Droplet-based microfluidic platform for protein crystallization

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Introduction

The main focus of protein crystallization has been in the production of high-quality protein crystals for 3D structure determination by X-ray diffraction. However, this is a complex and multiparametric process, involving thermodynamic and kinetic features, as well as the optimization of several variables, where there is not an accurate theory to substitute for empirical approaches (Giegé and McPherson 2006). In this context, droplet-based microfluidics allows the generation of hundreds of droplets, each one acting as a microreactor, enabling thus to perform high-throughput screening of protein crystallization experiments under identical conditions.

The main goal of the present work is to develop an easy-to-use and cheap droplet-based microreactor for protein crystallization. The first part of the study aims at obtaining a broad range of droplet size by testing different flow rate ratios between the dispersed and the continuous phases (Q_d/Q_c). To study the regime of droplet formation, a range of low values of the Capillary number (Ca) was studied ($Ca < 2 \times 10^{-4}$). This part covers also a numerical study with CFD (Computational Fluid Dynamics) for the prediction of the droplet size. In a second part, it is intended to perform multiple microbatch lysozyme trials to study the influence of the droplet size on the nucleation mechanism.

Materials and Methods

The experimental set-up consists of a flow-focusing geometry coupled to a 1 mm diameter Teflon tube fixed by an acrylic plate, which is jacketed to control the temperature. Droplets were generated at the intersection between the continuous (silicon oil 1 cSt) and dispersed (aqueous solution) phases. Droplet size measurements were then conducted by image analysis. Microbatch lysozyme crystallization trials were carried out by mixing an egg white lysozyme (60 mg·ml^{-1}) and a precipitant agent (sodium chloride 6% (w/v)) in a *T*-junction before the intersection between the continuous and dispersed phases. Both solutions were prepared in a 0.2 M phosphate buffer adjusted to pH 4.7. After approximately 20 h, the formed crystals were counted by microscopy. Afterwards, nucleation rates were determined using the double pulse technique (Galkin and Vekilov 1999). Regarding the numerical study, 2D numerical simulations were performed using a FEM platform together with the Level-Set (LS) technique to capture the dynamic shape of the fluid interface between the two liquid phases.

Results and Discussion

According to Figure 1, the developed droplet-based platform enabled to produce uniform and stable droplets with a very good frequency (up to 250 droplets per assay). It also allowed a large flexibility regarding the generated droplet volume (range of 0.9 - 18 µl). Figure 1 also shows a slight deviation between the numerical and the experimental results, probably due to the 2D geometry used in the simulations, which limits the prediction of the droplet curvature effects and the complex flow topology inside the droplet.

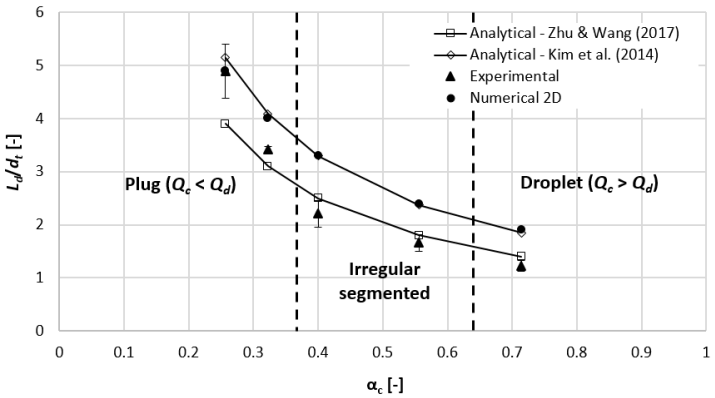

Figure 1. Scaled lengths of the dispersed phase (L_d) by Teflon tube diameter (d_t) as a function of the continuous fluid volumetric flow ratio (α_c) for squeezing flow regime and transition lines between regimes [The error bars are standard deviations from three independent experiments].

Lastly, correlations reported by Zhu and Wang (Zhu and Wang 2017) and Kim *et al.* (Kim et al. 2014) are in good agreement with experimental and numerical results, respectively (Figure 1). As to the lysozyme nucleation study, crystallization trials are currently being performed for further estimation of both the nucleation rate and induction time.

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