PROTEIN CRYSTALLIZATION: INDUSTRIAL SCALE-UP

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INTRODUCTION

Protein crystallization process involves big variety of parameters and it is highly dependent not only on the supersaturation but also on media and other external parameters, strongly influencing the development of the two critical steps, the nucleation and the growth. This means that obtaining crystals with a homogeneous size distribution is not an easy task. Nucleation determines the final number of crystals controlling also their final size. The seeding technique allows the nucleation barrier to be avoided and nucleation decoupled from the crystallization equation. Hydrogels, as crystallization media, have demonstrated their ability to produce higher quality crystals while introducing a control over the nucleation and growth, preventing seeds' precipitation at the same time.

For the future industrial process, selecting an appropriate crystallization method that having into account the feasibility of the process and crystals' quality is important, since industrial production of protein crystals is a challenge due to the inherent difficulty to have reproducible, controllable and cost effectiveness processes. In this way, batch method is the best option thanks to its simplicity and its capacity to be scalable to bigger volumes.

In this work, we have developed a method to obtain protein crystals with a narrow size distribution that could be easily transferable to industrial productions.

RESULTS AND DISCUSSION

Using hanging drop technique, we have determined the appropriate crystallization conditions. The selected conditions have to be found within the metastability zone (crystallization occurs after 48 hours or more) to prevent secondary nucleation in the gelled batch, allowing only seeds' growth. The most promising range was 50 – 35 mg/mL for protein and 3,5 – 2,5% (w/v) for sodium chloride (NaCl).

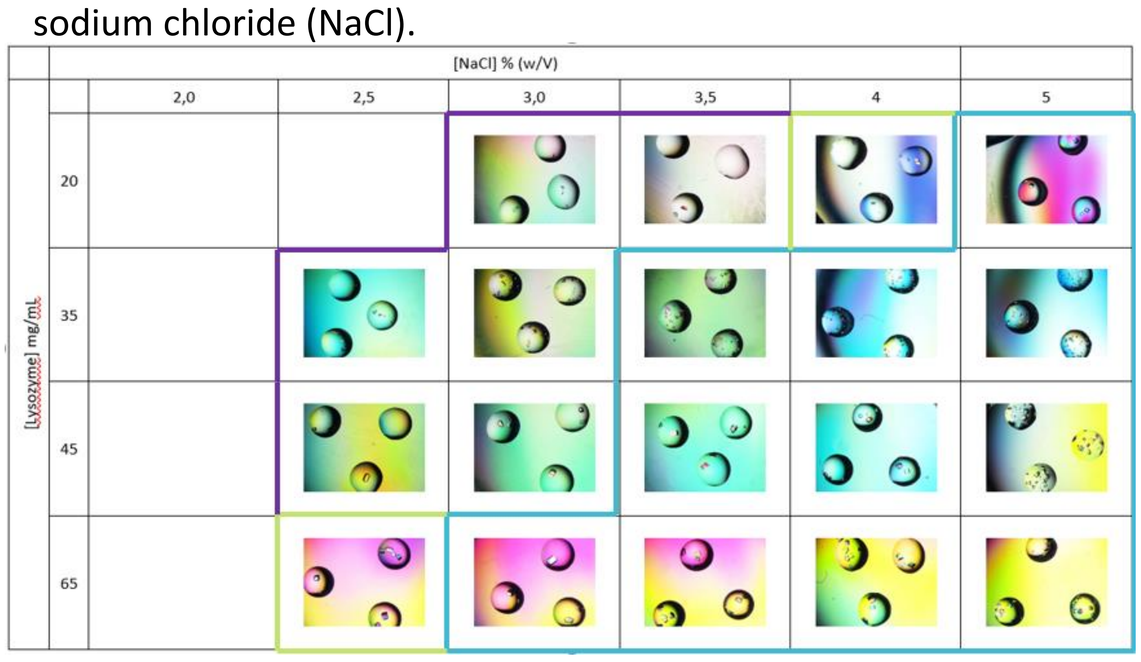

Fig 1. Screening of concentrations using hanging drop: crystals grown in 24 h (blue), crystals grown in 48 h (green), crystals grown in 1 week or more (purple).

Those concentrations were carried from hanging drop to micro batch to check the behavior of these conditions when changing technique and volume. We selected a narrow range where the best results were located for both gelled and not gelled batch. Seeds were prepared starting from crystals grown in a gel free batch and then grinded manually to obtain final seeds. They were seeded in a gelled batch metastable solution. We were able to scaled up this procedure from 100 µL to 100 mL (Fig 2).

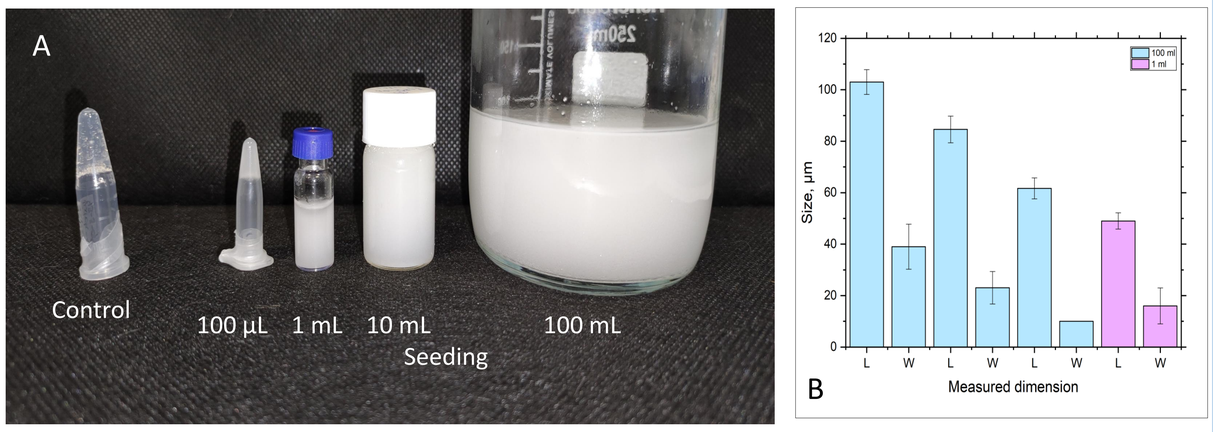

Fig 2. A) Scale-up visual results from 100 µL to 100 mL when the system is balanced and crystal growth has ceased. B) Measured size distribution.

Our goal is to have a fine control over the final results so, to know the process in depth, we are studying the influence of inicial size (and shape) of the seeds, obtained in different ways. We have employed different grinding times, methods and materials to study the role of seed characteristics in the final results. Fig 3 and 4 show seeds characterization to date.

	L, µm	W, µm
Mean	183,7	102,4
St. Dev.	30,3	30,7

Fig 3. Seeds' size before grinding taking by optical microscope.

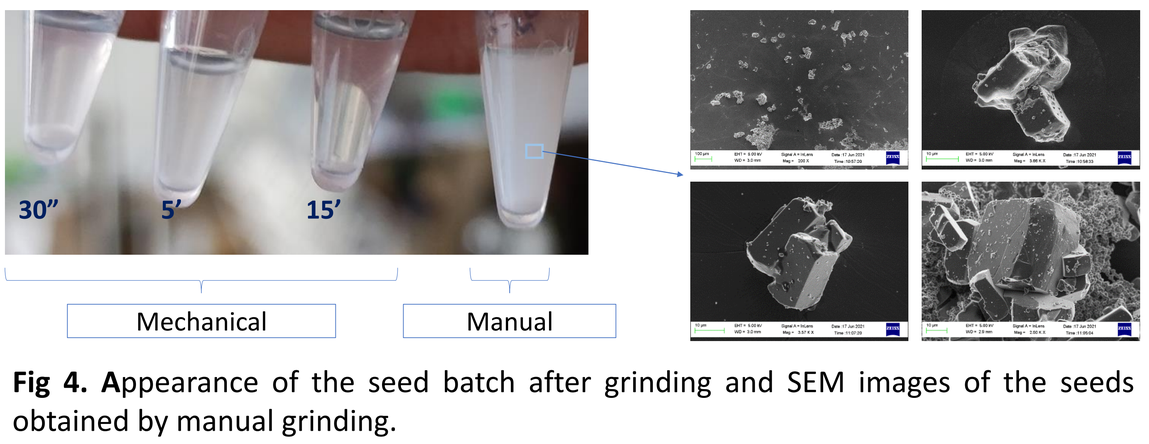

Fig 4. Appearance of the seed batch after grinding and SEM images of the seeds obtained by manual grinding.

CONCLUSIONS

Our preliminary results, presenting in this poster, lead us to stablish some conclusions.

- We have been able to reproduce and scale our seeding technique at different volumes (100 µL, 1 mL, 10 mL and 100 mL).
- Seeds act as crystallization nuclei and not only inducing the crystallization of metastable system in 24 h but also leading the system towards obtaining crystals with a more homogeneous size distribution.
- It is clearly demonstrate that there is a direct correlation between seeds´ initial size and crystals final size while keeping a narrow size distribution.
- Quantity of seeds is a control point. If we get smaller seeds, we will put more seeds per unit of volume, controlling this set point and starting nuclei number.

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