INTEGRITY Application note: A02-003A

A Comparison of the Integrity (Clarity Solubility Station) IR Measurement Probes.

Introduction

Phase diagrams are fundamental to the design and development of optimized crystallisation processes, ensuring the production of high quality crystals. The measurement of a sample solution's 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 STEM Integrity 10 reaction station, equipped with three different infrared transmission detectors, will use the turbidity measurement technique to determine the nucleation and solubility points of solution of lysozyme in a sodium acetate buffer, with a comparison being made on the % transmission (%T) data recorded by each probe.

This application note will also compare the current small in-situ IR probe and a new large in-situ probe when used to determine the nucleation and solubility points of a viscous solution.

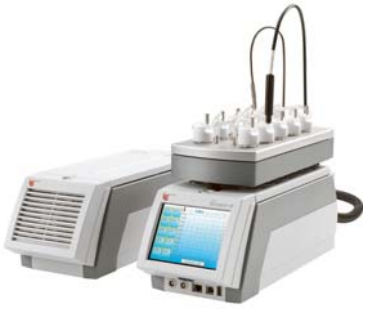

Figure 1. Electrothermal's Integrity 10 STEM Block (Clarity Solubility Station)

Experimental Methods

Nucleation and Solubility Point Determination - IR Probe Comparison

A sample of lysozyme was dissolved in 0.1 M sodium acetate buffer pH 5.0 and 4%(wt) sodium chloride (NaCl) to give a solution with a concentration of 20mg/ml.

The solution was heated and cooled in the STEM Integrity 10 reaction station in a controlled manner in order to determine the nucleation and solubility points. Turbidity measurements were collected using the optional non-intrusive IR probe (Part code - ATS10360), the optional small in-situ IR probe (Part code - ATS10230) and the optional large in-situ IR probe (Part code - ATS10395)

Figure 2. Image of an In-situ IR probe and a diagram of the probe's measurement principle

The Determination of the Nucleation and Solubility Points of a Viscous Solution

A 70% (w/w) sucrose solution was prepared to give a sample with a viscosity of approximately 480cP[(1)].

The solution was heated and cooled in the STEM Integrity 10 reaction station in a controlled manner in order to determine the nucleation and solubility points. Turbidity measurements were collected using the optional small in-situ IR probe (Part code - ATS10230) and the optional large in-situ IR probe (Part code - ATS10395).

Results

Nucleation and Solubility Point Determination - IR Probe Comparison

The solubility point was defined as the point at which the %T reached a stable plateau and the nucleation point was defined as the first point at which a sustained drop in %T was measured. The %T data recorded with each IR probe is shown in Figure 3.

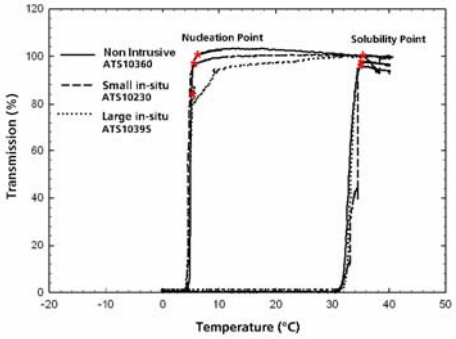

Figure 3. Turbidity change of lysozyme solution with temperature (20mg/ml).

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IR Probe	Nucleation Point (°C)	Solubility Point (°C)
Non Intrusive ATS10360	5.9	35.2
Small in-situ ATS10230	5.6	35.0
Large in-situ ATS10395	5.4	35.1
Std Deviation	0.25	0.10

Table 2. Determined nucleation and solubility points with different IR probes.

The experimental results show no significant differences when the nucleation and solubility points of the three different probes are compared. The standard deviation of the nucleation point is 0.25°C and the standard deviation of the solubility point is 0.1°C. As solubility is defined by thermodynamic principles, only a small variation in the observed results would be expected, but as the nucleation point is affected by experimental conditions such as the stirring rate, cooling rate, measuring probes etc. a greater variation in results would be expected.

The Determination of the Nucleation and Solubility Point of a Viscous Solution

An assessment of the nucleation and solubility points of a 70% sucrose solution was made and the %T values from each probe are shown in Figure 4.

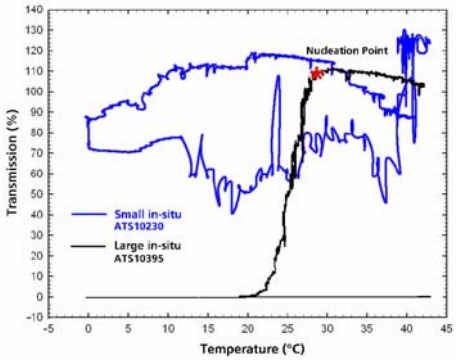

Figure 4. Turbidity change of lysozyme solution with temperature (20mg/ml); ATS10230 & ATS10395.

IR Probe	Nucleation Point (°C)	Solubility Point (°C)
Small in-situ ATS10230	Not Detected	Not Detected
Large in-situ ATS10395	28.2	Not Detected

Table 3. Determined nucleation and solubility points with different in-situ IR probes.

The %T data from the two in-situ IR probes shows that the small intrusive IR probe is unable to detect the nucleation or solubility point of the 70% sucrose solution while the large intrusive IR probe is only able to detect the nucleation point.

Conclusion

In the low viscosity lysozyme sample no significant difference was observed in the nucleation and solubility points that were determined with the IR probes tested during this study.

In the high viscosity sucrose sample the solubility point could not be determined with either of the IR probes tested. This is most likely due to inefficient mixing of the sample which meant that the sucrose crystals were not efficiently cleared from the light path of the probe. The nucleation point of the sucrose sample was only detectable with the large in-situ probe. The most likely explanation for this is again related to the inefficient mixing of the sample and the comparatively small opening to the light path found on the small in-situ probe. Further investigation work is required where more vigorous stirring mechanisms are employed to ensure the sample solutions are mixed effectively.

Acknowledgements

The content of this application note is adapted, with permission, from a report entitled **Electrothermal Integrity 10 - "IR – Probes"** written by Prof. Dr. J. Ulrich*.

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References

1. Neil L. Pennington, Charles W. Baker; Sugar, a user's guide to sucrose, (Chapman and Hall, London, 1990), p. 52.