Protein crystals as formulations for protein delivery

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Introduction

- Protein delivery has been attempted in several ways (eg. nanoparticles, chemical conjugation, freeze-drying) without achieving complete success.[1,2]
- Protein crystals as a form for protein delivery offer an increased stability to the protein, less susceptibility to degradation and elimination of aggregation issues.[3]
- Traditional crystallisation methods do not aim to produce crystals suitable for delivery (they aim to produce very few crystals of large dimensions) and are very hard to scale-up.

Aims

To produce a crystal population under:

- conditions suitable for direct formulation administration (i.e. using GRASS materials)
- to apply quick solvent evaporation method, allowing the production of a great number of small crystals in an easy to scale-up method.

Results

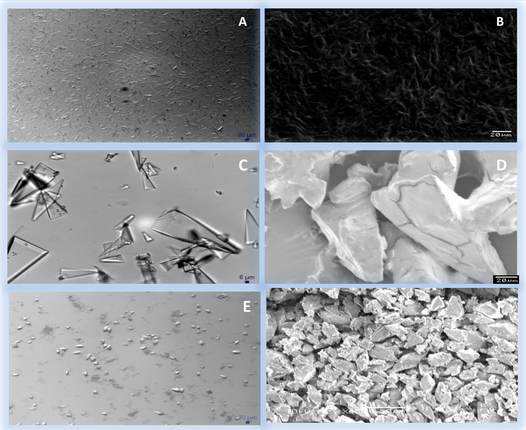

Figure 1. Crystallisation of chymotrypsinogen (A and B), pepsin (C and D) and thaumatin (E and F) using the rotary solvent evaporation method.
A, C and E – Light microscopy; **B, D and F** – Scanning Electronic Microscopy.

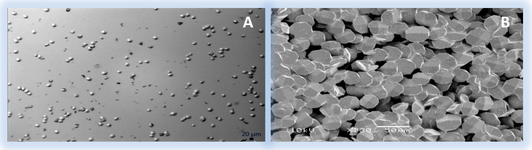

Figure 2 . Crystallisation of lysozyme using the rotary solvent evaporation method and PEG 5000.
A - Light microscopy; **B –** Scanning Electronic Microscopy. Average size of the crystal population: 23.19µm (Feret's diamater of light microscopy images).

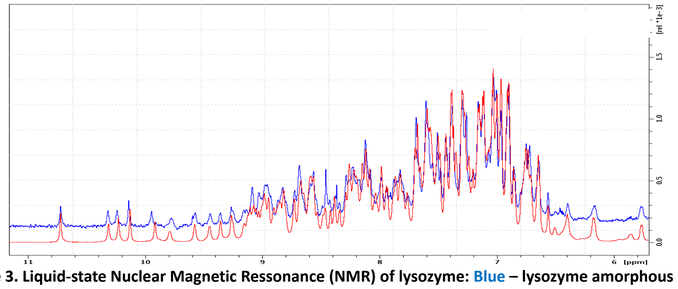

Figure 3. Liquid-state Nuclear Magnetic Ressonance (NMR) of lysozyme: Blue – lysozyme amorphous powder, Red – lysozyme crystals.
The similarities between the two spectra indicate that the structure of the protein was not affected by the crystallisation process.

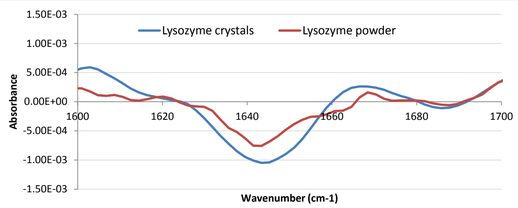

Figure 4 . Fourier Transformed Infrared Spectroscopy (FTIR) of lysozyme – second derivative.
Blue – lysozyme crystals **Red –** lysozyme powder

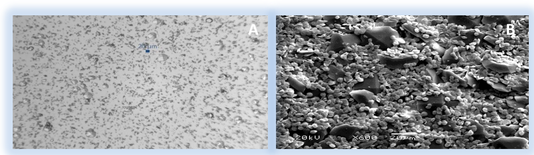

Figure 5. Lysozyme crystals produced by the rotray solvent evaporation method with polyvinylpirrolidone (PVP).
A – Light microscopy; **B –** Scanning Electronic Microscopy.
Average size of the population: 3.29µm (Feret's diamater of light microscopy images).

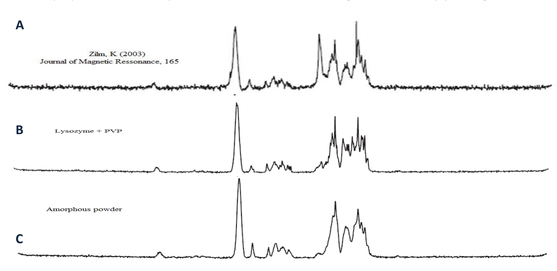

Figure 6. Solid-state NMR of lysozyme: A) lysozyme crystals with a high content of salt; B) lysozyme crystals with PVP; C) amorphous lysozyme powder.
T he higher resolution of the spectrum B, when compared to spectrum C, confirms the crystallinity of the population.

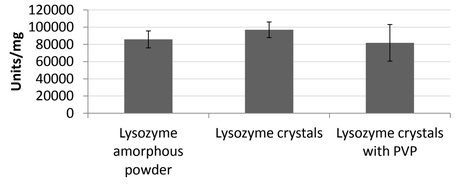

Figure 7. Activity of three different lysozyme formulations determined by enzymatic assay. (n=3).
Equivalent amouns of lysozyme used in eachformulation. The crystallisation process does not appreat to affect the activity of lysozyme.

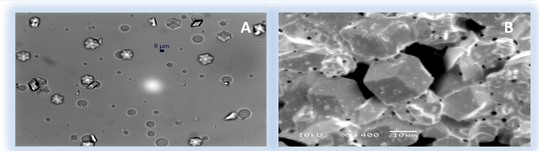

Figure 8. Crystallisation of insulin using the rotary solvent evaporation method and PEG 5000.
A - Light microscopy; **B –** Scanning Electronic Microscopy. Average size of the crystal population: 26.31µm (Feret's diameter of light microscopy images)

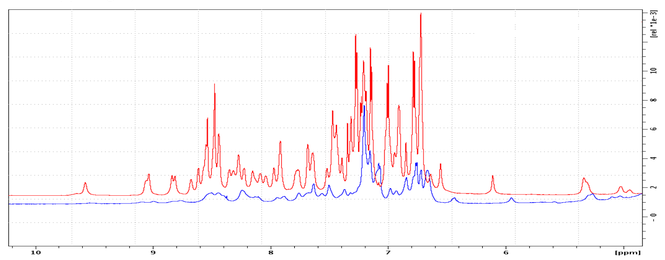

Figure 9. Liquid-state Nuclear Magnetic Ressonance (NMR) of insulin: Blue – insulin crystals. Red – insulin crystals.
The difference in the spectra indicates that insulin molecules may have undergone structural changes during the crystallisation process.

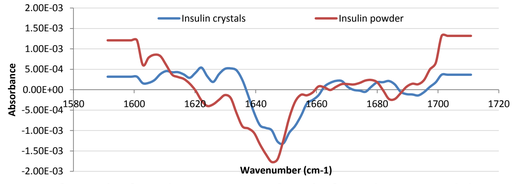

Figure 10. Fourier Transformed Infrared Spectroscopy (FTIR) of insulin – second derivative.
Blue – Insulin crystals **Red –** insulin powder

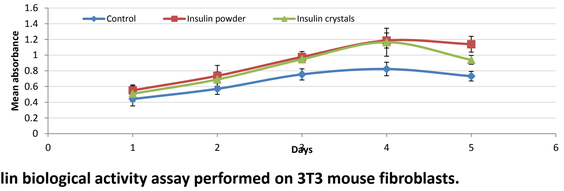

Figure 11 . Insulin biological activity assay performed on 3T3 mouse fibroblasts.
Insulin crystals show approximately 95% of the insulin powder activity. Cell viability assessed by MTS assay over a period of 5 days (n=8).

Concluding Remarks

- The quick solvent evaporation method was suitable for the production of crystals from different proteins using GRASS materials;
- Both analyzed proteins (lysozyme and insulin) did not seem to have lost their original activity after the crystallisation process;
- The addition of PVP to the lysozyme crystals showed a reduction in the crystal population with no effect on the crystallinity of the population.

References

1 - Rao, S. Self-nanoemulsifying drug delivery systems (SNEDDS) for oral delivery of protein drugs: I. Formulation development. International Journal of Pharmaceutics 362 (2008) 10–15
2 - Putney, S and Burke, P. Improving protein therapeutics with sustained-release formulations. Nat. Biotechnol. 16:153–157 (1998).
3 - Jen, A and Merkle, H. Diamonds in the Rough: Protein Crystals from a Formulation Perspective. Pharmaceutical Research; Vol. 18 Number 11, Nov 2001

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