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Solubility measurement of polymorphic compounds via the pH-metric titration technique

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Abstract

In drug development, the thermodynamically most stable form of a compound is preferred because metastable forms are prone to transform to the stable form during processing, formulation, or storage [Guillory, J.K., 1999. Generation of polymorphs, hydrates, solvates, and amorphous solids. In: Brittain, H.G. (Ed.), Polymorphism in Pharmaceutical Solids. Marcel Dekker, New York, pp. 183–226]. It is therefore important to discover and characterize the stable form as early as possible. One of the most important properties to determine is thermodynamic solubility. However, due to compound and time constraints this solubility value is usually not determined until late in discovery. This report explores the ability of the pH-metric titration method to measure intrinsic solubility of the stable form of compounds that exist in one or more polymorphic forms. One metastable form and the stable form of eight compounds were examined. Intrinsic solubility was measured via pH-metric titration. The technique was performed on a larger scale in order to monitor polymorphic form changes by powder X-ray diffraction. Shake-flask solubility and corresponding X-ray diffraction data of each form was also determined. The results of this study indicate that, in general, when starting with a metastable polymorph, the pH-metric titration method is able to achieve the solubility of the stable form by the third titration, while the traditional shake-flask solubility method is unable to consistently determine the stable form solubility.

Keywords: Solubility; Intrinsic; Polymorphs; Potentiometric; Titration

1. Introduction

In the pharmaceutical industry, the need for accurate solubility measurements of ionizable molecules is prevalent, extending from discovery through development. Early in discovery, solubility is typically measured using high throughput kinetic solubility measurements, which are designed to rapidly screen hundreds of compounds to determine if they have sufficient solubility for *in vitro* biological assays. However, these kinetic solubility values tend to be higher than thermodynamic solubility values since kinetic measurements are typically made from non-equilibrated solutions prepared from DMSO-solvated compounds (Lipinski, 2003). Later in discovery, precise thermodynamic solubility values are measured using crystalline material, since it is this solubility value that affects absorption, distribution, metabolism, and excretion (ADME) properties and formulation aspects of compounds (Glomme et al., 2005). Although crystalline material is used for this solubility measurement, it must be pointed out that at this stage the polymorphic form of this material is not typically identified as the stable form or a metastable form.

Determination of thermodynamic solubility is a much more rigorous exercise than determination of kinetic solubility. Thermodynamic solubility is generally determined by shaking solid compound in the solvent of interest over a period of 24 h or more (until equilibrium is achieved), filtering off the excess undissolved solid, and measuring the dissolved drug concentration in the filtrate. If the undissolved solid phase is the most stable form of the compound, the measured solubility value is the true equilibrium solubility of the compound in the solvent at the temperature of measurement. The most stable form of the compound will have the least solubility compared to the apparent solubility of any metastable or amorphous forms in which the compound might exist. However, it is not uncommon for the most stable form of the compound to appear later in development. Depending on the solubility of the compound and its dose, this can result in costly delays due to its impact on bioavailability and formulation (Morissette et al., 2003).

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Therefore, it is clear that once a compound with desirable activity has been identified in early discovery screens, it is important to determine the thermodynamic solubility of the stable form as early as possible. With the current paradigm of reduced costs and shortened timelines, solubility measurements that do not demand much compound or operator time are highly valuable. This report explores the utility of the pH-metric titration technique in this context. This technique has been previously described in detail (Avdeef, 1998). It is suitable for intrinsic solubility measurement and subsequent pH-solubility profile determination of ionizable compounds. To determine the thermodynamic solubility of a poorly soluble ionizable compound at a single pH, the best compound-sparing methods use at least 1 mg of compound, whereas, an entire pH-solubility profile may be determined with the same amount of compound using the pH-metric technique (Glomme et al., 2005). The theoretical basis for the pH-metric intrinsic solubility measurement is that any undissolved compound present in the titration mixture will shift the titration curve. The extent of this shift is a function of the amount of undissolved compound present in the titration mixture according to Eq. (1), where S_0 is the intrinsic solubility of the compound, ΔpK_a is the pK_a shift caused by the presence of undissolved compound in the titration mixture, and C is the total molar concentration of compound in the titration mixture.

$$-\log S_0 = \Delta pK_a - \log(C/2) \quad (1)$$

Good correlation between the intrinsic solubility derived from pH-metric titration and traditional shake flask solubility measurements has been reported, allowing acceptance of pH-metric titration data in regulatory submissions by the FDA (Avdeef et al., 2000). This report investigates the unique ability of the pH-metric titration system to measure the intrinsic solubility of the stable form of compounds that exhibit polymorphism, regardless of which polymorphic form is studied. Eight compounds that exist in one or more polymorphic forms were chosen for this study. One metastable form and the stable form of each compound were examined. Intrinsic solubility was measured for each form by cycling the compounds through three consecutive potentiometric titrations using the pH-metric titration technique. The technique was then simulated on a larger scale in order to collect enough precipitate to follow possible polymorphic form changes by powder X-ray diffraction analysis. In addition, shake-flask solubility and corresponding powder X-ray diffraction data of each polymorphic form was determined.

2. Experimental

2.1. Materials

Eight ionizable compounds were chosen for this study (Table 1). The selected compounds were known to exist in at least two polymorphic forms. Acetaminophen, Acetazolamide, Chlorpropamide, Sulfamethoxazole, and Sulfathiazole were obtained from Sigma (St. Louis, MO). Furosemide was obtained from ICN Biochemicals (Aurora, OH). Premafloxacin was obtained from Pfizer Inc. (Kalamazoo, MI). One proprietary compound, Pfizer Compound X, was also supplied by Pfizer Inc. (Ann Arbor, MI).

Table 1
Materials, required parameters for pH-metric titration, and polymorphic forms used

Compound	MW	pK_a	Compound type	Stable form	Metastable form	Solubility ratio (metastable/stable)	No. of known forms
Acetaminophen	151.16	9.42[a]	MA	I	II	1.3[e]	3[j]
Acetazolamide	222.25	7.2[b]	MA	A(II)	B(I)	1.1[f]	2[k]
Chlorpropamide	276.74	4.87[a]	MA	A	B	1.2[g]	3[l]
Furosemide	330.75	10.63, 3.52[c]	DA	A(I)	B(II)	1.0[f]	3[m]
Pfizer Compound X	416.48	2.66[a]	MA	A	B	3.0[h]	2[h]
Premafloxacin	403.45	6.31, 9.66[a]	MA, MB	III	I	23.1[f]	3[n]
Sulfamethoxazole	253.28	5.75[a]	MA	A(I)	B(II)	1.2[f]	4[o]
Sulfathiazole	255.31	7.14[d]	MA	III	I	1.7[i]	4[p]

[a] Determined by capillary electrophoresis.
[b] Parasrampuria (1993).
[c] Avdeef et al. (2000).
[d] Zhou et al. (2005).
[e] Sohn (1990).
[f] Pudipeddi and Serajuddin (2005).
[g] Burger (1975).
[h] Pfizer, unpublished data.
[i] Yu (1995).
[j] Burger (1982).
[k] Griesser et al. (1997).
[l] Simmons et al. (1973).
[m] Matsuda and Tatsumi (1990).
[n] Schinzer et al. (1997).
[o] Price et al. (2005).
[p] Anwar et al. (1989).

2.2. Methods

2.2.1. Physical characterization of polymorphs

2.2.1.1. Differential scanning calorimetry (DSC). The thermal behavior of the polymorphic forms of each compound was recorded with a TA Instruments Q1000 differential scanning calorimeter. The mass of each sample was approximately 1–3 mg and the heating rate was adjusted to 5 °C/min or 10 °C/min, as appropriate. Each sample was heated from ambient temperature to just past the melt in perforated, crimped aluminum pans while being purged with dry nitrogen.

2.2.1.2. Powder X-ray diffraction (PXRD). The powder X-ray diffraction patterns were acquired at ambient temperature with an AXS D8 Discover with GADDS (General Area Diffraction Detector System) diffractometer (Bruker, Madison, WI). This system uses Cu Kα radiation operating at 40 kV and 40 mA and a single Goebel mirror configuration. Data was collected over an angular range from 5° to 45° 2θ in continuous scan mode using a step size of 0.05° 2θ and a step time of 60 s. For the Acetazolamide samples only, the PXRD patterns were collected on a different X-ray diffractometer (Rigaku Ultima Plus, Tokyo, Japan). This system also uses Cu Kα radiation operating at 40 kV and 40 mA. Data was collected over an angular range from 3° to 50° 2θ in continuous scan mode using a step size of 0.02° 2θ and a step time of 0.6 s.

2.2.2. Preparation of polymorphs

2.2.2.1. Acetaminophen. Acetaminophen obtained from Sigma was confirmed to be form I by PXRD and DSC analyses (Nichols and Frampton, 1998). Acetaminophen form II was prepared by melting form I on several microscope slides and allowing the compound to recrystallize by slowly cooling to room temperature (Di Martino et al., 1997). Slow cooling was achieved by placing the slides in an oven heated to 100 °C, followed by shutting off the oven and allowing the samples to cool down to room temperature. The form II crystals were collected from the microscope slides and ground with a mortar and pestle. Polymorphic form was verified by PXRD and DSC analyses (Di Martino et al., 1997).

2.2.2.2. Acetazolamide. Acetazolamide obtained from Sigma was confirmed to be form A(II) by PXRD (Griesser et al., 1997) and DSC (Umeda et al., 1985) analyses. Acetazolamide form B(I) was prepared by recrystallizing approximately 9 g of form A(II) from 500 mL of refluxing water with slow cooling to room temperature (Griesser et al., 1997). Slow cooling was performed by slowly lowering the temperature on the hot plate until the solution reached room temperature. Form B(I) crystals were collected by vacuum filtration, dried in a vacuum oven at 60 °C for 2 h, and ground with a mortar and pestle. Polymorphic form was verified by PXRD and DSC analyses (Griesser et al., 1997).

2.2.2.3. Chlorpropamide. Chlorpropamide obtained from Sigma was confirmed to be form A by PXRD (Simmons et al., 1973) and DSC (Tudor et al., 1993) analyses. Chlorpropamide form B was prepared by dissolving 1.0 g of form A in 1.6 mL of hot benzene. The solution was removed from heat and all benzene was allowed to evaporate as the compound recrystallized to form B (Simmons et al., 1973). Polymorphic form was verified by PXRD (Simmons et al., 1973) and DSC (Tudor et al., 1993) analyses.

2.2.2.4. Furosemide. Furosemide obtained from ICN Biochemicals was confirmed to be form A(I) by PXRD (Abdallah et al., 1989) and DSC (Matsuda and Tatsumi, 1990) analyses. Furosemide form B(II) was prepared by adding excess form A(I) to *n*-butanol and then heating the solution to dissolve the compound. Any excess solid was removed by vacuum filtration. The solution was placed under nitrogen and the compound recrystallized as the *n*-butanol evaporated (Matsuda and Tatsumi, 1990). Form B(II) crystals were collected by vacuum filtration and allowed to dry at room temperature overnight. Polymorphic form was verified by PXRD (Abdallah et al., 1989) and DSC (Matsuda and Tatsumi, 1990) analyses.

2.2.2.5. Pfizer compound X. Compound X is a drug substance currently in development at Pfizer Global Research and Development. The two polymorphic modifications of this compound are labeled A and B.

2.2.2.6. Premafloxacin. Premafloxacin obtained from Pfizer Inc. was confirmed to be form III by PXRD and DSC analyses (Schinzer et al., 1997). Premafloxacin form I was prepared by dissolving 1.5 g of form III in methanol under reflux using a heat gun. Excess form I was removed by hot filtration (Schinzer et al., 1997). The filtrate was cooled in the freezer for 5 days. Form I crystals were collected by vacuum filtration and dried in the vacuum oven for 3 days. Polymorphic form was verified by PXRD and DSC analyses (Schinzer et al., 1997).

2.2.2.7. Sulfamethoxazole. Sulfamethoxazole obtained from Sigma was confirmed to be form A(I) by PXRD (Hartauer et al., 1992) and DSC (Price et al., 2005) analyses. Sulfamethoxazole form B(II) was prepared by dissolving 0.5 g of form A(I) in hot water, then cooling the solution in a metal beaker placed in acetone-dry ice. The solution froze and was allowed to thaw at room temperature (Yang and Guillory, 1972). Form B(II) crystals were collected by vacuum filtration. Polymorphic form was verified by DSC analysis (Luner et al., 2000).

2.2.2.8. Sulfathiazole. Sulfathiazole obtained from Sigma was confirmed to be form III by PXRD and DSC analyses (Anwar et al., 1989). Sulfathiazole form I was prepared by adding 1.6 g of form III to 50 mL of *n*-propanol, stirring the compound as the solution was heated to boiling, removing excess form III by vacuum filtration, and allowing the compound to recrystallize as the solution was cooled to room temperature (Khoshkhoo and Anwar, 1993). Form I crystals were collected by vacuum filtration and dried in the vacuum oven for 3 h. Polymorphic form was verified by PXRD and DSC analyses (Anwar et al., 1989).

2.2.3. Intrinsic solubility determination using the pH-metric titration technique

Solubility determination using the pSOL Model 3 system (*p*ION Inc., Woburn, MA) is based on a series of three automated potentiometric titrations (Avdeef et al., 2000). Titrations were conducted in a 25 mL test tube, placed in a jacketed circulating water bath maintained at a constant temperature of 25 °C. A magnetic stir bar was used for constant stirring of the solution throughout the titration. The titration mixture was blanketed by argon gas in order to minimize the effect of atmospheric carbon dioxide on the experiments. The *p*D-3 program (version 1.5) was used to perform the titrations. This program uses as input parameters the compound ionization type, molecular weight, pK_a (in pH range 2–10), and estimated solubility (or log P from which an estimated solubility is calculated), to set up the titration template. Depending on the intrinsic solubility of the compounds (S_0), which typically ranged from 5 μg/mL to 0.5 mg/mL, the compound requirement was 1–10 mg of compound. Accurate ionization constants (pK_as) were either obtained in-house by capillary electrophoresis or taken from reliable reports in the literature (Table 1).

At the start of the titration, the system predissolves the sample in either 0.5 M HCl or 0.5 M KOH, as appropriate. The titrator then adds an appropriate volume of 0.15 M KCl (also referred to as ionic strength adjusted, or ISA, water) to provide a background ionic strength, thereby diminishing the effect of changes in ionic strength during the titration. Compounds with an estimated S_0 greater than 500 μg/mL are not predissolved in acid or base since they immediately dissolve in the ISA water. The solution pH is then quickly changed to reprecipitate the compound (using acid or base) and the solution is stirred for 10–60 min (depending on the predicted S_0) to allow the precipitate to crystallize. Then the sample is titrated towards the pH of complete dissolution (i.e. weak acids are titrated from low to high pH and weak bases are titrated from high to low pH). A semi-micro combination pH electrode (*p*ION Inc.) is used for pH measurements. The instrument adjusts the data collection rate (based on the dissolution titration template constructed prior to the titration by the *p*D-3 program using the input parameters) to ensure complete equilibration at each pH, with very slow data collection near the point of complete dissolution (Avdeef et al., 2000). A typical titration usually takes 3–10 h, with poorly soluble compounds requiring greater assay time.

The principle behind the pH-metric intrinsic solubility measurement has been described (Avdeef, 1998). The *p*D-3 program calculates a mass of sample required for the titration (based on the experimental pK_a and an estimate of the intrinsic solubility of the compound) such as to ensure that in the pH range where the compound is unionized, an undissolved solid phase exists in the titration mixture. In the pK_a region, the presence of this undissolved material shifts the titration curve from one that would be obtained if all of the compound were in solution. The curve created by taking the difference between the two titration curves is called the Bjerrum difference curve (Avdeef, 1998). In the *p*S-3 program (version 1.5) following the titration, an approximate log S_0 value is determined from the Bjerrum curve. This log S_0 value must be refined using the *p*S-3 program's iterative least squares procedure to incorporate the exact weight of compound used, the titrant strengths, and electrode parameters. Understanding proper data refinement is key to accurate intrinsic solubility determinations using the pSOL-3 instrument. The goal of data refinement is to achieve the best goodness of fit (GOF $\leq$ 10) of the experimental titration data with the ideal titration curve in the presence of precipitate (Avdeef and Berger, 2001).

2.2.4. Monitoring polymorphic form change during pH-metric titration

Although the small amount of compound required for a typical pH-metric titration is usually considered an advantage of this method, in this study it was prohibitive in terms of allowing possible polymorphic form changes to be followed over the course of the titration. Therefore, experiments were run off-line to reproduce the pH changes of a typical pSOL intrinsic solubility determination on a larger scale so as to have sufficient solid for PXRD analysis. These experiments, it must be stated, were not exact replicates of the pH-metric experiments. The compound concentrations were approximately 10-fold greater and the temperature was not controlled. Approximately 20 mg of each polymorph was weighed into each of three screw-cap vials. Compound was dissolved with acid or base, as appropriate. Ionic strength adjusted water, 1–2 mL, was added. Compound was then reprecipitated (using acid or base depending on compound ionization type) and stirred to allow the precipitate to crystallize. At 30 min, one vial was removed from stirring, the precipitate was collected by vacuum filtration and then dried by placing the filter paper in a dessicator until analysis by PXRD. Sample was collected from the remaining two vials in the same manner at the 3- and 24-h time points.

2.2.5. Shake-flask solubility sample analysis

Thermodynamic solubility of the compounds was determined using an automated shake-flask (ASF) solubility determining system. Saturated solutions of each compound in 50 mM pH 6.5 phosphate buffer were prepared in screw-cap vials, vortexed, and affixed to a Glas-Col rotating bottle apparatus where the vials were rotated at 75% power for 24 h. The buffer was chosen because it is most commonly used for thermodynamic solubility determination in discovery. Undissolved material was separated by filtration and analyzed by PXRD. The concentration of dissolved compound in solution was measured by UV spectrophotometry and the final pH of the saturated solution was measured.

3. Results

3.1. Physical characterization of polymorphs

To ensure the identity and purity of each polymorph used in this study, the individual polymorphic forms were analyzed by X-ray crystallography and differential scanning calorimetry. The melting behavior observed for each polymorphic form agrees with the thermal data described in the literature for each polymorph. The powder X-ray diffraction patterns of each

polymorph correspond with the patterns found in the literature (see Section 2.2.2 for references).

3.2. Intrinsic solubility determination using the pH-metric titration technique

As described in Section 2.2.3, three intrinsic solubility values were determined from the three consecutive titrations performed on each polymorphic form over the course of each pH-metric titration experiment (Table 2). For five out of eight compounds, the intrinsic solubility values for each metastable form decreased with each successive titration (%RSD > 2.5%), indicating progressive conversion to a more stable form. In addition, for five out of eight compounds the intrinsic solubility values for each stable form remained constant throughout the experiment (%RSD < 2.5%), indicating that the polymorphic form had not changed. Furosemide, Compound X, Premafloxacin, and Sulfamethoxazole were the exceptions to these general trends and the behavior of these compounds will be addressed in Section 4.

3.3. Monitoring polymorphic form change during pH-metric titration

As detailed in Section 2.2.4, the pH-metric titration was simulated on a larger scale so that precipitate could be collected at various time points (30 min, 3 h, and 24 h) in order to follow polymorphic form change over the course of the titration. Powder X-ray diffraction analyses of the precipitate showed that for five out of eight compounds, the metastable form of each compound converted to the stable form within 30 min (Table 2). For example, the metastable form of Sulfathiazole, form I, converted to the stable form, form III, within 30 min as demonstrated in Fig. 1. In addition, for five out of eight compounds, the diffraction patterns of the stable forms did not change, demonstrating that the polymorphic form remained constant. For example, the

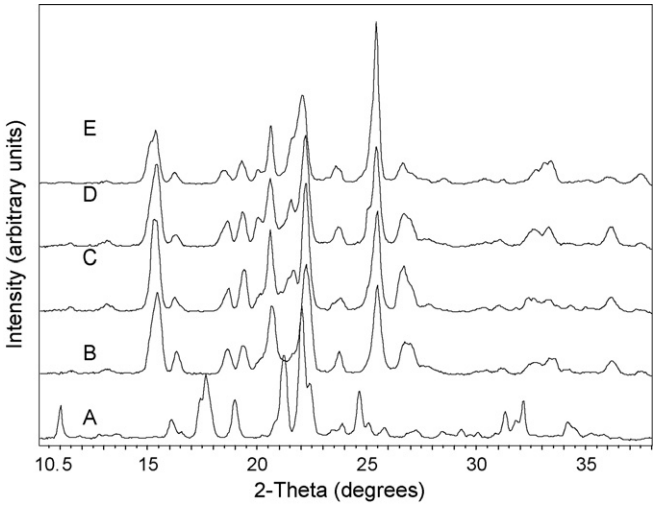

Fig. 1. Powder X-ray diffraction patterns of Sulfathiazole form I pH-metric titration scale-up samples: (A) form I initial, (B) form I at 30 min, (C) form I at 3 h, (D) form I at 24 h, and (E) form III.

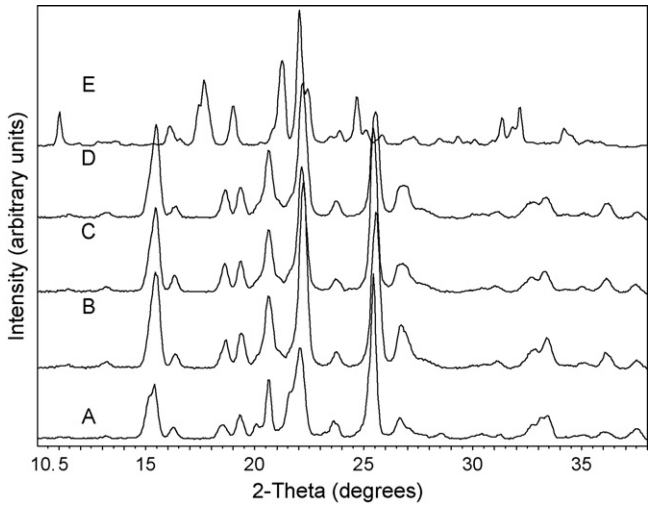

Fig. 2. Powder X-ray diffraction patterns of Sulfathiazole form III pH-metric titration scale-up samples: (A) form III initial, (B) form III at 30 min, (C) form III at 3 h, (D) form III at 24 h, and (E) form I.

stable form of Sulfathiazole, form III, did not change form over the course of the scale-up experiment, as demonstrated in Fig. 2. Compound X, Premafloxacin, and Sulfamethoxazole were the exceptions to these general trends and the behavior of these compounds will be addressed in Section 4.

While the results of this scale-up experiment are interesting and find an application as an approach to crystallizing stable forms of compounds, it must be recognized that the differences that were noted in the pattern and kinetics of form change between these simulated scale-up experiments and the actual pH-metric titrations arise from the fact that the scale-up experiments did not exactly imitate the pH-metric titration. Specifically, in the pH-metric titrations of the majority of the metastable forms, conversion to the stable form was not complete until the third titration (approximately 6 h after the start of the experiment), whereas, during the scale-up experiments, the majority of the metastable forms converted to the stable form within 30 min. There are several possible reasons for the faster conversion to the stable form during the scale-up experiment, namely the increased concentration of compound in the scaled-up solution in order to increase the percent recovered, the lack of temperature control, and the increased rate of pH changes employed. Despite the differences in the timeframe of conversion to the stable form, the scale-up experiment allowed collection of the precipitate for PXRD analyses and proved to be of value in generating the stable form quickly.

3.4. Shake-flask solubility sample analysis

As described in Section 2.2.5, thermodynamic solubility of the compounds was measured using an automated shake-flask (ASF) solubility determining system. Good agreement between shake flask solubility measurements and intrinsic solubility values determined using the pSOL system has been previously reported (Avdeef et al., 2000). Therefore, the main purpose of this experiment was to analyze the solid collected from the ASF solubility samples by PXRD in order to determine if the shake-

Table 2
Intrinsic solubility values and %RSD from pH-metric titration, polymorphic form changes observed during pH-metric titration scale-up and automated shake-flask solubility determination

Compound	Titration no.	$-\log S_0$ (stable)	S_0 (μg/mL) (stable)	$-\log S_0$ (metastable)	S_0 (μg/mL) (metastable)	%RSD of titrations 1–3 ($-\log S_0$) (stable)	%RSD of titrations 1–3 ($-\log S_0$) (metastable)	Form change during scale-up experiment (stable)	Form change during scale-up experiment (metastable)	Form change during ASF solubility experiment (stable)	Form change during ASF solubility experiment (metastable)
Acetaminophen	1	1.28	7900	1.06	13100	2.34	8.55	No form change	II_m to I_s within 30 min;	No form change	II_m to I_s
	2	1.25	8400	1.01	15000						
	3	1.31	7400	1.19	9800				No further change		
Acetazolamide	1	2.65	501.7	2.51	690.5	0.43	5.70	No form change	B_m to A_s within 30 min;	No form change	B_m to A_s
	2	2.65	499.3	2.77	376.2						
	3	2.67	477.3	2.78	367.6				No further change		
Chlorpropamide	1	2.92	333.2	2.92	331.1	2.36	3.49	No form change	B_m to A_s within 30 min;	No form change	B_m to A_s
	2	2.94	314.1	3.01	269.4						
	3	3.05	244.8	3.13	207.5				No further change		
Furosemide	1	4.76	5.7	4.89	5.4	0.65	1.87	No form change	B_m to A_s within 30 min;	No form change	No form change
	2	4.72	6.3	4.73	7.7						
	3	4.70	6.6	4.74	7.6				No further change		
Pfizer Compound X	1	5.22	2.5	6.13	0.307	5.98	0.81	A_s to B_m	No form change	No form change	No form change
	2	5.41	1.6	6.05	0.370			within 30 min;			
	3	5.86	0.582	6.04	0.377			No further change			
Premafloxacin	1	1.48	13400	1.48	13400	17.72	16.38	III_s to amorphous within 30 min;	I_m to amorphous within 30 min;	No form change	No form change
	2	1.82	6100	1.77	6900						
	3	2.12	3100	2.06	3500			No further change	No further change		
Sulfamethoxazole	1	2.67	536.9	2.90	316.5	3.28	0.20	A_s to hydrate within 3 h;	B_m to hydrate within 30 min;	A_s to hydrate	No form change
	2	2.78	416.5	2.90	318.9						
	3	2.85	362.0	2.91	311.9			No further change	No further change		
Sulfathiazole	1	2.89	331.6	2.67	552.3	0.20	4.13	No form change	I_m to III_s within 30 min;	No form change	I_m to III_s
	2	2.88	336.6	2.86	349.2						
	3	2.89	331.9	2.88	336.6				No further change		

s, stable form; m, metastable form.

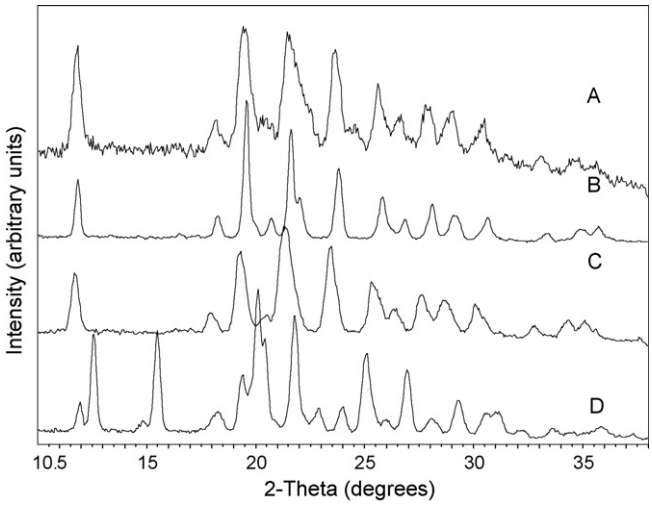

Fig. 3. Powder X-ray diffraction patterns of Chlorpropamide automated shake-flask (ASF) solubility samples: (A) form A ASF sample, (B) form A initial, (C) form B ASF sample, and (D) form B initial.

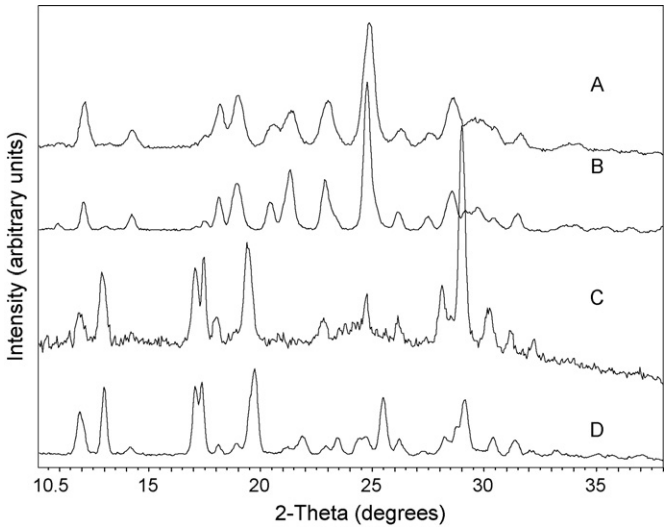

Fig. 4. Powder X-ray diffraction patterns of Furosemide automated shake-flask (ASF) solubility samples: (A) form A ASF sample, (B) form A initial, (C) form B ASF sample, and (D) form B initial.

flask method, like the pH-metric titration method, has the ability to determine the solubility of the stable form of the compound, irrespective of the polymorphic form of the starting material.

For the metastable forms of Acetaminophen, Acetazolamide, Chlorpropamide, and Sulfathiazole, PXRD analysis of the undissolved solid demonstrated that the metastable forms converted to the stable forms during the 24 h equilibration time. Therefore, the solubility of these metastable forms could not be determined. However the solubilities of the stable forms of these compounds could be measured using the ASF system, as confirmed by PXRD analysis. For example, the metastable form of Chlorpropamide, form B, converted to the stable form while the stable form, form A, did not change form during the 24 h equilibration, as demonstrated in Fig. 3.

For the metastable forms of Furosemide, Compound X, and Premafloxacin, PXRD analysis of the undissolved solid demonstrated that the metastable forms did not convert to the stable forms during the 24 h equilibration. Therefore, solubility values of both the stable and metastable forms of these compounds were measured using the ASF system. For example, both the stable and metastable forms of Furosemide did not change their respective polymorphic forms during the equilibration period, as demonstrated in Fig. 4. The metastable form of Sulfamethoxazole also did not convert to the stable form, so the solubility of the metastable form was determined. However, the stable form of Sulfamethoxazole converted to the hydrate, as evidenced by PXRD (Graf et al., 1982), so the solubility of the hydrate was determined using the ASF system.

3.5. Statistical analysis of data

In Section 3, we claim that in general, the pH-metric titration solubility values of the metastable forms of the compounds decrease over the course of the three titrations, while the solubility values of the stable forms of the compounds, in general, remain the same over the course of the three titrations.

First, to determine if the decrease noted with the metastable forms was significant, the Student's *t*-test was performed. It must be noted that Compound X, Premafloxacin, and Sulfamethoxazole were excluded from this test because of their exceptional behavior during the pH-metric titration that will be addressed in Section 4. For the purpose of this analysis, the sample data was normalized by using the ratio of metastable form solubility in titration one and stable form solubility and the ratio of metastable form solubility in titration three and stable form solubility. This was done to avoid scaling differences in solubilities among the compounds. Two hypothesis tests were run to evaluate this data. The tests sought answers to two questions:

(1) Is the ratio of metastable form solubility in titration 1 and stable form solubility equal to 1?
(2) Is the ratio of metastable form solubility in titration 3 and stable form solubility equal to 1?

For the first question, the result is p-value = 0.001 < 0.05 (level of significance), leading to the rejection of the null hypothesis (H_0). The actual mean ratio is not equal to 1. In other words, the solubility of metastable form in titration 1 is different than the solubility of stable form in titrations 1–3. For the second question, the result is p-value = 0.854 > 0.05 (level of significance), leading to the acceptance of the null hypothesis (H_0). The actual mean is equal to 1. In other words, the solubility of metastable form in titration 3 is the same as the solubility of stable form in titrations 1–3.

Secondly, to confirm that the solubility values of the stable forms of the compounds remain the same over the course of the three titrations, Dixon's test (Bolton, 1997) was performed. This test was used to identify any extreme solubility values within the pH-metric titration data of the stable forms of each compound. Using this test, none of the solubility values generated from any of the titrations of the stable forms were rejected as outliers at the 1% or even the 5% level of significance.

Lastly, the pSOL titration data for the stable and metastable forms of each compound was compared using the sum of least squares test. There is a much better correlation (sum of least squares = 0.074) between the values for the third titrations of both forms than there is between the first titration of the metastable form and the third titration of the stable form (sum of least squares = 0.670). This indicates that when studying a polymorphic material on the pSOL system, the solubility value determined by the third titration is likely to be the closest value to the solubility of the stable form.

4. Discussion

The results of this study show that the pH-metric titration technique for solubility measurement serves as a valuable tool to produce and measure the solubility of the stable form of ionizable polymorphic compounds using very small amounts of compound. When using the pH-metric titration method, if the intrinsic solubility was found to decrease over the course of the titration experiment (%RSD > 2.5%), it was predicted that the compound was converting from a metastable form to a more stable form. Conversely, when using the pH-metric titration method, if the intrinsic solubility was found to remain constant over the course of the titration experiment (%RSD < 2.5%), it was predicted that the compound was not changing form. For 10 out of the 16 polymorphs studied, this was found to be the case as seen from X-ray diffraction patterns from the scale-up experiment. Furosemide, Compound X, Premafloxacin, and Sulfamethoxazole were the exceptions to the general trends observed with the pH-metric titration data. However, the behavior of these compounds during the pH-metric titration may be explained using the results of the scale-up experiments.

The intrinsic solubility values of the metastable forms of Furosemide, Compound X, and Sulfamethoxazole remained constant over the course of the three titrations (%RSD < 2.5%). In the case of Furosemide, the metastable form converted to the stable form within 30 min during the scale-up experiment. Therefore, during the pH-metric titration, it is believed that the metastable form reprecipitated as the stable form prior to the start of each titration. In the case of Compound X, the metastable form did not convert to the stable form during the scale-up experiment. Based on this information, it appears that during the pH-metric titration, the metastable form reprecipitated prior to each titration. In the case of Sulfamethoxazole, the metastable form converted to the hydrate during the scale-up experiment. Therefore, during the pH-metric titration, it is believed that the metastable form reprecipitated as the hydrate prior to each titration.

The intrinsic solubility values of the stable forms of Compound X, Premafloxacin, and Sulfamethoxazole decreased over the course of the three titrations (%RSD > 2.5%). In the case of Compound X, the stable form converted to the metastable form during the scale-up experiment. Therefore, during the pH-metric titration, it appears that the stable form converted to the metastable form by the third titration since the solubility from the third titration of the stable form agrees with the metastable form solubility values. The reasons for this conversion from the stable form to the metastable form are not yet clear. In the case of Premafloxacin, the stable form converted to amorphous material during the scale-up experiment. Therefore, during the pH-metric titration, it is believed that the stable form converted to amorphous material prior to the first titration and then reprecipitated as the stable form by the third titration. In the case of Sulfamethoxazole, the stable form converted to the hydrate during the scale-up experiment. Therefore, during the pH-metric titration, it is believed that the stable form converted to the hydrate by the third titration. The conversion to the hydrate occurred more slowly during both the scale-up (within 3 h) and pH-metric titration of the stable form of Sulfamethoxazole. Similarly the conversion to the hydrate occurred more quickly during both the scale-up (within 30 min) and pH-metric titration of the metastable form of this compound.

5. Conclusion

In this study, the pH-metric titration technique has proven to be an excellent method for attaining the intrinsic solubility of the stable form of polymorphic compounds, regardless of the form of the starting material. Recently it has been shown that high solubility (at least 8 mM) is typically needed to ensure transformation of a metastable polymorph to the stable form (Miller et al., 2005). Whereas, this solubility target may or may not be attained using the traditional shake-flask method, by taking acidic compounds to very high pH and taking basic compounds to very low pH, this high solubility is undoubtedly achieved using the pSOL method. In this study, powder X-ray diffraction analysis of the solid collected from the ASF solubility samples demonstrated that four out of eight of the metastable forms converted to the least soluble form (stable form or hydrate) during the 24 h equilibration period, whereas, seven out of eight of the metastable forms (with the exception of Compound X) converted to the least soluble form over the course of the pH-metric titration experiment. One possible explanation is that during the pH cycling of the compounds over the course of the pH-metric titrations, microscopic regions of supersaturation lead to the formation of seeds of the stable forms of the compounds. In any case, these results demonstrate that when working with compounds that may exist in one or more polymorphic forms, the pSOL system has the ability to achieve the solubility of the least soluble form with greater confidence and using less time and sample than the traditional shake-flask method.

There are a few limitations to using the pSOL instrument. One limitation is the type of compound that may be analyzed. The pSOL method may only be used for compounds that are ionizable and are free forms, not salts. Another limitation is that although the method is automated and fast, the instrument lacks high-throughput, which may be needed in a discovery setting. Finally, in order to accurately interpret the pSOL titration data for compounds that have more than one polymorphic form, the method needs to be repeated on a larger scale to obtain supporting powder X-ray diffraction information. Characterizing physical form of the compound in real-time over the course of the pH-metric titration would vastly increase our understanding

of the exact behavior of polymorphic materials during the three dissolution–precipitation–titration cycles of the experiment.

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