Electrostatic correlations in semi-dilute zwitterionic polymer solutions result in highly thermoresponsive coacervates

Yury A. Budkov,1,2,a) Petr E. Brandyshev,1 and Nikolai N. Kalikin1,2

1) School of Applied Mathematics, HSE University, Tallinskaya st. 34, 123458 Moscow, Russia
2) G.A. Krestov Institute of Solution Chemistry of the Russian Academy of Sciences, 153045, Akademicheskaya st. 1, Ivanovo, Russia

Taking into account strong polymer concentration fluctuations and electrostatic correlations of charged groups of dipolar monomeric units of flexible polymer chains within the random phase approximation, we develop a molecular theory of self-coacervation in semi-dilute zwitterionic polymer solutions. We show that the interplay between these key features of such system can result in two qualitatively different coacervation regimes. In the first regime, where the electrostatic correlations are reduced to the effective Keesom attraction of the point-like dipoles, located on the monomeric units, the polymer concentration in the coacervate phase $c \sim 1/T^8$, while in the second regime, where the electrostatic correlations are described within the Debye-Hückel theory, $c \sim 1/T^2$. According to the Lifshitz hypothesis of "disconnected" monomers, we establish that in the regime of semi-dilute polymer solution, chain connectivity has an extremely weak effect on the electrostatic interactions of the dipolar monomeric units, i.e. their contribution to the total free energy can be described as the contribution of a fluid of unbounded dipolar particles. The predicted high thermal sensitivity of the coacervate polymer number density (or size of coacervate droplets) can be used to control purification of macromolecular solutions by the uptake of target biomacromolecules (liposomes and vesicles, for instance) by the zwitterionic coacervate droplets.

a)ybudkov@hse.ru
Coacervation plays an extremely important role in the macromolecular assembly in soft liquid-phase media with different functions. Coacervation is the result of spontaneous separation of a macromolecular solution into two immiscible liquid phases of low and high concentration – liquid-liquid phase separation. The latter is a common physical phenomenon, occurring in aqueous solutions of polyelectrolytes, surfactants, and biomacromolecules – systems that nowadays are extensively explored both theoretically and experimentally and have a number of applications in food, pharmaceutical, cosmetics, and chemical industries. On the other hand, in the 1930s, Alexander Oparin proposed the concept of coacervation in solutions of biomacromolecules underlying the origin of life. Since then it was not until 2009 that biophysicists established the role of the liquid-liquid phase separation in the formation of certain membraneless organelles. Today the widespread relevance of coacervates has led to fundamentally new functional biomaterials and created a new wave of modern materials, extending the boundaries of polymer physics and chemistry.

These days, the theory of coacervation is being exceedingly developed for solutions of polyelectrolytes, polyampholytes, and intrinsically disordered proteins, in which phase separation is driven by strong electrostatic correlations of ionic groups. Nevertheless, there are a number of macromolecular systems, whose thermodynamic behavior is determined by the dipole-dipole interactions of highly polar or polarizable monomeric units. Especially important among them are macromolecules valuable for materials science such as zwitterionic polymers whose monomeric units carry two oppositely charged ionic groups.

One of the most interesting effects driven by dipole-dipole interactions is the coil-globule transition of a single dipolar polymer chain. The latter can occur in dilute solutions of the aforementioned zwitterionic polymers. However, while in a dilute dipolar polymer solution sufficiently strong dipole-dipole interactions provoke a coil-globule transition of an individual polymer chain, in semi-dilute solutions such interactions must lead to a liquid-liquid phase separation (so-called self-coacervation). For the aforementioned industrial applications, it is important to study the phase behavior of such zwitterionic polymer solutions in the semi-dilute regime, where strong concentration fluctuations are expected to interplay with the electrostatic correlations of the dipolar monomeric units. From general considerations, superposition of both concentration fluctuations and electrostatic correlations of polar monomeric units must lead to high thermal sensitivity of the coacervation process. The latter, for example, would allow researchers to control the coacervate density (or size of
the coacervate droplets) by varying the temperature in rather narrow ranges.

However, to the best of our knowledge, there are only a few theoretical studies devoted to the investigation of the coacervation effect in polymer solutions, induced by the dipole-dipole attraction of the macromolecule polar monomeric units. In paper the authors addressed complex coacervation – the liquid-liquid phase separation of a solution of oppositely charged polyelectrolyte chains into a polyelectrolyte-rich complex coacervate phase and a dilute aqueous phase, based on the general premise of spontaneous formation of polycation-polyanion complexes. The complexes are treated as flexible chains consisting of dipolar and charged segments. Using a mean-field theory accounting for the entropy of all the dissociated ions in the system, electrostatic interactions between the dipolar and charged segments of the complexes and separated polyelectrolytes, and polymer-solvent hydrophobicity, the authors predicted the closed liquid-liquid phase diagrams with lower and upper critical points of such a system in terms of polyelectrolyte composition, added salt concentration, and temperature. In paper using similar mean-field theory, the authors described the complex coacervation formed by the liquid-liquid phase separation of a polyzwitterion and a polyelectrolyte. The authors demonstrated the potential of polyzwitterionic coacervates as a tool for pH-triggered release of pharmaceutically active compounds inside the gastrointestinal tract. It is important to mention that programmable coacervates based on zwitterionic polymers were proposed recently as promising materials for purification of soft nanoparticles such as liposomes and extracellular vesicles.

In this Letter, we will present a molecular statistical theory of self-coacervation in a semi-dilute dipolar polymer solution taking into account strong concentration fluctuations within the scaling theory and electrostatic correlations within the random phase approximation (RPA). We will describe the phase behavior of a polymer solution in a wide range of electrostatic interaction strength values elucidating the limiting coacervation regimes and crossover between them.

Let us consider a solution of flexible polymer chains, whose monomeric units carry two oppositely charged sites with charges $\pm q$ separated by, in the general case, the fluctuating distance described by the probability distribution function, $\omega(r)$ (the so-called intramolecular correlation function). We assume that the polymer chains are immersed in a polar solvent with the permittivity $\varepsilon$. We will consider the case of a semi-dilute polymer solution, where, as mentioned above, strong concentration fluctuations take place. The total free energy of
such dipolar polymer solution can be written as a sum of two basic terms:

\[ f = f_p + f_{cor}, \]  

where

\[ f_p = \frac{ck_B T}{N} \left( \ln \left( \frac{c\lambda^3}{N} \right) - 1 \right) + \alpha \left( \frac{B}{b^3} \right)^{3/4} \left( cb^3 \right)^{9/4} k_B T \]  

is the free energy of the basic polymer system – the semi-dilute solution of flexible chains without the electrostatic interactions – with the second virial coefficient \( B \), related to the repulsive interaction of the monomeric units, bond length of the chain \( b \), degree of polymerization \( N \gg 1 \), concentration of monomeric units \( c \), and numerical factor \( \alpha = \frac{32\pi}{243(16\pi/9)^{1/4}} \). The first term in the right hand side of eq. (2) is the free energy of ideal gas of the polymer coils (\( \lambda \) is the de Broglie thermal wavelength of the chain), whereas the second term is the free energy of the semi-dilute polymer solution. The correlation contribution to the free energy density within the RPA has the form

\[ f_{cor} = \frac{k_B T}{2} \int \frac{dk}{(2\pi)^3} \left( \ln \left( 1 + \frac{\kappa^2(k)}{k^2} \right) - \frac{\kappa^2(k)}{k^2} \right), \]  

where the screening function, which was first calculated by two of us in paper in the context

Figure 1. Schematic representation of the semi-dilute dipolar polymer solution model.
of the dipolar polymeric gel theory, takes the form

$$\kappa^2(k) = \kappa_D^2(1 - \omega(k)) \left(1 + \frac{1}{2}g(k)(1 - \omega(k))\right),$$  \hspace{1cm} (4)

where $$\kappa_D = \frac{1}{r_D^{-1}} = (2q^2c/\varepsilon k_B T)^{1/2}$$ is the inverse Debye length, attributed to the charged groups on the monomeric units and $$\omega(k) = \int d\mathbf{r} e^{-ik\mathbf{r}} \omega(\mathbf{r})$$ is the Fourier-image of the probability distribution function. The Fourier-image of the pair correlation function of the present basic polymer system is

$$g(k) \simeq \frac{c\xi^3}{1 + k^2\xi^2},$$  \hspace{1cm} (5)

where $$\xi$$ is the effective size of the polymeric blob (correlation length). Then, choosing the function $$\omega(k) = (1 + k^2l^2/6)^{-1}$$ which quite accurately mimics the case of the stiff dipole of length $$l$$ allowing us at the same time to obtain the analytical results, we arrive at

$$f_{\text{cor}} = -\frac{k_B T}{l^3} \sigma(y) - \frac{k_B T}{l^3} \delta(y, \gamma),$$  \hspace{1cm} (6)

where the first term describes the free energy of the disconnected dipolar monomers, whereas the second one describes the interplay of the chain connectivity, the excluded volume interactions, and the electrostatic correlations of the zwitterionic monomeric units; the auxiliary function $$\sigma(y) = \sqrt{6}(4\pi)^{-1} (2(1 + y)^{3/2} - 2 - 3y)$$ is expressed in terms of the electrostatic coupling parameter $$y = q^2l^2c/(3\varepsilon k_B T)$$. Using the expression obtained in ref. for the correlation length $$\xi = (9/16\pi)^{3/4}(B/b^3)^{-1/4}(cb^3)^{-3/4}b$$, we obtain $$\gamma = gl^2/(12\xi^2) = (9/16\pi)^{3/4}(cb^3)^{1/4}(B/b^3)^{-1/4}(l/b)^2/12$$. Taking into account that $$cb^3 \ll 1$$ for the semi-dilute polymer solution while for real macromolecules the relations $$B/b^3 \sim 1, l/b \sim 1$$ are satisfied, we conclude that $$\gamma$$ is very small ($$\gamma \ll 1$$). Thus, the cumbersome auxiliary function $$\delta(y, \gamma)$$ can be calculated in the first order approximation in $$\gamma$$ as follows

$$\delta(y, \gamma) = 3\sqrt{6}(4\pi)^{-1} (1 + y/2 - \sqrt{1 + y}) \gamma + O(\gamma^2).$$

Thus, in the semi-dilute solution regime this contribution is negligibly small, so that in what follows it will be safely omitted. Note that the extremely weak influence of the chain connectivity on the electrostatic interactions of the dipolar monomeric units is in accordance with the well-known Lifshitz hypothesis of ”disconnected” monomers. More specifically, within this hypothesis we can consider the contribution of the short-range attractive interactions between the monomeric units (dipole-dipole interaction in our case) to the total free energy to be the same as for the fluid of disconnected monomers.
To analyse the phase behavior of the polymer solution, let us calculate the osmotic pressure by the relation 
\[ \Pi = c \frac{\partial f}{\partial c} - f. \]

The result takes the form

\[ \Pi = \frac{c k_B T}{N} + \frac{5}{4} \alpha \left( \frac{B}{b^3} \right)^{3/4} c b^3 \left( \frac{k_B T}{b^3} \right)^{9/4} \sigma_1(y), \]

(7)

where \( \sigma_1(y) = \sqrt{6} (2\pi)^{-1} \left( 3y(1+y)^{1/2} / 2 - (1+y)^{3/2} + 1 \right). \)

Before numerical calculations, let us analyse the limiting regimes following from eq. (7). In the case of very long chains \((N \to \infty)\) and at \(y \ll 1\) or \(r_D \gg l\), we obtain

\[ \Pi \approx \frac{40\pi}{243} \left( \frac{16\pi}{9} \right)^{1/4} \left( \frac{B}{b^3} \right)^{3/4} \left( \frac{kb^3 b^3}{g^4} \right)^{9/4} \sigma_1(y). \]

(8)

In this case, the electrostatic correlations manifest themselves as effective interactions of point-like freely rotating dipoles – Keesom interactions. If the Keesom interaction is sufficiently strong, the second term in the right hand side of eq. (8) becomes bigger than the first one, leading to the coacervate formation. The condition of coacervate formation at \(N \gg 1\), \(\Pi = 0\), results in the following scaling law for the polymer concentration in the coacervate phase

\[ cb^3 \approx \frac{9}{16\pi} \left( \frac{81\sqrt{6}}{40} \right)^4 \left( \frac{l^3}{b} \right)^4 \left( \frac{B}{b^3} \right)^{-3} \left( \frac{l_B}{b} \right)^8, \]

(9)

where we have introduced the standard Bjerrum length, \(l_B = q^2 / (4\pi\varepsilon k_B T)\). Note that in this regime the polymer concentration in the coacervate is extremely thermally sensitive \((c \sim T^{-8})\). Such thermoresponsiveness of the coacervate is the result of the superposition of two physical effects: strong concentration fluctuations and Keesom dipole-dipole attractive interactions of the polar monomeric units. It is interesting to note that in this 'Keesom' regime, the polymer concentration in the coacervate also strongly depends on the dipole length and the second virial coefficient. In the opposite regime, \(y \gg 1\) or \(r_D \ll l\), we obtain

\[ \Pi \approx \frac{40\pi}{243} \left( \frac{16\pi}{9} \right)^{1/4} \left( \frac{B}{b^3} \right)^{3/4} \left( \frac{kb^3 b^3}{g^4} \right)^{9/4} \frac{k_B T}{24\pi r_D^2}. \]

(10)

In this case, the electrostatic interactions of the charged groups are the same as in poly-electrolyte solutions within the Voorn-Overbeek theory, which in turn is based on the Debye-Hueckel theory of dilute simple electrolyte solutions. Thus, in this case, we obtain the following scaling law for the polymer concentration in the coacervate

\[ cb^3 \approx \left( \frac{9}{4\pi^3} \right)^{1/3} \left( \frac{81}{20} \right)^{4/3} \left( \frac{b}{l^3} \right)^{-1} \left( \frac{l_B}{b} \right)^2. \]

(11)
Figure 2. Typical dependences of reduced osmotic pressure, $\Pi b^3/k_B T$ on the polymer concentration in the case of $N \gg 1$ corresponding to a homogeneous polymer solution (red solid line) and a polymer solution, where the coacervate phase coexists with pure solvent (blue dash-dotted line). The polymer concentration in the coacervate phase corresponds to the value at which $\Pi = 0$. The data are shown for $l/b = 1$, $B/b^3 = 1$.

We would like to note that the polymer concentration in the coacervate in this 'Debye' regime depends on the temperature much less ($c \sim T^{-2}$) than in the Keesom regime discussed above and does not depend on the dipole length.

Fig. 2 shows two typical dependences of the reduced osmotic pressure $\Pi b^3/(k_B T)$ on the reduced polymer concentration $cb^3$ corresponding to a homogeneous polymer solution ($l_B/b = 0.1$) and a polymer solution containing a coacervate phase coexisting with pure solvent ($l_B/b = 0.4$). The polymer concentrations at which $\Pi < 0$ and $\partial\Pi/\partial c < 0$ correspond
to the fully unstable states, at which $\Pi < 0$, and $\partial \Pi / \partial c > 0$ – to metastable states, and the equilibrium states of the coacervate phase are determined by the conditions $\Pi \geq 0$ and $\partial \Pi / \partial c > 0$. The latter condition allows us to calculate the equilibrium polymer concentration in the coacervate phase, $c_b^3$, as a function of electrostatic 'strength', $l_B/b$. Such a dependence is shown in the logarithmic scale in Fig. 3. As is seen, there are two qualitatively different Keesom and Debye regimes (discussed above) corresponding to $c_b^3 \sim (l_B/b)^8$ and $c_b^3 \sim (l_B/b)^2$ (see eqs. (9) and (11)), respectively, and a crossover between them. We would like to note that the chain connectivity effect on the electrostatic correlation contribution to the total free energy remains negligibly small even for $c_b^3 \sim 1, l_B/b \sim 1$ (in the crossover region and Debye regime) and, thus, can be neglected. Now we would like to discuss the parameters of real polymers, for which one can expect to observe coacervation experimentally. For instance, the dipole length of zwitterionic polymers can reach $l \simeq 0.5 - 0.7 \, \text{nm}$ (see, for instance, 16). Assuming that $b \simeq 1 \, \text{nm}$ and $B/b^3 \simeq 1$ we obtain quite reasonable concentration in the coacervate phase $c \simeq 50 \, \text{mM} - 0.15 \, \text{M}$ for an aqueous solution at $T = 300 \, K$ ($\varepsilon = 78\varepsilon_0$, $\varepsilon_0$ is the vacuum permittivity). Such an estimate should be considered rather rough because the second virial coefficient, $B$, and the segment length, $b$, for real polybetaines with complex molecular monomeric units must be clarified by experimental data treatment. Nevertheless, these estimates can be used as the starting point for possible experimental validation of the polyzwitterionic coacervate thermoresponsiveness discussed above.

In conclusion, taking into account strong polymer concentration fluctuations, excluded volume interactions, and electrostatic correlations of charged groups of dipolar monomeric units of the polymer chains within the RPA, we have developed a molecular theory of liquid-liquid phase separation in semi-dilute dipolar polymer solutions. We have established that the interplay of concentration fluctuations in the semi-dilute solution regime and electrostatic correlations can result in two qualitatively different coacervation regimes. In the first Keesom regime, where the electrostatic correlations manifest themselves as an effective Keesom attraction of point-like dipoles, the polymer concentration in the coacervate phase is inversely proportional to the eighth power of temperature, while in the second Debye regime, where the electrostatic correlations are described by the Debye-Hueckel limiting law, the polymer concentration is inversely proportional to the temperature squared. We have also found the region of crossover between these regimes. The predicted high thermoresponsiveness of the coacervate polymer concentration (or the size of the coacervate droplets) can be relevant to
aqueous solutions of zwitterionic polymers in terms of purification of macromolecular solutions through the uptake of the target macromolecules (such as intact liposomes, extracellular vesicles, and nanoalgosomes\textsuperscript{25}) due to their affinity to the coacervate droplets.

Acknowledgements. YAB dedicates this article to the memory of Igor Ya. Erukhimovich, an outstanding theoretical physicist who had great influence on him. YAB and NNK thank the Russian Science Foundation (Grant No. 22-13-00257) for financial support.

Figure 3. Polymer concentration in coacervate phase as a function of electrostatic ‘strength’, $l_B/b$, calculated from the condition $\Pi = 0$ illustrating the two coacervation regimes described by eq. (9) (blue dashed line) and (11) (green dash-dotted line) and crossover between them (red solid line). The data are shown for $l/b = 1, B/b^3 = 2$.
REFERENCES

1. Xiao X, Jia L, Huang J, Lin Y, Qiao Y. Small Amphiphile-Based Coacervation. Chemistry–An Asian Journal. 2022.
2. Oparin AI. The origin of life on the earth. 3rd Ed. Oliver Boyd, Edinburgh London; 1957.
3. Brangwynne CP, Eckmann CR, Courson DS, Rybarska A, Hoege C, Gharakhani J, et al. Germline P granules are liquid droplets that localize by controlled dissolution/condensation. Science. 2009;324(5935):1729-32.
4. Sing CE, Perry SL. Recent progress in the science of complex coacervation. Soft Matter. 2020;16(12):2885-914.
5. Borue VY, Erukhimovich IY. A statistical theory of globular polyelectrolyte complexes. Macromolecules. 1990;23(15):3625-32.
6. Kudlay A, Ermoshkin AV, Olvera de La Cruz M. Complexation of oppositely charged polyelectrolytes: effect of ion pair formation. Macromolecules. 2004;37(24):9231-41.
7. Ermoshkin A, Olvera de La Cruz M. A modified random phase approximation of polyelectrolyte solutions. Macromolecules. 2003;36(20):7824-32.
8. Budkov YA, Kolesnikov A, Georgi N, Nogovitsyn E, Kiselev M. A new equation of state of a flexible-chain polyelectrolyte solution: Phase equilibria and osmotic pressure in the salt-free case. The Journal of Chemical Physics. 2015;142(17):174901.
9. Shen K, Wang ZG. Polyelectrolyte chain structure and solution phase behavior. Macromolecules. 2018;51(5):1706-17.
10. Rumyantsev AM, Johner A, Tirrell MV, de Pablo JJ. Unifying weak and strong charge correlations within the random phase approximation: Polyampholytes of various sequences. Macromolecules. 2022;55(14):6260-74.
11. Lin YH, Song J, Forman-Kay JD, Chan HS. Random-phase-approximation theory for sequence-dependent, biologically functional liquid–liquid phase separation of intrinsically disordered proteins. Journal of Molecular Liquids. 2017;228:176-93.
12. McCarty J, Delaney KT, Danielsen SP, Fredrickson GH, Shea JE. Complete phase diagram for liquid–liquid phase separation of intrinsically disordered proteins. The journal of physical chemistry letters. 2019;10(8):1644-52.
13. Martin JM, Li W, Delaney KT, Fredrickson GH. Statistical field theory description of inhomogeneous polarizable soft matter. The Journal of chemical physics. 2016;145(15):154104.
14 Peng X, Liu H, Yin Q, Wu J, Chen P, Zhang G, et al. A zwitterionic gel electrolyte for efficient solid-state supercapacitors. Nature communications. 2016;7(1):1-8.
15 Lei Z, Wu P. Zwitterionic skins with a wide scope of customizable functionalities. ACS nano. 2018;12(12):12860-8.
16 Li M, Zhuang B, Yu J. Functional Zwitterionic Polymers on Surface: Structures and Applications. Chemistry–An Asian Journal. 2020;15(14):2060-75.
17 Gordievskaya YD, Budkov YA, Kramarenko EY. An interplay of electrostatic and excluded volume interactions in the conformational behavior of a dipolar chain: theory and computer simulations. Soft matter. 2018;14(17):3232-5.
18 Cherstvy A. Collapse of Highly Charged Polyelectrolytes Triggered by Attractive Dipole-Dipole and Correlation-Induced Electrostatic Interactions. The Journal of Physical Chemistry B. 2010;114(16):5241-9.
19 Schiessel H, Pincus P. Counterion-condensation-induced collapse of highly charged polyelectrolytes. Macromolecules. 1998;31(22):7953-9.
20 Kumar R, Fredrickson GH. Theory of polyzwitterion conformations. The Journal of Chemical Physics. 2009;131(10):104901.
21 Budkov YA, Kalikin NN, Kolesnikov AL. Molecular theory of the electrostatic collapse of dipolar polymer gels. Chemical Communications. 2021;57(33):3983-6.
22 De Gennes PG. Scaling concepts in polymer physics. Cornell university press; 1979.
23 Adhikari S, Leaf MA, Muthukumar M. Polyelectrolyte complex coacervation by electrostatic dipolar interactions. The Journal of chemical physics. 2018;149(16):163308.
24 Margossian KO, Brown MU, Emrick T, Muthukumar M. Coacervation in polyzwitterion-polyelectrolyte systems and their potential applications for gastrointestinal drug delivery platforms. Nature communications. 2022;13(1):1-11.
25 Paganini C, Capasso Palmiero U, Picciotto S, Molinelli A, Porello I, Adamo G, et al. High-Yield Separation of Extracellular Vesicles Using Programmable Zwitterionic Coacervates. Small. 2022:2204736.
26 Muthukumar M, Edwards S. Extrapolation formulas for polymer solution properties. The Journal of Chemical Physics. 1982;76(5):2720-30.
27 Borue VY, Erukhimovich IY. A statistical theory of weakly charged polyelectrolytes: fluctuations, equation of state and microphase separation. Macromolecules. 1988;21(11):3240-9.
28 Budkov YA. Statistical field theory of ion-molecular solutions. Physical Chemistry Chemical
29 Budkov YA. Nonlocal statistical field theory of dipolar particles in electrolyte solutions. Journal of Physics: Condensed Matter. 2018;30(34):344001.

30 Budkov YA. Statistical theory of fluids with a complex electric structure: Application to solutions of soft-core dipolar particles. Fluid Phase Equilibria. 2019;490:133-40.

31 Lifshitz I, Grosberg AY, Khokhlov A. Some problems of the statistical physics of polymer chains with volume interaction. Reviews of Modern Physics. 1978;50(3):683.

32 Grosberg AY, Khokhlov AR. Statistical physics of macromolecules. Amer Inst of Physics; 1994.

33 Budkov YA, Kalikin N, Kolesnikov A. Polymer chain collapse induced by many-body dipole correlations. The European Physical Journal E. 2017;40(4):47.

34 Overbeek JTG, Voorn M. Phase separation in polyelectrolyte solutions. Theory of complex coacervation. Journal of Cellular and Comparative Physiology. 1957;49(S1):7-26.