Small Molecule Crystallography

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Abstract

The Thermo Scientific NanoDrop™ 1000 Spectrophotometer was used to measure the solubility of paracetamol as a function of temperature. The instrument was found to rapidly provide reliable spectrophotometric data using minimal volumes of material. Paracetamol was used as a model small molecule active pharmaceutical ingredient (API) for which solubility data is important in the evaluation and development of the crystallization process.

Introduction

Crystallization processes are the predominant method of purification and solid form generation for most small molecule active pharmaceutical ingredients. Establishing and optimizing a crystallization process requires knowledge of the solubility behavior to identify the preferred solvent system and the appropriate mode of crystallization. Solubility data is also required during preformulation evaluations of APIs to anticipate if a molecule may have dissolution rate or solubility-limited absorption.

Generally solubility measurements are performed by analysis of the dissolved concentration of the API in equilibrium with the crystalline phase of interest. Solvent composition, temperature, ionic strength and pH are parameters that commonly affect solubility of APIs. Upon equilibration, the liquid phase is isolated from the solids in a slurry by filtration or centrifugation. The clarified liquor is then analyzed for dissolved concentration by the most appropriate method. For organic compounds such as APIs, HPLC provides a suitable detection system for determination of concentration, yet is more complex than required for relatively pure materials. Therefore, where separations are not required prior to concentration determination, spectrophotometric methods provide greater ease of use and a shorter analysis time per measurement.

Conventional spectrophotometry requires accurate dilution and tedious use of cuvettes. The NanoDrop 1000 instrument was used here in place of a conventional spectrophotometer in the measurement of paracetamol solubility as a function of temperature. The instrument provides a high-speed, UV/vis spectral scan from a 1 µl drop of solution. Cuvettes are not required and the system automatically measures samples at two path lengths to give greater dynamic range than a fixed path length spectrophotometer.

Materials

Equipment:
- NanoDrop 1000 Spectrophotometer
- Mettler Toledo AX205 balance
- Pipette, Rainin Instrument, LLC
- Vortexer, VWR
- 5°C Incubator, VWR Scientific
- 20°C Incubator, Echotherm chilling incubator, Torrey Pines Scientific
- 40°C and 60°C incubators, Thelco Laboratory Ovens

Materials:
- Millex® GV filters, Durapore® PVDF Membrane, 0.22µm

Reagents:
- Paracetamol, Ultrapure, Spectrum Lot UM1027
- Water, HPLC grade, Burdick and Jackson

Methods - Dynamic Range

To correlate absorbance with solution concentration of paracetamol, three aqueous standard solutions were accurately prepared at a concentration of 1 mg/ml, using 10-15 mg samples of paracetamol per standard. Mass was measured using a micro balance, and solvent volumes were dispensed with a pipette. Water was used as the solvent to dilute each solution to the standard concentration. The solutions were mixed with a vortexer until complete dissolution was apparent. Each 1 mg/ml standard solution was further diluted to cover a range for standard curve data generation including: 0.250, 0.125, 0.064, and 0.031 mg/ml. Three 1µl drops from each diluted standard were separately placed into the NanoDrop 1000 instrument to collect absorbance data. The absorbance of each drop was analyzed three times. That is, a total of nine measurements were made per diluted standard concentration. While the NanoDrop 1000 measures the UV/vis spectrum, only the absorbance data at 244 nm were used for the correlation between absorbance and concentration.

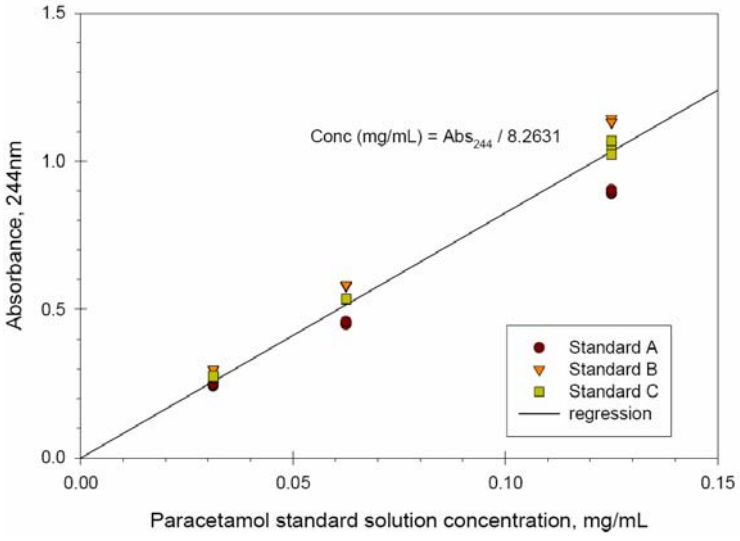

Figure 1: Standard curve for paracetamol in water for absorbance measured at 244 nm.

Solubility Experiments

Sixteen slurries of paracetamol in water were prepared, three each at 5°C, 20°C, 40°C, and 60°C. These slurries were prepared in 15 ml scintillation vials containing water and excess crystalline paracetamol solids (approximately 70 – 100 mg/ml of solids in water). All slurries were shaken periodically and equilibrated overnight in constant temperature incubators. Syringes and filters were stored in the appropriate incubator so as to minimize thermal gradients during sampling. During sampling, a small volume of slurry was withdrawn and rapidly passed through a filter, followed by rapid, accurate dilutions. For the slurries stored at 40°C and 60°C, nucleation occurred rapidly in the filtrate. To enable representative sampling, the filtrates were heated to 80°C for a short period to ensure nuclei had been dissolved. Sampling was performed with a pipette, followed by dilution with water. Diluted samples were analyzed for total concentration using the NanoDrop 1000 at 244 nm and the standard curve results. For each sample, the NanoDrop 1000 measurements were performed in triplicate. This provided nine measurements per temperature of interest and an ability to estimate errors of analysis related to the instrument as well as those related to sample preparation.

Discussion

The results indicate acceptable linearity of the standard curve over the typical absorbance range (Figure 1). More variability within the standard data was seen between separate standard solutions than between dilutions from the same standard. This indicates a level of precision of the instrument superior to that of the preparation of standard solutions. Likewise, this was seen in all but one case, in the standard deviation data of solubility measurements (Table 1). Greater variability was seen between mean values of measurements on separate samples than the variability seen for triplicate measurements on the same drop.

The solubility data of paracetamol in water (Figure 2) indicates a typical exponential dependence upon temperature. A straight line fits the data reasonably well on a log-linear plot. The extent of scatter of the concentration data at each temperature is low, and slightly greater at the highest temperature, yet still acceptable for the purpose of evaluating a cooling crystallization process option in a water solvent system. Typically, the 95% confidence interval about each concentration measurement taken from the same standard solution was in the range of 1 to 4%. This is an acceptable relative error for evaluating temperature dependence of solubility and for assessing crystallization processing options.

Conclusion

Solubility data for the paracetamol-water system was measured, indicating an exponential relationship with temperature over the range 5 – 60°C. The NanoDrop 1000 was found to be a reliable instrument for rapidly screening solubility of a small molecule API in support of crystallization process development studies.

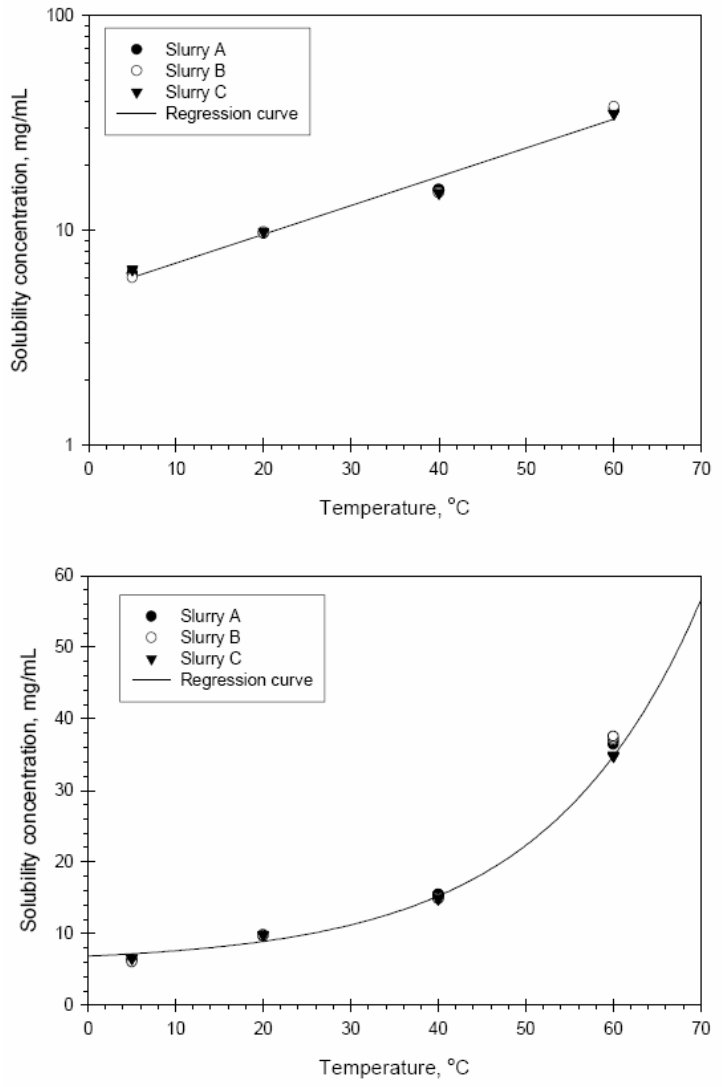

Figure 2: Paracetamol solubility in water with respect to temperature, log-linear and linear scales shown.

Table 1: Absorbance (244 nm) data for paracetamol filtrate samples after overnight equilibration. Standard deviations are given for measurements on same drop (triplicates) and for means from separate drops.

Temperature	Sample code	Dilution factor	Triplicate Nanodrop measurements (same drop)			std dev (same drop)	std dev (different drops)
			A_{244}	A_{244}	A_{244}		
5°C	P-5A	161.0	0.322	0.322	0.322	0.000	
	P-5B	161.0	0.311	0.312	0.308	0.002	
	P-5C	161.0	0.334	0.337	0.338	0.002	0.013
20°C	P-20A	161.0	0.497	0.496	0.497	0.001	
	P-20B	161.0	0.503	0.504	0.498	0.003	
	P-20C	161.0	0.502	0.560	0.502	0.033	0.013
40°C	P-40A	161.0	0.765	0.767	0.785	0.011	
	P-40B	161.0	0.792	0.793	0.790	0.002	
	P-40C	161.0	0.766	0.761	0.763	0.003	0.015
60°C	P-60A	322.0	0.935	0.946	0.953	0.009	
	P-60B	322.0	0.950	0.957	0.964	0.007	
	P-60C	322.0	0.894	0.896	0.891	0.003	0.034