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Modeling the depletion effect caused by an addition of polymer to monoclonal antibody solutions

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Abstract

We present a theoretical study of colloidal stability of the model mixtures of monoclonal antibody molecules and non-adsorbing (no polymer-protein attraction) polymers. The antibodies are pictured as an assembly of seven hard spheres assuming a Y-like shape. Polymers present in the mixture are modeled as chain-like molecules having from 32 up to 128 monomers represented as hard spheres. We use Wertheim's thermodynamic perturbation theory to construct the two molecular species and to calculate measurable properties. The calculations are performed in the osmotic ensemble. In view that no direct attractive interaction is present in the model Hamiltonian, we only account for the entropic contribution to the phase equilibrium. We calculate chemical potentials and the equation of state for the model mixture to determine the liquid-liquid part of the phase diagram. We investigate how the critical antibody number density depends on the degree of polymerization and the bead size ratio of the polymer and protein components. The model mixture qualitatively correctly predicts some basic features of real systems. The effects of the model 'protein' geometry, that is the difference in results for the flexible Y-shaped protein versus the rigid spherical one, are also examined.

Keywords

antibodies; phase separation; depletion; polymer

1. Introduction

Monoclonal antibodies (mAb) are the fastest-growing sector in the biopharmaceutical industry [1–3] but there are problems associated with their production, cleaning, and medical application. These complex molecules are prone to form aggregates, which may yield to amorphous precipitate, crystal formation, or the liquid-liquid phase transition [4–11].

Unfortunately the effects of additives, usually aqueous solutions of buffers and polymeric

co-solvents, on the protein aggregation are not well understood. Note that the process of protein aggregation is induced in the downstream processing [12], while this effect is strongly undesirable when pharmaceutical formulations are prepared [13].

It has long been known that colloid or protein precipitation can be induced by the non-adsorbing polymer such as polyethylene glycol (PEG); see for example [14–20]. When PEG or a similar substance is added to the solution, the colloids (or protein molecules) experience a net attraction, which may yield their precipitation. The theory explaining the origin of this effect (in literature also called depletion ‘interaction’) has been suggested by Asakura and Oosawa [14, 15]. For a review of large body of literature, discussing this effect, see for example [21–23].

Recently [24, 25], it has been suggested that depletion effect may serve as an indicator of the ‘colloidal’ stability of antibody solutions under various experimental conditions. Proteins can be destabilized by a temperature decrease, but the liquid–liquid phase separations induced by PEG, can be studied at room temperature, which represents a clear experimental advantage. Conditions of the so-called ‘colloidal’ stability, where proteins remain in their native form [26, 27] are examined in several recent papers [7, 8, 10, 25, 28–31]. Some of these studies use computer simulations to obtain the information about the relevant protein–protein interactions. Computer simulations are very useful in pointing out the details of interaction; while they can, for example, explain why some antibodies have higher viscosity than the others [32], they do not provide an easy way to determine thermodynamic properties and phase diagrams.

Experimental studies prove [33] that stability of mAb solutions depends, besides on the polyethylene glycol concentration, also on the pH of the solution and on the ionic strength of added salt. However, the mechanisms of the two types of interactions—depletion and electrostatic—are very much different. Bonina *et al* [34] examined experimentally the simultaneous effects of the PEG and various salts on the solubility of lysozyme in water. The solubilities were obtained as a function of experimental variables such as protein and electrolyte concentrations, electrolyte type, degree of polymerization of PEG, and pH of the mixture. An important finding of that study was that an addition of PEG does not qualitatively change the lysozyme–electrolyte interaction, including the ion-specific effects. The conclusion was that the two types of interaction can be, at least on the qualitative level, treated independently [34].

In the present contribution we propose a model for the two-component mixture containing non-adsorbing polymer and monoclonal antibody embedded in a continuum representing solvent. In experiments the buffer solutions in water (often with some salts added) are most often used as solvent. In our model both species, polymer and antibody molecules, are assumed to be composed of hard spheres. In other words, direct attractive antibody–antibody interactions, treated for example in [35], are not included in the model. In addition, there is no attractive polymer–polymer or polymer–antibody interaction present. In this way, only the entropic contribution to the free energy of the model mixture is accounted for. This allows us to examine effects of the, so called, depletion ‘interaction’ separately from other interactions that may cause protein precipitation. Theoretical models including both patchy

and isotropic interactions for fluid of spherical particles have been considered recently [36, 37].

The novelty of this work, comparing to similar previous studies, which are treating proteins as hard impenetrable bodies, arises from the effects of the specific antibody shape, treated as tangentially bonded hard-sphere beads and the model antibody flexibility. We are interested how the geometry (shape) of the protein molecule influences parameters of the liquid–liquid phase transition, caused by an addition of the model polymer. For this reason we construct an equivalent (of the same excluded volume) rigid spherical ‘protein’ to numerically investigate effects of the geometry differences. In continuation we first introduce the model of antibody–polymer mixture and present the thermodynamic perturbation theory of Wertheim (sections 2 and 3). These sections are followed by calculations of the liquid–liquid phase equilibrium (section 4). Extensive numerical results in which the influence of model parameters on the liquid–liquid phase diagram is examined, are presented in results and discussion section (section 5). Concluding remarks are summarized at the end of the manuscript (section 6).

2. Modeling the mixture

We consider a mixture of protein molecules mimicking monoclonal antibodies (mAbs) with the number density ρ_s and polymer molecules of the number density ρ_p . Each polymer molecule consists of n_p monomers, here presented by the tangentially bonded hard spheres, shown in figure 1 in green. Molecules modeling antibodies (in figure 1 shown in blue color) are represented by the tangentially bonded hard-sphere beads (see also figure 1 of [35]), forming three-arm star molecules with I_s -bead arms attached to the central sphere. Notice that $I_s = 2$ in our case. Hard-sphere diameters of the beads composing the model protein are σ_s while those composing the polymers are denoted by σ_p . For theoretical description it is convenient to view the model as a complete association limit of the $n_p + n_s$ component hard-sphere associating fluid model with m_{ia}^a sticky sites of the type α ($\alpha = A, B$), randomly placed on the surface of each particle of the type (a, i) forming the molecule. Here $n_s = I_s + 1$, the hard-sphere monomers are denoted by the pair of indices (a, i) , where $a = s, p$ and denote the type of the molecule, and $i = 1, \dots, n_a$ denote the type of the monomer in the molecule a . Following Baxter [38] we have

$$4\pi\bar{f}_{i\alpha j\gamma}^{ab}(r) = K_{i\alpha j\gamma}^{ab} \delta(r - \sigma_{ab}) \quad (1)$$

where $\bar{f}_{i\alpha j\gamma}^{ab}(r)$ is orientation averaged Mayer function for sticky interaction between the sites α and γ located on the particles of the type (a, i) and (b, j) and σ_{ab} is equal to $(\sigma_a + \sigma_b)/2$. It is assumed that this interaction happened only between the sites of different type, belonging to the monomers forming the molecules, i.e.

$$K_{i\alpha j\gamma}^{ab} = (\delta_{\alpha A} \delta_{\gamma B} \delta_{i, j-1} + \delta_{\alpha B} \delta_{\gamma A} \delta_{i, j+1}) \delta_{ab} K. \quad (2)$$

In addition we assume that the number of sites of the type a located on the hard-sphere monomer of species (a, i) , is

$$m_{ia}^a = \delta_{aA}(m_s \delta_{as} + \delta_{ap})\delta_{i1} + \delta_{aB}\delta_{in_a} + (1 - \delta_{i1})(1 - \delta_{in_a}) \quad (3)$$

and the number density of the monomers is

$$\rho_i^a = \delta_{as}(m_s - l_s \delta_{i1})\rho_s + \delta_{ap}\rho_p. \quad (4)$$

Here m_s is the number of arms in the protein molecule, i.e. for the model at hand $m_s = m_{1A}^s = 3$, $\delta_{a\gamma}$, δ_{ij} and δ_{ab} are Kronecker deltas. Notice that according to equation (2) the quantity $K_{i\alpha j\gamma}^{ab}$ assumes value zero for a not equal b , and K if $a = b$. With this choice of parameters the model at hand will be recovered by assuming the infinitely strong attraction ($K \rightarrow \infty$) between these sites. It is worth mentioning that use of the orientation averaged Mayer function in equation (1) introduces an extra feature into the model: the particles describing monoclonal antibodies and polymers possess certain internal flexibility, mimicking that of real molecules. The model molecules composing the mixture are illustrated in figure 1.

3. Thermodynamic perturbation theory of Wertheim

The thermodynamic perturbation theory of Wertheim [39, 40] was used to investigate the phase behavior of the athermal mixtures of large size hard-sphere colloidal particles and small bead size linear chain polymers in several earlier studies [41–43]. We applied the thermodynamic perturbation theory of the first order (TPT1) to study the phase behavior of Y-shaped antibody protein molecules in presence of polymer. Due to the single-bond approximation of this theory, thermodynamic properties of the model depend only on the number of the hard-sphere beads, composing the molecule. This approximation has been for branched polymers tested against the second order perturbation theory [44, 45] (TPT2). The latter approach accounts explicitly for the correlation between three consecutive beads and leads to good agreement with computer simulations [46]. Both versions of theories yielded very similar result for description of the star-like molecules with a small number of arms. Recently we started the Monte Carlo simulations for the neat mAbs fluid modeled in figure 1(b) of [35]. The osmotic pressure results from the TPT1 calculations are in good agreement with our preliminary simulations. We expect for the TPT1 to retain a similar accuracy in case of the polymer-protein mixtures.

According to Wertheim's perturbation theory of associating fluids [39, 40] Helmholtz free energy A of the associating hard-sphere fluid can be written as

$$A = A_{hs} + \Delta A_{assoc}, \quad (5)$$

where A_{hs} is the free energy of the reference hard-sphere fluid and A_{assoc} is contribution to the free energy due to bonding. Here the reference system is represented by the two-component hard-sphere mixture with the densities $\rho_s^{(\text{hs})} = (m_s l_s + 1)\rho_s$, $\rho_p^{(\text{hs})} = n_p \rho_p$ and hard-sphere sizes σ_s , σ_p , respectively. Remember that index p denotes polymer, while s denotes protein species. For A_{hs} we use the expression of Mansoori *et al* [47] and for A_{assoc} we have:

$$\frac{\Delta A_{\text{assoc}}}{k_B T V} = \sum_a \rho_a \sum_{i,\alpha} \left(\ln X_{i\alpha}^a - \frac{1}{2} X_{i\alpha}^a + \frac{1}{2} m_{i\alpha}^a \right), \quad (6)$$

where k_B is Boltzmann's constant, T absolute temperature, and V volume of the system. $X_{i\alpha}^a$ is the fraction of the particles of the type (a, i) , which are not bonded via the site of the type α . This fraction satisfies the following set of the mass action law-type of equations

$$\sigma_a^2 X_{i\alpha}^a \sum_j \rho_j^a \sum_\gamma X_{j\gamma}^a K_{i\alpha j\gamma}^{aa} g_{aa}^{(\text{hs})} + X_{i\alpha}^a - 1 = 0, \quad (7)$$

where $g_{aa}^{(\text{hs})}$ is the contact value of the radial distribution function of hard-sphere reference system. Taking into account the expression for $K_{i\alpha j\gamma}^{ab}$ (equation (2)), the solution of this set of equations can be reduced to the solution of the second order polynomial equation in the form

$$\sigma_a^2 \rho_i^a K g_{aa}^{(\text{hs})} (X_{iA}^a)^2 + [\sigma_a^2 K g_{aa} (\rho_{i+1}^a - \rho_i^a) + 1] X_{iA}^a - 1 = 0. \quad (8)$$

Here $i = 1, \dots, n_a - 1$ and

$$X_{(i+1)B}^a = \left(1 - \sigma_a^2 \rho_i^a K g_{aa}^{(\text{hs})} X_{iA}^a \right)^{-1}. \quad (9)$$

Pressure and chemical potential are obtained using the standard thermodynamic relations, i.e.

$$P = - \left(\frac{\partial A}{\partial V} \right)_{T, N_i^a}, \quad \mu_i^a = \left(\frac{\partial A}{\partial N_i^a} \right)_{T, N_j^a}, \quad (j \neq i). \quad (10)$$

Combining these relations and the expression for Helmholtz free energy (5) at the limit of the complete association ($K \rightarrow \infty$), we obtain

$$\beta\Delta P = \beta\Delta P_{\text{hs}} - m_s l_s \rho_s \frac{\rho_{\text{tot}}^{(\text{hs})}}{g_{ss}^{(\text{hs})}} \frac{\partial g_{ss}^{(\text{hs})}}{\partial \rho_{\text{tot}}^{(\text{hs})}} - (n_p - 1) \rho_p \frac{\rho_{\text{tot}}^{(\text{hs})}}{g_{pp}^{(\text{hs})}} \frac{\partial g_{pp}^{(\text{hs})}}{\partial \rho_{\text{tot}}^{(\text{hs})}}, \quad (11)$$

$$\beta\Delta\mu_s = (1 + l_s m_s) \beta\Delta\mu_s^{(\text{hs})} - l_s m_s \ln g_{ss}^{(\text{hs})} - (1 + l_s m_s) \left[m_s l_s \frac{\rho_s}{g_{ss}^{(\text{hs})}} \frac{\partial g_{ss}^{(\text{hs})}}{\partial \rho_s^{(\text{hs})}} + (n_p - 1) \frac{\rho_p}{g_{pp}^{(\text{hs})}} \frac{\partial g_{pp}^{(\text{hs})}}{\partial \rho_s^{(\text{hs})}} \right] \quad (12)$$

and

$$\beta\Delta\mu_p = n_p \left[\beta\Delta\mu_a^{(\text{hs})} - \frac{n_p - 1}{n_p} \ln g_{pp}^{(\text{hs})} - m_s l_s \frac{\rho_s}{g_{ss}^{(\text{hs})}} \frac{\partial g_{ss}^{(\text{hs})}}{\partial \rho_p^{(\text{hs})}} - (n_p - 1) \frac{\rho_p}{g_{pp}^{(\text{hs})}} \frac{\partial g_{pp}^{(\text{hs})}}{\partial \rho_p^{(\text{hs})}} \right], \quad (13)$$

where P and P_{hs} are the values of the pressure of the actual mixture and of the hard-sphere reference mixture in excess to their ideal gas values. Further, μ_a and $\Delta\mu_a^{(\text{hs})}$ are excess chemical potentials of the molecules in mixture and monomers of hard-sphere reference mixtures with respect to their ideal gas values, while $\rho_{\text{tot}}^{(\text{hs})} = \rho_s^{(\text{hs})} + \rho_p^{(\text{hs})}$. For P_{hs} and $\Delta\mu_a^{(\text{hs})}$ we are using expressions due to Mansoori *et al* [47] and for the contact values of the radial distribution functions $g_{aa}^{(\text{hs})}$ the Percus–Yevick expression. Ideal parts have to be added to obtain complete expressions for these two quantities.

4. Calculations of the phase equilibrium

Mixtures of proteins and polymers may under suitable conditions separate into the (i) ‘liquid’ phase (rich in protein and poor in polymer) and (ii) the ‘gas’ (polymer-rich and protein-poor) phase. To study the phase behavior of the model mixture we use the osmotic equilibrium approach (for illustration see figure 2.1 of [23]). We assume an existence of the protein-free solution, with the packing fraction of the polymer component equal to η_0 . This polymer solution is in equilibrium with the solutions containing protein–polymer mixtures. Due to the osmotic equilibrium conditions, chemical potential of the polymer component assumes the same value in all three compartments. In such situation the difference in pressure between pure polymer solution and protein–polymer mixture is equal to the osmotic pressure $\Pi(\eta_0, \rho_s, \rho_p)$,

$$\Pi(\eta_0, \rho_s, \rho_p) = P(\rho_s, \rho_p) - P_0(\eta_0), \quad (14)$$

where P_0 is the pressure of the pure polymer solution (no protein molecules present). The osmotic pressure is a function of the packing fraction of the pure polymer solution $\eta_0 = \pi n_p \rho_p^{(0)} \sigma_p^3 / 6$ and the number densities of the components ρ_s and ρ_p in the mixture phases. The phase equilibrium conditions for the polymer–protein mixtures can now be formulated in terms of the osmotic pressure P and chemical potential μ_s of proteins (mAb molecules). The equilibrium between the protein-rich (here denoted as ‘liq’) and protein-poor (denoted as ‘gas’) solutions is established when the chemical potential μ_s of the protein component and the osmotic pressure are the same in both solutions:

$$\Pi(\eta_0, \rho_s^{(\text{gas})}, \rho_p^{(\text{gas})}) = \Pi(\eta_0, \rho_s^{(\text{liq})}, \rho_p^{(\text{liq})}), \quad (15)$$

$$\mu_s(\eta_0, \rho_s^{(\text{gas})}, \rho_p^{(\text{gas})}) = \mu_s(\eta_0, \rho_s^{(\text{liq})}, \rho_p^{(\text{liq})}). \quad (16)$$

By solving the set of equations (15) and (16) we obtain number densities of the polymer and protein components in the coexisting phases as a function of η_0 .

5. Results and discussion

Numerical results are presented in reduced units, but in the actual calculations σ_p (polymer bead diameter) was fixed to 0.3 nm.

5.1. Liquid–liquid phase separation

To introduce the results we first present the graphs showing the reduced chemical potential of the model protein, $\beta\mu_s$ and the reduced osmotic pressure $\Pi^* = \Pi\sigma_s^3 / K_B T$ in the mixture as a function of the reduced protein number density ρ_s^* equal to $\rho_s \sigma_s^3$. These results are shown in figure 2.

Notice that in this particular case the phase separation is caused by a non-adsorbing polymer so the crucial parameter is the volume occupied by polymer, η_0 , in the pure polymer solution. The latter solution is in equilibrium (with respect to the polymer component) with the two protein–polymer mixtures. When the protein-rich and protein-poor phases are also in equilibrium in between (above the critical η_0 value) we observe the typical van der Waals loops. The Newton–Raphson method is used to solve the set of equations (15) and (16) and to compute the number densities of polymer and protein components in the two coexisting phases. The packing fraction η_0 is, together with the pertaining reduced chemical potential $\beta\mu_p$, indicated on the graphs.

The resulting three-dimensional phase diagram is shown in figure 3. It is described by η_0 , ρ_s , and ρ_p having three projections: η_0 versus ρ_p^* (green curve), η_0 versus ρ_s^* (blue curve) and ρ_p^* versus ρ_s^* (black curve). The red curve is the sum of these three curves. These separate projections are later shown also in figures 4–6. In this and other plots the continuous lines

present the liquid phase (protein-rich) and the broken lines the gas (protein-poor) phase. Note that for high densities our system may undergo the fluid-solid phase transition. For this reason all the calculations have been terminated at total packing fraction of the system

$$\eta_{\text{tot}} = \pi[(l_s m_s + 1)\sigma_s^3 + n_p \sigma_p^3]/6 \text{ being around } 0.45.$$

Perhaps the most important experimental parameter affecting the phase separation is the degree of polymerization of the polymer component, here denoted by n_p . From theory [14] and experiment [17] it is expected for the onset of the phase separation to depend on the degree polymerization in such a way that longer polymers produce stronger net attractive interaction. This expectation is confirmed in figure 4, where higher degree of polymerization n_p yields lower polymer critical η_0 value. In addition, the critical value of the protein number density ρ_s^* decreases with an increase of n_p . Figure 5 indicates that equilibrium η_0 value depends strongly on the protein to polymer bead size (σ_s/σ_p) ratio. For larger bead-size ratios, smaller packing fraction of the solute η_0 is needed to precipitate the protein. An increase of the σ_s/σ_p ratio, namely, increases the surface area of the model mAb molecule and accordingly yields a somewhat stronger depletion. As we see, also the critical ρ_s^* value decreases with an increasing bead-size ratio.

In figure 6 we present the graph ρ_p^* (reduced number density of polymers present in the polymer-protein mixture) as a function of the reduced protein number density, ρ_s^* . The tie lines, connecting the points being in equilibrium, are shown by the dotted lines. Our calculations apply to three values of the degree of polymerization, equal to: $n_p = 32, 64$, and 128 . The slopes of the tie lines are negative in this representation and become less steep (smaller difference in the ρ_p^* of the coexisting phases) with an increase of the degree of polymerization n_p of the polymer component. The calculations show that critical ρ_s^* and ρ_p^* values decrease by the increasing degree of polymerization n_p of polymer. In figure 7, the influence of the bead-size (σ_s/σ_p) ratio on the ρ_p^* versus ρ_s^* graph is presented. As we see the tie-lines become less steep if σ_s/σ_p ratio decreases.

Figures 6 and 7 contain perhaps the most important results of the study so it would be useful to compare the calculations with experimental data. It is known from literature [48, 49] that correct slopes of the tie lines strongly depend on the protein-protein interaction. Unfortunately, experimental data for the antibody-PEG mixtures showing tie-lines are, to our best knowledge, unavailable so we compared our calculations with the measurements performed on globular proteins. One such example is bovine γ D crystallin [50] and the other bovine serum albumin [51]. In both experimental cases the tie-lines on the ρ_p^* versus ρ_s^* graph have negative slopes in qualitative agreement with our calculations. Annunziata *et al* [50] presented a graph (see their figure 2) in which they simultaneously varied the degree of polymerization and temperature of the measurements, so the effect of the degree of polymerization cannot be explicated from the plots they published. In addition they used PEG oligomers with very low molecular mass.

5.2. Second virial coefficient

The second virial coefficient, B_2 , quantifying the binary solute–solute interaction in dilute solutions, is one of the most important measurable quantities in protein solutions. It is known that value of this parameter can be used as an indicator of the crystallization [52] as also, that low B_2 values are indicative for high viscosity [53, 54]. In our case the coefficient is defined as

$$\frac{\beta\Pi}{\rho_s} = 1 + B_2\rho_s + O(\rho_s^2), \quad (17)$$

and can be obtained from the osmotic pressure equation as explained elsewhere [55]. In theory it is convenient to use reduced (dimensionless) second virial coefficient, here defined as $B_2^* = B_2\sigma_s^3$.

The results for the reduced second virial coefficient are for different values of the model parameters shown in figure 8. The upper panel of this figure presents B_2^* as a function of η_0 at various values of n_p . The bottom panel of the same figure is similar in spirit, but the model parameter here is the bead-size (σ_s/σ_p) ratio. As expected, the reduced second virial coefficient, B_2^* , decreases with an increase of η_0 ; the decrease (destabilization of the solution) is stronger for higher degrees of polymerization. In the lower panel we see how the sizes of the beads composing the components, affect the stability of the mixture of interest. Larger σ_s/σ_p ratio yields less stable solution, as already shown in figure 5. Numerical results are consistent with those presented in the previous subsection.

The trends in protein phase behavior have their origin in the effective protein–protein interaction, which is in turn reflected in the second virial coefficient. The second virial coefficient by definition depends only on temperature, that is in our model only on η_0 . But each value of η_0 in figure 4 as also in figure 5 applies to two equilibrium ρ_s^* values, both associated with the same B_2^* value. Accordingly, it is possible to plot the graph B_2^* versus ρ_s^* . This is a version of the phase diagram, first suggested by Haas and Drenth [56] (H–D graph) and presented in several subsequent papers (for mAbs solutions see, for example, [31]). These graphs are for our model shown in figures 9 and 10, providing an alternative presentation of the results shown in the section 5.1.

5.3. Influence of the shape of the model protein on the phase diagram

While globular proteins can often be approximated as impenetrable spheres or ellipsoidal particles, antibodies, in contrast, are big, flexible and Y-shaped. First in figure 11 we show the part of the phase diagram presenting η_0 as a function of ρ_s^* for two different geometries.

In red we show the result for the model antibody, already presented in figure 4, while in black we show the result for hard spherical protein. The excluded volume of both particles is the same, equal to $7\pi\sigma_s^3/6$. As we see the curves are considerably different, the red one having a smaller η_0 value (approximately 0.015 versus 0.018). This reflects a somewhat

lower stability of the Y-shaped proteins toward the polymer induced precipitation. The effect seem to be a consequence of the larger surface area of the model mAb molecules in comparison with spherical model, what may lead to stronger depletion. Large difference between the results for the two protein geometries is observed in case of the critical density. This value is $\rho_s^* = 0.025$ in case of the model antibody, while for the spherical hard object representing the protein it is around 0.06. The result is consistent with experimental findings: it is known from literature [7, 10] that globular proteins have several times higher critical concentrations than solutions of monoclonal antibodies. Because the excluded volumes of both types of molecules (spherical and Y-shaped) are the same in our model, the difference in ρ_s^* is probably caused by the fact that flexible (Y-shaped) molecules can interpenetrate and this way make denser clusters.

In figure 12 we show ρ_p^* as a function of ρ_s^* , together with the corresponding tie-lines. Again the results for the Y-shaped antibody are shown with red color and those of the spherical model by black. The shapes of both graphs are similar: the tie-lines are having negative slopes for both examples but in the case of the model antibody they are steeper. In addition, the instability region is smaller in case of protein pictured as hard globule. Notice that for globular proteins, such as bovine γ D crystallin [50] and bovine serum albumin [51], negative slopes of the tie lines were found experimentally.

6. Conclusions

In this study we present numerical results for the liquid–liquid phase separation in the model mixture of antibodies and chain-like (polymer) molecules. The model system mimics the conditions in the antibody–polyethylene glycol mixtures. The phase separation is induced by the so-called depletion effect, caused by an addition of the non-adsorbing polymer to antibody solution. Because no direct attractive interaction between the antibody molecules is included in the model Hamiltonian, only the entropic contribution is accounted for in this calculation. With this respect, the calculation serves as the limiting case, describing the situations where direct attractive interactions between the antibody molecules are weak in comparison to the depletion effect. In support of this approximate model we quote experimental results suggesting [34], that depletion and electrostatic interaction can be, at least on the qualitative level, treated independently.

An important novelty of this work in comparison to the previous studies is the fact that the model protein (mAb) molecule has a realistic shape and flexibility. This is in sharp contrast with previous models treating proteins as rigid bodies. In conjunction with our model we use Wertheim's thermodynamic perturbation theory to calculate the liquid–liquid phase separation curves under various conditions. We varied the ratio in the size of the beads composing both components, the degree of polymerization of the polymer (from short oligomers with 32 beads to the longest one with 128 beads), and calculated the liquid–liquid phase diagrams, including the tie-lines. We also calculated the second virial coefficient and constructed the H–D graphs [56].

Unfortunately, there is not much experimental data published for the antibody–polyethylene mixtures that could serve as tests of our calculations. Numerical results indicate that despite its simplicity the model can reproduce qualitatively some important features of the actual mixtures. For example, the model coupled with Wertheim’s thermodynamic perturbation theory correctly predicts the influence of the degree of polymerization on the antibody stability in solution. Further, the tie-lines for the liquid–liquid phase separation graph appear to have correct slopes. The calculations suggest that the bead-size ratio of the two components and the degree of polymerization of the polymer are the main model parameters influencing the solution stability. For proteins pictured as rigid spherical bodies the critical density is considerably larger than for Y-shaped particle as experimentally and theoretically proven before [7, 10]. Calculations presented in this work represent the first step toward more realistic modeling of the antibody-polymer mixtures. Inclusion of other forces, mimicking directional and electrostatic interactions between proteins, is possible and currently a subject of investigation.

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Figure 1. Schematic presentation of the model mAb (blue) and PEG polymer (green) molecules, composing the mixture. In black we show the A-type and in red B-type sites.

Figure 2.

Top panel: the reduced osmotic pressure Π^* as a function of the reduced protein number density ρ_s^* for various values of η_0 . The top curve is supercritical, the middle one (red) applies to the critical η_0 and the bottom one (blue) to conditions where phase separation takes place. The open circles connected by the line denote the equilibrium densities, while the full circles mark the critical conditions. The corresponding $\beta\mu_p$ and η_0 values are given in the graphs; $n_p = 128$ and $\sigma_s/\sigma_p = 7$. Bottom panel: the reduced chemical potential $\beta\mu_s$ as a function of the reduced protein number density ρ_s^* at various values of occupied volume η_0 of the pure polymer compartment. Notation as for the top panel.

Figure 3.

Phase diagram where the principal axes are η_0 , ρ_s^* , and ρ_p^* . Continuous parts of the curves denote the protein-rich and the broken ones the protein-poor phases being in equilibrium; $n_p = 128$ and $\sigma_s/\sigma_p = 7$. Projections η_0 versus ρ_p^* are shown by the green curve, η_0 versus ρ_s^* by the blue curve, and ρ_p^* versus ρ_s^* by the black one.

Figure 4. Phase diagram: η_0 versus ρ_s^* projection at various degrees of polymerization (n_p values). Again the continuous part of the curve denotes the protein-rich and the broken one the protein-poor phase; $\sigma_s/\sigma_p = 7$.

Figure 5.

Phase diagram: η_0 is given as a function of ρ_s^* , for the σ_s/σ_p ratios indicated in the figure; $n_p = 64$. Notation as for the previous figure.

Figure 6.
 ρ_p^* as a function of ρ_s^* at various degrees of polymerization n_p values; $\sigma_s/\sigma_p = 7$. Dotted lines, connecting the protein-poor and protein-rich phases, represent the tie-lines.

Figure 7.

ρ_p^* as a function of ρ_s^* for different values of the σ_s/σ_p ratio; $n_p = 64$. The bead-size ratios are indicated on the graph, other as for figure 6.

Figure 8. B_2^* as a function of η_0 for different values of the degree of polymerization n_p ($\sigma_s/\sigma_p = 7$, top panel) and the σ_p/σ_s ratio ($n_p = 64$, bottom panel). The values of these parameters are indicated on the graph.

Figure 9.

A version of the H–D phase diagram [56]: B_2^* as a function of ρ_s^* for the degrees of polymerization indicated on the graph; $\sigma_s/\sigma_p = 7$.

Figure 10.

The same as for figure 9 for different σ_s/σ_p ratios as denoted on the graph; $n_p = 64$.

Figure 11.

Phase diagram η_0 versus ρ_s^* presented for two different protein geometries. In red we show the result for Y-shaped antibody molecule and in black the result for the spherical molecule having the same excluded volume. η_p value is 128 and $\sigma_s/\sigma_p = 7$ in this case. Again the continuous part of the curve denotes the protein-rich and the broken one the protein-poor phase.

Figure 12.
 ρ_p^* as a function of ρ_s^* at n_p and σ_s/σ_p as above. The color code as for figure 11. Dotted lines, connecting the protein-poor and protein-rich phases, are the tie-lines.