Electrothermal

Bibby Scientific

INTEGRITY

Application note: A02-002A

The Determination of the Metastable Zone Width - Case Study Lysozyme.

Introduction

Phase diagrams are fundamental to the design and development of optimized crystallization processes, ensuring the production of high quality crystals. The measurement of a sample solutions solubility and nucleation point can take a long time when using classical techniques. In the case of proteins this is a particular problem as the determination cannot be carried out simultaneously by one single method.

In this application note a rapid, reliable and reproducible determination method to determine the metastable zone width of lysozyme in sodium acetate buffer solution is presented. The method uses an STEM Integrity 10 reaction station, equipped with infrared transmission detectors to allow the saturation and crystallisation points to be determined using the turbidity measurement technique.

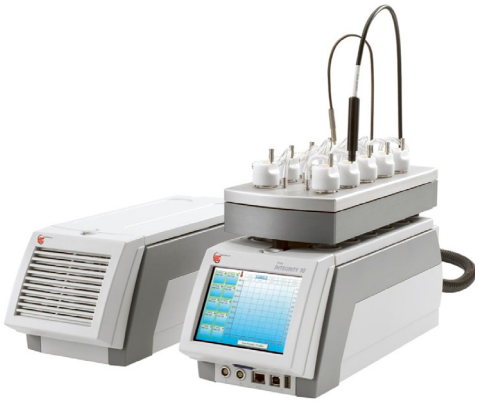

Figure 1. Electrothermal's Integrity 10 STEM Block (Clarity Solubility Station)

Experimental Methods

Hen egg white lysozyme was purchased from Fluka (CAS-No 9001-63-2) and dissolved in 0.1 M sodium acetate buffer pH 5.0 and 4%(wt) sodium chloride (NaCl) to give solutions with concentrations ranging from 10mg/ml to 40mg/ml.

The solutions were heated and cooled in the STEM Integrity 10 reaction station in a controlled manner at 0.1°C/min in order to determine the nucleation and solubility points. Turbidity measurements were collected using the optional in-situ IR probes (Part code - ATS10230).

Results

The solubility point was defined as the point at which the %transmission reached a stable plateau and the nucleation point was defined as the first point at which a sustained drop in %transmission was measured, as shown in Figure 2.

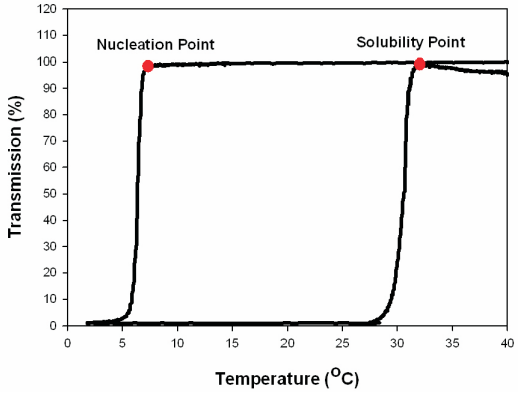

Figure 2. Turbidity change of lysozyme solution with temperature (15mg/ml).

The nucleation and solubility points were determined for a number of solution concentrations as shown in Figure 3.

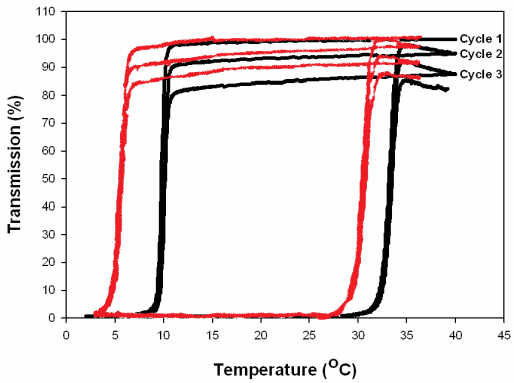

Figure 3. Turbidity change of lysozyme solution with temperature; 12mg/ml & 20mg/ml.

info@electrothermal.com
www.electrothermal.com
Tel: +44 (0) 1702 303350

The nucleation and solubility points, as determined from the turbidity measurements, were plotted along with literature data for the solubility of lysozyme in the pseudo-phase diagram shown in Figure 4.

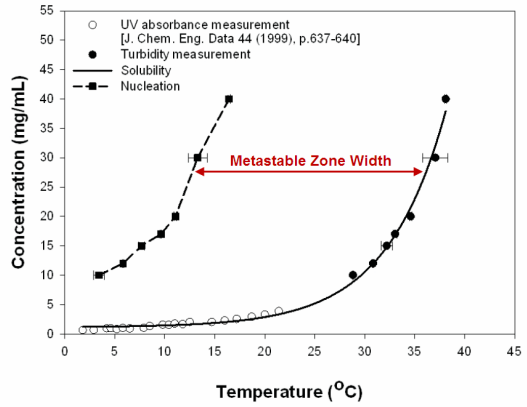

Figure 4. Pseudo-phase diagram of lysozyme protein (4% NaCl, 0.1M Sodium acetate buffer pH 5.0).

The construction of a diagram of this type allowed the metastable zone to be easily identified.

Conclusion

The nucleation and solubility points for systems with different protein concentrations were determined by the use of a turbidity measurement technique. The method is characterized by good reproducibility and reliability. The results from this investigation were coupled with data reported in literature to extend the known phase diagram and the data obtained in this study showed a good correlation. The use of this technique allows the metastable zone width to be determined within a few hours and with higher precision when compared to other classical methods.

Acknowledgements

The contents of this application note are taken directly from a poster presentation of the same name given by S. Maosoongnern[1], D. Pertig[2], V. Diaz Borbon[2], J. Ulrich[2].

[1]School of Chemical Engineering, Suranaree University of Techology, Nakhon Ratchasima 30000, Thailand

[2]Martin-Luther-Universitität Halle-Wittenberg, Zentrum für Ingenieurwissenschaften, Verfahrenstechnik / TVT, D-06099 Halle (Saale), Germany