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Determination of water content in pharmaceutical hydrates by differential scanning calorimetry

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Summary

Thermogravimetric analysis (TGA) and Karl Fischer titrimetry (KFT) are the most commonly used techniques for determination of the water content of pharmaceutical solids. We here report a novel method of determining water content of drug hydrates by differential scanning calorimetry (DSC). The method is based on the hypothesis that the enthalpy of binding of n moles of water molecules in the hydrate (enthalpy of dehydration, ΔH_d) is the same as that of n moles of water molecules in liquid water ($n\Delta H_v$), where ΔH_v is the enthalpy of vaporization of water. From the literature value of ΔH_v and the ΔH_d value for each dehydration endotherm the number of moles of water associated with each endotherm was calculated. This approach was applied to several non-ionic drug hydrates, such as hydrates of ampicillin, carbamazepine, caffeine and theophylline, and to several metal salts of nedocromil. The results obtained by DSC agree well with the KFT and TGA data. Since enthalpy is a state function, the applicability of the above hypothesis does not necessarily support any particular mechanism of dehydration. The above hypothesis was also tested using the dehydration endotherms in the literature for a variety of organic and inorganic hydrates. For those hydrates which give more than one dehydration endotherm by DSC, it is hypothesized that each dehydration endotherm corresponds to a specific binding state or location of water molecules in the crystal lattice. Using this method, it is possible to apportion the water content in each location. The DSC method described cannot be applied when the dehydration endotherm overlaps another peak, such as a polymorphic change, melting, sublimation or decomposition, but can, in principle, be extended to other solvents of crystallization in organic and inorganic solvents. The method may complement the TGA and KFT methods and provide additional information about water binding in pharmaceutical solids.

Introduction

During the process of crystallization, solvent may become incorporated into the crystal structure. The presence of solvent in the crystal structure usually modifies the pharmaceutical properties such as solubility, dissolution rate, bioavailability, chemical stability, powder flow, and compaction (Haleblian and Macrone, 1969; Haleblian, 1975; Zografi and Kotney, 1986). Therefore, the characterization and analysis of the solvent of crystallization (stoichiometric or nonstoichiometric) are important in preformulation studies.

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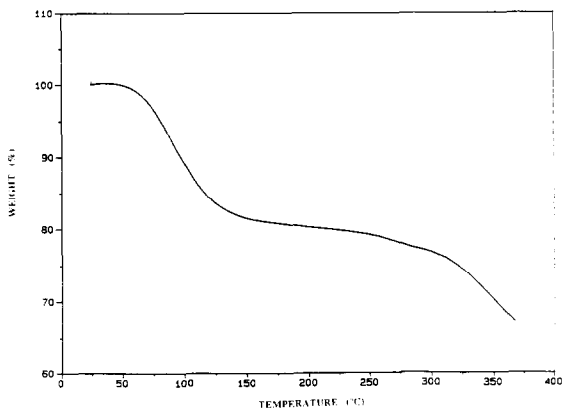

Fig. 1. TGA thermogram of nedocromil nickel. It is difficult to decide a cut-off point on the TGA step.

Hydrates form when water is the solvent of crystallization. The most commonly used techniques for quantitation of water content in hydrates are thermogravimetric analysis (TGA), Karl Fischer titrimetry (KFT) and evolved gas analysis (EGA). All these methods are limited to some extent in their application. For example, in the case of TGA, it may be difficult to decide the cut-off point on a TGA step (Fig. 1) or TGA may not differentiate between various states of binding or water location in a hydrate (Fig. 2). Although KFT provides an accurate determination of water in many compounds, interference from side reactions is frequently encountered mainly due to the oxidizing-reducing properties of the iodine-iodide couple involved in the KFT reaction (Mitchell and Smith, 1980). KFT and EGA determine total water content and therefore do not differentiate between adsorbed moisture and water of crystallization. These limitations of the various methods used for water content determination indicate that there is a need to develop a complementary method without these disadvantages.

Differential scanning calorimetry (DSC) has been widely used for identification of solvates (Ford and Timmins, 1989). The objective of this study is to develop and evaluate the use of DSC for the determination of water stoichiometry in pharmaceutical hydrates, including nedocromil salts.

Nedocromil salts are 9-ethyl-4,6-dioxo-10-propyl-4H,6H-pyrano[3,2-*g*]quinoline-2,8-dicarboxylates (Freer et al., 1987). Nedocromil sodium (Tilade®) is used in the treatment of reversible

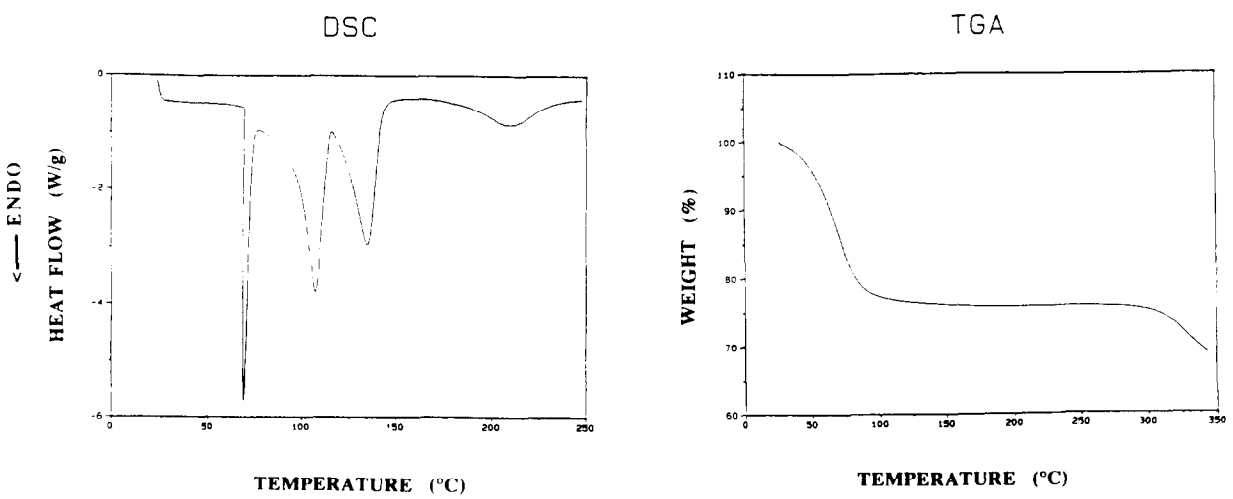

Fig. 2. DSC curve and TGA thermogram of nedocromil sodium. The DSC curve shows four desolvation endotherms, whereas the TGA thermogram shows only one desolvation step.

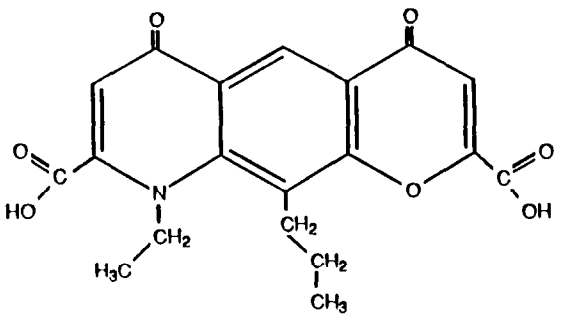

Fig. 3. Molecular structure of nedocromil acid.

obstructive airways diseases such as asthma. The molecular structure of nedocromil acid is shown in Fig. 3.

Theoretical Background and Hypothesis

States of water in hydrates

Water is a highly polar molecule and can therefore interact strongly with ions or polar molecules by ion-dipole or dipole-dipole interactions, respectively. Due to such interactions, water may become adsorbed onto the surface of a crystal (water of hygroscopicity) (Parrott, 1970) or may occupy a position within the lattice (Mitchell and Smith, 1977a). Since the hydrogen bond energy between water molecules in ice or liquid water does not differ greatly from the energy of one hydrogen bond between the solid and water, clustering of water molecules can occur on the surface even before a complete monolayer is formed (Zografi, 1988).

The water held in crystals may be stoichiometric (water of crystallization) or nonstoichiometric. Many stoichiometric hydrates, such as the hydrates of mercaptopurine, caffeine, and theophylline, exhibit different powder X-ray diffraction patterns upon dehydration. On the other hand, dehydration of certain other hydrates does not change the crystal structure significantly, hence they yield very similar powder X-ray diffraction patterns upon dehydration. Among these are the hydrates of cephalexin and cephaloglycin (Clark, 1963; Byrn, 1982).

When the water is present in nonstoichiometric proportions, the molecular water may form hydrogen-bonded networks which, as in ice, are nearly tetrahedrally coordinated (Narten and Levy, 1969). These networks contain cavities capable of accommodating other molecules. Clathrates with water molecules as the host exemplify this type of interaction. Sometimes the water molecules are not part of the crystal structure but are present as guests in a cage formed by molecules other than water. In this case, the nonstoichiometric water is present in cavities with sufficiently large spacings or windows between the cages that water can pass from one cavity to another and leave the lattice without affecting its overall structure (Mitchell and Smith, 1977a). The nonstoichiometric water could also be present as layers (Haleblian, 1975).

Desolvation

The desolvation endotherm in DSC includes such steps as the breakage of the solvate bonds and vaporization of the solvent. Upon desolvation the crystal structure may rearrange through the breakage and formation of intermolecular forces which include Van der Waals interactions and/or hydrogen bonds, in which case the rearrangement of the desolvated structure will also be included in the desolvation process. The area under the desolvation peaks in the DSC curve yields the heats (i.e. enthalpies) of the desolvation transition (Mitchell and Smith, 1977b). Thus, the process of desolvation in an open or crimped pan DSC is

$$D \cdot nA_{solid} \rightarrow D'_{solid} + nA_{gas} \qquad \Delta H_d \qquad (1)$$

which can be divided into following steps,

$$D \cdot nA_{solid} \rightarrow D_{solid} + nA_{solid} \qquad \Delta H_{diss} \qquad (2)$$

$$D_{solid} \rightarrow D'_{solid} \qquad \Delta H_t \qquad (3)$$

$$A_{solid} \rightarrow A_{liquid} \qquad \Delta H_f \qquad (4)$$

$$A_{liquid} \rightarrow A_{gas} \qquad \Delta H_v \qquad (5)$$

where D is the solid drug, A denotes the solvent of crystallization, $D \cdot nA_{solid}$ is the solvate and D'_{solid} represents the final desolvated solid. The loss of solvent from the solvate may simply involve breaking the D-A and A-A interactions without a rearrangement of D molecules; the solid so obtained is D_{solid}. The unsolvated solid (D'_{solid}) in Eqns 1 and 3 is formed not only by the loss of solvent from the solvate through breaking the D-A and A-A interactions, but also by the rearrangement of molecules in the desolvated crystal lattice through the weakening (or breaking) of certain D-D interactions and/or the strengthening of other D-D interactions and/or the formation of new D-D interactions. Thus, D_{solid} is a polymorph of D'_{solid}. According to the nomenclature of Byrn (1982), $D \cdot nA_{solid}$ is a pseudopolymorphic solvate of D, and is a polymorphic solvate of D'.

ΔH_d is the heat of desolvation which can be calculated from the area under the DSC desolvation endotherm(s). ΔH_{diss} is the heat of dissociation of the solvate to yield D_{solid} and the solvent in the solid state. ΔH_t is the heat of transition of D_{solid} to D'_{solid}, ΔH_f denotes the the heat of fusion of A and ΔH_v is the heat of vaporization of A. Thus, ΔH_d in Eqn 1 is related to the other enthalpy changes by the summation of Eqns 2–5 as follows

$$\Delta H_d = \Delta H_{diss} + \Delta H_t + n\Delta H_f + n\Delta H_v \qquad (6)$$

The magnitude of ΔH_d will vary with the experimental conditions and the state(s) of binding of the solvent in the solvate. For example, at a temperature below the critical temperature of A in hermetically sealed pans, A will remain as a liquid so that the vaporization process represented by Eqn 5 will not occur and the term $n\Delta H_v$ must be removed from Eqn 6. ΔH_d is then given by

$$\Delta H_d = \Delta H_{diss} + \Delta H_t + n\Delta H_f \qquad (7)$$

Dehydration

Let us now consider the specific case in which the solvating solvent is water. In the solid state (ice) each water molecule forms O-H---O hydrogen bonds with four other water molecules; each water molecule donates two hydrogens and accepts two hydrogens. In the liquid state of water, there are a large number of broken, as well as strained, hydrogen bonds. Theoretical analysis has shown that the average number of hydrogen bonds per molecule in liquid water is about two (Stillinger, 1980). Thus, Eqn 2 will correspond to a practical situation only when water is largely present in an 'ice-like' structure after dissociating from the hydrate. This situation will occur in those hydrates in which the probability of each water molecule forming hydrogen bonds with four other water molecules is greatest, e.g. when water is present as a host in certain clathrates, or when water is present in layers, or when a large number of water molecules are present in an exoclathrate matrix (Nelson et al., 1989). In all other cases, which include most of the pharmaceutical hydrates, each water molecule in the hydrate forms a maximum of three hydrogen bonds with other water molecules. In these cases water will separate as a liquid upon dehydration and Eqns 2 and 4 may be combined to yield

$$D \cdot nA_{solid} \rightarrow D_{solid} + nA_{liquid} \qquad \Delta H_l \qquad (8)$$

where the applicable enthalpy change ΔH_l represents the heat of dissociation of the hydrate to yield the dehydrated structure (D_{solid}) and water in its liquid state. Thus,

$$\Delta H_l = \Delta H_{diss} + n\Delta H_f \qquad (9)$$

In this case, Eqn 6 reduces to

$$\Delta H_d = \Delta H_l + \Delta H_t + n\Delta H_v \qquad (10)$$

A similar equation has been applied by Tanaka and Negita (1980) to calcium oxalate.

Since energy is required to overcome the interactions between D_{solid} and A_{liquid}, ΔH_l is usually a positive quantity (endothermic). The magnitude of ΔH_l depends upon the extent and strength of these interactions. The interactions are relatively weak in the case of caffeine monohydrate and

theophylline monohydrate and are somewhat stronger in the case of ampicillin trihydrate. When new bonds are formed between the drug molecules, energy is released, hence in most cases ΔH_t is a negative quantity (exothermic). The magnitude of ΔH_t depends upon the type of interactions that may occur between molecules of D upon dehydration of the hydrate. Therefore, the energy (ΔH_l in Eqn 8) required to overcome the interactions between the water molecules and the D molecules in $D \cdot nA_{solid}$ may be compensated by an equivalent amount of energy liberated (ΔH_t in Eqn 3) during the formation of new bond(s) in the rearranged dehydrated solid (D'_{solid}). Thus, $\Delta H_l \approx -\Delta H_t$, and Eqn 10 will approximate to

$$\Delta H_d \approx n\Delta H_v \tag{11}$$

Eqn 11 may be referred to as the vaporization hypothesis and may apply to those hydrates in which (i) the crystal structure rearranges upon dehydration so that $\Delta H_l \approx -\Delta H_t$ in Eqn 10 or (ii) the crystal structure does not rearrange (i.e. $D'_{solid} = D_{solid}$ and $\Delta H_t = 0$) and there is no change in energy when the hydrate desolvates to give D_{solid} and liquid water according to Eqn 8 (i.e. $\Delta H_l = 0$).

Eqn 11 will not apply to the hydrates in which the heat of dissociation (ΔH_l) is not compensated by the heat of transition (ΔH_t). This situation will arise when there is a significant interaction between the molecules of water and the anhydrate (D_{solid}) in Eqn 8, but the crystal structure does not rearrange upon dehydration, i.e. $\Delta H_t = 0$ in Eqn 3. This situation will also arise when there is a significant amount of adsorbed water in the hydrate.

For those hydrates in which water molecules are present in liquid-like structures, we have seen that $\Delta H_l \approx -\Delta H_t$ and Eqn 10 is applicable, from which Eqn 11 is derived. By analogy, for those hydrates in which water molecules are present in ice-like structures, $\Delta H_{diss} \approx -\Delta H_t$ and Eqn 6 is applicable, which then reduces to

$$\Delta H_d \approx n\Delta H_f + n\Delta H_v \tag{12}$$

Since, $\Delta H_f + \Delta H_v = \Delta H_s$, where ΔH_s is the heat of sublimation of ice, Eqn 12 can be written as

$$\Delta H_d \approx n\Delta H_s \tag{13}$$

Eqn 13 may be termed the sublimation hypothesis. We show that the vaporization hypothesis is applicable to all the hydrates examined with the exception of copper sulfate pentahydrate and calcium gluceptate USP. However, even in these exceptional cases, neither does the sublimation hypothesis apply. It is therefore evident that the sublimation hypothesis does not apply in the present work.

The present study is therefore based almost exclusively on the vaporization hypothesis (Eqn 11). The underlying assumption is that each desolvation endotherm in DSC is a quantitative measure of water content corresponding to a particular binding energy or location in the solid.

Method of calculation

Based on the vaporization hypothesis (Eqn 11), the following method of calculation was formulated for the determination of the number of moles of water per mole of the anhydrate (n). Since we do not know the value of n and therefore do not know the molecular weight of the hydrate, $D \cdot nA_{solid}$, we determine the enthalpy changes per unit mass (Δh) instead of per mole (ΔH). The relationships between these quantities are:

$$\Delta H_d = \Delta h_d \cdot (M_s + nM_w) \tag{14}$$

$$\Delta H_v = \Delta h_v \cdot M_w \tag{15}$$

where Δh_d is the specific enthalpy of dehydration (J/g of hydrate), Δh_v denotes the specific enthalpy of vaporization of water (2261 J/g of water, Stark and Wallace, 1976), M_s is the molecular weight of the anhydrate and M_w represents the molecular weight of water (18.016).

Substituting Eqns 14 and 15 into Eqn 11 affords

$$n = \frac{\Delta h_d \cdot M_s}{(\Delta h_v - \Delta h_d) \cdot M_w} \tag{16}$$

Thus, the stoichiometric number, n, represents the number of moles of water, A, per mole of D in the hydrate, $D \cdot nA_{solid}$, and was calculated from the specific enthalpies and molecular weights using Eqn 16.

In the cases of copper sulfate pentahydrate and calcium gluceptate USP, the number of moles of water per mole of the anhydrate (n) was also calculated by applying the sublimation hypothesis (Eqn 13). For sublimation, the following equation (Eqn 17) is analogous to Eqn 15:

$$\Delta H_s = \Delta h_s \cdot M_w \tag{17}$$

where Δh_s is the specific enthalpy of sublimation of water (as ice) (2830 J/g of water, Riddick et al., 1986), ΔH_s denotes the molar enthalpy of sublimation of water (as ice) and M_w has been defined above. Substituting Eqns 14 and 17 into Eqn 13 yields

$$n = \frac{\Delta h_d \cdot M_s}{(\Delta h_s - \Delta h_d) \cdot M_w} \tag{18}$$

This equation enables the stoichiometric number (n) of the hydrate to be calculated based on the sublimation hypothesis (Eqn 13).

Materials and Methods

Preparation of the hydrates

Nedocromil sodium was obtained from Fisons plc, Loughborough, U.K. Manganese, cobalt, nickel and zinc salts of nedocromil were prepared from nedocromil sodium. Manganese chloride tetrahydrate (MCB, Norwood, OH), cobalt chloride hexahydrate, nickel nitrate hexahydrate and zinc chloride (Mallinckrodt, Paris, KY) were the sources of the metal ions. An aqueous solution of one of the latter salts was added with constant stirring to an equimolar quantity of an aqueous solution of nedocromil sodium. The precipitated nedocromil salt was thoroughly washed with cold water. The salts were recrystallized twice from water and were allowed to dry in the atmosphere at room temperature (22°C).

Ampicillin trihydrate was obtained from Sigma Chemical Co. (St. Louis, MO). From anhydrous caffeine (N.Y. Quinine Co., NY) and theophylline (Sigma, St. Louis, MO) the hydrates were obtained by recrystallization from water and were dried in the atmosphere at room temperature (22°C).

Methods used for water content determination

Differential scanning calorimetry (DSC) A differential scanning calorimeter (DuPont, Model 910) equipped with a data station (Thermal Analyst 2000, DuPont Instruments, Wilmington, DE) was used to record the DSC curves of the samples. The temperature axis and the cell constant of the DSC cell were calibrated with indium (8 mg, 99.999% pure, peak maximum at 156.6°C and heat of fusion = 28.4 J/g).

The heating rate and the type of pans are the two most important instrumental factors which may affect the measured enthalpy changes and the measured temperature in DSC. A heating rate of 5–10°C/min with a nitrogen purge was employed throughout the study. Eqn 5 is applicable only if crimped or open pans are used because these pans allow the water vapor to escape. Crimped pans were used in the present work. The mass and particle size of the sample will influence the attainment of thermal and thermodynamic equilibrium throughout the DSC experiment (Van Dooren and Müller, 1984). A sample of 4–6 mg was weighed onto an aluminium pan. If the rate-limiting step were the diffusion of water vapor through the solid sample rather than the release of water from the crystals, the desolvation endotherms would be expected to be broader than those obtained in the present work. Furthermore, as the maximum variation in the enthalpy values did not exceed 5%, differences of particle size were judged not to be influential in the present experiments.

Thermogravimetric analysis (TGA) Each sample was placed in an aluminium pan and heated at a rate of 5–10°C/min under nitrogen purge in a thermogravimetric analyzer (Model 951, DuPont Instruments, Wilmington, DE) linked to a data

station (Thermal Analyst 2000, DuPont instruments, Wilmington, DE).

Karl Fischer titrimetry (KFT) The percent moisture content was also determined by KFT using a Mitsubishi Moisture Meter (Model CA-05, Mitsubishi Chemical Industries Ltd, Tokyo, Japan).

Hot stage microscopy The dehydration events postulated in DSC were confirmed by hot stage microscopy. A small amount (< 1 mg) of sample was placed in high-temperature silicone oil (Aldrich Chemical Co., Milwaukee, WI) and heated at a constant rate on a hot stage (Mettler FP80, Mettler Instrument Corp., Hightstown, NJ) under a microscope (Wild-Leitz M3Z, Wild Heerbrug, Switzerland). The liberation of bubbles due to the escape of water vapor from the solid enabled dehydration to be observed and its temperature to be determined.

Results and Discussion

The vaporization hypothesis (Eqn 11) was tested using literature data (Table 1). The number of moles of water per mole of anhydrate (i.e. n) was calculated using Eqn 16. In most cases, the stoichiometry so determined agrees well with the reported or theoretical stoichiometry. In the case of testosterone, Frøkjær and Anderson (1974) have shown that the contribution of the enthalpy of dissociation to the enthalpy of dehydration is

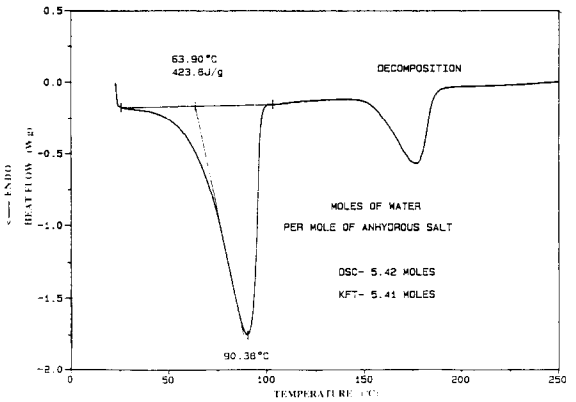

Fig. 4. DSC curve of nedocromil manganese. The desolvation endotherm is well separated from the decomposition endotherm. The water stoichiometry determined by the vaporization hypothesis agrees well with that determined by Karl Fischer titrimetry.

significant (about 22%). This means that the ΔH_l term in Eqn 10 is not compensated by the ΔH_t term, so that Eqn 11 does not apply. The variation observed in some other values in Table 1 could be due to the fact that these data were collected under other conditions and for other purposes than those identified in the present paper.

Fig. 4 shows the DSC thermogram of nedocromil manganese. Hot stage microscopy confirmed that the endotherm at 90°C corresponded to dehydration. The heat of dehydration derived from the area under this curve was used to calcu-

TABLE 1

Test of the hypothesis, ΔH of desolvation = $n\Delta H$ of vaporization (Eqn 11), using literature data

Compound	Moles of water/mole of anhydrate (n)		Reference
	Reported	Calculated (DSC data)	
Mercaptopurine	1	0.94	Niazi (1978)
Urapidil	1	0.85	Botha et al. (1988)
Testosterone	1	1.37	Frøkjær and Anderson (1974)
$(Co(NH_3)_5)Cl_3$	1	1.09	Mori et al. (1960)
$(Co(NH_3)_5)Br_3$	1	1.03	Mori et al. (1960)
$(Co(NH_3)_5)(NO_3)_3$	1	1.04	Mori et al. (1960)
$(Cr(NH_3)_5)Cl_3$	1	0.66	Tsuchiya et al. (1969)
$(Cr(NH_3)_5)Br_3$	1	0.97	Tsuchiya et al. (1969)
$(Cr(NH_3)_5)I_3$	1	1.15	Tsuchiya et al. (1969)

TABLE 2

Further tests of the vaporization hypothesis (Eqn 11 using DSC) by comparison with KFT

Hydrate	DSC	KFT
	Moles [a] [SD] ($n = 3$)	Moles [a] [SD] ($n = 3$)
Nedocromil Mn	5.45 (0.03)	5.41 (0.18)
Nedocromil Co	7.10 (0.23)	7.44 (0.02)
Nedocromil Ni	7.65 (0.17)	7.15 (0.15)
Nedocromil Zn	4.45 (0.10)	4.76 (0.07)
Ampicillin	3.20 (0.08)	3.31 (0.04)
Carbamazepine	2.09 (0.06)	2.06 (0.03)
Caffeine	1.04 (0.00)	0.90 (0.06) [b]
Theophylline	0.84 (0.13)	0.81 (0.08) [b]

[a] Number of moles of water per mole of anhydrate.
[b] $n = 5$.

late the number of moles of water per mole of anhydrous nedocromil manganese (Eqn 16). The water content was also determined by KFT. Similarly, the water content was determined by DSC and KFT for other nedocromil salts. The results are summarized in Table 2. In general the DSC and KFT results are in good agreement.

Table 2 also presents the stoichiometry of four hydrates of widely used pharmaceuticals determined by DSC and KFT. The DSC results agree well with the KFT data.

In the hydrates of caffeine and theophylline the water molecules are present in tunnels parallel to the c crystal axis along which the hydrates desolvate (Sutor, 1958a,b; Byrn, 1982). Byrn (1982) has shown that these hydrates desolvate readily and have low dehydration temperature thresholds. This observation indicates that the enthalpy of dissociation (ΔH_1) is small; in these cases the vaporization hypothesis (Eqn 11) is applicable.

The crystal structure of ampicillin trihydrate (James and Hall, 1968) shows that there is a significant interaction between the molecules of ampicillin and water. However, in this case, the energy required to break the hydrogen bonds between the drug and water molecules (mainly ΔH_1 in Eqn 8) is probably compensated by the energy released in the formation of new hydrogen bonds (mainly ΔH_t in Eqn 3) between the donor and the acceptor groups present in the ampicillin molecule.

The crystal structure of carbamazepine dihydrate (Reck and Dietz, 1986) shows that each water molecule is linked to one carbamazepine molecule by one hydrogen bond and to other water molecules by three hydrogen bonds. In the crystal structure of anhydrous carbamazepine (Himes et al., 1981), on the other hand, no new hydrogen bond between two drug molecules is formed to compensate for that between each drug molecule and a water molecule in the dihydrate. However, on dehydration, an increase in the strength of the Van der Waals interactions may occur. Since the vaporization hypothesis applies to carbamazepine dihydrate, the ΔH_t term in Eqn 3, which in this case arises mainly from Van der Waals interactions, evidently compensates for ΔH_1 in Eqn 10.

The dehydration of nedocromil sodium occurs in four steps as seen in Fig. 5, presumably due to four binding energies, each corresponding to a different energy state or location in the crystal

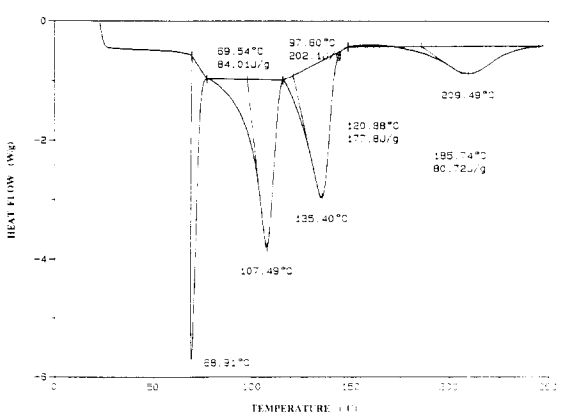

Fig. 5. DSC curve of nedocromil sodium. Since the desolvation endotherms are quite well separated, it is possible to apportion the water stoichiometry to each endotherm corresponding to a particular binding state of water in the hydrate.

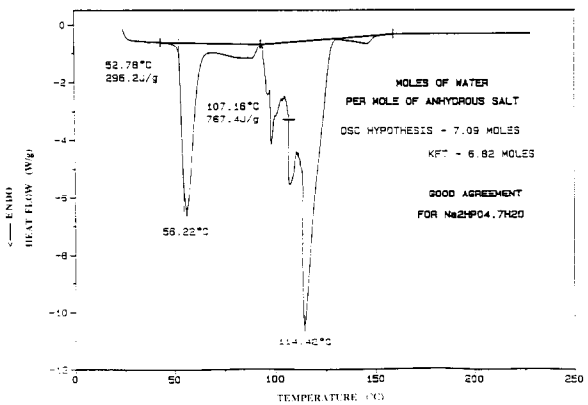

Fig. 6. DSC curve of disodium hydrogen phosphate heptahydrate ($Na_2HPO_4 \cdot 7H_2O$). The water stoichiometry determined by DSC assuming the vaporization hypothesis (Eqn 11) agrees well with that determined by Karl Fischer Titrimetry.

lattice. Each endotherm can be considered to represent the enthalpy of dehydration corresponding to that energy state or location. Using the vaporization hypothesis, the stoichiometry of the water molecules in each state could be calculated. The results are listed in Table 3. The total number of moles of water obtained by the DSC method agrees closely with that from KFT.

Using DSC, the vaporization hypothesis was also tested on sodium phosphate heptahydrate, copper sulfate pentahydrate and calcium gluceptate USP. The DSC and KFT results for disodium hydrogen phosphate heptahydrate (Fig. 6) are in good agreement.

The DSC, TGA and KFT results for copper sulfate and calcium gluceptate USP are shown in Table 4. In the case of copper sulfate pentahydrate, TGA was the only method which gave a stoichiometric number close to the theoretical value of 5. The anomalous behavior in KFT can be partly attributed to the reaction of Cu^{2+} with the Karl Fischer reagent (Mitchell and Smith, 1980) and partly to experimental error. During titration of the water of crystallization of $CuSO_4 \cdot 5H_2O$ with the Karl Fischer reagent, hydriodic acid and methyl hydrogen sulfate are reactive intermediates. The hydriodic acid simultaneously reduces Cu^{2+} liberating 0.5 mole of iodine. Therefore, the net reaction of $CuSO_4 \cdot 5H_2O$ with iodine in the Karl Fischer reagent corresponds to 4.5 moles of water instead of 5 as shown below.

$$CuSO_4 \cdot 5H_2O + 4.5I_2 + 5SO_2 + 5CH_3OH \rightarrow CuI + 8HI + 5HO \cdot SO_2 \cdot OCH_3 + H_2SO_4 \quad (19)$$

In contrast, the DSC method, using the vaporization hypothesis, overestimated the water stoichiometry of $CuSO_4 \cdot 5H_2O$ (Table 4) indicating that the ΔH_1 and ΔH_t terms in Eqn 10 are substantial in magnitude and do not compensate each other. The discrepancy in the DSC results for $CuSO_4 \cdot 5H_2O$ could also be due to its decomposition and to the chemical reaction of either the sulfuric acid so formed or the Cu^{2+} with the aluminium pans which are then oxidized.

In the case of calcium gluceptate USP, KFT was the only method which gave a stoichiometric number close to the theoretical value of 3.5. The DSC and TGA methods gave higher values of the

TABLE 3

Distribution of water among the four desolvation endotherms of nedocromil sodium

Peak temperature (°C)	ΔH of each desolvation endotherm (J/g)	Water in each energy state determined using the vaporization hypothesis, Eqn 11 (moles/mole of anhydrous salt)
68.9	84.0	1.14
107.5	202.1	2.78
135.4	177.8	2.44
209.5	80.7	1.11
	Total moles of water from DSC	7.49
	Moles of water from KFT	7.70

TABLE 4

Comparison of values of water content determined by stoichiometry, KFT, TGA, DSC (vaporization hypothesis, Eqn 11) and DSC (sublimation hypothesis, Eqn 13)

Compound	Moles of water/mole of anhydrate				
	Stoichiometry	KFT	TGA	DSC (vaporization)	DSC (sublimation)
$CuSO_4 \cdot 5H_2O$	5.00	3.92	4.81	8.20	5.61
Calcium gluceptate $\cdot 3.5H_2O$	3.50	3.47	4.08	7.92	5.96

water stoichiometry because dehydration occurs during decomposition. In other words, the dehydration endotherm could not be separated from the decomposition endotherm in DSC and, similarly the two weight losses could not be separated in TGA.

Application of the sublimation hypothesis (Eqn 18) to the DSC data for $CuSO_4 \cdot 5H_2O$ and calcium gluceptate USP yielded respective values of 5.61 and 5.96 moles of water per mole of anhydrous salt. These results suggest that the ΔH_{diss} term in Eqn 6 is not compensated by the ΔH_t term, so that Eqns 12 and 13 do not apply. Furthermore, for both these hydrates, the measured ΔH_d value obtained from DSC is abnormally large, probably as a result of decomposition and/or chemical reaction during dehydration, as mentioned above.

Determination and comparison of the desolvation enthalpies of more organic hydrates are currently under investigation. We are also planning to extend these studies to other solvents of crystallization in various other solvents.

Conclusions

(1) A novel DSC method for the determination of water content and stoichiometry of hydrates has been evaluated. Thus, the original hypothesis holds water.
(2) The model, which assumes that ΔH of desolvation of n moles of water $\approx n\Delta H$ of vaporization of water, has been successfully applied to literature data for some hydrates and some anomalous behavior explained.
(3) The results obtained from the DSC method agree well with those from Karl Fischer titrimetry.
(4) Using this new method, it was possible to apportion the water content to each binding state or location.
(5) This method could provide an insight into water binding in solid hydrates.
(6) The method cannot be applied when the dehydration peak overlaps another peak such as polymorphic change, melting, sublimation and decomposition.

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