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The model Lysozyme–PSSNa system for electrostatic complexation: Similarities and differences with complex coacervation

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ABSTRACT

We review, based on structural information, the mechanisms involved when putting in contact two nano-objects of opposite electrical charge, in the case of one negatively charged polyion, and a compact charged one. The central case is mixtures of PSS, a strong flexible polyanion (the salt of a strong acid, and with high linear charge density), and Lysozyme, a globular protein with a global positive charge. A wide accurate and consistent set of information in different situations is available on the structure at local scales (5–1000 Å), due to the possibility of matching, the reproducibility of the system, its well-defined electrostatics features, and the well-defined structures obtained. We have related these structures to the observations at macroscopic scale of the phase behavior, and to the expected mechanisms of coacervation. On the one hand, PSS/Lysozyme mixtures show accurately many of what is expected in PEL/protein complexation, and phase separation, as reviewed by de Kruif: under certain conditions some well-defined complexes are formed before any phase separation, they are close to neutral; even in excess of one species, complexes are only modestly charged (surface charges in PEL excess). Neutral cores are attracting each other, to form larger objects responsible for large turbidity. They should lead the system to phase separation; this is observed in the more dilute samples, while in more concentrated ones the lack of separation in turbid samples is explained by locking effects between fractal aggregates.

On the other hand, although some of the features just listed are the same required for coacervation, this phase transition is not really obtained. The phase separation has all the macroscopic aspects of a fluid (undifferentiated liquid/gas phase) — solid transition, not of a fluid–fluid (liquid–liquid) one, which would correspond to real coacervation). The origin of this can be found in the interaction potential between primary complexes formed (globules), which agrees qualitatively with a potential shape of the type repulsive long range attractive very short range.

Finally we have considered two other systems with accurate structural information, to see whether other situations can be found. For Pectin, the same situation as PSS can be found, as well as other states, without solid precipitation, but possibly with incomplete coacervation, corresponding to differences in the globular structure. It is understandable that these systems show smoother interaction potential between the complexes (globules) likely to produce liquid–liquid transition. Finally, we briefly recall new results on Hyaluronan/Lysozyme, which present clear signs of coacervation in two liquid phases, and at the same time the existence of non-globular complexes, of specific geometry (thin rods) before any phase separation. These mixtures fulfill many of the requirements for complex coacervation, while other theories should also be checked like the one of Shklovskii et al.

1. Introduction

This paper aims at a brief review, based on structural information, of the mechanisms involved when putting in contact two nano-objects of opposite electrical charge, in the case of one negatively charged polyion, and a compact positively charged one. The central case will be the case of PSS Na, a strong flexible polyanion (the salt from a strong polyacid, with a high linear charge density), and Lysozyme, a globular protein with a global positive charge. This system will be central in this paper because we have gathered a large amount of consistent data in different situations. We will then compare the conclusions concerning such a system to the expected mechanisms of coacervation.

Prior to proceeding to the description of the mechanisms, it is worth reminding the meaning of coacervation itself to set the basis of our purpose, since several definitions can be found in the literature or on the Web. As recalled in the excellent review by De Kruif et al [1], *"coacervation is the separation into two liquid phases in colloidal*

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systems", as defined by the International Union of Pure and Applied Chemistry (IUPAC 72 a, [2]). A particular class of coacervation, firstly introduced by Bungenberg, de Jong, and Kruyt [3], is the "complex coacervation" that stands for *the coacervation caused by the interaction of two oppositely charged colloids* (IUPAC 72 b, [2]).

In colloidal or polymer science, liquid–liquid phase separations can occur in suspensions of a single type of colloids, depending of the shape of the interaction potential between colloids. We thus can imagine that complex coacervation can be described as a two-steps process involving first the formation of complexes, made of the two oppositely charged colloids, and second their phase separation, above certain concentrations, in two liquids phases containing both complexes with the same structure, one with a high content and the other with a poor content. The formation of complexes before coacervation has indeed been discussed as a prerequisite in several theoretical descriptions of the process [3–12] from Overbeck [3] to Veis [5–8] and more recently Tainaka [11,12]]. Another view by Shklovskii has been proposed recently, addressing the case of two components different in size: it considers the formation, mainly in a unique step, of either «single complexes» of specific geometry, or bigger aggregates (called condensates), depending on the stoichiometric ratio, with possible coexistence [13].

In colloidal science, of the two mechanisms of liquid–liquid phase separations in suspensions of a single kind of spherical colloids have been largely studied in the 80s and 90s, owing to the possibility to finely tune the interparticle potential between objects by 'simple' physicochemical parameters (ionic strength, polymer, etc..); that makes colloidal suspensions perfect model systems to study the physics of phase separations in general [14]. These studies may provide a framework for the description of simple "coacervation", which should be also phase separation in suspension, the word having been introduced earlier by a different community, made of polymerists, biologists and biochemists. Theoretical works have shown that the phase behavior of assemblies of spherical colloids is governed only by the relative intensity and relative range of the attractions and repulsions in the potential between colloids [15,16]. The authors considered a very general interaction potential, sum of two Yukawa potentials, one attractive and one repulsive to calculate the Π–Φ phase diagrams (Fig. 1) where Π is the osmotic pressure and Φ the volume fraction (the only condition imposed on this potential is that the repulsive component is dominant at very short range to avoid trivial irreversible aggregation of particles). We will first recall that the term "Fluid" means here a dispersed homogeneous phase of objects in the solvent; at low concentration it is similar to a gas of atoms or molecules, at larger one to a liquid phase of atoms or molecules. When there are long-ranged attractions in the system (case 1 in Fig. 1), there is (i) a fluid phase at low volume fraction and high pressure, (ii) at low volume fraction and low pressure there are two fluid phases possibly coexisting, called gas and liquid phases by reference to atomic/molecular systems, and finally (iii) at high volume fraction there is a solid phase, with a critical point and triple point. Otherwise (case 2 in Fig. 1), for long-range repulsions, the diagram reduces to (a) a fluid phase (no transition between gas and liquid, no critical point) at high pressure and low volume fraction and (b) a crystal phase at high pressure and high volume fraction, both phases coexisting at low pressure.[2] The first type of diagram – (i, ii, iii), case 1 – is observed quite always in atomic/molecular systems which present the long-ranged attractions in the potential necessary to get gas–liquid transitions; but it is rare in colloidal systems, because these long ranges are often missing. An exception is systems with steric short range stabilization [14,17,18] and long range attraction through depletion: these systems usually involve polymer chains which large sizes can provide large enough size ranges for the depletion. On the contrary, for systems with electrostatic stabilization, one usually observes the second kind of diagram — (a, b), case 2 in Fig. 1). To our knowledge, the only case where liquid–liquid transitions have been observed in electrostatic systems is in suspensions of magnetic nanoparticles [19] when magnetic dipolar attractions interactions, specific to the system, have a sufficient range to allow phase transitions. In this case even the critical point has been measured [20]. If one adds short ranged attractions in the interparticle potential, *e.g.* Van der Waals (VdW) interactions, this leads to irreversible aggregation. The macroscopic trend therefore will be precipitation into a "solid" phase. We will better use the term precipitation for dense precipitates similar to a crystalline phase; when the solid phase formed is less dense and first gives flocs or flakes, as frequent for colloidal systems, we can also use the term flocculation. The colloidal objects often form, when in attractive regime, fractal aggregates. The fractal dimension of the aggregates depends on the way of aggregation process and lie between 1.78 for Diffusion Limited Colloidal Aggregation [21] to 2.1 for Reaction Limited Colloidal Systems [21]. For electrostatic systems, the repulsive electrostatic repulsive barrier has to be overcome, so there is a Reaction Limitation and one gets usually RLCA aggregation process.[3]

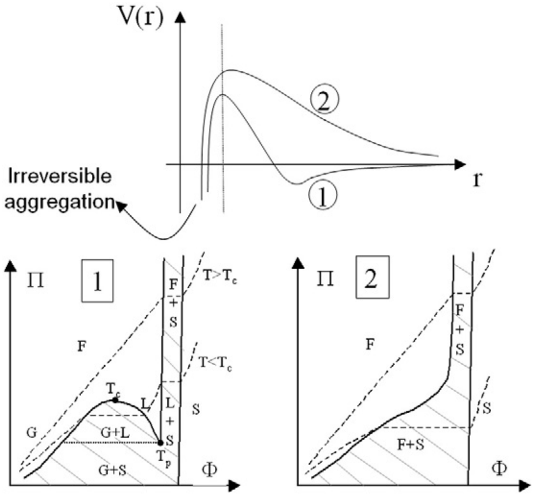

Fig. 1. Top: two shapes, noted 1 and 2, of the potential between two particles. Bottom: corresponding phase diagrams (osmotic pressure Π versus volume fraction fΦ) for colloid suspensions, for each shape of potential 1 and 2: F fluid (Gas or Liquid undifferentiated, no phase transition), G gas, L liquid, T_c critical temperature, T_p triple point. Full lines separate the one phase stability domains and the two-phase coexistence domains (hatched areas). In each diagram, dashed lines represent two exemplary paths, crossing these domains.

To what extent can these considerations on transitions in simple colloidal systems be applied to a suspension of two kinds of objects? In such a ternary system (the two colloids of opposite charge, plus the solvent containing the ions), there exists a multiplicity of interactions. However, there are encouraging similarities with binary systems. An example is found in the nice and very comprehensive review (~25 different systems) on proteins–polysaccharides coacervation of Kruif *et al* [1] who, comparing close systems that may *a priori* undergo a liquid–liquid transition, remarks that '*if one of the polyions is a strong polyelectrolyte (we postulate) a precipitate is formed rather than a liquid coacervate phase*'. Since a precipitate corresponds to a fluid–solid transition, while a coacervate corresponds to a liquid–liquid one, this is an encouraging similarity with the two final states in the phase behavior of simple colloids, either fluid–fluid or solid precipitate, and it points out the role of the intensity of electrostatics interactions. This similarity is the fundus of the present paper. The authors of [1] have also sorted out some trends in the case of liquid–liquid transitions that we

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[2] **Note 1**: Please note that a third kind of diagram is also predicted, symmetrical to the first diagram, with a single fluid phase and two crystalline solid phases of the same symmetry, possibly coexisting with high volume fraction, with a critical point and triple point fluid-solid-solid. It has never been observed experimentally to our knowledge.

[3] **Note 2**: In some cases RCLA fractal dimension can be obtained after rearrangement of a structure having first a DLCA fractal dimension.