SHORTER COMMUNICATION

AUTOMATED MEASUREMENT OF METASTABLE ZONES FOR PHARMACEUTICAL COMPOUNDS[†]

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It is important to know under what conditions crystals will grow but not nucleate when developing crystallization processes for the manufacture of pharmaceutical compounds. Owing to lengthy, personnel-intensive techniques, this data is often not available. In order to encourage the use of such data in the development programme an automated method of metastable zone determination is proposed. This method combines the use of a turbidity meter with an automated laboratory reactor. The validity of the automated method has been shown by reproducing a solubility curve for potassium nitrate, and in addition the effect of the turbidity meter sensitivity was studied. The method was then used successfully to measure the metastable zone for a pharmaceutical intermediate, thus providing useful information to aid the development of the crystallization process for that compound.

Keywords: metastable zone; solubility curve; turbidity; crystallization; pharmaceutical compounds.

INTRODUCTION

Crystallization is the most common method of formation of pharmaceutical solids. In most cases the goals of high purity and high yield can be met readily using cooling crystallization. Additionally cooling crystallizations can be controlled to give product of the correct physical form which filters and dries readily.

To this end, the relative rates of nucleation and crystal growth must be controlled (Wood, 1997). Specifically, the aim is for an initial period of nucleation to generate sufficient small crystals, followed by a period of growth in the absence of further nucleation. This requires knowledge of the conditions of concentration and temperature for nucleation and growth.

Solubility Curves and Metastable Zones

Crystal growth on pre-existing nuclei will occur once the solution is supersaturated, i.e. the concentration of the solute is higher than the solubility. A schematic solubility curve is shown in Figure 1. Crystal growth will occur in any region above the solid line.

Nucleation will only occur if the supersaturation is sufficient to overcome the unfavourable energy associated with the creation of new interfaces when the solid phase first forms. This supersaturation, sometimes referred to as the supersolubility, is shown by the dashed line in Figure 1. At all concentrations above this line, nucleation will occur.

At concentrations in between the dashed and solid lines, crystals will grow but fresh crystals will not nucleate. This is the 'metastable zone', which is the ideal region for crystal growth once the nuclei have formed. The width of the metastable zone affects the likelihood of unwanted nucleation, and the applicability of seeding (Wood, 1997).

Experimentally the solubility may be determined by heating up a suspension of solid and solvent until dissolution is complete. Hence the solubility curve is sometimes called the 'clear point' curve. The 'clear point' may be detected by eye or using optical probes (Wood, 1997; Riesen, 1999; Barrett and Glennon, 2002). There are several other methods of determining solubility data, which mostly rely on sampling a saturated solution at a known temperature and then analysing for the solute either gravimetrically or using spectroscopy (Wood, 1997; Fujiwara *et al.*, 2002).

The supersolubility can be determined by cooling a solution and detecting the first appearance of solid; hence this curve is sometimes called the 'cloud point' curve. This can be done visually, or using optical instruments to detect turbidity (Riesen, 1999; Barrett and Glennon, 2002; Fujiwara *et al.*, 2002) or using ultrasonics (Ulrich and Titiz-Sargut, 2002).

The solubility curve is a thermodynamic property of the solute–solvent system. In practice, at very fast heating rates it is possible to overshoot, but at slower rates the measured clear point should be constant irrespective of heating rate.

The supersolubility curve is not a thermodynamic quantity, and will vary with cooling rate. For comparative purposes it is important to measure cloud points at constant cooling rates. A more thorough approach is to determine the

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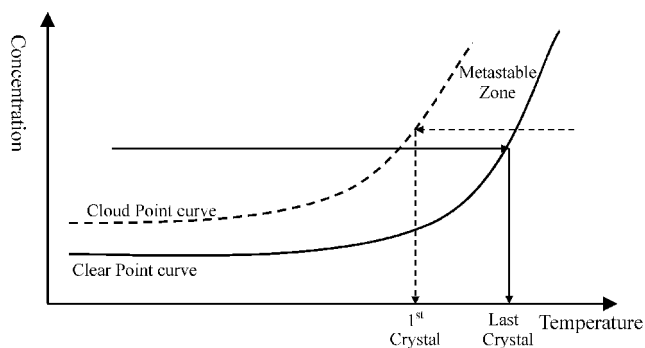[†]Copyright of this paper remains with AstraZeneca plc.

Figure 1. Diagram of solubility curve showing metastable zone.

cloud point at a range of cooling rates and extrapolate to infinitely slow cooling (Riesen, 1999; Barrett and Glennon, 2002).

Turbidity

The presence of solid particles, such as crystals, in a liquid changes its optical properties. Turbidity is a measure of the amount of particles in a liquid. The turbidity probe transmits near-infrared light into a liquid; the light is then scattered by any particles present and returned to the analyser, which provides a relative measure of the liquid turbidity (cloudiness). This technology can be used to detect crystal formation (cloud point determination).

EXPERIMENTAL

In order to measure metastable zones more easily an HEL (Hazard Evaluation Laboratory Ltd, Barnet, UK), the Auto-lab reactor was adapted for use with a Mettler Toledo FSC402 turbidity meter. The turbidity meter acted as an 'experimentalist's eyes' to note the cloud and clear points and hence allow the points to be generated automatically over a continuous 48 h period.

Figure 2 shows a schematic diagram of the Autolab. It was possible to set heating and cooling profiles controlled by the reactor or jacket temperature and to charge solvent to the reactor, allowing the concentration to be lowered. The turbidity meter output was recorded by the Autolab along with the reactor temperature and mass of solvent added.
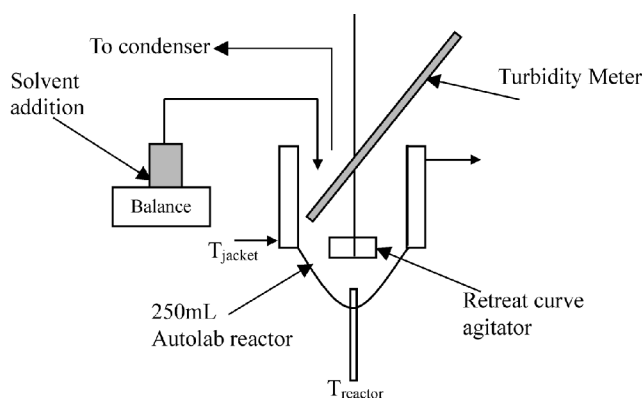

Figure 2. Schematic diagram of the Autolab reactor.

To measure the metastable zone automatically an Autolab programme was developed. This consisted of the following steps:

(1) start stirring the initial mixture at room temperature;
(2) heat to the required temperature (often the boiling point of the solvent) to bring about complete solution; a rate of 0.5°C/min was used during this work;
(3) cool to the temperature at which it is known the solute is out of solution; a rate of −0.25°C/min was used for this work;
(4) add the required amount of solvent to obtain the next, lower, concentration.

Steps 2–4 are then repeated as required; this is limited by the reactor volume.

The turbidity measurement was done using an FSC402 turbidity meter with the light source provided by an 880 nm infrared light emitting diode. The meter uses a backward scattering method of turbidity measurement and gives an output between −999 and +9999, but the Autolab only records values between 0 and 100.

The probe has two parameters that can be adjusted; the gain alters the probe sensitivity and the offset allows the probe to be zeroed. If the gain is too high then the probe is too sensitive to noise. If the gain is too low the probe will not respond to small increases in turbidity. The gain has to be optimized before commencing measurements, as discussed below in the Results section. In order to zero the probe, the reactor is filled with the solvent that is to be used, at room temperature. The probe offset is then adjusted to bring the probe output down to a value around zero. It is usually best to choose a value of around 10 to allow for decreases in reading due to temperature or other external factors such as the background light.

The initial concentration of solution is then charged to the reactor; the required solvent is supplied to the pump feed for the solvent additions and the programme is started. After 48 h the programme will have finished and the Autolab data can be exported to Excel (or Wincalc), where the data is analysed to obtain the clear and cloud points. A typical Autolab output after analysis in Excel is shown in Figure 3 (a turbidity of 100 indicates that the solution is cloudy and a turbidity of 7 indicates that it is clear). A decrease in turbidity from 100 to 7 indicates that the crystals have dissolved and the temperature at which this occurs gives the clear point at that concentration. In the same manner, an increase in turbidity from 7 to 100 indicates that crystals have come out of solution and gives the cloud point.

This method is versatile; the Autolab programme can be changed whilst it is running should extra steps be required. The range of concentrations that can be used depends on the size of the Autolab reactor. A 250 mL reactor was used for this work, which gave a minimum solvent volume of 150 ml (to ensure the probe tip was covered) and a maximum working volume of 300 ml. The Autolab used was fitted with a retreat curve agitator; optimization of agitator type and speed could help to ensure all solids were suspended.

As discussed previously, the temperatures at which the clear and cloud point are determined are a function of the rate of heating or cooling, respectively. In order to see the significance of this effect different heating and cooling rates were used to measure the clear and cloud point for
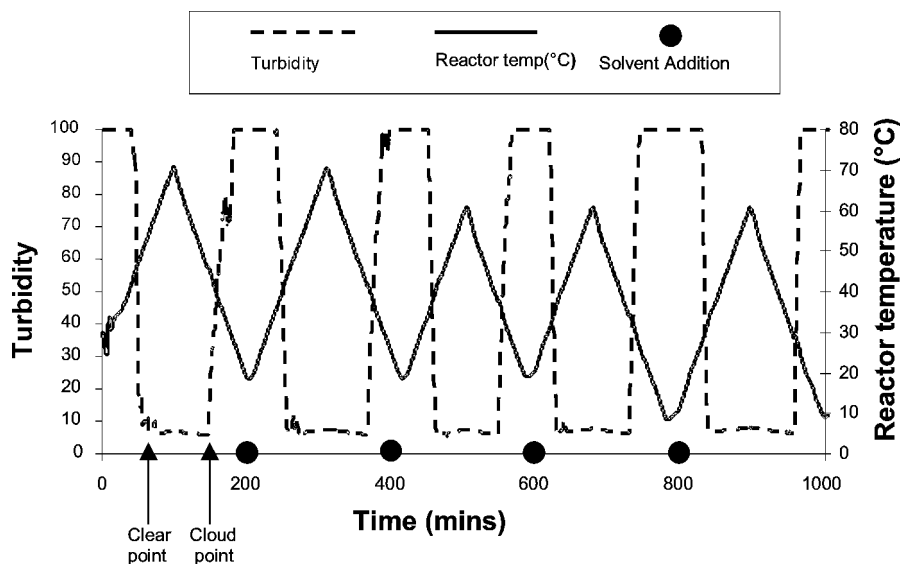
Figure 3. Autolab output: metastable zone for KNO_3 in H_2O. Meter gain $= 370.26$; offset $= 17.13$.

compound A (an active pharmaceutical ingredient, which is an organic molecule with molecular weight >300) at one concentration. The results are shown in Figure 4 where the clear and cloud point temperatures are plotted as a function of the heating or cooling rate used. It can be seen that the clear point is not significantly affected by the rate of heating (a variation of $4°C$ when varying the heating rate from 0.25 to $1.0°C/min$) but, as expected (see the Introduction), the cloud point varies by as much as $13°C$ when the cooling rate is varied from -0.5 to $-0.1°C/min$. A cooling rate of $-0.25°C/min$ was selected for this work based on practical considerations for the completion of the work.

Points to Note

- It was necessary to use an initial volume of 150 ml of solvent in order to cover the probe tip. This then dictated the use of around 20 g of the solid under consideration, a quantity that can be difficult to obtain during the development of pharmaceutical products.
- Some of the solid tended to re-crystallize on the reactor walls above the liquid level leading to inaccuracies in the

concentration. This problem was minimized by heating to reflux in order to wash solids off the walls.

RESULTS AND DISCUSSION

Validation of Turbidity Meter

In order to confirm the clear and cloud points predicted by the turbidity meter a literature comparison was conducted. Solubility data was measured for potassium nitrate (Figure 5) using the turbidity method described above with a meter gain of 740.52. The curves show good agreement with literature values (Perry and Green, 1999; Lange, 1961). It can be seen that potassium nitrate has a narrow metastable zone (about $3°C$).

The effect of the 'gain' (or sensitivity) of the probe on the clear and cloud points determined by the turbidity probe was investigated. Table 1 shows the measured clear and cloud points for one concentration (63.2 g $KNO_3/100$ g H_2O) of potassium nitrate at four different gains, ranging from 185 to 1480. Over the range of concentrations studied (48–80 g $KNO_3/100$ g H_2O), the gain was seen to have a significant effect on the temperature at which the clear and clouds points were determined; a range of up to $2.5°C$ was
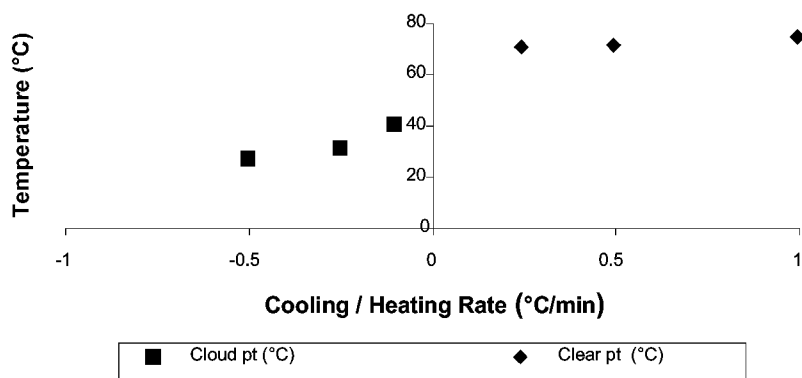

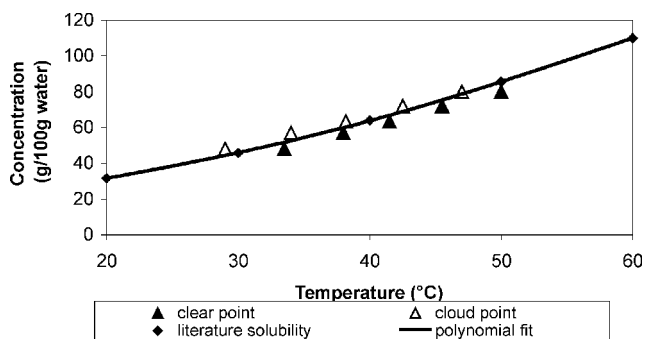*Figure 4.* Effect of heating/cooling rate on measured clear and cloud points of compound A in acetonitrile.

Figure 5. KNO$_3$ solubility curve—literature comparison (Perry and Green, 1999; Lange, 1961).

Figure 6. Metastable zone of X in acetonitrile.

observed at different gains. At the concentration chosen in Table 1, the gain had little effect on the clear point and an increase in gain increased the temperature at which the cloud point was detected. The lower the gain (less sensitive), the more crystals were required before the probe declared the cloud point and hence it was declared at a lower temperature. It is therefore important to consider what information is required from the probe and select an appropriate gain.

When determining metastable zones it is advisable to operate at a higher gain to obtain a temperature as close as possible to the true clear and cloud point. A value of 1000 has been used to determine the metastable zone of compound X.

The turbidity meter response was compared with an operator's judgement of when the cloud point occurred using compound A. The turbidity meter gain was 740.52. The measured cloud point was detected at 49.8°C and the operator judged the cloud point to be at 53°C. In this case the operator detected the appearance of a crystal 12 min before the turbidity meter picked it up. This importance of this discrepancy depends on the accuracy at which the data is required.

Generating Metastable Zone Data for Pharmaceutical Compounds

In order to find a solution to crystallization problems experienced during the processing of pharmaceutical intermediate X, the metastable zone was determined and the effect of methanol (a residual solvent suspected of causing poor yields) on the solubility was investigated. Compound X is an organic molecule with molecular weight >300. Prior to commencing this work the minimum solvent volume necessary to dissolve the material at reflux (81°C) was determined, by visual inspection with step-

wise solvent addition, to be 100 mg/mL, which was the composition employed at the start of the turbidity probe experiment.

Figure 6 shows the metastable zone data for X in acetonitrile. It can be seen that the product has a wide metastable zone width of about 40°C. The clear and cloud point measurements at 100 mg/mL concentration were repeated using the same heating and cooling rates. The repeated clear point shows good agreement (±1°C) while the repeated cloud point differs by 10°C and perhaps shows a better fit with the rest of the data. The difference in cloud point could be due to some undissolved solid on the vessel wall acting as a seed. The clear point data are in good agreement with the solubility determined previously by eye.

Having defined the metastable zone using the automated method (the whole process took less than 48 h with approximately 2 h of supervised work), it was possible to investigate the effect of methanol. A concentration of 100 mg/mL X in acetonitrile was used as the starting material. Four aliquots (0.5–3.5 g) of methanol were added and the clear and cloud points determined at each methanol concentration (Table 2). The points for 4.4% w/w of methanol are an average of at least three measurements and the repeatability was found from this to be ±11% for the clear point and ±6% for the cloud point.

Over the addition of 5.4 g methanol (0–4.4% w/w), the metastable zone width varies from 38.6 to 40.4°C. This indicates that methanol does not significantly affect the metastable zone or the solubility of X in acetonitrile at this composition. By increasing the proportion of methanol in the solution and finding that the metastable zone width was unaffected by this, the process chemistry team

Table 1. Effect of turbidity meter gain on KNO$_3$ metastable zone.

Turbidity meter gain	Clear point temperature (°C)	Cloud point temperature (°C)
185.13	41.4	36.8
370.26	41.2	37.3
740.52	41.5	38.2
1480	41.0	38.3

Table 2. Effect of methanol on the metastable zone of 100 mg/mL of X in acetonitrile.

Methanol added (% w/w)	Metastable zone width (°C)	Clear point (°C)	Cloud point (°C)
0	40.4	79.5	39.1
0.4	40.1	78.7	38.6
0.9	38.6	73.3	34.7
1.6	40.3	78.4	38.1
4.4	38.6	69.1	30.5

could discount the presence of methanol as a likely source of crystallization problems and focus their resources on other potential causes. It would be expected that a residual solvent that was affecting the crystallization process would have a noticeable effect on the width of the metastable zone and a significant effect on clear and cloud point temperatures.

The measured metastable zone width for compound X was in the range 38–40°C. This is similar to that for compound A, but much larger than the value measured here of 2–3°C for potassium nitrate. There is a lack of published data on metastable zone widths for large organic molecules, but values up to 26°C have been reported for potash alum (Ulrich and Titiz-Sargut, 2002).

CONCLUDING REMARKS

An automated method has been developed for measuring metastable zones by combining an Autolab reactor with a turbidity meter. Generating a solubility curve for potassium nitrate validated the turbidity meter data. The measured data showed good agreement with literature values, which increased confidence in its use. The effect of the turbidity meter gain was studied and it was found to be important to select the correct gain for each application.

An example of the automated method being used to measure metastable zone data for a pharmaceutical ingredient was presented. The method was then used to discount the presence of a residual solvent as the cause of poor crystallization experienced by the process chemistry team.

Using the turbidity meter in conjunction with an Autolab reactor enabled metastable zones to be measured in an automated fashion. This led to significantly reduced operator input compared with other methods, and should encourage the use of metastable zone data in future development of crystallization processes.

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