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## Salt-dependent complex formation in lysozyme-alginate mixture

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The associative interaction between oppositely charged macromolecules proceeds through either liquid-liquid phase separation (LLPS) forming complex coacervates or liquid-solid phase separation (LSPS) forming aggregates. In this work, we investigated the assembly of the basic protein lysozyme (LYS) with the negatively charged polysaccharide alginate (ALG) at pH 7 under varying conditions of mixing ratios, total concentrations, and ionic strengths.<sup>1</sup>

The droplet-based millifluidic device coupled with optical microscopy gave an extensive qualitative analysis of the phase behaviour of the system by probing different experimental conditions.<sup>2</sup> Grey level analysis associated with the droplet millifluidic experiment allowed both the quantification and the qualitative definition of phase separation. We constructed a three-dimensional phase diagram, incorporating salt, LYS, and ALG concentrations as coordinates, offering a detailed depiction of monophasic regions, liquid-solid and liquid-liquid phase separation domains, and areas of coexistence of both solid and liquid phases. The thermodynamic characterization of the formation of different LYS/ALG assemblies was carried out using isothermal titration calorimetry (ITC) where distinct ITC profiles were associated with coacervation and aggregation. The interaction affinity ( $K_a$ ) for aggregation was three orders of magnitude higher than for coacervation, without significant change in binding stoichiometry. Structural differentiation of various assemblies in the nanometer range was achieved through small-angle X-ray scattering (SAXS) experiments. To gain deeper insights into the mechanisms underlying both LSPS and LLPS processes, further investigation of additional polyelectrolyte couples is needed, facilitating a more comprehensive understanding of these phase separation phenomena.

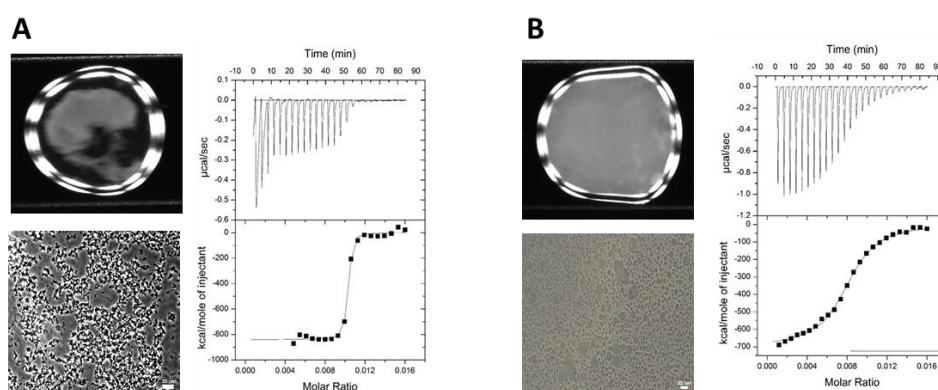


Figure 1. Droplet image, optical microscopy image, and ITC thermogram obtained for aggregation (A) and coacervation (B).

[1] Vakeri et al., *Food Hydrocolloids*, **2024**, submitted.

[2] Amine, C. et al, *Food Hydrocolloids*, **2017**, 70, 134-142; **2019**, 92, 94-103.