# NONSPHERICAL MICROFLUIDIC DROPLETS WITH CONTROLLED MORPHOLOGY TO INDUCE RAPID PROTEIN PHASE TRANSITION

**Giuseppina Simone[1], Paolo A. Netti[1,2]**

[1] *CRIB University of Napoli "Federico II", Italy*

[2] *Center for Advanced Biomaterials for Health Care@CRIB, Istituto Italiano di Tecnologia*

**ABSTRACT**

High throughput, compartmentalization with a deep control of the single volume represents the main advantages of microdroplet systems. Herein, we present immiscible liquid mixing within nanoliter droplet-based microfluidic devices for protein phase transition. In doing so, we create biphasic droplets with tunable internal structures and phase with near-equilibrium drop-in-drop morphology and we control the protein phase. The microfluidic device permits to investigate the morphology of the protein and the phase transition from gelation to crystallization.

**KEYWORDS**

Droplet generator, phase transition, ternary diagram

**INTRODUCTION**

The formation of microdroplets has been widely investigated for several interesting application [1, 2]. In fact the system allows to reproduce thousand of different experimental conditions in highly compartmentalized volumes. To date droplet generators are used when it is required to analyze huge amount of information with the control of the single microsystems (as single cell or single molecule, or reaction condition uniquely addressable and indexed).

Phase transition of proteins (coacervation, gelation and crystallization) may be initiated in a number of different ways [3, 4]. A less common application is the use of microdroplets for controlling the phase transition of the molecules. In particular it is possible to crystallize the proteins [5] to reduce the time of the process and increase the yield. Here, we demonstrate immiscible fluid mixing at microscale, within nanoliter droplet in microfluidic devices to control the phase transition of the proteins.

**EXPERIMENT**

The microfluidic network (top and bottom) was milled in Plexiglas© (w=300 μm, h~250 μm, and L~150 mm) (Figure 1). There were 11.5 sinusoids in the channel, which began at x~20 mm. The protein (A1) and precipitant (A2) flowed in through two different aqueous channels and met at the junction to form the slugs. A carrier fluid (B) transported the slugs and was immiscible to the aqueous solutions to avoid chemical exchange with the slugs (no evaporation or loss of chemicals).

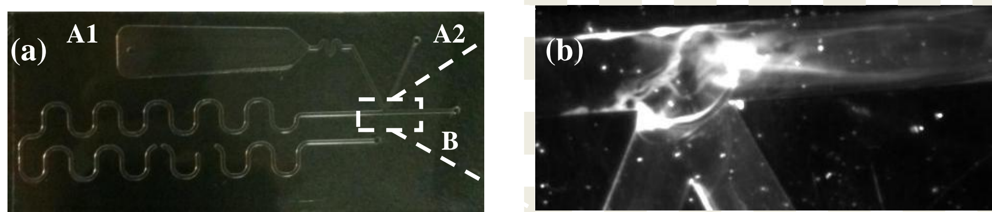


Figure 1: (a) View of the microfluidic system. (b) Mixing at the orifice.

Gelatin-B and ethanol were used as protein and precipitant. At the inlet the gelatin concentration may be varied between 5 and 35 v:wt% and the ethanol concentration between 10 and 100 v:v%. Gelatin viscosity increased with the concentration and limited the range of the protein concentration. The flow rate of the injected streams was varied between 0.2 and 2 μl $min^{-1}$. The volumetric flow rate of B to total aqueous streams, QB:(QA1+QA2), was maintained at 1.5. The temperature of the process was 37°C to keep the gelatin in the liquid phase.

**RESULTS**

Three different phases were collected at the exit of the microfluidic channel by varying the concentration of the gelatin and of the solvent.

Those phases spontaneously separated within each droplet via decomposition into two immiscible phases: an ethanol-rich outer phase and a gelatin-rich inner phase. Figure 2a shows the ternary diagram of the multiphase system as a function of the system gelatin-ethanol-water. The range of the concentration of the ternary diagram gelatin-ethanol-water ranged between 0:0.6-0:0.55-0.6:0.9 respectively.

The inner gelatin-rich phase was 'stirred' within the outer ethanol-rich phase (Figure 2b). During the formation of the droplet the gelatin and the ethanol came in contact and instantaneously the separation of inner and outer phases occurred. Once the droplet was formed and is stable the gelatin rich phase travels close to the walls.
Each droplet is equivalent to an intensively stirred picoliter flask with the moving oil walls. The high rate of the

shear within this confined reaction flask and the high surface-to-volume ratios can influence the particle properties and is also responsible of the chaotic mixing occurring inside it. At the end, the flask is completely stirred and this guarantees that the reaction occurs uniformly within the droplet. In particular, the ethanol concentration determined the formation of three different phases: the phase I, the gel (ethanol below 25%), the phase II, the crystals (ethanol above 25%) and the phase III, the coacervates (ethanol below 25% but gelatin above 40%) (Figure 2c). Differences due to the morphology were clear during the dynamic movement of the droplets along the channels.

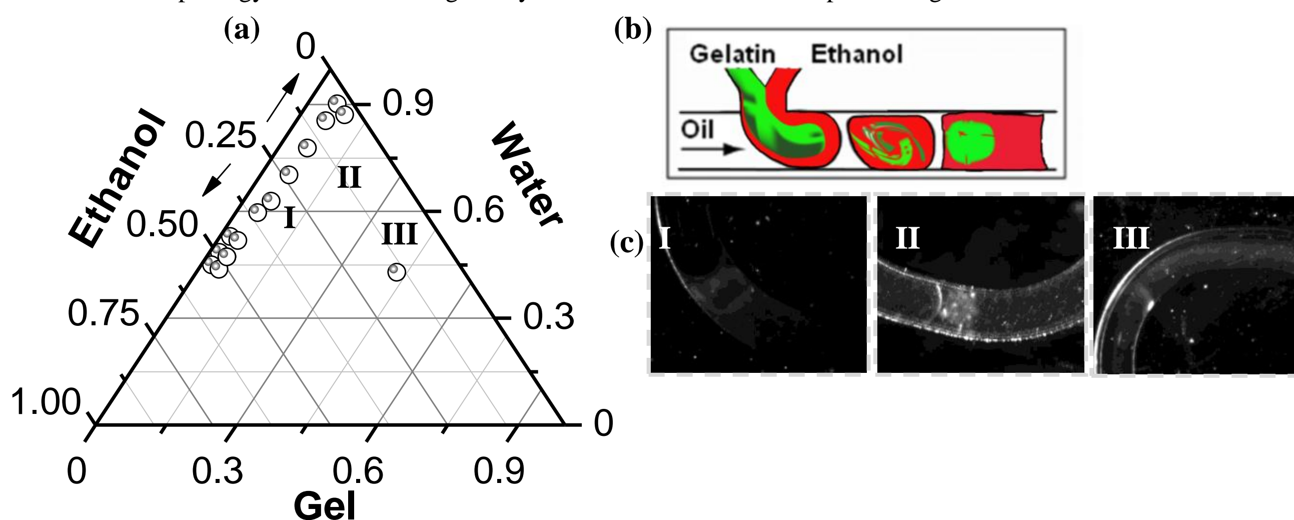


Figure 2: (a) Ternary diagram of Gelatin at pH 5 (isoelectric point). (b) Schematization of the mixing. (c) I: homogeneous transparent gel with no upper liquid-coacervate; II: crystals; III: homogeneous transparent gel with upper liquid.

A dynamic morphology diagram is shown in Figure 3, which maps the influence of flow velocity, and gelatin:ethanol composition. The transitions between the different regions on the map were correlated to the flow velocity. We observed that the role of the flow velocity was fundamental to determine the morphology of the protein. Droplets with the same concentration led different morphologies whether flowed at different velocity.

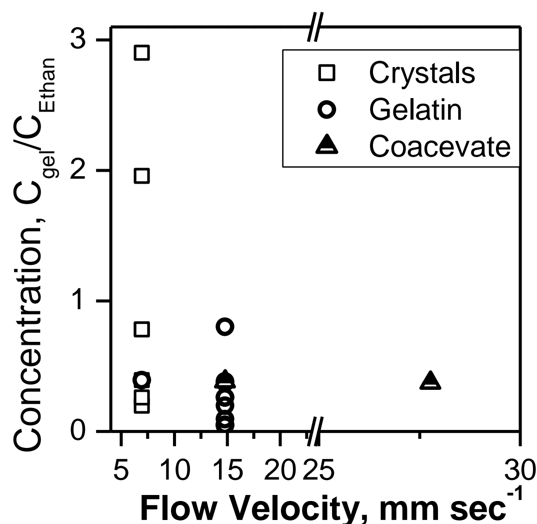


Figure 3: a) Morphology map as a function of concentration and flow velocity.

We observed that in correspondence of a concentration ratio Cgel/Cethanol of 0.4 by varying the flow velocity from 7.5 to 26.5 mm s$^{-1}$ the gelatin could be sorted out as gelatin, coacervate or crystal.

Furthermore, the morphology of gelatin and crystals changed along the channel as well. The snapshots reported in Figure 4 referred to the ratio Cgel/Cethanol=0.4 respectively at 7.5 mm sec$^{-1}$ and 15 mm sec$^{-1}$.

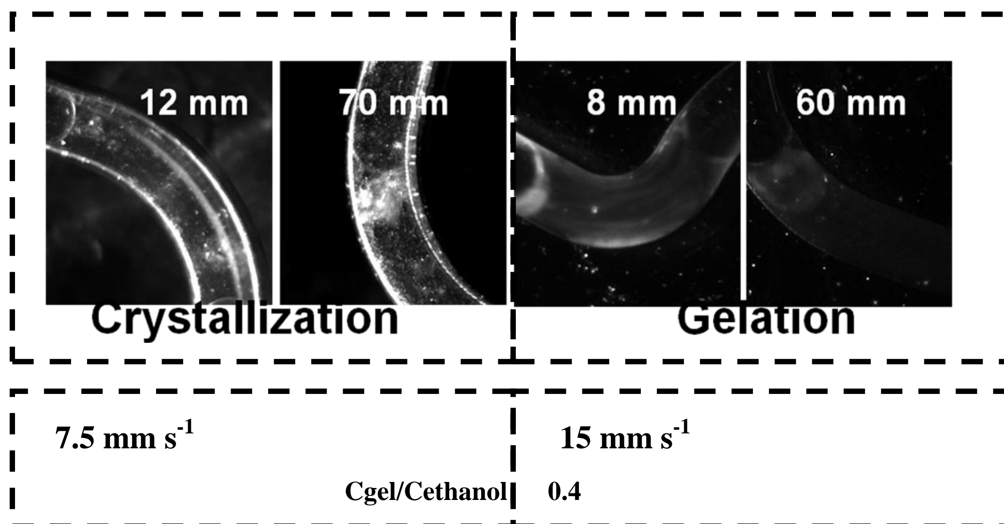


Figure 4: Morphology of the gelatin along the channel.

**CONCLUSIONS**

Phase transition of proteins in highly controlled two-phase droplets may be induced by tuning the composition of the droplets. This study enables the creation of droplets with tunable spatial heterogeneity, structure and morphology that can be exploited in biochemical investigations.

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**CONTACT**

Giuseppina Simone giuseppina.simone@unina.it