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Application of isothermal microcalorimetry in preformulation. I. Hygroscopicity of drug substances

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Abstract

Isothermal microcalorimetry was evaluated for the determination of the critical relative humidity (cRH) for three drug substances possessing different hygroscopic properties. Two extremely water-soluble substances (flupentixol,2HCl and Lu 25–109, water solubility over 1000 mg/ml) and a hydrophobic substance (sertindole, approximately 10 μg/ml) were examined. The technique was evaluated with respect to sample size, duration of exposure time and temperature for the substance Lu 25–109, and further tested with the other compounds. The cRH values determined by isothermal microcalorimetry showed results similar to various weighing methods. This, together with the high sensitivity of the technique, which will enable a determination within hours, and the small sample amount needed, makes isothermal microcalorimetry a valuable screening technique for new drug candidates. The temperature effects on the cRH for Lu 25–109 showed a linear van't Hoff plot, but with a heat of interaction with water differing from the enthalpy of water condensation, however. By results obtained from solution calorimetry, this deviation was accounted for by the heat of formation of a saturated solution in the water layer surrounding the solid particles.

Keywords: Hygroscopicity; Microcalorimetry; Preformulation; Water vapor sorption

1. Introduction

Water absorption by pharmaceutical substances or excipients can influence the chemical stability and physical properties of the materials. The chemical stability of a solid drug can change due to enhanced degradation caused by dissolution of the drug substance in the absorbed water layer surrounding the individual particles (Leeson and Mattocks, 1958; Van Campen et al., 1983). Excipients or additives might influence the degradation taking place in the absorbed water layer. Physical

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parameters such as binding, flow and compactibility properties can be markedly altered as the water content of the materials changes. Sebhatu et al. (1994) showed that the good filler/binder properties of amorphous lactose were lost during water absorption as the lactose crystallises.

The isothermal microcalorimeter technique has previously shown potential for measuring physical changes due to water absorption. Briggner et al. (1994), and Buckton et al. (1995) have employed the technique for detection of amorphous contents in crystalline substances. Angberg et al. (1992a,b) have studied the incorporation of water into various types of anhydrous lactose powders.

The aim of this study was to evaluate and use the isothermal microcalorimeter technique as a tool for hygroscopicity determination of drug substances. The influence of sample size, temperature and exposure times on the critical relative humidity (cRH) were investigated. The technique is non-specific, as a sum of every ongoing heat-associated process—endothermic and exothermic—is measured. The results, therefore, have to be verified by more specific analytical methods.

2. Materials and methods

2.1. Materials

Lu 25–109, sertindole and flupentixol,2HCl were all synthesised at H. Lundbeck A/S, purity over 99%. K_2SO_4, KNO_3, KCl, NaCl, NaBr, $Mg(NO_3)_2$, $MgCl_2$ and CH_3COOK used for the saturated salt solutions to maintain controlled humidity were all reagent grade (E. Merck, Darmstadt, Germany). The quality of the N_2 gas used for the microcalorimeter experiments was at least 99.999%, and the H_2O content no more than 3 ppm (supplied by Hede Nielsen A/S, Denmark, associated with Air Liquide).

2.2. Microcalorimetry

The heat changes were measured using a heat conduction microcalorimeter (Thermal Activity Monitor (TAM), Thermometric AB, Sweden). A complete description of the TAM has been reported by Suurkuusk and Wadsö (1982). The commercially available RH-perfusion cell accessory for the TAM (Model 2250-100) was used to control the RH within the sample vessel. The probe is similar to the one designed by Bakri (1993). Dry N_2 was passed into the RH-cell with a flow rate of $2.5–2.8 \pm 0.1$ ml/min utilising a Hewlett Packard mass flow controller. The flow rate was measured before and after each experiment with a flowmeter (model ADM 1000 Intelligent flowmeter, J and W Scientific, Scantec AB, Sweden). The gas inlet was subsequently split into two different tubes within the top of the RH-cell compartment, one delivering dry gas to the sample vessel, and the other delivering gas, which has been saturated with water by passage through two humidifying chambers. The actual RH in the sample vessel was achieved by accurately controlling the proportion of dry to saturated gas. In a standard microcalorimeter experiment, 50 or 100 mg of sample (drug substance) was used. The RH-perfusion cell was allowed to temperature equilibrate in three positions for 5, 5 and 10 min, respectively, before lowering into the measuring position of the TAM. The RH was kept constant at 30% until a stable heat flow signal was reached (i.e. signal within the range of -1 to $+1$ μW) or for a maximum of 10.5 h. The RH was then increased in a linear ramp from 30 to 100% over the following 18 h. For some experiments the RH was maintained at 100% for up to 4 h at the end of the experiment. The heat flow change arising when the moisture interacts with the solid sample was measured as a function of time, and as the RH changes with time, the heat flow (μW) was also recorded as a function of the RH. The TAM was calibrated with empty stainless steel ampoules before each series of experiments (1000 μW calibration for experimental temperatures 21–35°C and 3000 μW calibration for 40–45°C). The TAM was housed in a thermostated room maintained at 21 ± 1°C. To eliminate or minimise water condensation within the outlet tubes of the RH-cell, the outlet temperature was kept above the TAM temperature.

Due to the large water vapour sensitivity of Lu 25–109, the drug was pre-equilibrated at 21°C and 33% RH, whereas flupentixol,2HCl and sertindole were used without further equilibration.

The area under the heat flow curve (AUC) was calculated using the baseline fitting programs in Origin 3.5 (MicroCal Software).

2.3. Conventional weighing

Eight controlled humidity environments were created by equilibrating various saturated salt solutions in hygrostats at 21 ± 1°C (Nyqvist, 1983). Lu 25–109 and sertindole were pre-equilibrated at about 20% RH, while flupentixol,2HCl was dried in vacuum at 40°C for 2 days. Approximately 1 g (Lu 25–109 and flupentixol,2HCl) or 500 mg (sertindole) was accurately weighed into open glass vials and placed in the various hygrostats. The moisture uptake was measured simply by following weight gain as a function of time utilising a Mettler AE 163 balance.

2.4. Dynamic vapour sorption analysis

The hygroscopicity of flupentixol,2HCl and Lu 25–109 was examined in a Dynamic Vapour Sorption Analyser (DVS-1, Surface Measurement Systems, London, UK). Approximately 7 mg of sample was used together with the following experimental conditions: gas flow 150 ml N_2/min, ramp 0–98% RH at a rate of 10% RH/h at 30.0°C. The experiments were performed at the Shirley Institute, Manchester, UK.

2.5. Sorptiometer instrument

The sorptiometer was designed and built at the Department of Pharmaceutics, Royal Danish School of Pharmacy. A weight balance registers the sample weight automatically within the range -200 to $+200$ mg with a sensitivity of 10 μg. For small sample amounts, a sensitivity of 1 μg can be obtained within the range -20 to $+20$ mg. The sorptiometer is operated via a computer which controls and registers the actual temperature, the RH and the sample weight simultaneously. Between 1800 and 2500 datasets are registered throughout the pre-set experimental period.

The desired RH (pre-set in the controlling computer program) surrounding the sample is achieved by supplying either dry nitrogen or nitrogen which is saturated with water vapour at the experimental temperature. The supply of dry vs. saturated nitrogen is controlled by the difference between the actual and the desired RH (the maximal flow of nitrogen is 70 ml/min during the period of adjustment and 5–10 ml/min when the desired RH has been achieved), if the RH surrounding the sample is lower than the RH pre-set by the computer (the desired RH), water vapour saturated nitrogen is supplied until the desired RH is achieved. The flow rate of the saturated nitrogen supplied is regulated by the magnitude of the difference between the actual and the desired RH, e.g. if the difference is big, saturated nitrogen is supplied at a high flow rate whilst a smaller flow rate is used for smaller differences. On the other hand, if the actual RH surrounding the sample is higher than the desired RH, dry nitrogen is supplied in the same way as exemplified above. When the desired RH has been achieved, a flow of nitrogen with the correct RH (achieved by a valve mixing the dry and saturated nitrogen in the correct proportion) is supplied to the sample chamber (flow rate 5–10 ml/min). For practical purposes, the dynamic range for the sorptiometer is within 5–85% RH at temperatures between 15 and 45°C.

A sample amount between 10 and 20 mg was used in each experiment. The drug substance was allowed to equilibrate for 600 min at the RH preset as the start conditions. A stepwise increment in the RH was then applied with a rate of 0.2% RH/8 min or 0.2% RH/16 min.

2.6. Karl Fischer titration

The total moisture content was measured by Karl Fischer titration (KF titration) by usage of a Metrohm 701KF Titrino and 703 Ti Stand instrument with Hydranal® composite 5 as titrant and Hydranal® Eichstandard 5,00 (Riedel–de Haën). In every case, double measurements utilising 50 mg of drug substance each were performed.

2.7. Solution calorimetry

The heats of solution and dilution were determined for Lu 25–109 using the Thermometric 2225 Precision Solution Calorimeter for the TAM. A total of 100 mg of Lu 25–109 was accurately weighed into a glass vial using a five-digit balance (Mettler AE 163). The vial containing the dry powder was closed with a silicone rubber stopper, sealed with wax and stored at room temperature until used. To some samples 15, 22.5 or 30 μl of water, respectively, were added before closure. These samples were stored for 6–8 h at 45°C in order to slightly increase solubility and then equilibrated at 35°C for 3–6 days before usage. It was checked that vials stored at elevated temperatures had not leaked before measurement. A sample vial was placed in the reaction vessel containing 100.00 ml of ultrapure water. The heat of reaction (wetting, solution, dilution or a combination hereof) was determined at 35°C with a mixing rate of 500 rpm, after a calibration of 4–8 J.

3. Results and discussion

The applicability of the microcalorimetric method to estimate cRH was evaluated at 35°C using Lu 25–109, and furthermore tested with the two drug substances flupentixol,2HCl and sertindole. Lu 25–109 was chosen as a model substance, as previous studies at H. Lundbeck A/S have indicated the RH to be a critical stability parameter. The effect of temperature on the cRH was investigated in the range 21–45°C for Lu 25–109, and within 21–35°C for the other substances. Blank experiments were performed with an empty reaction vessel at 21 and 35°C. The heat flow signal was stable and within a few μW up to 90% RH. Above 90% RH, the heat flow signal increases to about 15 μW for both temperatures. This blank signal is negligible compared to the heat flow signal obtained for a standard experiment with Lu 25–109 at 35°C (Fig. 1). Thus, the experimental heat flow curves were not corrected for blank signals.

As the TAM is positioned in a room at 21 ± 1°C, which is lower than the calorimeter temperature, a risk of water condensation in the gas outlet tube exists. In order to minimise heat flow signals caused by such water condensation, the influence of the RH-cell outlet temperature was examined with the TAM operated at 25°C (linear ramp from 10 to 100% RH over 18 h). Increasing the outlet temperature decreased the blank heat flow signal, but was not able to eliminate blank signals above 90% RH. For experiments performed within the temperature range 21–35°C, the gas outlet temperature was 50°C, while the outlet temperature was 60°C for the 40–45°C experiments. Using a similar system, Hansen et al. (1996a) found blank heat flow signals up to 100 μW, most likely due to this system using a heated gas stream to obtain the desirable RH. The increased heat flow signal was suggested to be caused by water adsorption onto the surface of the sample ampoule. We found, however, no significant different blank signals by changing from a 4 ml stainless steel ampoule to a 2 ml vessel. Additional experiments in our laboratory have shown that the O-rings used for sealing between the reaction vessel and the RH-cell compartment sorb water vapour, resulting in an increased heat flow signal at elevated RH. Thus, we consider the blank signal at high RH to be due to water vapour interacting with such O-rings.

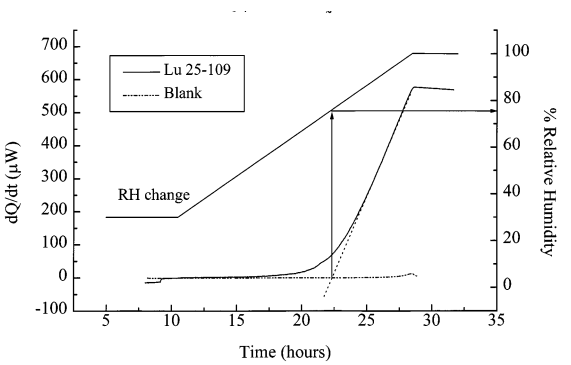

Fig. 1. Heat flow signals (μW) obtained as a function of time for 50 mg of Lu 25–109 together with a corresponding blank experiment at 35°C. Ramp conditions: linear increase in RH from 30 to 100% over 18 h. The cRH is defined as the RH where the linear part of the heat flow curve intercepts the baseline.

Table 1
Critical relative humidities (cRH) for Lu 25–109 at various temperatures, determined by different methods (n = no. of repetitions)

Temp. (°C)	Method	Conditions	Sample amount (mg)	n	cRH ± S.D. (%)
21.0	Weighing	Hygrostats with controlled RH	1000	1	86.0
21.0	TAM	Ramp 18 h, 30→100%	50	3	83.5 ± 2.1
21.1	Sorptiometer	Steps 0.2% RH/8 min, 10→85%	20	3	83.1 ± 0.3
24.9	Sorptiometer	Steps 0.2% RH/16 min, 40→85%	10	2	81.8 ± 0
25.0	TAM	Ramp 18 h, 30→100%	50	1	78.0
30.0	DVS-1	Ramp 10%/h, 0→98%	7	1	84.0
30.1	Sorptiometer	Steps 0.2% RH/8 min, 10→85%	20	3	78.0 ± 0
35.0	TAM	Ramp 18 h, 30→100%	50/100	10	75.4 ± 0.9
35.9	Sorptiometer	Steps 0.2% RH/16 min, 40→85%	12	4	74.0 ± 0.5
40.0	TAM	Ramp 18 h, 30→100%	50	3	74.5 ± 0.1
40.0	Sorptiometer	Steps 0.2% RH/8 min, 10→85%	20	3	71.0 ± 0.1
45.0	TAM	Ramp 18 h, 30→100%	50	3	70.8 ± 0.4
45.0	Sorptiometer	Steps 0.2% RH/8 min, 10→85%	10	3	68.9 ± 0.7

A typical heat flow curve obtained for 50 mg of Lu 25–109 at 35°C is shown in Fig. 1. The heat flow signal is at baseline level up to about 55% RH (after 17 h). The heat flow signal then increases as the RH increases and water condenses on the surface of the drug substance. Assuming that no other physical or chemical phenomena is significant in the reaction vessel, the heat flow signal is proportional to the rate of moisture uptake (Hansen et al., 1996a). We defined the cRH as the RH-value where the linear part of the heat flow curve intercepts the baseline. However, the moisture adsorption starts below this point, but at a much slower rate. This method was found to give identical results as compared to other methods (Table 1). The results obtained in the conventional weight gain experiment for Lu 25–109 performed at 21°C (Fig. 2) showed that up to several weeks were necessary to reach equilibrium. The standard TAM experiments were performed with a linear ramp time of 18 h, i.e. at non-equilibrium conditions. The heat flow signal obtained at any RH represents the initial moisture uptake rate. The time needed to obtain this rate by the conventional weighing experiment (the dotted lines in Fig. 2) depends on the sensitivity of the weight balance used. For the TAM investigations, the experimental set-up will provide the information of the cRH within 1 day. The influence of the ramp rate (70% RH/18 h ~ 3.89% RH/h) was examined by changing the duration of the ramp period (equivalent to 99 h). No significant change in the cRH was found, however, for the various ramp conditions. It is therefore concluded that the ramp rate is not critical for the determination of the cRH in the experimental set-up used.

As the microcalorimeter measures the sum of all processes (endothermic and exothermic) going on during an experiment, verification is needed to demonstrate that the heat flow signals are caused only by water condensation onto the drug substance. For this reason, five experiments with the ramp stopped between 90 and 100% RH were

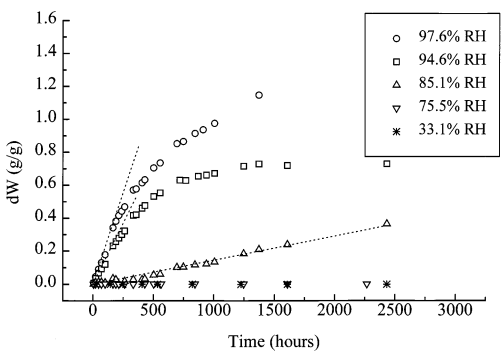

Fig. 2. Conventional weighing experiment showing weight gain obtained for Lu 25–109 as a function of time when stored at 21°C and various RH values. The dotted lines represent the initial rate of moisture uptake for the 97.6, 94.6 and 85.1% RH conditions.

Table 2
Energy output and amount of water condensed for 100 mg of Lu 25–109 at 35°C

Exp. No.	Microcalorimetry			KF titration Water condensed (mg)	Weighing Water condensed (mg)
	Exp. stopped (% RH)	AUC (J/100 mg)	Water condensed[a] (mg)		
1	90.0	3.51	1.48	0.82	1.27
2	91.5	3.25	1.47	—	1.66
3	97.8	6.81	2.82	—	3.00
4	98.0	6.90	2.96	2.96	3.50
5	98.5	6.48	2.72	2.10	2.60

[a] Assuming no processes other than water condensation are taking place.

performed with approximately 100 mg of Lu 25–109. Immediately following the experiments, the water content of each sample was determined by weighing and/or KF titration (Table 2). The area under the heat flow curve (AUC) from the start of the ramp until the experiment was stopped is a direct measurement of the total amount of heat produced. If this energy change is entirely due to water condensation, the theoretical amount of water present in the sample can be calculated from the AUC and the enthalpy change for water condensation at 35°C ($\Delta H_{cond} = -43.572$ kJ/mol). Table 2 lists the determinations of total water content in the samples together with the calculated water content based on the heat flow versus time AUC values. The calculated amount of water does agree with the measured amount. It is noted that water levels less than approximately 2.5 mg per 100 mg of Lu 25–109 is near the sensitivity level of the KF titration method. Thus, the relatively large variation obtained for those experiments is expected. In this respect, the sensitivity of the five-digit balance is much better. Based on the good agreement between TAM experiments and conventional water determinations (Table 2), it was concluded that the heat flow signal is completely dominated by water condensation. The TAM is considered suitable for hygroscopicity studies.

The effect of different sample size was examined in the range 10–250 mg drug substance. The weight-corrected AUC during a ramp period of 30–90% RH was calculated. Only minor or insignificant effects of sample size were observed, indicating that only the top surface of the samples was exposed to the water vapour. This agrees with the observations reported by Hansen et al. (1996a,b), who found a small effect on sample size (up to 100 mg). The microcalorimeter technique has been used by others (Sheridan et al., 1995; Puddipeddi et al., 1996) to investigate the wettability of α-lactose and p-hydroxybenzoate or the hygroscopicity of sodium benzoate, respectively. In these two studies, a pronounced effect of sample size on water uptake was observed. The reason for this apparent inconsistency is most likely due to different experimental conditions. In those two studies the total heat change (J) of a sample exposed to stepwise increments of the RH is measured. The heat flow signal was allowed to return to baseline after a peak before a new step in RH was applied. As the heat flow signal is proportional to the moisture uptake rate, a heat flow signal at baseline indicates that the sample is at equilibrium under the given conditions, i.e. the entire sample has been exposed. By doing relative fast ramp experiments as in the present study, however, equilibrium is never reached and, due to the rapidly changing moisture conditions, mainly the upper layer will respond.

In order to investigate the influence of temperature on cRH, a series of experiments were performed utilising Lu 25–109 as a model substance. Fig. 3 shows the heat flow curves for 50 mg of Lu 25–109 at various temperatures when exposed to an 18 h linear ramp. Fig. 4 illustrates the weight

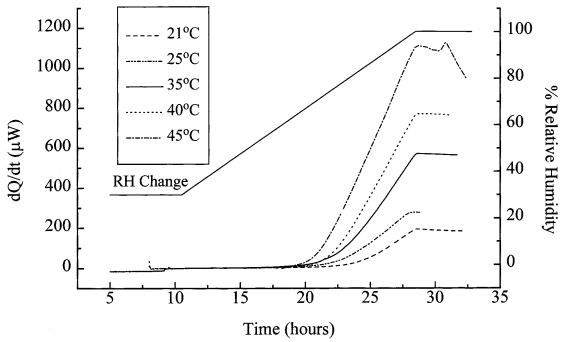

Fig. 3. Heat flow vs. time curves obtained for 50 mg of Lu 25–109 at various temperatures. Ramp conditions: linear increase in RH from 30 to 100% over 18 h.

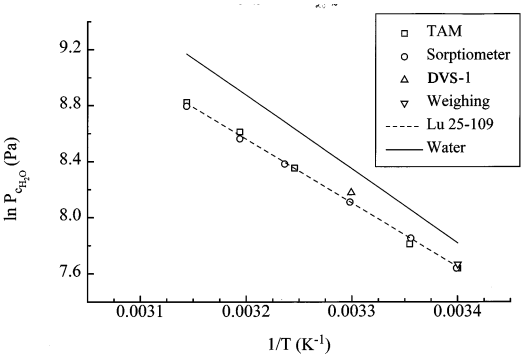

Fig. 5. The van't Hoff plot of the critical relative humidities (cRH) for Lu 25–109 expressed as water vapour pressures (Pa) together with the theoretical curve for saturated water vapour pressures in the temperature range 21–45°C.

gain for Lu 25–109 measured with the sorptiometer at various temperatures. The cRH for these curves was defined and determined similarly to the cRH obtained from the heat flow curves, where the linear part of the curve intercepts the baseline. The cRH values dependence of temperature is clearly illustrated in both figures. Table 1 lists the results of the cRH determination with the various methods at the examined temperatures. The corresponding van't Hoff plot is shown in Fig. 5. The ΔH for the adsorption process between Lu 25–109 and water estimated from the slope of the van't Hoff plot is -37.92 ± 0.37 kJ/mol water ($\Delta H_{\text{van't Hoff,Lu 25–109}}$). This is significantly different from the enthalpy for water condensation also calculated from the van't Hoff plot ($\Delta H_{\text{van't Hoff,water}} = -43.73$ kJ/mol). The $\Delta H_{\text{van't Hoff,Lu 25–109}}$ equals the sum of the heat of condensation of water and the heat of solution for formation of a saturated solution, i.e. $\Delta H_{\text{van't Hoff,Lu 25–109}} = \Delta H_{\text{van't Hoff,water}} + \Delta H_{\text{van't Hoff,sat. solut.,Lu 25–109}}$ (all parameters represented by the unit kJ/mol water). The heat of solution for formation of a saturated solution of Lu 25–109 ($\Delta H_{\text{van't Hoff,sat. solut.,Lu 25–109}}$) calculated from the above equation must therefore equal 5.81 ± 0.37 kJ/mol water. Additionally, we have estimated $\Delta H_{\text{sat. solut.,Lu 25–109}}$ by a two-step process using solution calorimetry:

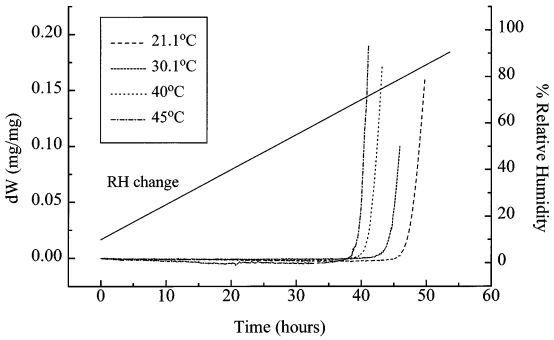

Fig. 4. Weight change vs. time curves obtained for Lu 25–109 in the sorptiometer at various temperatures. Ramp conditions: stepwise increase 0.2% RH/8 min from 10 to 85% RH.

1. Solid drug → Dil. solution (~ 1 mg/ml) ΔH_1
2. Sat. solution → Dil. solution (~ 1 mg/ml) ΔH_2

1 + (− 2): Solid drug → Saturated solution

$$\Delta H_{\text{sat.solut., Lu25–109}} = \Delta H_1 + (-\Delta H_2)$$

As Lu 25–109 possesses an extremely high water solubility, it was difficult to make a saturated solution usable for the solution calorimeter experiments. The solid drug was therefore weighed into the solution calorimeter vials and various amounts of water were subsequently added (15, 22.5 or 30 μl). After equilibration as described in Section 2.7, it was observed that within the vials

Table 3
Enthalpy of solution (ΔH_{sol}) for Lu 25–109 at 35°C in the solid state and with various amounts of water added (n = no. of repetitions)

Water added at 21°C (μl)	Conc. (g Lu 25–109/ml water)	$\Delta H_{sol} \pm$ S.D. (kJ/mol Lu 25–109)	n
0	—	30.19 ± 0.52	4
15	6.7	12.58 ± 1.80	3
22.5	4.4	5.63 ± 0.24	3
30	3.3	6.16 ± 0.16	3

containing 22.5 and 30 μl of water solutions were formed at both 45 and 35°C. The samples with 15 μl of water added remained, however, a suspension. The heat of solution values for the different conditions are listed in Table 3. From the results obtained with the 22.5 and 30 μl water-added samples, it is concluded that the heat of dilution is independent of concentrations in the concentration range examined. An average of the results is therefore used ($\Delta H_2 = 5.89 \pm 0.34$ kJ/mol Lu 25–109 ($n = 6$)). As $\Delta H_1 = 30.19 \pm 0.52$ kJ/mol Lu 25–109, the heat of solution for formation of a saturated solution, $\Delta H_{\text{sat. solut.,Lu 25–109}}$, estimated from the solution calorimeter experiments, is 24.30 ± 0.27 kJ/mol Lu 25–109. The same parameter estimated from the van't Hoff plot ($\Delta H_{\text{van't Hoff,sat. solut.,Lu 25–109}} = 5.81 \pm 0.37$ kJ/mol) had the unit kJ/mol water; therefore, the concentration of the saturated solution has to be calculated in order to convert the parameter to the unit kJ/mol Lu 25–109 (Eqs. (1)–(6)). By visual inspection of the vials, the concentration of a saturated solution of Lu 25–109 in water falls within the range $\sim$4.4–6.7 g/ml at 35°C. Further, the actual concentration of the saturated solution can be estimated from the results obtained with the 15 μl water-added samples. The ΔH_{sol} for these samples is a combination of solution and dilution, as only part of the drug substance was dissolved in the 15 μl of water added. A part of the ΔH_{sol} determined for these samples is caused by dilution of the dissolved drug substances (5.89 ± 0.34 kJ/mol Lu 25–109). The rest ($\Delta H_{\text{sol,solid drug}}$) is due to dissolution of the solid drug. The number of moles of Lu 25–109, which was present as a solid in the suspension (but was dissolved during the process), n_{solid}; the number of moles dissolved in the 15 μl of water, $n_{dissolved}$; the solubility of Lu 25–109 in water and, finally, the mole fraction of Lu 25–109, $X_{\text{Lu 25–109}}$, are calculated for the three 15-μl-added experiments in Table 4.

From the mole fraction of Lu 25–109 ($X_{\text{Lu 25–109}}$), the proportion of the amount of water and the amount of Lu 25–109 is calculated:

$$X_{\text{Lu 25–109}} = 0.22 \quad \text{(from Table 4)} \tag{1}$$

$$X_{\text{Lu 25–109}} = n_{\text{Lu 25–109}}/(n_{\text{Lu 25–109}} + n_{H_2O}) \tag{2}$$

$$n_{H_2O}/n_{\text{Lu 25–109}} = (1 - 0.22)/0.22 \tag{3}$$

in order to convert the heat for formation of a saturated solution calculated from the van't Hoff plot ($\Delta H_{\text{van't Hoff,sat. solut.,Lu 25–109}}$) from the unit kJ/mol water to kJ/mol Lu 25–109:

$$\Delta H_{\text{van't Hoff,sat. solut.,Lu 25–109}} = \Delta H_{\text{van't Hoff,sat. solut.,Lu 25–109}} * n_{H_2O}/n_{\text{Lu 25–109}} \tag{4}$$

(kJ/mol Lu 25–109) = (kJ/mol water) $\times$ *(mol water/mol Lu 25–109)

$$\Delta H_{\text{van't Hoff,sat. solut.,Lu 25–109}} = 5.81 \text{ kJ/mol water} * (1 - 0.22)/0.22 \tag{5}$$

$$\Delta H_{\text{van't Hoff,sat. solut.,Lu 25–109}} = 20.60 \text{ kJ/mol Lu 25–109} \tag{6}$$

Based on the standard deviation from the individual factors used for the calculation of $\Delta H_{\text{van't Hoff,sat. solut.,Lu 25–109}}$ (Eq. (4)), the standard deviation for the calculated factor can be estimated to 2.35 kJ/mol. The heat of solution for formation of a saturated solution obtained

Table 4
The calculation of the solubility of Lu 25–109 in water at 35°C expressed as g Lu 25–109/ml water and as the mole fraction of Lu 25–109 ($X_{\text{Lu 25–109}}$), estimated from the solution calorimeter results obtained for the 15 μl-water-added samples (n = no. of moles)

Exp. no.	Conc. of suspension (g Lu 25–109/ml water)	ΔH_{sol} (kJ/mol Lu 25–109)	$\Delta H_{\text{sol,solid drug}}$ (kJ/mol Lu 25–109)	n_{solid} (10^4 mol)	$n_{\text{dissolved}}$ (10^4 mol)	Solubility (g Lu 25–109/ml water)	$X_{\text{Lu 25–109}}$
1	6.616	14.35	8.46	1.117	2.091	4.31	0.20
2	6.801	10.75	4.86	0.659	2.638	5.44	0.24
3	6.671	12.65	6.76	0.900	2.334	4.81	0.22
$X \pm$ S.D.		12.58 ± 1.80				4.85 ± 0.57	0.22 ± 0.02

$\Delta H_{\text{sol,solid drug}} = \Delta H_{\text{sol}} - \Delta H_2$; $n_{\text{solid}} = (\Delta H_{\text{sol,solid drug}} \cdot n_{\text{total}})/\Delta H_{\text{sat.solut.,Lu 25–109}}$; $n_{\text{dissolved}} = n_{\text{total}} - n_{\text{solid}}$; Solubility $= (n_{\text{dissolved}} \cdot M_{\text{W}})/V_{\text{H}_2\text{O}}$; $X_{\text{Lu 25–109}} = n_{\text{Lu 25–109}}/(n_{\text{Lu 25–109}} + n_{\text{H}_2\text{O}}$, $\rho_{\text{H}_2\text{O}}(21°\text{C}) \approx 0.9980$ g/ml.

from the solution calorimetry experiments ($\Delta H_{\text{sat. solut.,Lu 25–109}} = 24.30 \pm 0.27$ kJ/mol Lu 25–109)[1] is in good agreement with the value estimated from the van't Hoff plot and the solubility calculations (20.60 ± 2.35 kJ/mol Lu 25–109)[2]. Thus, the aberration observed between the ΔH for water condensation and the ΔH for water interacting with Lu 25–109 can be explained by the heat associated with the formation of a saturated solution on the outer surface of the solid particles. The heat flow signal obtained is a sum of all heat-associated processes taking place. The interaction of water vapour with powder surfaces is a complicated reaction which might involve more processes than can be accounted for by the overall difference in enthalpy change. The interaction might be explained by a number of steeps, including condensation of water molecules followed by water–solid interaction (wetting), dissolution of solid in the condensed water layer surrounding the particle, and dilution.

The heat flow curves obtained for approximately 50 mg of each drug substance investigated at 35°C are shown in Fig. 6. The heat flow curve for sertindole is insignificant as compared to a blank experiment, indicating that no moisture sorption is taking place at any RH. This was also the case when investigated at 21°C. The conventional weighing for sertindole showed no weight change after 75 days of storage at 21°C and 97.6% RH. When examined in the sorptiometer, no differences from a blank experiment were observed. It was therefore concluded that sertindole is a non-hygroscopic substance in the temperature range 21–35°C. For flupentixol,2HCl, the heat flow curve illustrates a moisture uptake from the very beginning of the ramp—although small, significant from baseline signal. The heat flow signal increases rapidly when the RH has passed the cRH as seen for Lu 25–109 as well. A similar curve shape is obtained for experiments performed with the DVS-1 analyser (30°C), sorptiometer (21°C) and for the conventional weight

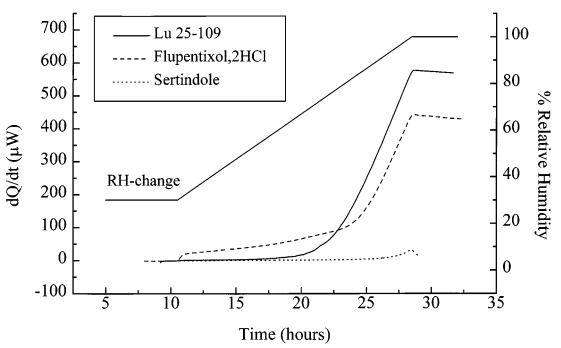

Fig. 6. Heat flow vs. time curves obtained for approximately 50 mg of each of the substances Lu 25–109, flupentixol,2HCl and sertindole, at 35°C. Ramp conditions: linear increase in RH from 30 to 100% over 18 h.

[1] The 95% confidence band for the parameters is $[\underline{d}; \bar{d}] = [d - t_{0.975}(f) \cdot s(d); d + t_{0.975}(f) \cdot s(d)]$, giving [23.7; 25.0].

[2] The 95% confidence band for the parameters is [15.8; 25.4].

Table 5
Critical relative humidities (cRH) for flupentixol,2HCl at various temperatures, determined by different methods (n = no. of repetitions)

Temp. (°C)	Method	Conditions	Sample amount (mg)	n	cRH ± S.D. (%)
21.0	Weighing	Hygrostats with controlled RH	1000	1	90.0
21.0	TAM	Ramp 18 h, 30→100%	50	3	89.2 ± 0.9
21.1	Sorptiometer	Steps 0.2% RH/8 min, 10→85%	20	3	84.1 ± 0.3
30.0	DVS-1	Ramp 10%/h, 0→98%	7	1	87.7
35.0	TAM	Ramp 18 h, 30→100%	50	3	83.7 ± 0.8

gain experiment (21°C), when plotting the rate of moisture sorption as a function of RH. A good agreement between the different methods of analysis exist for the two more-or-less hygroscopic substances investigated (Tables 1 and 5). A van't Hoff plot based on the results obtained with flupentixol,2HCl shows a $\Delta H_{\text{van't Hoff,flup.}} = -42.03 \pm 1.06$ kJ/mol water, which is not significantly different from the heat of water condensation (-43.73 kJ/mol water). Thus, although extremely water soluble, the heat associated with the interaction between water and flupentixol,2HCl does not indicate the formation of a saturated water layer surrounding the solid particles.

4. Conclusion

Knowledge of the cRH at various temperatures is important in relation to decision on the storage conditions both in manufacturing departments and during long-term stability studies. Isothermal microcalorimetry was found useful in the evaluation of hygroscopic properties of drug substances at various temperatures. Additionally, the small amount of compound needed makes the microcalorimeter technique a valuable tool in the preformulation phase of drug development.

The critical relative humidities obtained for three different compounds were in good agreement with those obtained by other methods such as sorptiometry and conventional weighing. For the hydrophilic compound Lu 25–109, it was demonstrated that the heat flow signal measured during increasing humidity primarily is due to condensation of water vapour onto the surface of the solid compound. The influence of temperature on cRH for Lu 25–109 was found to follow the van't Hoff equation, yielding a change of enthalpy of -37.92 kJ/mol. This deviates from the enthalpy for water condensation by approximately 6 kJ/mol, which may be explained by the heat associated with the formation of a saturated solution in the water layer surrounding the solid particles. It is, however, not possible on the basis of the heat flow signal to distinguish between heat changes originating from various processes such as: (i) condensation of water; (ii) drug–water interaction (wetting); (iii) dissolution; and/or (iv) dilution taking place.

The microcalorimetric response showed no significant effect on sample size, indicating that the heat flow signal is dominated by interaction of the water vapour with the upper layer of the powder bed. The duration of the exposure time (ramp period) did not seem to influence the cRH determination either.

For the less hygroscopic substance, flupentixol,2HCl, and the non-hygroscopic compound, sertindole, the results obtained by means of the microcalorimeter technique were consistent with those obtained by means of the various weighing methods.

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