



COACERVATE RHEOLOGY: APPLICATIONS OF DYNAMICS

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ABSTRACT

Investigation of the mechanisms of complex formation between polyelectrolytes and oppositely charged colloids, and of coacervate structures formed at different length scales represents an active and important research area. Although mechanisms of coacervation have been deduced from observations of self-organized microscopic structures, the true relationship between coacervation mechanism and the resultant structure remains to be further explored. There is still a need for more connection between experimental and theoretical work. Experimental efforts have largely focused on phase behavior as the primary point of comparison, however this is not always a direct probe of the molecular-level features such as charge correlations, charge density effects, excluded volume, and specific ion effects. This makes it difficult to distinguish between VO theory and more sophisticated theories, which as a result are not often compared to experiment.

For all, little is understood about the mechanism and kinetics of complex coacervation, few theories are quantitatively supported by experiment, phenomenology alone fails to predict coacervate stability, and equilibrium behavior is sometimes not confirmed. For example, to the author's knowledge the rheological behavior of coacervates has not been widely studied from a theoretical perspective. This is despite the regular use of rheology as a tool to characterize coacervates.

There has also been increasing interest in understanding the rheological properties of coacervates^[1,2] in order to improve and optimize applications. For this purpose, a series of biopolymers^[3,4] and/or synthetic polymer systems^[5] have been considered previously. Complex coacervates typically exhibit a viscoelastic response,^[6] and rheological studies have shown that the viscoelasticity and viscosity of coacervates are affected by a number of parameters, including salt concentration, mixing stoichiometry, and chain length.

Encapsulation and Release

In addition to characterization of the properties of the base material, rheology has been used to understand the impact that a guest molecule has on the structure of a complex coacervate. Tiwari *et al.*, examined both the encapsulation and release of salbutamol sulfate, a bronchodilator used in the treatment of asthma and other chronic airway diseases, from coacervates formed from a pair of protein molecules, gelatin-A and gelatin-B.^[7] Linear viscoelastic characterization showed that drug encapsulation caused a significant decrease in the storage

modulus (G'), suggesting that the coacervate matrix was disturbed and weakened by the presence of the drug, and a slight increase in the loss modulus (G''), suggesting a commensurate fluidization of the material. Such changes in material properties can be critical for modeling of release processes for more advanced therapeutics.

Connection to Layer-by-Layer Films and solid PECOX

The associative electrostatic interactions driving complex coacervation are an obvious parallel to those used to drive the formation of layer-by-layer (LbL) films. LbL films are assembled by the sequential deposition of oppositely-charged polyelectrolytes on a surface.^[8] While complex coacervation is an equilibrium phase separation, LbL films are a kinetically-trapped assembly. Such films can be formed onto a variety of surfaces, including bubbles and droplets to create capsules for delivery. LbL assembly can be used either as a method for creating a capsule, or as a layered structure that allows for the direct release of therapeutics upon disassembly. Careful design of the layered structures can enable both spatial and temporal control over the release of therapeutics. Alternatively, the materials of the films themselves could be harnessed as active coatings, such as antibacterial and/or antifouling surfaces.^[9] While LbL films can be formed as free-standing films, they are much more commonly presented as coatings. However, recent reports have demonstrated the utility of bulk PECOX solids as well. PECOX may be extruded into large articles if sufficiently plasticized with salt and water ("saloplasticity") has introduced yet another processing dimension.^[10,11] This idea of salt-driven

plasticization, or ‘saloplasticity’ has opened up a tremendous range of opportunities for using salt to process polyelectrolyte complex materials as liquid coacervates, and then solidify the materials by the removal of salt, and a range of accounts describing ultracentrifuged^[12,15] or extruded materials,^[10] spin-coated films,^[16,17] 3D printed structures,^[18] and electrospun fibers^[19,20] have been reported. However, thus far saloplastic materials have only been reported for synthetic polymer systems. Expanding this class of materials to include biopolyelectrolytes such as proteins, hyaluronic acid, chitosan, or chondroitin sulfate has tremendous potential to further enhance their utility.

Self-Healing and Structural Recovery

Another interesting material property conferred by the non-covalent nature of the molecular interactions defining complex coacervates is the potential for self-healing and structural recovery after complete network disruption.^[2,21] This can be assayed by the application of a large strain (*i.e.*, 100% oscillatory shear strain, well beyond the linear range for the material) to break the sample, followed by continuous measurements back in the linear strain regime to track the ability of the material to recover its original elasticity, and the requisite timescale to do so. An example of this type of experiment is given for a fish gelatin/sodium montmorillonite coacervate system.^[21]

From an initially high value for the equilibrium storage modulus (G'), the application a high strain decreases the modulus of the material, breaking the internal elastic network of electrostatic interactions and converting it into a liquid-like material. Five minutes after the application of this high strain, the sample was able to recover 20-30% of its original modulus – a process that could be modeled by an exponential function. Examination of the effects network disruption in the example by Qazvini *et al.*,^[21] suggested that the liquid-like behavior was not the result of free-flowing individual nanoplatelets, but rather the result of domain fracturing. Upon removal of strain, these domains are then able to quickly recover the percolated elastic behavior of a network to give a solid-like response, followed by a slower rearrangement of the gelatin network. This self-healing capability suggests that these types of materials could be easily processed as low viscosity materials, for example as thin films or injectables, followed by a recovery time to allow for the formation of an elastic gel.

Microrheology Techniques

Elbaum-Garfinkle *et al.*, recently reported the use of microrheology techniques to coacervate-like materials formed as a result of the self-interaction of LAF-1, a DDX3 RNA helicase found in the P granules of *Caenorhabditis elegans*.^[22]

Here, confocal fluorescence microscopy was used in conjunction with particle tracking methodologies to

measure the viscosity of coacervate-like droplets of LAF-1 both with and without RNA. A more detailed treatment of this type of microrheological characterization is beyond the scope of the current work. However, adoption of these types of techniques has the potential to enable the analysis of a vast array of coacervate-based materials that would otherwise be inaccessible to larger-scale rheological measurements.

CONCLUSION AND FUTURE PERSPECTIVES

There is a need from the theoretical side to expand the palette of models relevant to the coacervate-driven assembly that is at the forefront of experimental work in the field. Joint efforts will expedite the convergence on an overall picture of coacervation; this will hopefully enable polymer scientists to fully exploit their complexity to make truly exciting and biologically inspired materials. Further levels of understanding require incorporation of knowledge regarding the molecular structure of coacervate-based materials, and the development of more detailed theories and models. For example, to the author's knowledge the rheological behavior of coacervates has not been widely studied from a theoretical perspective. This is despite the regular use of rheology as a tool to characterize coacervates.^[23] Hybrid techniques, such as simultaneous small-angle X-ray or small-angle neutron scattering, coupled with rheology (rheo-SAXS/SANS),^[1] rheo-optics,^[24,26] calorimetry, and/or other methods have tremendous potential to further expand our understanding of this class of materials. Subsequent interpretation of the resulting data will require the further development and validation of theoretical predictions to couple observation with molecular models. For instance, the systematic linear viscoelastic analysis of polymer chemistry effects on complex coacervation would enable further testing of the “sticky” Rouse model proposed by Spruijt *et al.*^[6,26] Such efforts could also be coupled with advancements in the modeling of coacervation in general.

It is important to note, that the vast majority of experimental and theoretical efforts have focused on understanding small-amplitude oscillatory shear (SAOS) data because of the strong theoretical background of such measurements. However, from an applications perspective, most processing operations subject materials to deformations that are well beyond the limits of linear viscoelasticity. Extension of theoretical treatments of polymer behavior into the non-linear large-angle oscillatory shear (LAOS) regime, has tremendous potential to elucidate critical mechanisms related to the large-scale disruption and rearrangements of polymer networks. The application of such non-linear techniques to the characterization of complex coacervate-based materials is an open challenge for the future.

Notes

The authors declare no competing financial interest.

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