Meier, Kuhn & others, 1971; Shafer, 1972). It was also identical with the spectrum of U.S.P. acetohexamide reference standard dried at 105° for 3 h (m.p. 183–185°).

The infrared spectrum of polymorph B exhibited the same wavelength maxima as the preparation obtained when the United States Pharmacopeia XIX (U.S.P.) identification test was carried out on a commercial sample of acetohexamide tablets, the infrared spectrum being determined of the residue from evaporation of a chloroform extract of the tablets. This suggested that the U.S.P. identification test would not indicate which polymorph had been used in the tablet preparation. To verify this, the U.S.P. test was carried out using each polymorph separately in place of the tablet sample, and also, more simply, residues were obtained by evaporating a chloroform solution of each polymorph to dryness. The residue in each case exhibited maxima at the same wavelengths as polymorph B.

Differential scanning calorimetry (DSC) of crystals of polymorph A produced a peak at 185·5°, which was also obtained when the sample was ground thoroughly before scanning. With crystals of polymorph B, however, two endothermic peaks were obtained at 172·5° and 182·5°. These were attributed to solid-solid transition and melting respectively, since when polymorph B crystals were scanned so as just to produce the first peak (at 172·5°) and then removed from the DSC pan, they exhibited the infrared spectrum of polymorph A. The unexpectedly low melting point found for crystals of polymorph B (182·5°) was confirmed by repeated experiments on a number of samples of each polymorph, and probably indicated either that the transition to form A was incomplete or that it was accompanied by slight decomposition of the sample. To indicate whether this would be possible without changes appearing in the infrared spectrum, various mixtures of forms A and B were prepared. It was found that the spectrum of polymorph A was not significantly altered by the presence of up to 10% by weight of polymorph B. When crystals of polymorph B were ground to a fine powder, DSC scans for the powder did not show any solid-solid transition peak, but produced a single peak at 185·5° indicating that transition to polymorph A had

nevertheless still taken place. To confirm this, and because acetohexamide decomposes on melting, samples of the finely powdered crystals of polymorph B were scanned up to a temperature of 175°. On removal from the DSC pan, the powder was found by infrared examination to have undergone transition to form A.

Shenouda (1970) has noted that grinding can change a polymorphic form of sulphathiazole. With acetohexamide, however, the elimination of the transition peak by grinding may be due only to an effect on the thermal conductivity of the sample so that the transition takes place more gradually. It was not found possible to alter the infrared spectrum of polymorph B to that of A by grinding, and grinding in itself is therefore not sufficient to produce the transition.

The identification of polymorphic form in a commercially available sample of tablets containing 500 mg of acetohexamide per tablet was carried out by extracting a powdered tablet with 30 ml of water, to remove a yellow colouring material and any other water-soluble tablet excipient present, filtering and washing the residue well with water. The residue which was white in colour was dried in the oven at 105°. Its infrared spectrum was the same as that of polymorph A.

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Estimation of the plasma protein binding of drugs by size exclusion chromatography at medium pressure (150 lb in^{-2}: 1·03 MPa)

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The most reliable method of estimating the degree of protein binding of a drug is to separate the unbound drug, before its assay, from the drug/protein mixture. However, the commonly used methods of separation suffer from the disadvantage that either the total drug concentration or the total protein concentration is altered during the separation process. For example, if a drug/protein mixture is dialysed, then the total drug concentration on the protein side of the membrane will decrease and a new equilibrium will be established. Similarly in ultrafiltration, the protein concentration will increase at the point of filtration, this being the point at which unbound drug is being separated from the mixture. Therefore a method is needed, in which it is certain that the concentrations of protein and total drug remain at their original concentrations, when the estimation of degree of binding is performed. A method which satisfies this criterion is frontal analysis using size exclusion chromatography (Nichol & Winzor, 1964; Cooper & Wood, 1968; Burke, 1969). The principle underlying this method is as follows (a complete treatment of analysis of results from frontal analysis is given by Nichol & Winzor, 1972). A column is packed with size exclusion material of pore size sufficiently small that only free drug can permeate into the pores while protein (and protein-bound drug) are excluded. A sample of the mixture to be analysed is applied continuously to the column. As the front moves down the column, unbound drug is retarded with respect to protein and protein-bound drug. To maintain an equilibrium, bound drug will then dissociate from the protein in the region not yet reached by unbound drug. At the void volume of the column (the only volume in the column available to protein), protein and remaining protein-bound drug will begin to elute. At a stage shortly after the elution volume of the unbound drug (the volume in the column available to unbound drug) has been reached the composition of the eluate will be identical to the composition of the sample, which is still being continuously applied. Under these steady state conditions the total concentration of drug (unbound + bound) can be determined by assay for drug in the eluate.

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