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Protein crystallization in a droplet-based microfluidic device: Hydrodynamic analysis and study of the phase behaviour

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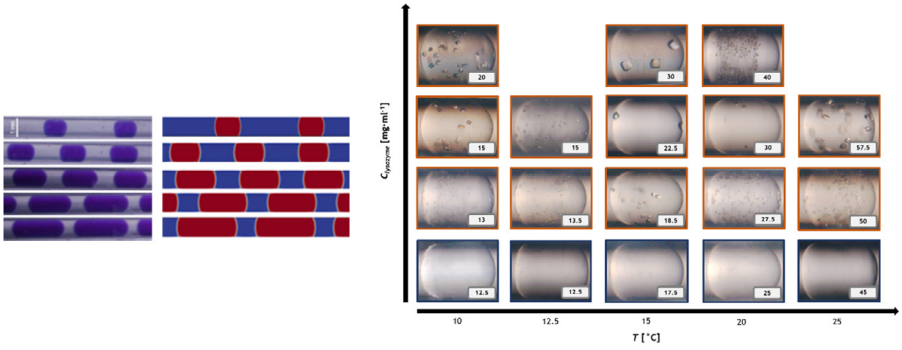
HIGHLIGHTS

- Systematic study of protein phase behaviour in a droplet-based microreactor.
- Numerical prediction of droplet sizes in a flow-focusing geometry.
- Parametric study to quantify the influence of the droplet volume on nucleation.
- Parametric study of the influence of the droplet volume on the phase diagram limits.

GRAPHICAL ABSTRACT



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ABSTRACT

This work reports a cheap and easy-to-use droplet-based microfluidic platform for the study of protein crystallization, offering the possibility to characterize the protein phase behaviour, and the effect of volumetric and interfacial phenomena on the crystallization mechanism. We conducted a parametric study supported by comparison with literature data, to quantify the influence of the droplet volume on the thermodynamic (solubility data) and kinetic (metastability data) parameters, using lysozyme as a model protein. Experiments were performed in a tubular microreactor at low Capillary numbers (4.1×10^{-5}–2.3×10^{-4}), resulting in a broad range of droplet sizes. The droplet formation in a flow-focusing geometry was also numerically studied using CFD and a correlation for the droplet size was developed. Subsequently, the lysozyme phase behaviour and the possible mechanisms associated with the nucleation process were evaluated. While crystallization in small volume droplets is usually characterized by a low nucleation probability and correspondingly low number of crystals, we did not observe this in our experiments. A potential explanation for this is the complex and stochastic mechanism of nucleation, including the competition between monomers and oligomers in solution.

1. Introduction

Since the pioneering work of Friedrich Hünefeld in 1840, who accidentally discovered protein crystals in blood samples (Giegé, 2013), researchers from different scientific fields are trying to produce high-quality protein crystals for three dimensional (3D) structure determination by X-ray crystallography (Vorontsova et al., 2015; Leng and Salmon, 2009; Haeberle and Zengerle, 2007; Maeki et al., 2016; Yang et al., 2010; Shui et al., 2007; Teh et al., 2008). Proteins are the building blocks of all cells in all living organisms and their function is dependent on their structure,

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Nomenclature

Roman symbols

C	concentration [mol·m^{-3}]
Ca	Capillary number (Ca $= \mu u/\sigma$) [–]
Co	Courant number (Co $= \lvert\vec{u}\rvert\Delta t/\Delta x$) [–]
d	diameter [m]
Δt	time-step [s]
Δx	cell size [m]
d_t	Teflon tube diameter [m]
e	wall wetting film thickness [m]
f_σ	surface tension force [kg·m^{-2}·s^{-2}]
g	gravity acceleration [m·s^{-2}]
L	length [m]
$\hat{n}$	unit surface normal vector [–]
Q	flow rate [m^3·s^{-1}]
P	probability [–]
S	supersaturation [–]
T	temperature [K]
u	velocity [m·s^{-1}]
V	volume [m^3]

Greek symbols

μ	dynamic viscosity [Pa·s]
α	marker function (volume fraction) [–]
ρ	density [kg·m^{-3}]
σ	surface tension [N·m^{-1}]
κ	local surface curvature [–]
θ	contact angle [°]

Subscripts

c	continuous phase
d	dispersed phase
i	phase (i = c, d)
nucl	nucleation
t	Teflon tube
0	initial

Abbreviations

CFD	Computational Fluid Dynamics
CFL	Courant-Friedrichs-Lewy
CSF	Continuum Surface Force
MULES	Multidimensional Universal Limiter with Explicit Solution
MZW	metastable zone width
OF	OpenFoam®
PFA	perfluoroalkoxy alkane
PIMPLE	Pressure Implicit Method for Pressure Linked Equations
VOF	Volume-of-Fluid method

therefore determining their 3D structure is of importance. This is a crucial insight for fundamental research in biochemistry for the identification and design of new drugs and pharmaceutical products, and to understand biological systems at a molecular level (Walsh, 2015). However, crystallization of proteins is a complex and multiparametric process, involving thermodynamic and kinetic features, as well as the optimization of several variables (*i.e.* temperature, pH, and concentrations of protein and precipitant agent, among others), where there is not an accurate theory to substitute for empirical approaches (Leng and Salmon, 2009; Yang et al., 2010).

Crystallization is a two-step physical process in which the supersaturation is the driving force. The first step (nucleation) is the formation of nuclei, followed by the growth of these nuclei to a macroscopic scale (crystal growth). Compared to crystal growth, nucleation is more difficult to address theoretically and experimentally due to its stochastic nature and the high supersaturation levels to overcome the critical activation free energy barrier (Vorontsova et al., 2015; Leng and Salmon, 2009; Haeberle and Zengerle, 2007; Maeki et al., 2016; Yang et al., 2010; Shui et al., 2007; Teh et al., 2008). Associated with the highly stochastic nature of protein nucleation, it is often a matter of trial-and-error to successfully crystallize a protein. This implies testing a large number of potential crystallization conditions with large amounts of protein of interest. Since proteins are usually only available in low quantities, high-throughput experimental methodologies for the acquisition of relevant thermodynamic and kinetic data, as well as more systematic crystallization strategies are needed. An example of a systematic strategy for monitoring nucleation and crystal growth is using seeding, which allows a better control of crystal size and the reduction of crystallization time (Leng and Salmon, 2009; Schieferstein et al., 2018; Gerdts et al., 2006; Revalor et al., 2010).

Crystallization processes can be situated in a phase diagram, which indicates which state (liquid, crystalline, or amorphous precipitate) is stable when varying crystallization parameters. Fig. 1 depicts the protein concentration as a function of adjustable parameters (e.g. precipitant agent concentration). In the labile zone (nucleation zone) the spontaneous formation of new nuclei is occurring, whereas in the metastable zone crystal growth is favoured, with a low probability for the formation of new nuclei. In addition, there is a stable zone (below the solubility curve), where the protein is soluble, and a precipitation zone, where the formation of an amorphous solid phase is observed (Chayen and Saridakis, 2008).

The information on protein phase behaviour is fundamental for a systematic design of crystallization experiments, which contributes to a more accurate prediction of protein crystallization conditions. However, most of the studies were focused on the solubility instead of the metastability data (Forsythe et al., 1999; Howard et al., 1988). The effect of different parameters on the metastability limit was reported in a few number of publications. For example, the effect of salt concentration was studied by Crespo et al. (2010) and Castro et al. (2016), the temperature effect

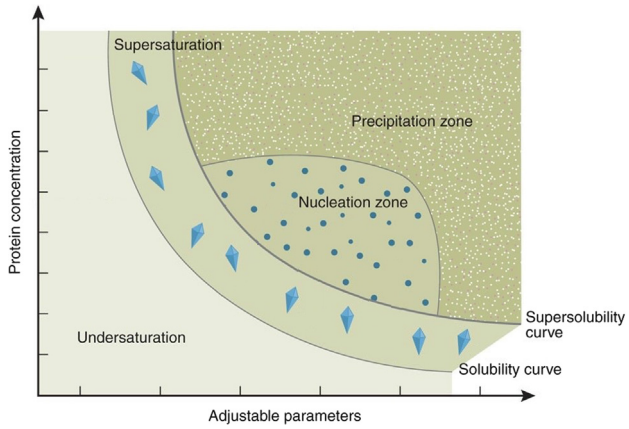

Fig. 1. Representation of the phase behaviour of a biological macromolecule: Stable (undersaturation), metastable (below the nucleation zone), nucleation and precipitation zones [Adapted from Chayen and Saridakis (2008)].