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RESEARCH PAPER

A Novel Method for Determining the Solubility of Small Molecules in Aqueous Media and Polymer Solvent Systems Using Solution Calorimetry

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ABSTRACT

Purpose To explore the application of solution calorimetry for measuring drug solubility in experimentally challenging situations while providing additional information on the physical properties of the solute material.

Methods A semi-adiabatic solution calorimeter was used to measure the heat of dissolution of prednisolone and chlorpropamide in aqueous solvents and of griseofulvin and ritonavir in viscous solutions containing polyvinylpyrrolidone and N-ethylpyrrolidone.

Results Dissolution end point was clearly ascertained when heat generation stopped. The heat of solution was a linear function of dissolved mass for all drugs (<10% RSD, except for chlorpropamide). Heats of solution of 9.8 ± 0.8, 28.8 ± 0.6, 45.7 ± 1.6 and 159.8 ± 20.1 J/g were obtained for griseofulvin, ritonavir, prednisolone and chlorpropamide, respectively. Saturation was identifiable by a plateau in the heat signal and the crossing of the two linear segments corresponds to the solubility limit. The solubilities of prednisolone and chlopropamide in water by the calorimetric method were 0.23 and 0.158 mg/mL, respectively, in agreement with the shake-flask/HPLC-UV determined values of 0.212 ± 0.013 and 0.169 ± 0.015 mg/mL, respectively. For the higher solubility and high viscosity systems of griseofulvin and ritonavir in NEP/PVP mixtures, respectively, solubility values of 65 and 594 mg/g, respectively, were obtained.

Conclusion Solution calorimetry offers a reliable method for measuring drug solubility in organic and aqueous solvents. The approach is complementary to the traditional shake-flask method, providing information on the solid properties of the solute. For highly viscous solutions, the calorimetric approach is advantageous.

KEY WORDS crystal lattice · drug-polymer dispersions · heat of solution · mixed solvents · solubility · solution calorimetry

INTRODUCTION

Drug solubility is one of the most important physicochemical parameters necessary for the development of pharmaceutical products. Aqueous solubility is a fundamental parameter as it has direct influence on dissolution rate in aqueous media and drug absorption. The importance of drug solubility extends beyond aqueous solubility. Solubility measurements in different types of solvents are an integral part of drug discovery, preformulation and formulation efforts. Due to its simplicity, the shake-flask method is the preferred, often unstated way for measuring solubility. A sufficiently large amount of the solid solute to produce a saturated solution is mixed with the solvent of interest. The mixture is then shaken in a closed container (the "flask") for a period of time (often between 24 to 72 h.) until equilibrium is reached. A portion of the liquid from the obtained suspension, free from excess solid is separated by filtration in order to determine the concentration of the solute in the liquid. The assay is often carried out by HPLC/UV or LC/MS. The shake-flask method is the gold standard for solubility determinations; it is a straightforward approach that provides high quality solubility data. However, formulation scientists often find themselves in situations where obtaining reliable solubility values with the shake-flask method is challenging. For example, when the solvent of interest is

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highly viscous, a few complications arise. Highly viscous solvents make it difficult to anticipate the equilibration time in solubility studies. In fact, even if the typical 24 to 72 h. equilibration time actually applies to a viscous solution, it is often necessary to make the assessment for the particular case by monitoring concentration as a function of time (1). This situation greatly increases the time and resources needed to measure the solubility (2). Furthermore, separation of the liquid aliquot destined for assay from the solid solute becomes more challenging for viscous solutions because a simple step like filtration does not work well in these situations. Issues related to accurate measurements of solubility are easily defined but are nevertheless challenging to solve. Such issues translate into uncertainty about the solubility value. Uncertainty about solubility values resulting from relatively high variability among measurements often arises in cases where the solubility of the drug in the solvent of interest is quite low. In these situations, the shake-flask procedure per se does not present any particular difficulties. The problem resides on the consistency of the results among replicate measurements. The solubility of every organic drug compound will be determined in a variety of solvents throughout the course of drug development. Sound decisions regarding formulation approach and drug exposure considerations depend to a good extent on timely and reliable knowledge of solubility. We present a calorimetry based method for measuring the solubility of drugs in various solvents. Because of its underlying principle, the method is theoretically applicable to every solubility measurement. However, the advantages of the method become more practical in situations where the use of the shake-flask method becomes challenging or unreliable as in the case of highly viscous solvents or when information is required about the physical properties of the material.

Here we explore the potential use of isothermal solution calorimetry for establishing drug solubility. Solution calorimetry measures the heat change generated from the dissolution of a solid or liquid solute by a solvent. The enthalpy of solution (ΔH_{SOL}) can thus be determined. This quantity reflects the combined enthalpic contributions from the breaking of the solute-solute interactions, and the formation of solute-solvent interactions. In the case of solid crystalline solutes, the former contribution corresponds to breakage of the crystal lattice, ΔH_{latt}, namely,

$$\Delta H_{latt} = \Delta H_m + \int_{T}^{T_m} \Delta C_p dT \tag{1}$$

where ΔH_m is the enthalpy of melting of the solute, T_m ant T denote the melting and experimental temperature (expressed in Kelvins), respectively, and ΔC_p is the difference in heat capacity between the solid and the liquid forms of the solute.

The second contribution to the observed heat of solution corresponds to the enthalpy of solvation, ΔH_{solvat}, so that the measured heat of solution is given by

$$\Delta H_{SOL} = \Delta H_{latt} + \Delta H_{solvat} \tag{2}$$

The first term on the right hand side of Eq. 2 is a property exclusively of the solid solute, while the second term depends on the (liquid) solute-solvent combination. The quantities in Eq. 2 vary with temperature. For simplicity, the temperature dependence notation is not shown in the expression, considering that all experiments in this study were conducted isothermally. The type of breakdown presented in Eq. 2 has been successfully used for assessing the proportion of amorphous content in partially crystalline samples (3–5). It should be pointed out that although not explicitly represented in Eq. 2, the total enthalpy of solution measured encompasses the heat evolved from the initial wetting of the solid solute by the solvent (4,6). This particular contribution depends on the (solid) solute-solvent combination, as well as on the attributes of the actual sample (i.e., the particular lot) being tested. Sample to sample variations in attributes such as specific surface area will result in variation in enthalpy of wetting. For any given solvent, creating a saturated solution at a given temperature, involves dissolving the solute at this temperature up to the maximum possible concentration of the solute. This leads to a maximum and constant concentration that also corresponds to a constant value of ΔH_{SOL}. Solution calorimetry equipment is highly sensitive, providing highly accurate measurements (7). The technique does not lend itself to mixing arbitrarily large amounts of solute with a solvent. Therefore, the method discussed here uses gradual additions of small amounts of solute in order to locate the saturation point. Incremental additions of solute to the solvent result in a straight line increase in the magnitude of ΔH_{SOL} until a plateau is reached when the concentration of the solute equals the solubility value. One important consideration is that the calorimetric measurement eliminates the need to establish equilibration time. Thanks to the high sensitivity of the instrument, equilibrium is ascertained when the evolution of heat from the sample comes to a stop. This approach takes advantage of the sensitivity and accuracy of the instrumental technique employed, while providing information on the quality and self consistency of the determination.

Isothermal calorimetry has several applications in the pharmaceutical sciences due to its sensitivity and ability to study samples under highly controlled conditions. A comprehensive review on this subject is provided by O'Neill and Gaisford (7). Solution calorimetry has been successfully applied in the detection and characterization of polymorphs, through the effect of polymorphism on heat of solution (8,9). Several investigators have shown the accuracy of solution calorimetry in quantification of the degree of crystallinity of pharmaceuticals

(3,10,11). Hogan and Buckton (5) reported that quantification of the amorphous content of lactose was accurate to within ±0.5%. More recently, Kayaert *et al.* (12) showed the utility of solution calorimetry for monitoring rapid drug dissolution from nanosuspensions.

The objective of this work is to explore the application of solution calorimetry for the direct determination of drug solubility in situations where the shake-flask method presents difficulties. We apply the method to the two cases discussed above. One is the case where the solvent is highly viscous due to the presence of a polymer. The other is the case of drugs with low aqueous solubility. As example of viscous solvents, we use mixtures of polyvinyl pyrrolidone (PVP) and N-ethylpyrrolidone (NEP) with PVP content of 20% (w/w) and 40% (w/w) PVP as model. In addition to serving as model of a viscous solvent, this system is of interest as PVP is commonly used in research and in solid dispersion formulations aimed at improving drug solubility. Measurement of drug solubility using the traditional shake-flask method and analysis by HPLC-UV is a challenge in this viscous system as analysis and even the attainment of equilibrium are difficult to ascertain. Here we report on the experimental conditions necessary for measuring the solubility of two model drugs, griseofulvin and ritonavir in viscous solvent systems. The choice of these drugs, in turn, covers two situations: one where the drug (griseofulvin) is not extremely soluble in the viscous PVP-NEP solvent, and one where the drug (ritonavir) is very highly soluble in the solvent. We also investigate the use of solution calorimetry for measuring the solubility of poorly water soluble drugs in aqueous systems. The model drugs for this part of the study are prednisolone and chlorpropamide. These drugs provide representative examples of a non-electrolyte and a weakly acidic drug, respectively. The results are compared with solubility measurements obtained using the shake-flask method with HPLC-UV analysis. The advantages, and conditions for optimal applications of the calorimetric method presented are discussed.

MATERIALS AND METHODS

Materials

Polyvinyl pyrrollidone, MW 2000–3000 (Kollidon® 12 PF) and N-ethylpyrrolidone (NEP) were a gift from BASF (Florham Park, New Jersey, USA). Griseofulvin was purchased from Hawkins (Minneapolis, MN, USA). Ritonavir was obtained as a generous gift from Clinton Health Access Initiative. Prednisolone and chlorpropamide (≥98% purity) and sodium lauryl sulfate (SLS) were purchased from Sigma Aldrich (St Louis, MO, USA). Analytical grade HCl was obtained from VWR (Radnor, PA, USA).

Solution Calorimetry Methods

The heat of solution was determined at 298.16 K using a Thermometric 2225 Precision Solution Calorimeter in combination with the 2227 Thermal Activity Monitor (TA Instruments, New Castle, DE, USA). This is a semi-adiabatic calorimeter which records temperature change upon reaction. The details of operation have been discussed in detail elsewhere (13,14). Briefly, the calorimeter is equipped with a glass vessel where a known amount of the solvent of interest is placed. A sealed glass ampoule containing a precisely known amount (m) of the solute sample is placed inside the vessel. The ampoule containing the solute is broken thus allowing the mixing of solute and solvent and the heat absorbed or released by the dissolution process (Q_{SOL}) is recorded. The sequence of events taking place during the experiment are: a) Baseline stage: Thermal equilibration as the temperature of the calorimeter vessel approaches that of the surrounding heat-sink. b) Pre-break calibration: Electrical heat-temperature calibration whereby a precisely known amount of heat is supplied to the liquid in the reaction vessel using an electrical calibration heater that is part of the instrument. c) Break: The ampoule held within the calorimetric vessel is broken and the solute is released into the solvent. d) Post-break calibration: Electrical heat-temperature calibration is performed on the resulting solution. The accompanying temperature change is recorded for assessing the amount of heat evolved with additional baseline stages performed between the steps listed in the above sequence. All experiments were conducted at least in sets of three separate determinations and the average $\Delta H_{SOL} = Q_{SOL}/m$ values are reported. The reproducibility of the solution calorimetry method can be established from the collection of all individual readings; the extent to which each individual reading yields the same ΔH_{SOL} value, is a measure of the reproducibility of the method (see discussion below). The performance of the instrument was verified by means of an external calibration with KCl and purified water (Milli-Q®, 18.2 MΩ·cm), which provides the most widely accepted test reaction (13,15). The performance test used 100 mg of KCl, dried at 420 K for 4 h hr. prior to use, and 25 mL of water (16).

Shake Flask Solubility and Concentration Determination using HPLC-UV

Aqueous solubility measurements were performed by adding excess drug to the solvent system and agitating for 24 to 36 h at 25°C on a thermal rocker set at 75 rocks/min (Lab Line Instruments, Melrose Park, IL). At different time points, excess drug was removed by filtration with a 0.45 μm polyethersulfone filter. Saturation solubility of the drugs was achieved within 24 h and it was confirmed that no drug adsorption onto the filters took place. The concentration of drug in the

saturated solution was determined by reversed-phase HPLC analysis with UV detection. After undergoing suitable dilutions, aliquots of the drug solution were injected into an Agilent 1200 Infinity series HPLC comprising a 1260 Infinity quaternary pump, 1260 Infinity thermostated column compartment, 1260 standard autosampler and a 1260 Infinity diode-array detector. The detector was interfaced via a PC with ChemStation software. Separation of prednisolone was achieved with a C_8 column (5 μm, 4.6×250 mm, Water Symmetry, Waters, MA) maintained at 40°C during the separation. The mobile phase used for the analysis consisted of 68.8% (v/v) water, 25% (v/v) peroxide-free tetrahydrofuran and 6.2% (v/v) methanol, and the flow rate was 1 mL/min. The injection volume was 20 μL and detection wavelength 254 nm. Drug retention time was 9.9 min. Separation of chlorpropamide was achieved with a C_{18} column (5 μm, 4.6×150 mm, Agilent Eclipse Plus, DE). The mobile phase used for analysis comprised 50% (v/v) acetonitrile and 50% (v/v) dilute glacial acetic acid (1 in 100) and the flow rate was 1 mL/min. The injection volume was 50 μL and detection wavelength 240 nm. Drug retention time was 3.6 min. Standards and calibration curves for both drugs were prepared in their corresponding mobile phases. The relative standard deviation for all drugs was less than 2%.

Solubility determinations for griseofulvin in NEP-PVP present additional experimental difficulties because they involve very viscous solutions. Since considerably longer equilibration times were used, the assay was used to ensure that no chemical degradation took place during the solubility experiments. The samples were shaken for an equilibration time of 4 days using a magnetic stirrer. After equilibration, the mother liquor was decanted, centrifuged using an Eppendorf Centrifuge 5804 (Eppendorf AG, Germany), filtered through 13 mm HPLC 0.45 μm filter (Alltech Associates, IL, USA), and diluted for HPLC assay. HPLC analysis was performed for determining the solubility values as well as to ensure the chemical stability of the samples after the equilibration time. The analytical method used an Agilent 1100 series equipped with an auto-sampler, and UV–VIS detection. Peak areas were determined using Agilent 1100 ChemStation for LC3D systems (Agilent, USA). The column used was Eclipse XDB-C 18 4.6 mm×25 cm (Zorbax Columns, USA); a 45:55 (v/v) mixture of acetonitrile with 45 mM KH_2PO_4 (adjusted to pH 3.0 with H_3PO_4) was used as mobile phase at a flow rate of 1.0 mL/min at 25°C. A standard concentration curve was established for quantitative determination.

RESULTS AND DISCUSSION

Figure 1-top shows a representative graph of the calorimetric signal obtained in these experiments. The area under

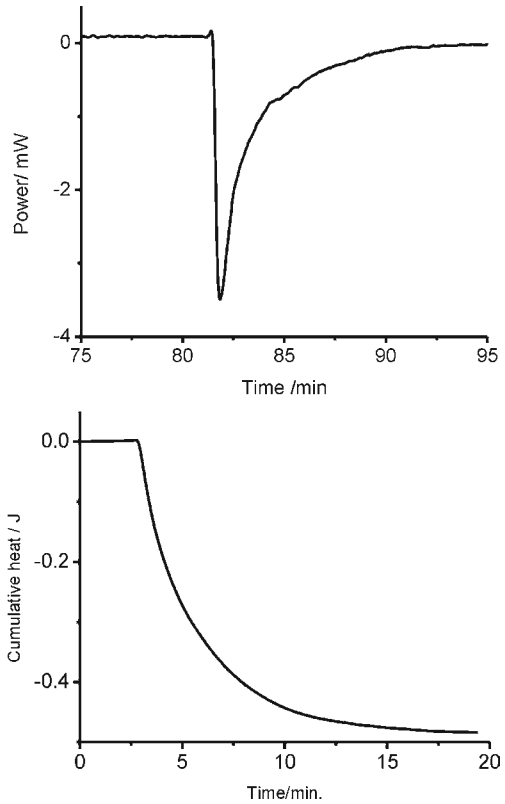

Fig. 1 Calorimetric signal curves obtained from the dissolution of griseofulvin in a mixture of 40% (w/w) PVP in NEP. *Top*: Heat flow trace curve. *Bottom*: Cumulative heat of dissolution as a function of time.

endothermic peak corresponds to the heat of solution (Q_{SOL}) of the sample. The enthalpy of solution (Eq. 2) corresponds to:

$$\Delta H_{SOL} = \frac{Q_{SOL}}{m} \tag{3}$$

where m is the mass of solid solute dissolved. The first term on the right hand side of Eq. 2 is positive (endothermic) for crystalline organic solutes and greater in magnitude than the second term, which can be either positive or negative, and corresponds to the heat of mixing. The peak in Fig. 1-top has a baseline to baseline shape, with area Q_{SOL}, providing a visual indication of the point where the dissolution process has come to completion. In this sense, the assignment of the (baseline) boundaries of the signal peak to integrate, is analogous to the procedure followed when integrating a peak obtained from a DSC thermogram. Another useful representation of the data is provided in Fig. 1-bottom, where the cumulative heat of solution is plotted as a function of time. The heat evolution of the dissolution process, represented by the curve, is enclosed between the two horizontal portions of the graph. It should be pointed out that theoretically, every separate calorimetric determination using the same solute in a given solvent should give the same ΔH_{SOL} value. This means that the

reproducibility of the solution calorimetry method is best assessed by looking at the consistency of the ratio Q_{SOL}/m, among separate measurements involving varying values of m.

Prednisolone was used as model of a drug with low aqueous solubility for our measurements. The results, plotted in Fig. 2, show the Q_{SOL} values obtained with increasing amounts of solute (m) placed inside the sealed ampoule. The first (ascending) portion of the plot corresponds to the situation where m is a sufficiently small amount of solid solute as to be completely solubilized in the solvent. For sufficiently small values of m (below the solubility limit), a constant slope of Q_{SOL}/m is observed in the form of a straight line in the ascending portion of the plot. Figure 2 was obtained with m=1 mg, 2 mg and 3 mg of the drug in the ampoule and 25 g of water in the vessel (W_s=25 g). The values and standard error for the Q_{SOL}/m values obtained for the solutes used in this study are listed in Table I and it is represented with dashed lines in Fig. 2 for prednisolone. In an ideal system, where there is no source of heat other than the dissolution process under study, the reading from the instrument corresponds exactly to the heat of solution of the sample. In such an ideal system, a straight line with zero intercept and a slope equal to the ΔH_{SOL} is obtained. Actual calorimetric measurements however, involve additional sources of heat, that while controlled, result in deviations from ideal readings. For example, the breakage of the ampoule involves a heat release measured in advance as 32±2 mJ. Another source of background heat is that of the stirring provided during the measurements. These acknowledged sources of heat that are an inherent part of solution calorimetry and the equipment is designed to keep them constant. They affect the intercept but not the slope (i.e., ΔH_{SOL}) of the line of the plotted data. Another source of heat

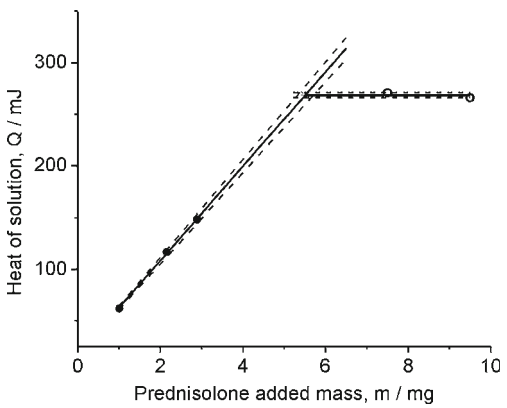

Fig. 2 Enthalpy of dissolution of prednisolone in water. *Closed symbols*: measurements where the entirety of the solid solute was dissolved. *Open symbols*: measurements where the amount of solute resulted in a saturated solution. The m value where the two straight lines cross corresponds to the amount of solute (m^*) that saturates the solvent contents in the vessel. The box demarcated by the *dashed lines* represent the uncertainty for the solubility value.

Table I Enthalpy of Solution and Variability Values for the Drug-Solvent Systems Included in the Study

	Q_{SOL}/m (J/g)	Std. error (J/g)	RSD%
Griseofulvin	9.8	0.8	8
Ritonavir	28.8	0.6	2
Prednisolone	45.7	1.6	3
Chlorpropamide	159.8	20.1	13

is the interaction between the solvent and the surface of the solid solute during the initial wetting preceding dissolution. The heat evolved by the wetting of the solute is a function of its surface properties. Surface heterogeneity, in the form of different energy of active sites or different degrees of surface crystallinity lead to variations in wetting energy (17,18). Variability in surface properties of powders is common and can be detected by solution calorimetry (19). These effects result in deviations from the theoretically constant ΔH_{SOL} value but provide valuable information in terms of lot-to-lot variability. The data in the ascending line in Fig. 2 exhibit consistent values of the Q_{SOL}/m ratio. The corresponding enthalpy of solution, ΔH_{SOL}=45.7±1.6 J/g. The flat portion of the profile corresponds to the heat of solution obtained when the amount of solute exceeds that needed to form a saturated solution (Δh_{SOL}). The notation ΔH_{SOL} and Δh_{SOL} is used here to distinguish between the enthalpy of solution (reflecting complete dissolution of the amount m of solute) and heat of solution measurements reflecting partial dissolution of the solid drug placed in the sealed ampoule, respectively. Placing m=7.5 and 9.5 mg of the drug in the glass ampoule resulted in partial dissolution of the samples with average heat of solution value Δh_{SOL}=268.5 mJ. When the amount of drug is sufficiently large to generate a saturated solution, the value of Δh_{SOL} is the same for different values of m, resulting in the flat portion of the plot shown in Fig. 2. The point where the ascending and flat portions of the graph cross, corresponds to the value of m (m^*), which is the minimum necessary to produce a saturated solution (or the maximum that can be completely dissolved in the amount W_s of solvent). From Fig. 2, the value (centered within the error range) of m^* is 5.5 mg, which for W_s=25 g (~25 mL) corresponds to a solubility value of 0.23 mg/mL for prednisolone in water. The solubility value obtained from the solution calorimetry method is a single point calculated from the crossing of two straight lines. The uncertainty about the prediction is given by the standard error of the Q_{SOL}/m slope, calculated from the set of calorimetric measurements. The data presented in Fig. 2 can be used for obtaining the solubility value (S) according to the following expression:

$$S = \frac{\Delta h_{SOL}}{\Delta H_{SOL}\ W_s} \tag{4}$$

The obtained solubility value is in very good agreement with the solubility value of 0.212 ± 0.013 mg/mL, determined by the shake-flask method and HPLC assay ($p > 0.05$).

The experimental procedure for obtaining the solubility value of prednisolone by the calorimetric method is as simple (or as intricate) as the HPLC assay method. Thus the point is not whether the calorimetric method is a suitable replacement for the shake-flask/HPLC-UV assay approach. The question has more to do with the type of information that the different methods provide, in addition to a solubility value. Provided that the appropriate analytical method is used, the chemical assay based approach has the potential advantage of being stability indicating. Thus providing information about chemical impurities and degradation products present in a particular sample. However, even when chemical purity is not an issue, lot-to-lot variability in the dissolution performance of pharmaceutical compounds is often observed. This is a common situation when the synthesis and crystallization schemes evolve from drug discovery to pilot scale synthesis and subsequently to large scale manufacturing. Changes in the solid state properties of crystalline compounds are frequently encountered. From these considerations, the chemical assay based and solution calorimetry approaches to solubility measurement are complementary. The former provides useful information reflecting variations in chemical composition, whereas the latter provides equally useful information about variations in physical properties. As stated above, in the case of variability among solid samples of the compound of interest, physical variability will be reflected on the uncertainty about the slope of the ascending portion of Fig. 2 (ΔH_{SOL}), as well as on the kinetics of dissolution captured in the heat-time profile of Fig. 1-bottom (20).

Knowledge of the solubility of a drug in water is a fundamental parameter to discovery and formulation scientists. The importance of the solubility properties of drugs however, is by no means restricted to neat water as the solvent. Knowledge of the solubility and dissolution behavior of drugs in aqueous media resembling gastric or intestinal fluids for example is an important aspect of drug development. The dissolution behavior of weak bases is of special interest because the pH dependence of their solubility/dissolution behavior can have a significant impact on absorption and bioavailability (21,22). We selected chlorpropamide as example of a weak electrolyte in this study. Chlorpropamide is also poorly soluble in water but it has the additional characteristic that its powder is not easily wet by plain water. The solvent used for the solubility determinations of chlorpropamide consists of 0.1 M HCl with 0.015% (w/v) SLS. The surfactant concentration used was below its critical micelle concentration (CMC) so as to act as a wetting agent and not as an active micellar solubilizer (23). The vessel was loaded with $W_s = 25$ g (~25 mL) of the solvent medium for each measurement. The amounts of chlorpropamide placed in the sealed ampoules for complete dissolution were $m = 0.9$ to 1.45 mg. For $m = 9$ mg and $m = 12$ mg, the amounts of drug in the sealed ampoule were sufficient to exceed the saturation concentration of the drug in the solvent. The results are shown in Fig. 3. From the slope of the ascending portion of the plot, a value of $\Delta H_{SOL} = 159.8 \pm 20.1$ J/g is obtained. The crossing point of the two lines is enclosed within a rhomboid-shaped box demarcated by the dashed lines. The box is a graphical representation of the uncertainty of the solubility value for chlorpropamide obtained using this method. The two segments of the plot cross at $m^* = 3.95$ mg and $\Delta h_{SOL} = 551.5$ mJ. From these results, the obtained solubility value for chloropropamide is 0.158 mg/mL, which is in agreement with the solubility value of 0.169 ± 0.015 mg/mL obtained from the shake-flask method with HPLC-UV detection.

In addition to the complementary information on the physical properties of the sample, the calorimetry based method becomes advantageous over the shake-flask method in situations where filtration and ascertaining equilibration times become challenging. This is frequently the case when working with highly viscous solutions, as those consisting totally or in part of polymers. In recent years, there has been a significant level of interest on the study of polymer based dispersions as means for formulating poorly soluble drugs. Polyvinylpyrrolidone is one of the polymers commonly used for such type of investigations. In this study, PVP was used to create a viscous solvent system presenting some challenges for solubility measurements. Various mixtures of PVP-NEP were used in order to explore solubility measurements in different systems using solution calorimetry. For griseofulvin, the viscous solvent used consisted of a solution of 40% (w/w) PVP in NEP. For ritonavir, the viscous solvent used consisted of a

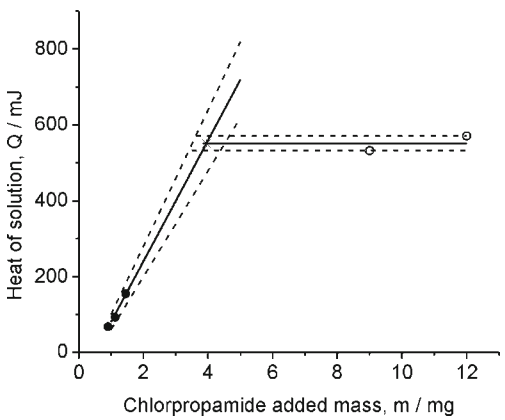

Fig. 3 Enthalpy of dissolution of chlorpropamide in 0.1 M HCl/0.015 (w/v) % SLS. *Closed symbols*: measurements where the entirety of the solid solute was dissolved. *Open symbols*: measurements where the amount of solute resulted in a saturated solution. The m value where the two straight lines cross corresponds to the amount of solute (m^*) that saturates the solvent contents in the vessel. The *dashed lines* demarcate the uncertainty for the value.

solution of 20% (w/w) PVP in NEP. Using griseofulvin as model drug, a second experimental challenge arises, in addition to that of the viscosity of the solvent. Even though griseofulvin is poorly soluble in water, it has a relatively high solubility in the PVP-NEP mixture. This means that the type of simple solubility determinations illustrated in Figs. 2 and 3 cannot be directly applied to a system like griseofulvin in PVP-NEP. When the solvent of interest is capable of dissolving large amounts of drug, we run into a situation where the total capacity of the sealed ampoule is less than the amount of drug needed for producing a saturated solution (i.e., $m<m^{*}$). In practical terms, this means that placing increasingly larger amounts of drug into the ampoule will provide the sought after Q_{SOL}/m value and its standard error. However, because of the high solubility of the drug, the carrying capacity of the ampoule will be reached with an amount of drug that is much smaller than that necessary to produce a saturated solution. Due to the physical constrains of the instrumentation, it is not possible to use increasingly large amounts of solute without restriction until a saturated solution is obtained (as is the case with a poorly soluble drug like prednisolone). For a highly soluble solute, Q_{SOL}/m values are therefore obtained from measurements confined to the low concentration (relative to the solubility limit) range. Such an instrumental limitation however, does not preclude the use of solution calorimetry for assessing the solubility of highly soluble solutes. With a slight modification, the calorimetry based method can still be applied for solubility determinations in this type of situation. The approach in this case consists in dissolving a known amount of the drug in the solvent and using the resulting mixture as background solution of known concentration C_1 as the solvent placed in the vessel. The heat of solution of dissolving an amount m of solid solute in the background solution is then measured. For these experiments, the vessel was first loaded with W_s=17 g of a background solution (solution **1**) of the drug in the solvent mixture (40% w/w PVP in NEP) containing a concentration, C_1=43 mg/g. The heat of solution values resulting from dissolving solute amounts of m=50, 75 and 100 mg into solution **1** were determined. The three samples completely dissolved into solution **1**, thus giving the Q_{SOL}/m value and standard error for this system. The results are shown in Fig. 4. A linear plot showing a ΔH_{SOL} value of 9.82±0.8 J/g was obtained. The background concentration of 43 mg/g results in a situation where the capacity of the glass ampoule was insufficient to attain a saturated solution. Therefore, the same general procedure was then repeated using a second background solution (solution **2**) with a higher background concentration of the drug, C_2=58 mg/g. Using solution **2**, the systems was pushed through the solubility limit by placing larger amounts (m=100 and 150 mg) of drug into sealed ampoule. These two samples resulted in saturated solutions (assessed by the persistence of

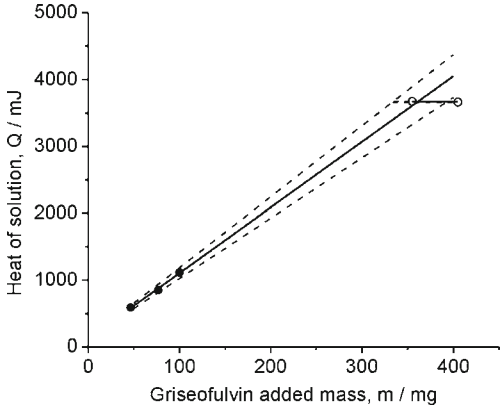

Fig. 4 Enthalpy of dissolution of griseofulvin in a solvent medium composed of 40% (w/w) PVP in NEP. *Closed symbols*: measurements of dissolution of the drug against a background concentration of 43 mg/g. *Open symbols*: measurements of dissolution of the drug against a background concentration of 58 mg/g. The plotted *open symbols* coordinates correspond to Eqs. 6 and 7. The *dashed lines* mark the uncertainty for the measurements.

suspended powder after heat flow had stopped), giving heat of solution readings, δh_{SOL}, of 1.17 and 1.16 J for the 100 and 150 mg samples, respectively. From these data, the solubility of the drug is given by

$$S = C_2 + \frac{\delta h_{SOL}}{\Delta H_{SOL}\, W_s} \tag{5}$$

In order to maintain consistency through all of the examples presented in this report, we use the notation δh_{SOL} in Eq. 5 instead of Δh_{SOL} as with the examples of prednisolone and chlorpropamide. Both δh_{SOL} and Δh_{SOL} denote heat of solution readings in which the solid sample in the glass ampoule is not completely dissolved (i.e., a saturated solution is formed). The notation Δh_{SOL} is used here to identify those systems where a saturated solution is obtained by mixing the contents of the ampoule with pure solvent contained in the vessel. In contrast, δh_{SOL} is used to identify those systems where the saturated solution is obtained by mixing the contents of the ampoule with a background solution of the same solute contained in the vessel. Applying Eq. 5 gives a solubility value of 65 mg/g, which is in agreement with the value of 60.1 to 60.9 mg/g, determined with the shake-flask method and HPLC-UV detection. The time for the measurements, up to the end point when heat flow stopped, was of 12–14 min.

It is possible to plot the data from griseofulvin in similar fashion as done for prednisolone and chlorpropamide shown in Figs. 2 and 3, respectively. The procedure requires a correction for the background concentration placed in the vessel prior to conducting the calorimetric determinations. Accordingly, for the samples resulting in a saturated solution, the corrected amount of drug "added" (m_{corr}) comprises the

drug placed in the ampoule plus the excess drug previously dissolved in the background solution:

$$m_{corr} = m + (C_2 - C_1) W_s \quad (6)$$

in similar fashion, the correction necessary for arriving at Δh_{SOL} is given by

$$\Delta h_{SOL} = \delta h_{SOL} + (C_2 - C_1)\, W_s\, \Delta H_{SOL} \quad (7)$$

When the liquid in the vessel is pure solvent, $C_1 = C_2 = 0$ and $\delta h_{SOL} = \Delta h_{SOL}$ and $m_{corr} = m$. The values of m_{corr} and Δh_{SOL} are shown as open circles in Fig. 4, thus providing a graphic representation analogous to Figs. 2 and 3. The significance of Eqs. 6 and 7 however, is not limited to facilitating graphical representation. The results indicate that starting with a background concentration of zero and small m values, experiments made using increasingly high background concentrations, can be combined to produce a highly detailed description of the heat of solution properties of the drug spanning from highly diluted to highly concentrated solutions. One practical consideration however, has to do with the availability of drug to use for producing an increasingly populated straight line of Q *vs.* m, since drug availability is an issue frequently faced by drug development scientists.

Ritonavir was chosen as a model drug for this study because it presents a particularly challenging system, resulting from a practical concentration issue. Ritonavir is very highly soluble in NEP and in 20% (w/w) PVP in NEP, such that this drug can be considered as being freely soluble in the solvent. Solubility determinations in these situations consume large amounts of the active. This situation highlights the concern about economizing in the use of the active during drug development. We used ritonavir to test the ability of the calorimetric method for determining solubility while trying to keep a low use the use of drug. The approach consisted in using small amounts of drug to determine ΔH_{SOL} and the strictly necessary larger amounts for determining δh_{SOL}, and hence arrive to the solubility value (Eq. 5). This situation requires the use of substantially different (different order of magnitude) background concentrations. The background concentrations used were $C_1 = 67.5$ and $C_2 = 590$ mg/g for (background) solution **1** and solution **2**, respectively, with $W_s = 17$ g. The results are shown in Fig. 5. Given the large span between of background concentrations used to collect the data in the ascending and the plateau portions of the graph, the information is presented in the insets of the figure. Dissolution of amounts $m = 20$–70 mg of ritonavir into solution **1** (low concentration inset) gave $\Delta H_{SOL} = 28.75 \pm 0.6$ J/g. Dissolution of $m = 102$ and 120 mg of the drug into solution **2** gave δh_{SOL} readings between 2.1 and 2 J/g, respectively. The saturation data (Eqs. 6 and 7) are shown as open circles in the high concentration inset of Fig. 5. From Eq. 5, the solubility value

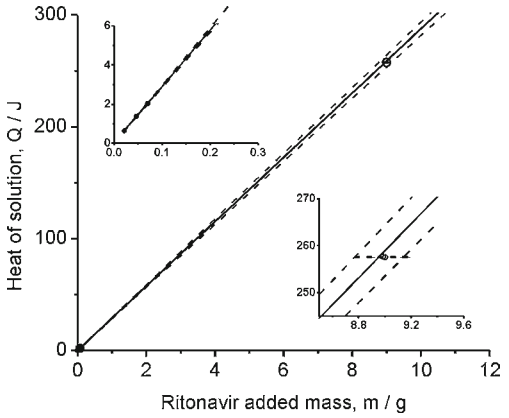

Fig. 5 Enthalpy of dissolution of ritonavir in a solvent medium composed of 20% (w/w) PVP in NEP. *Top* inset (*closed symbols*): measurements of dissolution of the drug against a background concentration of 67.5 mg/g. *Bottom* inset (*open symbols*): measurements of dissolution of the drug against a background concentration of 590 mg/g. The plotted *open symbols* coordinates are given by Eqs. 6 and 7. The *dashed lines* mark the uncertainty for the measurements.

for ritonavir in 20% (w/w) PVP/NEP is 594 mg/g. The example presented in Fig. 5 illustrates one of the most challenging types of solubility determinations for drugs. The data in Fig. 5 cover the very low and very high end of concentrations and hence do not result in a visually appealing plot. However, the important piece of information from the figure is that the heat of solution data obtained when pushing the concentration to the saturation limit, falls within the standard error of the Q_{SOL}/m value determined at the much lower concentrations. This means that despite the instrumental limitations and the experimental challenges posed by this type of extreme case system, the solution calorimetry method can still be used for measuring solubility. It is pertinent to mention that for a system like ritonavir in 20% (w/w) PVP/NEP, the high solubility of the drug and ensuing high viscosity of the solution make the shake-flask method virtually impractical, posing some remarkable experimental difficulties for sample preparation. In this type of situation, the calorimetric method is definitively advantageous.

Solubility determinations involving high concentrations require some practical considerations with every method of analysis. For example, high solute concentrations require dilution of the sample in order to bring the concentration within the range of the HPLC-UV assay. With the solution calorimetry method, high solubility values makes it necessary to go the opposite way; using increasing levels of background concentration in order to produce (i.e., cross over into) a saturated solution when the contents of the glass ampoule are emptied into that solution. The situation has to do with the limited physical capacity of the glass ampoule holding the solid solute. In order to determine the solubility, a saturated solution needs to be attained. If the contents of the ampoule are completely dissolved, a new reading of ΔH_{SOL} will be obtained (on the ascending portion of the plot) but the plateau portion will

remain unpopulated. This means that the analyst will need to select a sufficiently high background concentration such that the solute content of the ampoule is sufficient to exceed (cross over) the solubility limit. For drugs that have low solubility in the solvent, the solution calorimetry method is quite straightforward. Heat of solution determinations using increasing amounts of solute in the ampoule give the entire profile. From the measurements where the drug is completely dissolved, each one provides a separate determination of ΔH_{SOL}. From this set, the mean value and standard error are obtained. Measurements resulting in saturated solutions provide the plateau required to assess the solubility value. It is noteworthy that the greatest uncertainty observed (RSD > 10%), was for chlorpropamide. This is the model solute for which the wetting of the solid presents the most issues. As discussed, this is an important factor related to the properties of the solid solute, as well as to the surface-solvent interactions affecting heat of solution measurements (4,6,17–19).

CONCLUSIONS

Solution calorimetry offers a reliable and simple approach for measuring the solubility of organic compounds. We have shown that the approach provides a practical and reproducible method for measuring drug solubility in systems where the shake-flask method may be experimentally challenging, even impractical, such as in the case of a highly concentrated, highly viscous system like PVP/NEP. In addition to the solubility values, the cumulative heat-time profiles can provide important information on the solid state properties of the drug. This method successfully works in organic as well as aqueous solvent systems and for drugs with low and high solubility. It offers an attractive approach for solubility measurements at the early stages of the drug development process where limited quantities of drug are available or analytical assays are yet to be developed. Moreover, this approach does not require the compound under investigation to possess a specific functional group, e.g., a chromophore or a fluorophore for detection by an analytical assay.

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