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Thermochimica Acta 417 (2004) 239–243
thermochimica acta
www.elsevier.com/locate/tca

Ranking of polymorph stability for a pharmaceutical drug using the Noyes–Whitney titration template method

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Received 10 June 2003; received in revised form 16 July 2003; accepted 16 July 2003

Available online 8 February 2004

Abstract

A further refinement to the screening process of candidate selection in early drug development is the selection of a polymorphic form on the bases of solid state stability. The Noyes–Whitney titration template method has been used routinely by others to determine the intrinsic solubility of sparingly soluble materials. This method uses potentiometric measurements whilst titrating over a pH range to determine the pH-solubility profile of a drug substance. Using a novel modification to the conventional Noyes–Whitney titration template method, this paper describes an application for the determination of the relative stability between polymorphic forms of materials. Such an assessment can be deduced from the change in Gibbs energy that accompanies the physical changes in materials when going from a solid to a solution phase and will be shown to be derived from the intrinsic solubility measurements. In addition, it will be shown that solution calorimetry was used to good effect to help in the interpretation of the solubility results.

Three crystalline polymorphic modifications, a hydrate and two anhydrate forms, and an amorphous form of a pure drug substance currently in development in GSK were ranked in terms of physical stability. Stability measurements were made as a function of temperature and a phase diagram over a narrow temperature range was constructed.

Keywords: Polymorphism; Intrinsic solubility; Noyes–Whitney titration template; Solution calorimetry; Gibbs energy

1. Introduction

Pharmaceutical drugs are commonly crystalline materials and are therefore subject to polymorphism. Urgency to market dictates rapid drug development leading to an early selection of a polymorph to take into development. Where there are two or more forms to choose from, selection primarily is made on the basis of solid state stability, solubility as it affects rate, and developability. Such selection can be significantly aided if information about the solid state activity or surface energy can be learnt from physical chemical properties at the outset of the drug development process. Bio-availability, which is prerequisite to having an efficacious product, can be highly dependent on the polymorphic form selected. Bio-equivalence (i.e. equivalent time course and total deliverable dose) of two or more polymorphic forms may be deduced from knowing the change in Gibbs energy for the conversion of one form into another [1].

Where the change in Gibbs energy for the conversion of one form to another is small, bio-equivalence is likely. For example, the Gibbs energy change for the conversion of chloramphenicol palmitate form B to form A is $-3.24\,\mathrm{kJ\,mol^{-1}}$ [2]. In contrast, the change in Gibbs energy for the conversion of mefenamic acid form II to form I is $-1.05\,\mathrm{kJ\,mol^{-1}}$ [2]. In vivo studies of these drug substances show that the two forms of mefenamic acid are bio-equivalent, whereas the two forms of chloramphenicol palmitate are not [2].

In addition to bio-equivalence, the change in Gibbs energy for polymorphic conversion provides a means of measuring the relative solid state stability of each of the known polymorphic forms and hence the propensity for a given form to change. This can be determined conveniently by measuring the Gibbs energy change associated with the conversion of each form to a different common phase such as a solution phase.

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0040-6031/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.tca.2003.07.022

It is the aim of this paper to investigate the solubility of three crystalline polymorphs and an amorphous form of a drug substance currently in development within GlaxoSmithKline using a newly introduced technique and to report its ability to disclose the unique property of the several forms. From these measurements an appreciation of the bio-equivalence of the forms to aid form selection was envisaged. As will be detailed in this paper, such changes in Gibbs energy can be determined from solubility measurements using the Noyes–Whitney titration method [3]. The solid state stability and the potential bio-equivalence of three crystalline polymorphs, two anhydrates and one hydrate, and an amorphous form of a drug substance currently in development in GSK were determined.

2. Theoretical

The solubility of a material is a statement of equilibrium. Where the solid that is at equilibrium with the solution is a meta stable form there can not, strictly, be an equilibrium between the meta stable solid and the solution [4]. However, where the measurement of the intrinsic solubility is time dependent, the solubility of meta stable forms may be captured before there is conversion to more stable forms. Initial high solubility may be the consequence of crystal energy or possibly supersaturation, but in the Noyes–Whitney titration method supersaturation is minimised. From Eq. (1) it can be shown that the change in Gibbs energy associated with the dissolution of a solid is proportional to the equilibrium constant that equates the ratio of the solid in solution and solid not in solution;

$$\Delta G^{\theta} = -RT\ln(K) \tag{1}$$

where R is the gas constant and T is the temperature. The equilibrium constant, K, which is the ratio of the forward and reverse rate constant can be defined as;

$$K = \frac{a_{\text{solution}}}{a_{\text{solid}}} \tag{2}$$

where a_{solid} is the activity of a solid and is unity and a_{solution} is the activity of the solution. It is assumed that the activity of the solution is the same as the concentration of the solution in these dilute systems. Therefore the equilibrium constant in Eq. (1) has the same value as the intrinsic solubility. Measuring the intrinsic solubility allows a direct calculation of the change in Gibbs energy.

Intrinsic solubility measurements can be made as a function of temperature to create a phase diagram. Monotropic (where a single form is predominantly stable over the temperature range) and enantiotropic (where the relative stability of a polymorph changes at a given temperature) behaviour can be deduced. Note that monotropic and enantiotropic behaviour relates to the temperature range up to the melt temperature of the lowest melting form. In most cases this will be above 100 °C which is unobtainable using the Noyes–Whitney template method due to solvent evaporation.

3. Experimental method

Three crystalline forms of the study drug substance were identified by PXRD and polarised light microscopy and was shown to be >99% pure. Form I being the form of choice for development as a tableted product. The amorphous form was deduced to be amorphous by PXRD and polarised light microscopy and was assessed as being predominantly amorphous. The parent molecule is a weak base with pK_a of 8.10. Solubility data for the different forms were collected potentiometrically using the pSOL Model 3 instrument (pION Inc., Woburn, MA, USA) and analysed using the accompanying pSOL software version 1.4.

The Noyes–Whitney titration template is a potentiometric-derived method. For weak acid or base drugs it is relatively fast, based on sound theoretical grounding, and has a large dynamic range. The method requires an accurate pK_a value/s to be known, have drug in excess of solubility, and an estimated solubility. A titration curve is predicted which serves as a template to "teach" the instrument how to conduct the titration based on Noyes–Whitney dissolution, e.g. very slow titrant addition in the pH region of precipitation. Bjerrum Difference Plots [5] are constructed from the titrations, i.e. average number of protons bound versus pH. These provide approximate solubilities that are then refined via iterative least squares.

The principle of the method is to initially dissolve the solid drug in a buffer that is at a pH that will give "theoretically" infinite solubility. A basic drug is therefore dissolved in an acid buffer at pH 4 and an acid drug dissolved in a buffer at pH 14. An acid or basic solution is then titrated stepwise (in 0.1 pH steps) changing the pH from one extreme to another. As the pH of the solution approaches the pK_a of the drug, the drug will start to precipitate. From the accurately known pK_a, the number of protons bound and free can be calculated assuming complete solubility. The number of free protons at a given amount of titrant added is measured experimentally with a very high precision. Any difference between the calculated and measured protons bound is related to the solubility of the material studied. Each experiment takes about eight hours to complete and consists of three dissolution cycles to aid in the accurate determination of intrinsic solubility.

In this study it is disadvantageous to initially dissolve the solid, as the polymorphic form would be lost. A modification was thus made to the standard method in that the drug sample was not dissolved at the onset of the experiment. This was achieved by adjusting the start pH of the solution so that there was minimal solubility at the start of the study and, essentially, the procedure was to run the standard method in reverse. In the case of the drug studied, as the pH decreases, more and more of the solid dissolves. Close to

the pK_a, titrant is added in very small increments; 0.02 μl. When the solid is completely gone, additional titrant causes a marked change in pH. A total of 1.7 ml of a 0.15 M solution of KCl was initially added to the solid and the pH adjusted to pH 14. Sample masses in the region of 1.5 mg were used and the solubility determined at seven different temperatures, 15, 20, 22, 25, 27, 30 and 35 °C (±0.2 °C) using a jacketed cell and a circulating water bath. The system was run under an argon atmosphere to prevent dissolution of atmospheric carbon dioxide and stirred using a Teflon bar. The solution was initially stirred for 5 min. The pH of the solution was then stepped down in 0.1 pH increments to pH 4.0 with a minimum of 0.02 μl titrated. The instrument was calibrated using this new method by measuring the intrinsic solubility of atenolol (melting point 152 °C). The solubility values obtained were consistent with literature values of 13.5 mg ml^{-1} [6].

Heats of solution measurements were made using a 2225 Precision Solution Calorimeter together with a 2277 Thermal Activity Monitor (Thermometric, Jarfalla, Sweden). Approximately 80 mg of sample was dispersed into 100 ml 0.5 M KCl at pH 3.0 at 25 °C with a stir rate of 200 RPM. An amount of solid is used which is less than the saturation solubility of form III.

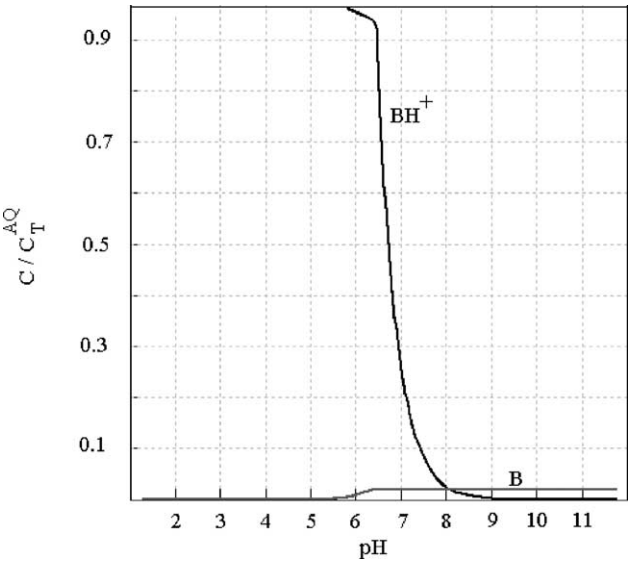

Fig. 2. Shows a pSOL output for the calculated solubility profile of Form I drug substance. The area under the grey line represents the solubility of non ionised drug molecules as a function of pH (B) and the area under the black line represents the solubility of the ionised molecules (BH^+). C/C_T represents the relative concentration of each species BH^+ and B in the aqueous phase as a ratio of the total concentration of drug molecules in solution, C_T.

4. Results/discussion

Intrinsic solubility measurements of the three crystalline forms indicate that the drug substance has an exothermic heat of solution, seen by a decline in solubility as temperature is increased. This was confirmed by a solution calorimetric study that shows an exothermic heat of solution, see Fig. 1. Thus the solubility of the solid will decrease as temperature is increased. Measurements of the intrinsic solubility were made using the modified method described earlier and the results are shown in Table 1. Fig. 2 shows a typical output from the pSOL software package. Replicates of solubility measurements were made for each form at each temperature. The errors associated with replicates were better than 10% of the mean. During the three consecutive cycles of the pSOL experiment it was found that the solubility of all forms tended towards that of form III. Solid was isolated during each cycle and analysed by polarised light microscopy, where it was shown that each form, except form III, precipitated as an amorphous solid. These findings were reported at the British Pharmaceutical Conference, 2001 [7].

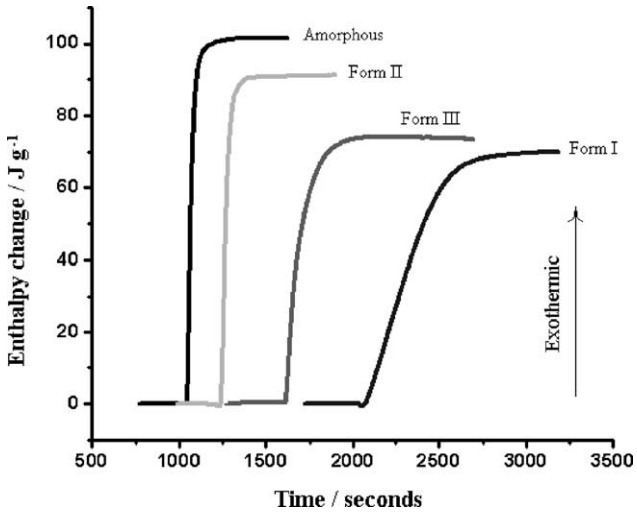

Fig. 1. shows the calorimetric trace of a solution calorimetric study for the dissolution of various forms of the drug substance in 0.5 M KCl at pH 3.0. The magnitude of the signal gives the enthalpy change for the dissolution and the *x*-axis gives the rate of dissolution.

Of the crystalline forms I to III, form III has the largest positive Gibbs energy change for dissolution over the temperature range 288–310 K and so is identified as the most stable polymorph, see Fig. 3. The change in Gibbs energy associated with the inter-conversion of form I (the least stable crystalline form) and form III (the most stable crystalline form) can be found from the difference between the change in Gibbs energy for the dissolution of the two forms. At 298 K, $\Delta\Delta G$ for the dissolution of form I and III is -3.98 kJ mol^{-1}. It is likely, therefore, at a pH close to the pK_a of the drug substance, form I will have a higher bio availability compared with form III. The amorphous form has the same intrinsic solubility profile and hence Gibbs energy change as that of form II. On further investigation, it was found that the amorphous form rapidly converts to a more stable form (Form II) when in contact with water. This was confirmed by PXRD analysis of the amorphous form after it had been slurried in water for 30 min at 298 K. It is

Table 1
shows the intrinsic solubility of the three crystalline forms and amorphous form of the drug substance

T (K)	Form I		Form II		Form III		Amorphous Form	
	(μg ml^{-1})	ΔG (kJ mol^{-1})	(μg ml^{-1})	ΔG (kJ mol^{-1})	(μg ml^{-1})	ΔG (kJ mol^{-1})	(μg ml^{-1})	ΔG (kJ mol^{-1})
288	80	21.6	48	22.8	15	25.6	50	22.7
293	50	23.1	30	24.4	10	27.0	30	24.4
295	35	24.2	20	25.6	8	27.9	20	25.6
298	25	25.2	12	27.1	5	29.2	12	27.1
300	20	26.1	10	27.8	3	30.8	10	27.8
303	12	27.5	8	28.5	2	32.0	8	28.5
308	8	29.1	4	30.9	2	32.7	4	30.9

The changes in Gibbs energy have been calculated from Eq. (1).

interesting to note that it converts to form II and not to the most stable form, form III. This observation is consistent with the Ostwald step rule that provides a rationale for amorphous materials crystallising to a meta stable crystalline form [8]. The solution calorimetric study of the amorphous form shows the enthalpy change for dissolution to be the largest and most negative, consistent with it being the least stable form. The large exothermic enthalpy change associated with the dissolution of the amorphous form largely reflects the enthalpy change of neutralisation of the base with the acid. The crystalline forms have a lower exothermic heat of solution (i.e. a larger endothermic heat of solution) reflecting the contribution from the crystal lattice energy.

During the solution calorimetric study the solid does not come into contact with the solvent prior to dispersion. Dissolution of the amorphous solid is very rapid at pH 3.0 and there is little chance to detect intermediates as the amorphous form dissolves. In contrast, the pSOL method requires the solid to be in contact with solvent for a considerable time at pH's distant from where precipitation can occur owing to the slow progression of the pH change. Amorphous drug substances in general will change to a more stable crystalline form under these conditions [9].

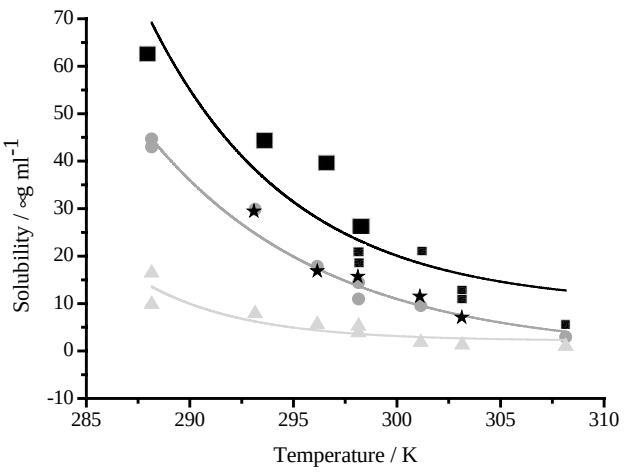

Fig. 3. The intrinsic solubility of the polymorphic forms of a drug substance, and an amorphous form, as a function of temperature. (■) Form I, (★) form II, (▲) form III, (●) amorphous form.

Table 2
A comparison of the enthalpies of dissolution determined by the van't Hoff isochore and measurements made by solution calorimetry

Polymorphic form	van't Hoff enthalpy change (kJ mol^{-1})	Heat of solution (kJ mol^{-1})
Amorphous	−101	−102.0
Form I	−82	−70.1
Form II	−104	−91.2
Form III	−115	−74.4

$N = 3$ for solution calorimetric measurements with an associated error of better than 2% of mean.

The results of the solution calorimetric study for form I was somewhat unexpected. A heat of solution for form I was expected between that for the amorphous form and form II to corroborate the pSOL observations. As the heat of solution was close to that of form III and the rate of dissolution significantly slower than any other form, we speculate that at pH 3, form I converts to form III during the dissolution process.

The van't Hoff enthalpy change for each polymorph was determined from the van't Hoff isochore, Eq. (3) [10] whilst making the familiar assumption that ΔH is independent of temperature over the temperature range studied [11].

$$\ln\left(\frac{K_{T_1}}{K_{T_2}}\right) = \frac{-\Delta H^\circ}{R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right) \quad (3)$$

where R is the gas constant. The calculated van't Hoff enthalpy change was compared with the enthalpy change measured by solution calorimetry, see Table 2.

The values of $\Delta_v H$ were found to be similar to but not exactly the same as $\Delta_{dis} H$. $\Delta_{dis} H$ is measured at pH 3.0. The pH at which intrinsic solubility is measured from which $\Delta_v H$ is determined is somewhat higher and close to pH 8. The discrepancy between $\Delta_{dis} H$ and $\Delta_v H$ may therefore be explained in terms of the ionisation of the dissolved molecule.

5. Conclusion

Results from the pSOL experiments show that the amorphous form is the least morphologically stable form, form I being the least stable crystalline form and form III is the

most stable form (up to 310 K). A $\Delta\Delta G$ of $-3.98\,\mathrm{kJ\,mol^{-1}}$ for the inter-conversion of form I to form III indicates these two forms are not bio-equivalent. The choice of form I to take into drug development was undoubtedly made on the bases of bio availability and not physical stability. Solubility studies show that form I will have greater bio availability than the other crystalline forms but will also have the greatest propensity for change. Solution calorimetry indicates that at pH 3 (conditions comparable to the stomach) form I probably converts to form III having a similar dissolution rate as form III. Careful formulation (with a strong case for enteric coating of the tablet) and rigorous stability testing ensures that the progression from the meta-stable to a stable form does not impact on product quality during the shelf life of the product. However the relative physical stability of forms is ignored at the peril of a successful drug product [12].

Measurements of intrinsic solubility are conveniently performed by the pSOL instrument and with considerable accuracy even for very sparingly soluble materials. Some puzzlement was initially caused when comparing the change in Gibbs energy of the amorphous form to that of form I (see Fig. 3). The pSOL data in isolation suggested that the amorphous solid has a greater physical stability than the crystalline form I, which could not be satisfactorily rationalised. The solution calorimetric study gave a crucial clue to the way the amorphous material physically changed during the initial stages of the pSOL experiment, giving rise to a pSOL result that is consistent with the solubility of form II. Comparative studies of the pSOL method to the traditional shake flask method have been made by others [13], where it was shown that the pSOL method gave significant advantages over traditional methods and is regarded by some as the “Gold Standard” for solubility analysis [13]. However, the setting up, running and data interpretation of the pSOL requires considerable appreciation and understanding. Results can be obtained by casual usage but meaningful results require careful thought and experimentation.

Acknowledgements

One of the authors, RW, is grateful to Professor Tony Beezer, Medway Sciences, University of Greenwich, for helpful discussions of this work.

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