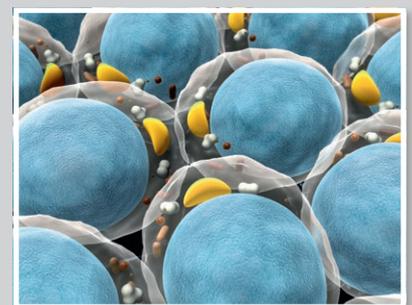
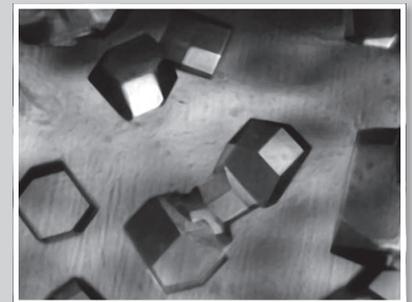
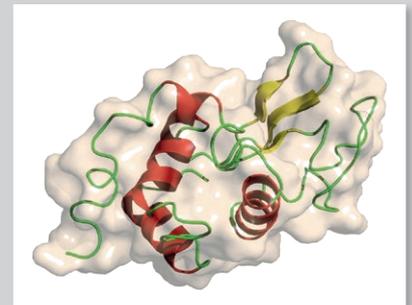


# Stability of protein formulations and their importance

Proteins are essential for life. They are, among other, responsible for structure and functioning of living cells, they are elements of growth and repair, and they help us fight infections. We find them in our food, (biological) drugs, cosmetics and others.

In solution, proteins are prone to associate. This can lead to protein crystallization, to the formation of amorphous aggregates, or to the liquid-liquid phase separation where the two liquid phases differ in the concentration of the protein. Instability of protein solutions can be both desired (e.g. protein isolation, crystallization for structure determination) and undesired (e.g. causing diseases such as cataract of the eye lens or Alzheimer's disease; short shelf-life of biological drugs, etc).

Phase transition in protein formulations may appear due to many factors: type of the protein and its concentration, pH and temperature of the solution, type and amount of additives (e.g. salts, sugars). The **Crystalline** instrument offers the possibility to quickly obtain data on the stability of protein formulations by making use of the real time digital camera. This information is vital in many industrial areas.



## Case study: Stability of lysozyme formulations

Lysozyme is a widely studied protein. Its aqueous solutions exhibit complex phase behaviour dependent on the solution's composition (i.e. protein concentration, type of buffer, additives) and external conditions such as temperature, pressure. In this study, the **Crystalline** instrument was used to monitor the phase changes upon cooling and heating aqueous-phosphate buffer lysozyme solutions in presence of different additives such as sodium chloride (NaCl), sodium bromide (NaBr), sodium nitrate (NaNO<sub>3</sub>), maltose, trehalose and PEG 10 000 (polyethylene glycol). Cloud temperature point,  $T_{cloud}$ , was determined upon cooling the solution. Usually the solution becomes opaque at  $T_{cloud}$ . On the other hand, the clear temperature point,  $T_{clear}$ , was determined by heating the sample. The chosen heating/cooling rate was 0.1 °C/min. By making use of the real time digital camera and transmissivity of the **Crystalline** instrument, the clear and cloud temperature points were accurately and easily determined.

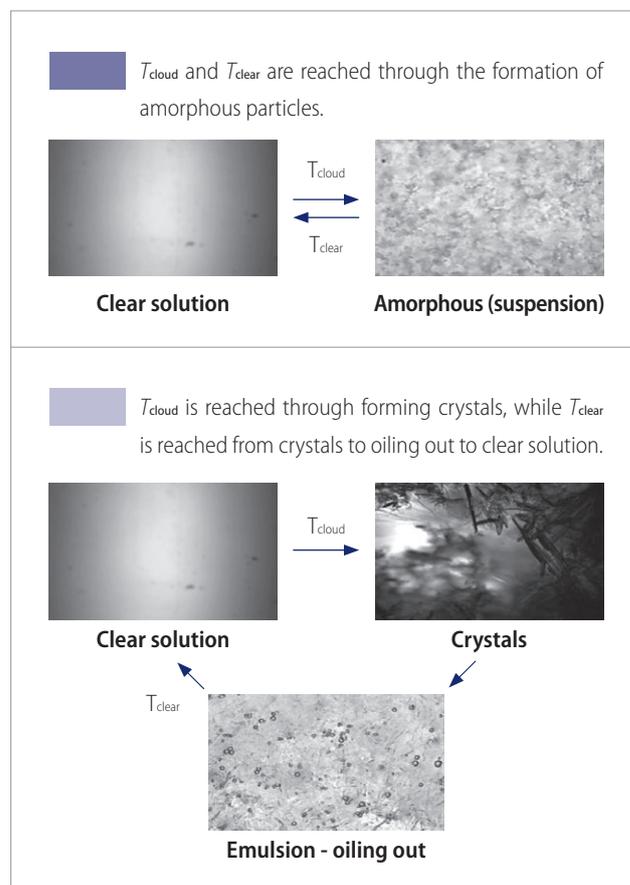


	NaCl	NaBr	NaNO <sub>3</sub>
<b>Salt</b>			
<b>+ TREHALOSE</b>			
<b>+ MALTOSE</b>			
<b>+ PEG 10 000</b>		*	*

amorphous particles
  crystals

\*  $T_{clear}$  was partly achieved. After experiment was finished, solution was not totally transparent (small particles inside it).

**Table 1:** Phase changes observed when using salts (NaCl, NaBr and NaNO<sub>3</sub>) and salts in combination with trehalose, maltose and PEG 10 000.



**Figure 1:** Types of phase transitions observed during heating/cooling cycles.

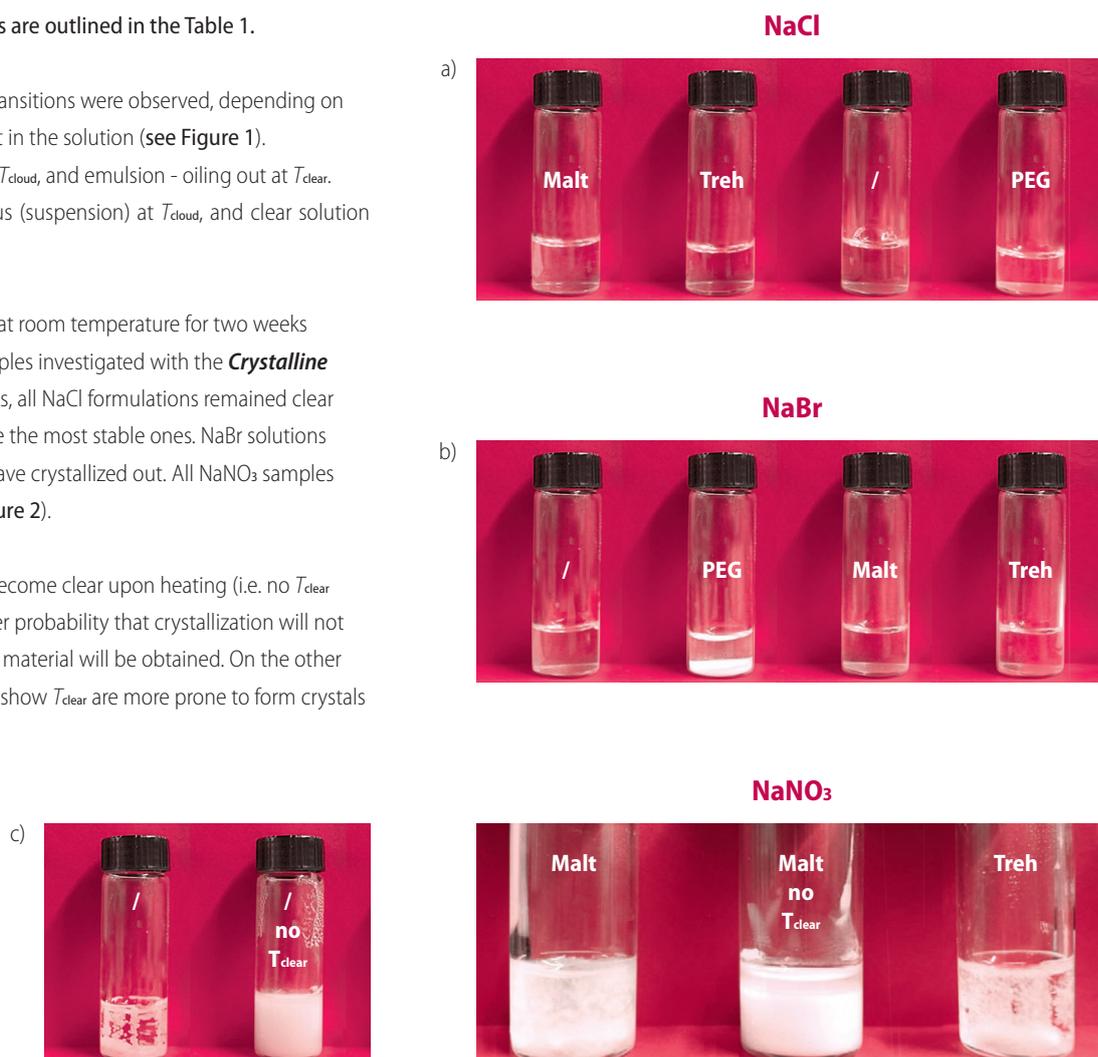
## Results and conclusions

- $T_{\text{cloud}}$  and  $T_{\text{clear}}$  decrease when salts are added to lysozyme solutions. Lower values of  $T_{\text{cloud}}$  and  $T_{\text{clear}}$  indicate better stability of the protein formulations. NaCl showed the best results followed by NaBr and  $\text{NaNO}_3$ .
- Addition of sugars (maltose and trehalose) decreases the  $T_{\text{cloud}}$  and  $T_{\text{clear}}$  (i.e. stabilizes the formulation) while an addition of PEG 10 000 (polyethylene glycol) increases the  $T_{\text{cloud}}$  and  $T_{\text{clear}}$  (i.e. destabilizes the formulation).
- Addition of sugars and salts to a lysozyme solution showed the best results for NaCl followed by NaBr and  $\text{NaNO}_3$ . Maltose and trehalose showed the same behaviour.

The observed phase changes are outlined in the Table 1.

- Two main types of phase transitions were observed, depending on the type of additive present in the solution (see Figure 1).
  - (1) Formation of crystals at  $T_{\text{cloud}}$ , and emulsion - oiling out at  $T_{\text{clear}}$ .
  - (2) Formation of amorphous (suspension) at  $T_{\text{cloud}}$ , and clear solution at  $T_{\text{clear}}$ .
- Additional stability studies at room temperature for two weeks were performed on all samples investigated with the **Crystalline** instrument. After two weeks, all NaCl formulations remained clear solutions and seemed to be the most stable ones. NaBr solutions in combination with PEG have crystallized out. All  $\text{NaNO}_3$  samples contained crystals (see Figure 2).
- Formulations that do not become clear upon heating (i.e. no  $T_{\text{clear}}$  is detected) there is a higher probability that crystallization will not occur and only amorphous material will be obtained. On the other hand, formulations that do show  $T_{\text{clear}}$  are more prone to form crystals (see Figure 2c).

*Technobis Crystallization Systems is highly grateful to MSc Tadeja Janc, Prof. dr. Vojko Vlachy and dr. Miha Lukšič (Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia) for providing the information for this application note.*



**Figure 2:** Two weeks stability studies at room temperature

## Crystalline

### Operating parameters

#### Crystalline SE

- 8 Reactors with a working volume of 2.5 - 5 mL
- 8 Independently controlled temperature zones
- Temperature range -25°C to 145°C in 4 reactors\*
- Temperature range -20°C to 145°C in 8 reactors\*
- Linear and non-linear temperature profiles supported
- 0° - 20°C/min heating/cooling rate
- Temperature control accuracy: 0.1°C
- Variable overhead stirring in each reactor
- Variable magnetic stirring in each reactor
- Stirrer speed: 0 - 1250 rpm
- Turbidity measurement in each reactor
- Reflux, slow evaporation, anti-solvent and seeding capabilities

\*When ambient temperature is 21°C ± 2°C and chiller cooling capacity at 18°C is about 1180 watt.

#### Crystalline PV

- **Crystalline SE**
- 4 or 8 parallel visualization probes
- Sample illumination: front and back pulsed lighting
- Automatic light intensity control
- Each camera is individually controlled and programmable
- Image analysis software: particle size distribution and shape

#### Crystalline RR

- **Crystalline SE**
- 4 parallel real time Raman probes
- Correlation of Raman spectra with temperature profiles and turbidity signals
- Software interface for spectroscopic analysis
- Compatible with any type of Kaiser RAMAN Spectrometer



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