ELSEVIER

International Journal of Pharmaceutics 118 (1995) 1-10

international journal of pharmaceutics

Research Papers

Processing effects on crystallinity of cephalexin: characterisation by vacuum microbalance

B.A. Hendriksen [a,*], M.S. Preston [b], P. York [b]

[a] *Pharmaceutical Research, Lilly Research Centre, Eli Lilly and Co, Erl Wood Manor, Windlesham, Surrey GU20 6PH, UK*
[b] *Postgraduate Studies in Pharmaceutical Technology, School of Pharmacy, University of Bradford, Bradford BD7 1DP, UK*

Received 5 April 1994; revised 26 July 1994; accepted 30 August 1994

Abstract

Cephalexin is known to exist in several pseudopolymorphic or solvated forms, and to be slowly hydrolysed by water, and accordingly the study of its interaction with water is particularly important. Three common processing techniques - milling, spray drying and freeze drying - are examined for their influence on the interactions with water, which are probed by measuring the water sorption isotherms on a vacuum microbalance. In addition, water sorption isosteres are determined directly to quantify the sorption heats as a function of moisture content. Crystalline cephalexin shows discreet formation of mono and dihydrated forms, and their formation is easily reversible, indicating that water plays little part in the stabilisation of the lattice. Spray and freeze drying both lead to a form of cephalexin which continuously sorbs water without formation of a stoichiometric hydrate, and does not reversibly dehydrate; this indicates the sample is highly disordered and that the water can access a wide range of lattice sites which play a significant part in the overall solid state stability. Ball-milled samples are intermediate, with a partially discreet monohydrate formation as the vapour pressure is raised, which is partially reversible as the pressure is reduced. The increased disorder over the crystalline sample allows more sites to become accessible to the water samples.

Keywords: Gravimetry; Enthalpy of sorption; Isostere; Degree of crystallinity; Crystal engineering; Processing effect

---

1. Introduction

Water can interact with a crystalline or amorphous drug in many ways. Many reviews have been written on the states of water associated with solids (Zografi, 1988), on the effect on solid dosage forms (Carstensen, 1988, et al., 1990), and on the stability of drugs (Ahlneck and Zografi, 1990), and moisture sorption uptake (Van Campen et al., 1983a,b,c). Chemical stability, crystal structure, powder flow, compaction and dissolution rate are some of the physicochemical properties that can be affected where residual water is associated with a drug in the solid state. Residual water exists as a result of exposure to an atmosphere containing water vapour or due to processing involving the use of water, such as

---

[*] Corresponding author.

SSDI 0378-5173(94)00282-7

spray drying, wet granulation or aqueous film coating. It is important to characterise the behaviour of the drug as this water affects many chemical and physical properties. Improvements in solubility and bioavailability can be brought about by the use of common processing techniques such as milling, spray drying and freeze drying. These changes are brought about by altering particle size, shape, area, and degree of crystallinity; in this work these processes are applied to crystalline cephalexin. Cephalexin is known to exist in several pseudopolymorphic or solvated forms, and is known to undergo hydrolysis in the presence of moisture. Thus, characterisation is particularly important as this water affects many chemical and physical properties.

This work attempts to examine the use of three common processing techniques – milling, spray drying and freeze drying – on the physicochemical and hydration properties of cephalexin, chosen as a model drug. Hydrate stability is of pharmaceutical consequence because processing operations involving thermal energy such as drying or compaction could partially remove water of crystallisation to an unknown extent or cause a mixture of hydrated and dehydrated forms to exist within the formulations.

Moisture sorption isotherms are determined in the present work to quantify the affinity of the samples for water as a function of relative pressure; in particular, they are determined using a vacuum microbalance, with computer control and automated data acquisition. Sorption isotherms may be obtained at two or more temperatures to examine the relationship between reciprocal temperature and log pressure (Czanderna, 1980). In an equation analogous to the Arrhenius equation this relationship simply yields the sorption heat, ΔH.

$$\mathrm{d}\ln P/\mathrm{d}(1/T) = -\Delta H/R$$

where P is the vapour pressure of water in mmHg, R denotes the gas constant and T is the absolute temperature. Data analysis involves constructing an isostere (line of constant mass) with one datum point for each isotherm. A novel and potentially superior strategy, adopted in the present work, is to determine the isostere directly by experimentally constraining the sample weight to remain constant while the sample temperature is varied between limits.

2. Materials and methods

2.1. Materials

2.1.1. Crystalline cephalexin monohydrate

Crystalline material was prepared by dissolving the technical material (100 g) in 160 ml of 1.12 M hydrochloric acid, with stirring, and the pH adjusted to 1.3–1.5 with ammonia solution. Disodium edetate, carbon black, and filter aid were added and the mixture stirred for 40 min. The mixture was filtered through a glass filter paper (GF/B, Millipore) and the cake was washed with a weak acid solution. The combined filtrate and wash were stirred and heated to 45–50°C with a hot water bath. The solution was rapidly adjusted to pH 3 with 15% v/v ammonia solution when precipitation occurred. Further ammonia solution was added to adjust the slurry to pH 4.5–4.6 which was then stirred for 15 min. The slurry was filtered and washed with water, then dried in an air oven at 40°C overnight before being passed through a 1180 μm sieve to remove large lumps.

2.2.2. Ball-milled cephalexin

10 g of crystalline cephalexin was placed in a 400 ml ceramic ball mill chamber, which was sealed and set on rotating rollers preset at 50 rpm (Pascall Engineering Co. Ltd, Crawley, UK). This sample was milled for 24 h.

2.2.3. Spray-dried cephalexin

A solution of crystalline cephalexin (8 g/l) was spray dried in a Buchi Minispray 190 (Buchi Laboratory-Techniques, Switzerland), with an inlet temperature of 125°C, an outlet temp of 70°C, and a pump rate of 5 ml/min.

2.2.4. Freeze-dried cephalexin

1.5 g of crystalline cephalexin was dissolved in 100 ml of water at room temperature, mixed with carbon black and stirred for 30 min. The solution was filtered and the filtrate frozen at −30°C for

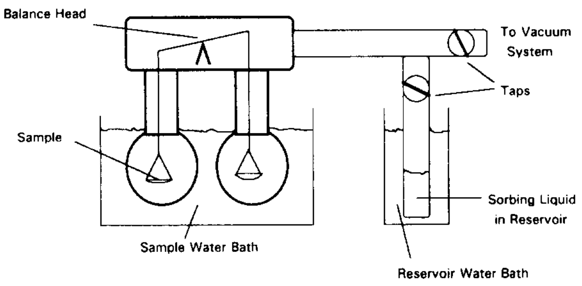

Fig. 1. Schematic diagram of vacuum microbalance arrangement.

1–2 h. The frozen mass was lyophilised in a Speedivac Centrifugal Freeze Drier (Model 5PS, Edwards, Crawley, UK) under a vacuum of < 0.5 Torr for 24 h. The material was stored under vacuum until used.

2.2. *Methods*

Moisture sorption studies were carried out gravimetrically on a vacuum microbalance (Fig. 1). Two sample pans were suspended centrally in two 250 ml round bottomed flasks, which were immersed in a controllable water bath (10–60°C), from the beam of a vacuum microbalance head (1 g; CI Electronics, UK). The vacuum balance head was connected to a high vacuum system which comprised an oil diffusion pump (Diffstak Mk 2, 63/105M, Edwards, UK), a backing pump, a Baratron 128A absolute pressure transducer measuring up to 100 mmHg and Pirani and Penning gauges to measure approximate pressures. A reservoir tube containing either water or a strong lithium chloride solution (in principle any solvent or salt solution could be used) was connected via a tap to the microbalance head. This reservoir tube was in turn immersed in a controllable water bath (−2 to 28°C) Temperature transducers were used to monitor temperatures of the sample and reservoir. Signals from the transducers as well as from the Baratron and balance head were computer logged on an IBM XT (IBM Systems, UK). Purpose written software monitored temperatures, masses and pressures, to provide the basis of controlling the temperatures of the sample and reservoir baths.

The sample was placed on the sample pan and weighed in air. The vacuum was gently applied and the sample heated to 50°C; evacuation continued until the mass was constant and the pres-

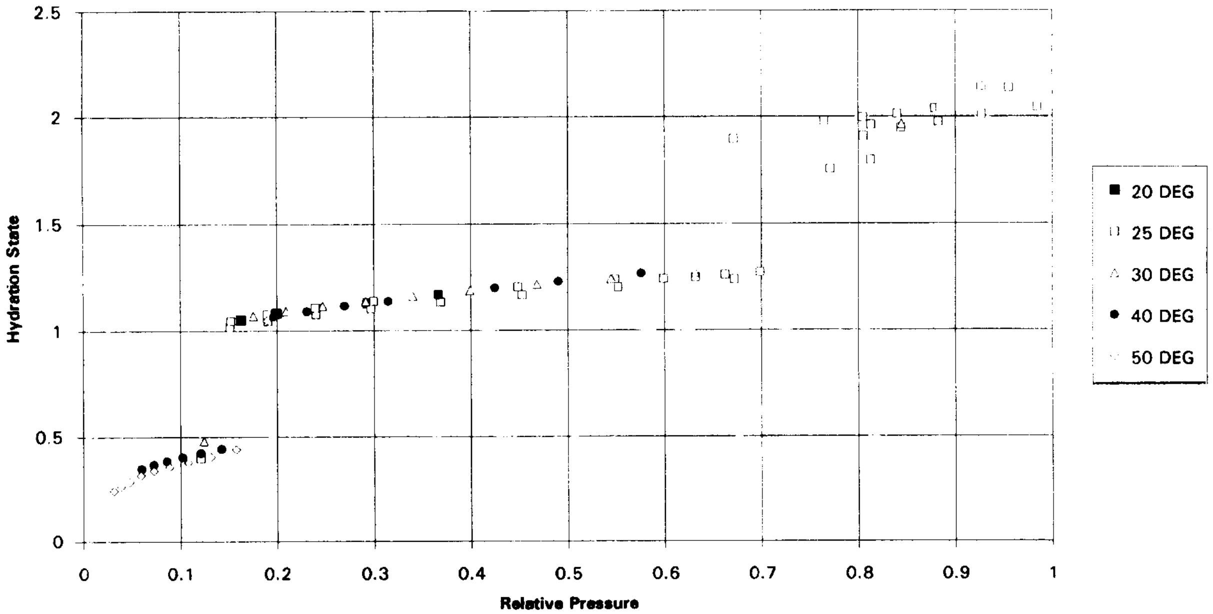

Fig. 2. Isotherms of water sorption on crystalline cephalexin over a wide range of relative pressures and temperatures, showing monohydrate and dihydrate formation.

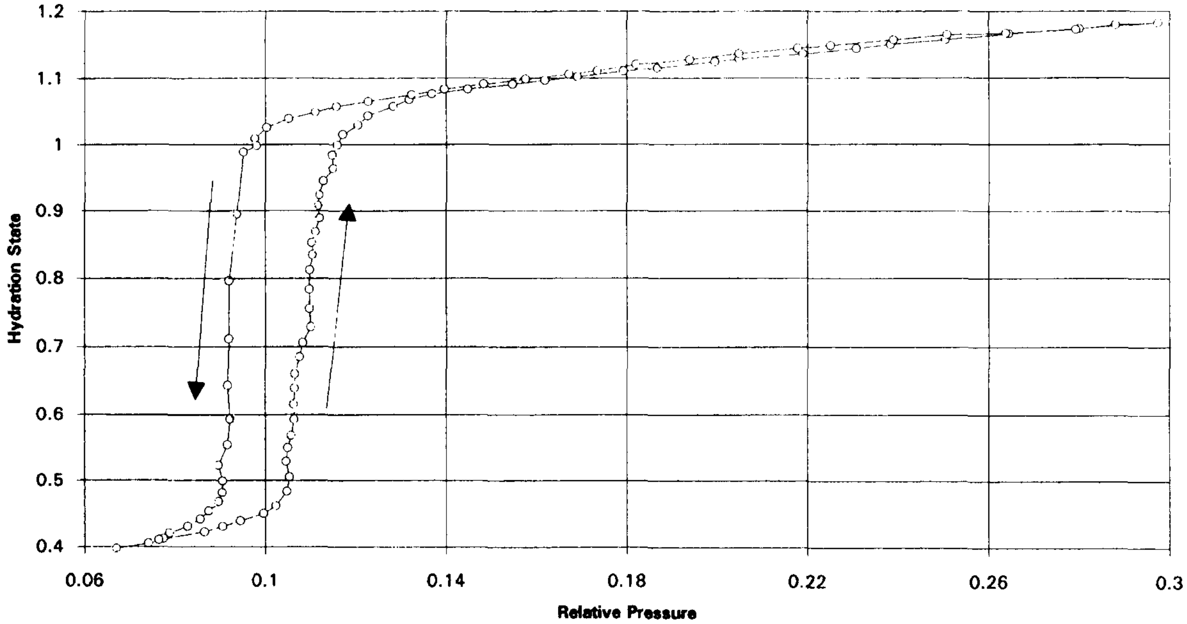

Fig. 3. Adsorption and desorption isotherm of water on crystalline cephalexin at 16°C, showing reversibility.

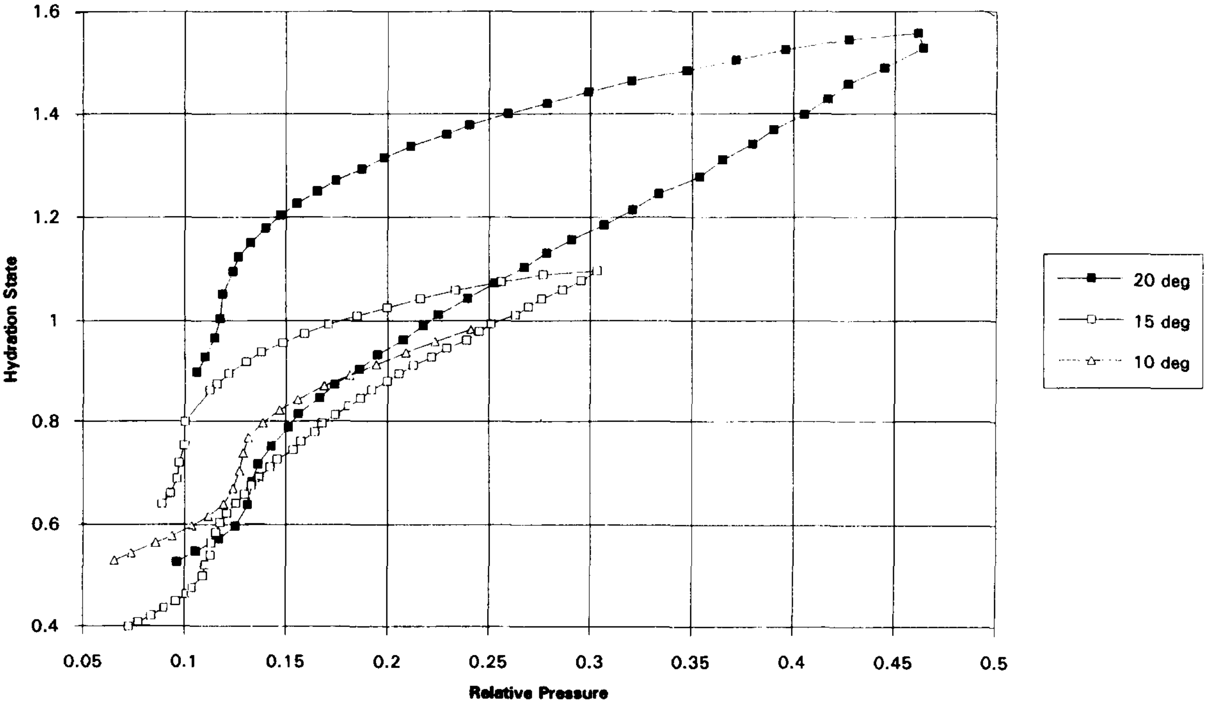

Fig. 4. Isotherms for water sorption on ball-milled cephalexin.

sure reduced to about 5×10^{-6} mmHg. The temperatures of the sample and reservoir were adjusted to predetermined values and the tap between them opened. The reservoir temperature was reversibly ramped from −2 to 28°C in 0.25°C stages. Thereafter, the sample temperature was changed and the process was repeated. The mass of the sample increased or decreased as water was sorbed or desorbed, respectively.

The software was written such that any changes in mass above a default value, were monitored. When detected, the ramping of the reservoir temperature, and thus the water vapour pressure and relative humidity, was postponed until equilibrium had again been reached. Data were stored electronically for subsequent manipulation. Results were typically presented as isotherms with hydration state plotted as a function of relative pressure. In addition isosteres were determined directly by running at a constant mass of sample; in this case the mass signal was made the basis of adjusting the reservoir temperature, i.e., as mass became too large, reservoir temperature was decreased, lowering relative humidity and thus lowering mass again.

Specific surface areas were determined with a Flowsorb Model 2300 (Micromeritics Instrument Corp, Norcross, USA) using nitrogen as adsorbate. Relative pressures of nitrogen in nitrogen/helium mixtures were obtained in the range 0.05–0.3 using a Tylan gas mixer (Tylan UK Ltd, Westlea). The powders were outgassed for at least 12 h in a 30% nitrogen atmosphere at ambient. Each sample was run in duplicate and the mean taken.

3. Results

3.1. Water sorption isotherms

Water sorption isotherms for each sample are presented in Fig. 2–6. In the case of crystalline cephalexin the relative humidity was increased in

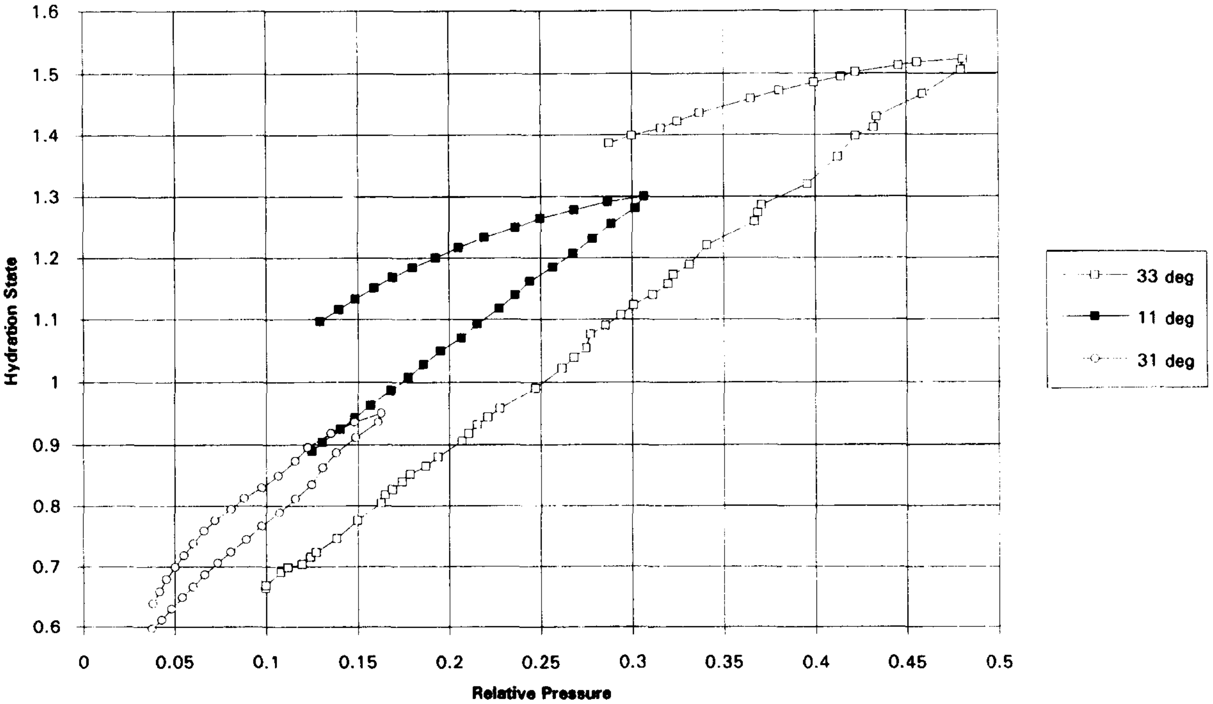

Fig. 5. Isotherms for water sorption on spray dried cephalexin.

one experiment to 100% (Fig. 2) to allow dihydrate formation to be observed, but in all other experiments relative humidity did not exceed 56%.

Desorption isotherms were measured for most samples. For crystalline cephalexin (Fig. 3) the desorption isotherm was seen to be very close to the absorption isotherm. Ball-milled and spray-dried cephalexin gave divergent desorption isotherms as clearly shown in Fig. 4 and 5 respectively. Only the absorption isotherm is shown for the freeze-dried sample (Fig. 6). As this sample was particularly light and fluffy, only a small mass could be placed on the balance pan, rendering the experiment less precise than with other samples.

3.2. Water sorption isosteres

Representative examples of directly determined isosteres are presented in Fig. 7 (spray-dried cephalexin) and Fig. 8 (crystalline cephalexin). Enthalpies of sorption calculated from these and other isosteres are presented in Table 1.

3.3. Specific surface areas

These are presented in Table 2.

4. Discussion

Visual examination of the water sorption isotherms underlines the physical differences between the samples. In the isotherm extending over a wide range for crystalline cephalexin (Fig. 2) there is an increase in the water content at RH 15% corresponding to the formation of the monohydrate. The hydration state remains stable until RH 70% when there is another rise which corresponds to the formation of the dihydrate. There is no significant hysteresis upon reducing the humidity, as the dehydration data are almost

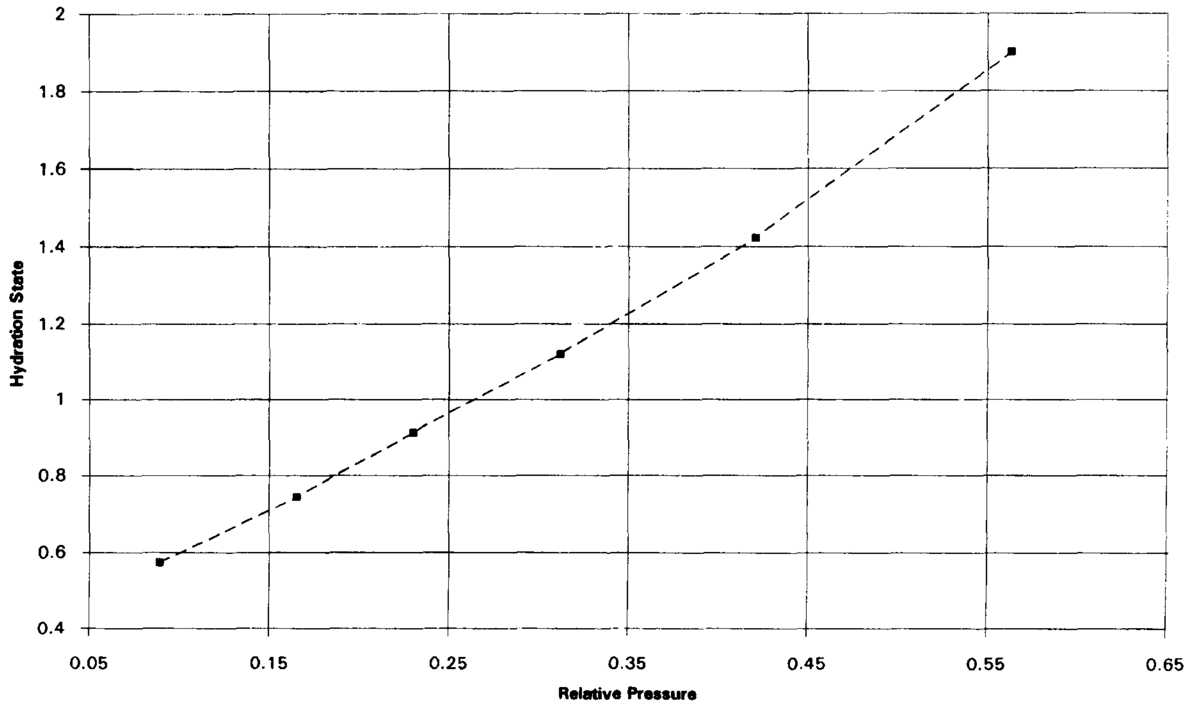

Fig. 6. Isotherm for water sorption on freeze dried cephalexin.

superimposed upon the original hydration data. This is clearly shown in Fig. 3 which shows monohydrate formation followed by dehydration in detail for one sample at 16°C. This suggests that the water plays little part in the stabilisation of the crystal lattice, and may be found in tunnels or other easily accessible sites such as are found in caffeine (Byrn, 1982).

The isotherms for ball-milled cephalexin (Fig. 4) show a small increase equivalent to monohydrate formation between RH 12% and 15% followed by a gradual increase in the water content of the sample on increasing relative humidity. Hysteresis is observed on dehydration, which suggests that the water is located in different positions, some of which require a higher driving force to be removed. This is caused by the increased disorder in the milled sample which allows more molecular sites to become accessible to the water molecules.

In spray and freeze-dried cephalexin (Fig. 5 and 6) there is a gradual increase in water content but no step or inflexion point at the water content equivalent to the formation of a monohydrate. The isotherms for spray-dried cephalexin also exhibit hysteresis. This gradual increase in water content again is thought to be caused by the increased lack of lattice order allowing more of the sites to be accessible to the water molecules.

The samples were examined by X-ray powder diffractometry (Preston, 1992) and it was noted that ball-milled, spray-dried and freeze-dried samples showed progressively reduced crystallinity, with the latter two showing largely amorphous characteristics.

Kontny et al. (1987) described the adsorption of water on freshly crystallised sodium chloride before and after grinding the samples to introduce defects. The freshly crystallised sample exhibited reversible adsorption/desorption isotherms. However for the ground sample, the uptake of water was much greater and adsorption/desorption hysteresis occurred. The ground sample showed a significant decrease in surface area as the relative pressure increased. It was concluded that water vapour was taken up in

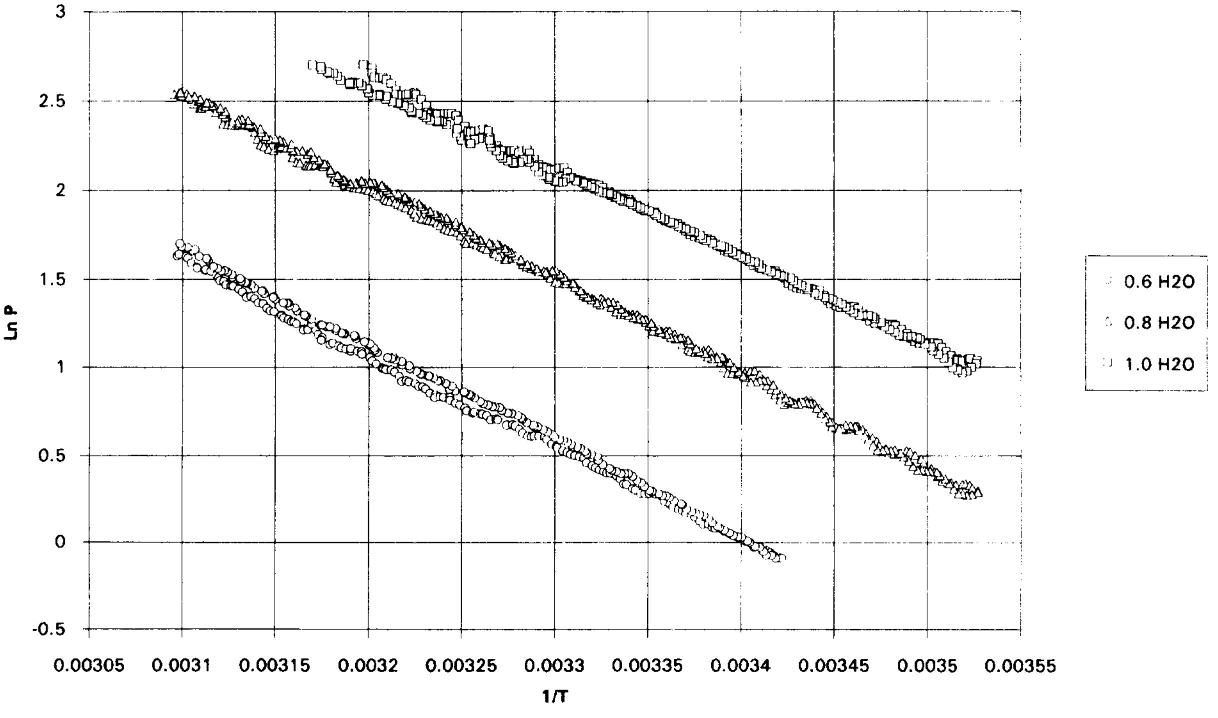

Fig. 7. Isosteres for water sorption on spray dried cephalexin at several water contents.

Table 1
Isosteric sorption heats (kJ mol^{-1}) for cephalexin samples as a function of moisture content, mol water per mol cephalexin

Sample	Moisture content	Heat of adsorption
Crystalline	0.4	42.6 ± 0.2
	0.45	46.2 ± 0.3
	0.775	50.5 ± 0.7
	1.15	42.4 ± 0.1
	1.15	43.0 ± 0.1
Ball-milled	0.4	44.3 ± 0.3
	0.7	50.1 ± 0.2
	1.0	46.5 ± 0.2
	1.3	46.2 ± 0.4
Spray-dried	0.6	44.3 ± 0.2
	0.8	43.7 ± 0.1
	0.9	46.7 ± 0 2
	1.0	40.5 ± 0.1
	1.2	45.2 ± 0.2

Table 2
Surface areas of cephalexin samples by nitrogen adsorption

Sample	Surface area (m^2 g^{-1})
Crystalline	1.50 ± 0.03
Ball-milled	4.90 ± 0.11
Spray-dried	3.56 ± 0.14
Freeze-dried	8.62 ± 0.12

greater amounts by the ground sample because of defects introduced into the crystals by grinding. It was assumed that water was able to exist at a defect site in a higher metastable thermodynamic state which allowed enough mobility in the sodium and chloride ions to allow recrystallization.

The sorption isotherms obtained in this study provide evidence that the samples with reduced crystallinity show different physical characteristics suggesting that they exist in different disordered states. Again the ball-milled sample showed intermediate behaviour between the crystalline sample and the spray- and freeze-dried samples. Attempts at quantifying this low level of crystallinity by measuring the increase in water content occurring on monohydrate formation, and

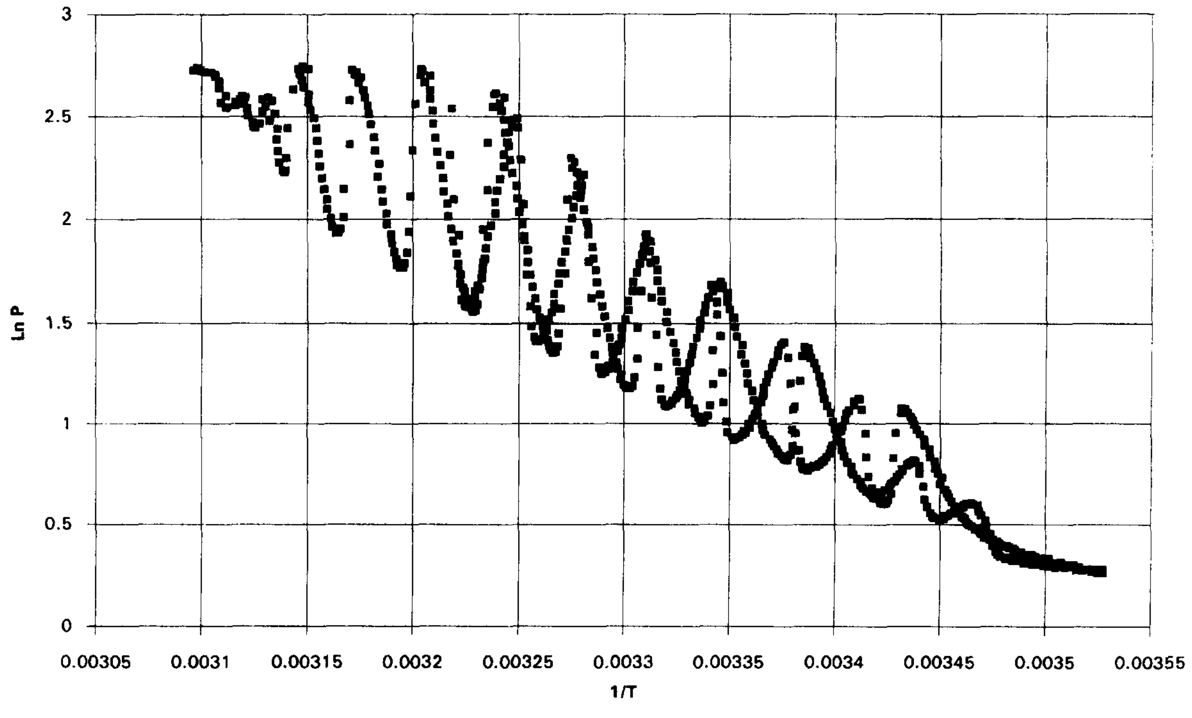

Fig. 8. Isostere for water sorption on crystalline cephalexin at water content of 0.775 mol/mol.

comparing it with that for crystalline cephalexin, were not successful.

The surface areas calculated using nitrogen sorption and application of the BET equation are shown in Table 2. As expected surface areas increased on processing, with the highest area calculated for the totally noncrystalline freeze-dried cephalexin.

In order to probe further the differences between the samples, water sorption isosteres were determined, to calculate the heat of sorption of the water to the samples. Fig. 7 and 8 show isosteres determined for cephalexin by simultaneously varying the relative vapour pressure and temperature required to keep the moisture content constant at the following values: (1) before monohydrate formation (0.6 mol, Fig. 7, spray-dried); (2) approaching monohydrate formation (0.8 mol, Fig. 7, spray-dried and 0.775 mol, Fig. 8, crystalline); (3) after monohydrate formation (1.0 mol, Fig. 7, spray-dried)

The isosteres show a good correlation between vapour pressure and the temperature before and after monohydrate formation, but a poor relationship when approaching monohydrate formation in the case of crystalline material. The poor correlation was not apparent in ball-milled and spray-dried samples since a distinct hydrate does not form.

Table 2, which lists the sorption heats calculated from the gradients of the isosteres, show that values varied in the range 40–50 kJ mol^{-1}; thus they are in the same order of magnitude as the condensation heats of water, which in the range 0–50°C vary from 45 to 43 kJ mol^{-1}. It is noted that during the period when samples were approaching monohydrate formation, the sorption heats were about 50 kJ mol^{-1} in the cases of both crystalline cephalexin (at 0.775 H_2O) and ball-milled cephalexin (at 0.7 H_2O), whereas at lower and at higher hydration states the heats were lower and close to the heat of water condensation value. It is possible that at these intermediate hydration states the water is sorbing at molecular sites of relatively high energy, but the differences are not great. In the disordered spray-dried sample values were on average even lower, with one value dropping to only 40 kJ mol^{-1}. Whilst this is difficult to explain, Yamanaka et al. (1990) similarly found that isosteric adsorption heats of water onto alumina pillared clay showed that a shallow maximum at intermediate extents of sorption coupled with isosteric heats slightly below the condensation heat of water. Direct calorimetric determination of dissolution heats would clarify the energetics of water sorption in these systems.

Studies on moisture uptake by the samples have been able to probe the differences in the locations and interactions of the water. The data showed that the ball-milled sample was intermediate between the crystalline and spray-dried sample in its adsorption characteristics. This is considered significant to the ultimate solid state stability of the samples since cephalexin degrades by hydrolysis. The direct determination of isosteres has provided data based on a multiplicity of datum points, leading to good confidence. If extended to a wider range of pressures than those studied in the present work it may become possible to elucidate variation of adsorption heat with temperature.

References

Ahlneck, C. and Zografi, G., The molecular basis of moisture effects on the physical and chemical stability of drugs in the solid state. *Int. J. Pharm.*, 62 (1990) 87–95

Byrn, S.R., *Solid State Chemistry of Drugs*, Academic Press, New York, 1982, p. 156

Carstensen, J.T., Effect of moisture on the stability of solid dosage forms. *Drug Dev. Ind. Pharm.*, 14 (1988) 1927–1969

Carstensen, J.T, Gerhardt, A., Morris, T. and Nikfar, F., Effect of moisture on solid dosage forms. Can the Arrhenius equation be used as a predictor? *Drug Dev. Ind. Pharm.*, 16 (1990) 2267–2281

Czanderna, A.W., Chemisorption studies with the vacuum microbalance. In Czanderna A.W. and Wlosky S.P. (Eds), *Microweighing in Vacuum and Controlled Environments*, Elsevier, Amsterdam, 1980, pp. 182–183

Kontny, M.J. Grandolfi, G.P. and Zografi, G., Water vapour sorption of water soluble substances: studies of crystalline solids below their critical relative humidities. *Pharm. Res.*, 4 (1987) 104–112

Preston, M.S., Processing and Hydration effects on the Pharmaceutics of Cephalexin. Ph.D Thesis, University of Bradford (1992).

Van Campen, L., Amidon, G.L. and Zografi, G., Moisture sorption kinetics for water soluble substances: 1 Theoretical considerations of heat transport control. *J. Pharm. Sci.*, 72 (1983a) 1381–1388

Van Campen, L., Amidon, G.L. and Zografi, G., Moisture sorption kinetics for water soluble substances: 2 Experimental verification. *J. Pharm. Sci.*, 72 (1983b) 1388–1393

Van Campen, L., Amidon, G.L. and Zografi, G., Moisture sorption kinetics for water soluble substances: 3 Theoretical and experimental studies in air. *J. Pharm. Sci.*, 72 (1983c) 1394–1398

Yamanaka, S., Malla, P.B. and Komarneni, S., Water adsorption properties of Alumina pillared clay. *J. Colloid Interface Sci.*, 134 (1990) 51–58

Zografi, G., States of water associated with solids. *Drug Dev. Ind. Pharm.*, 14 (1988) 1905–1926