Colloid-Polymer Mixtures in the Protein Limit

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We computed the phase-separation behavior and effective interactions of colloid-polymer mixtures in the "protein limit," where the polymer radius of gyration is much larger than the colloid radius. For ideal polymers, the critical colloidal packing fraction tends to zero, whereas for interacting polymers in a good solvent the behavior is governed by a universal binodal, implying a constant critical colloid packing fraction. In both systems the depletion interaction is not well described by effective pair potentials but requires the incorporation of many-body contributions.

Adding polymers to suspensions of micro- and nano-particles induces depletion interactions that profoundly affect their physical properties. This phenomenon has important scientific and (bio)technological applications. Polymers such as polyethylene glycol are routinely added to protein solutions to enable protein crystallization [1,2], a poorly understood process and of great importance in structural biology [3]. In cell biology depletion interactions are key in the process of macromolecular crowding [4]. Food and paint production are among the industrial sectors where depletion phenomena play a role.

In this Letter we focus on mixtures of hard sphere (HS) colloids with a radius R_c and nonadsorbing polymers with a radius of gyration R_g, in the regime where $q = R_g/R_c > 1$. This is often called the nanoparticle or protein limit, because in practice small particles such as proteins or micelles are needed to achieve large size-ratios q (of course, HS's are a very crude model for proteins). Whereas the opposite "colloid limit" ($q \lesssim 1$) has been well studied, the physics in the protein limit is less established. This imbalance is partially due to the lack of well-characterized experimental model systems for the protein limit and partially to a poor theoretical understanding. The colloid limit can be well described within the framework of effective depletion pair potentials [5,6], in contrast to the protein limit, where the interactions cannot be reduced to a pairwise form [7,8]. Nevertheless, for biological and industrial applications, this regime is at least as important as the colloid limit.

One of the first theoretical treatments of colloid-polymer mixtures in the protein limit was by de Gennes [9], who showed that the insertion free-energy $F_c^{(1)}$ of a single hard, nonadsorbing sphere into an athermal polymer solution scales as

$$\beta F_c^{(1)} \sim (R_c/\xi)^{3-1/\nu} \qquad (1)$$

when $R_c < \xi$, with the polymer correlation length $\xi(\phi_p) \sim R_g \phi_p^{-\nu/(3\nu-1)} \approx R_g \phi_p^{-0.77}$ [10]. Here, $\beta = 1/k_BT$ is the reciprocal temperature, $\nu \approx 0.59$ is the Flory exponent, and $\phi_p = \rho_p \frac{4}{3}\pi R_g^3$ is the polymer vol-ume fraction for a polymer number density ρ_p, so that $\phi_p \approx 1$ at the crossover from a dilute to a semidilute solution [10]. The prefactors can be calculated by renormalization group (RG) theory [11], yielding $\beta F_c^{(1)} \approx 4.39\phi_p q^{-1.3}$, which has been verified by computer simulations for large q [12]. Based on this description of $F_c^{(1)}$, de Gennes [10] and Odijk [8] predicted extensive misci-bility for colloid-polymer mixtures in the large q limit if $R_c < \xi$. However, it is well known that protein-polymer mixtures do phase separate [13,14]. Recently, Odijk *et al.* [15] suggested that a poor solvent could facilitate phase separation. Sear [16] altered the form of $F_c^{(1)}$ to include effects when $R_c \gg \xi$, and also predicted phase separation with a truncated virial theory. The same author recently proposed an alternative theory [17] where the colloids induce depletion attractions between the polymers, lead-ing to a poorer effective solvent and eventually phase separation. Mean-field cell model calculations also pre-dict demixing [18]. Another promising approach uses integral equation techniques [19] to predict spinodal curves and critical points. However, all these theories suffer from several uncontrolled approximations leading to different predictions for the causes and properties of the phase separation. To clarify this situation, we per-formed computer simulations with as few simplifying assumptions as possible, on which we report in this Letter.

We have recently used a coarse-graining technique [20] to study the colloid limit, and found quantitative agree-ment with experimental fluid-fluid binodals [21], and significant qualitative differences between interacting (IP) and noninteracting (NIP) polymers. Here, we study the same athermal model of HS colloids and nonadsorb-ing polymers in the protein limit, and calculate, for the first time, the full fluid-fluid binodals by direct simula-tion. The results for the IP and NIP show even larger qualitative differences, and many-body depletion inter-actions must be invoked to understand the phase behavior.

The simulation model consists of polymers on a simple cubic lattice mixed with HS colloids. The interacting

polymers in a good solvent are modeled as self-avoiding walks (SAW) of length L, which have a radius of gyration $R_g \sim L^\nu$. The noninteracting polymers are modeled as random walks, for which $R_g \sim L^{0.5}$. In both models there is an excluded-volume interaction between the colloidal HS and the polymer segments. The simulations were performed on a D^3 lattice with periodic boundary conditions, where $D = 48$ and $D = 100$ for the NIP and IP system, respectively. Throughout this Letter, we use the lattice spacing as the unit of length. For the NIP the colloidal HS diameter was $\sigma_c = 5.5$ and the polymer length was $L = 50, 100, 200,$ and 500, corresponding to $q = 1.03, 1.45, 2.05,$ and 3.2, respectively. For the IP $L = 2000$, and $\sigma_c = 10, 14,$ and 20, yielding $q = 3.86, 5.58,$ and 7.78, respectively. Colloidal positions have continuous values, but when we calculated the interaction between colloid and polymer the colloids were shifted such that they occupied a constant number of lattice sites to prevent spurious attractive positions for single colloids (other lattice effects, although unavoidable, are expected to be small). Thermodynamic state points were calculated in the grand-canonical ensemble, i.e., at fixed volume V, colloid chemical potential μ_c, and polymer chemical potential μ_p using Monte Carlo (MC) techniques. The NIP were sampled using an (exact) lattice propagation method [22,23], while the IP configurations were generated using translation, pivot moves, and configurational bias MC [24] in an expanded ensemble to facilitate insertion of long chains [25]. Typical simulation lengths were 10^9 Monte Carlo moves per state point. In order to determine the liquid-liquid binodals we first estimated the coexistence line by scanning a series of μ_c for several values of μ_p and locate the μ_c for which a sudden density change occurred. Subsequently 8–10 (μ_p, μ_c) coexistence state points were simulated simultaneously using parallel tempering [25]. When the estimated coexistence points are sufficiently close to the true binodal and to each other, and near the critical point, this scheme results in proper ergodic sampling of both phases. If necessary, the chemical potentials were adjusted towards coexistence. We used the multiple histogram reweighting [25,26] technique to determine the precise location of the (μ_c, μ_p) coexistence line, and the phase boundaries in the (ϕ_c, ϕ_p) plane, where $\phi_c = \rho_c \frac{4}{3}\pi R_c^3$ is the colloid volume fraction, with ρ_c the colloid number density.

Figures 1 and 2 contrast the calculated phase diagrams for NIP and IP for several size-ratios q. First, we note that both models show extensive immiscibility, in agreement with experiment [14]. Second, the two systems exhibit striking differences: for the NIP, the critical colloid volume fraction ϕ_c^{crit} tends to zero with increasing size-ratio q, while the IP exhibit a nearly constant value of ϕ_c^{crit}. For both systems the critical polymer concentration ϕ_p^{crit} increases with increasing q. The phase separation occurs well into the semidilute regime for the IP, again in qualitative agreement with experiment [13]. Structural proper-
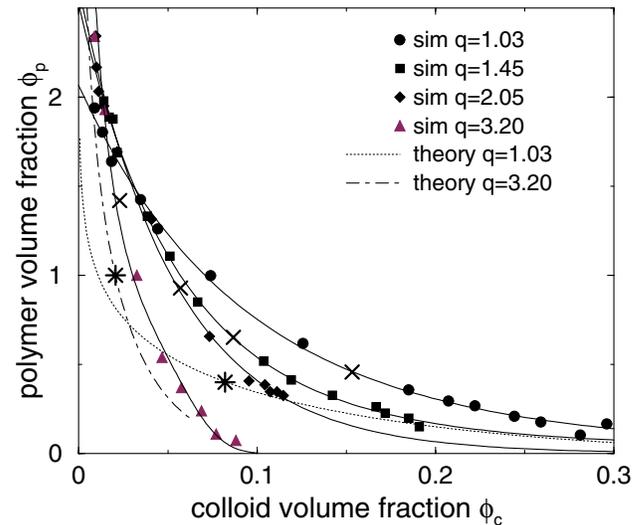
FIG. 1 (color online). Fluid-fluid binodals for a mixture of noninteracting polymers and HS colloids with different size-ratios q, in the (ϕ_c, ϕ_p) plane. The polymer volume fraction is defined as $\phi_p = \rho_p \frac{4}{3}\pi R_g^3$, and can thus be higher than 1. Crosses indicate the estimated critical point, obtained by extrapolating the calculated phase boundaries. The full lines are a guide to the eye. Dashed lines denote the simple theory described in the text, with stars marking the critical points.

ties of semidilute polymer solutions are independent of polymer length, being instead determined by the correlation length ξ, which is a function of the monomer density $c = L\rho_p$. This suggests analyzing the phase
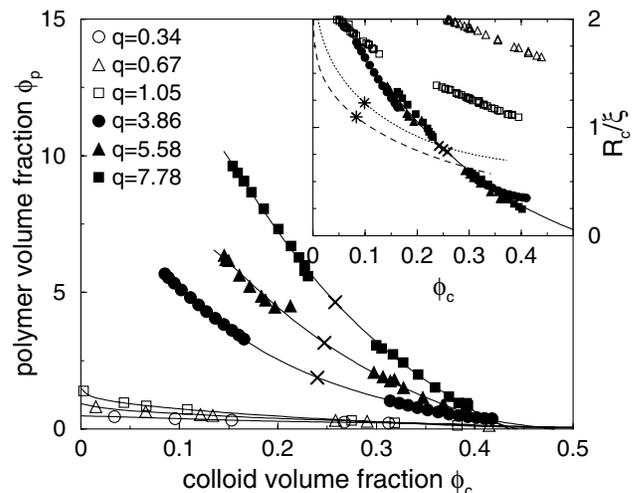
FIG. 2. Fluid-fluid binodals for a mixture of interacting polymers and HS colloids at different size-ratios q. Filled symbols are direct simulation data. The open symbols are the colloid limit ($q \lesssim 1$) results from Ref. [21]. Solid lines are a guide to the eye. Inset: The same binodals plotted in a reduced polymer density representation. The dotted curve corresponds to a simple theory for the universal binodal when polymers are in a good solvent while the dashed line is for polymers in a poorer solvent. Crosses and stars as in Fig. 1.

behavior of the polymer-HS mixture in terms of the ratio R_c/ξ [16]. Indeed, when the phase lines in Fig. 2 are rescaled with an accurate prescription for $\xi(\rho_p)$ [12], the binodals nearly collapse onto a "universal binodal," as shown in the inset of Fig. 2. This explains why the critical colloid packing fraction is nearly constant in the simulations. Similarly, ϕ_p^{crit} scales as $\phi_p^{\text{crit}} \sim q^{(3\nu-1)/\nu} \approx q^{1.3}$. For comparison, we have also included results for $q \lesssim 1$ from Ref. [21] in Fig. 2. These results do not exhibit the same scaling behavior, since they are not in the semi-dilute regime.

The differences between NIP and IP phase behavior can be rationalized with some simple theories. Consider a Helmholtz free-energy F of the form $\beta F/V = f = f_c^{\text{HS}} + f_p + f_{cp}$. Here, the HS free-energy f_c^{HS} is given by the accurate Carnahan-Starling expression [27], and the polymer free-energy f_p for either IP or NIP solutions is well understood [10]. The contribution due to the HS-polymer interactions f_{cp} is nontrivial. A first approximation truncates after the second cross-virial coefficient, yielding $f_{cp} \sim \rho_c F_c^{(1)}$. For NIP the insertion free-energy $F_c^{(1)}$ is exactly known [11], so that f_{cp} takes the form $f_{cp}^{id} = \rho_p \phi_c [1 + (6q/\sqrt{\pi}) + 3q^2] \equiv \rho_p \phi_c \hat{b}_{cp}$, which defines the reduced cross-virial coefficient $\hat{b}_{cp}$. Since f_{cp}^{id} grows with increasing q, immiscibility sets in at lower colloid packing fraction ϕ_c. The theory can be improved by realizing that the polymers exist only in the free volume left by the colloids [28]. Simply taking this free volume to be $1 - \phi_c$ is an adequate first approximation for the protein limit. The trends for the binodal lines calculated from this simple theory, shown in Fig. 1, agree qualitatively with the simulations. For example, the critical point shifts to smaller ϕ_c and larger ϕ_p for increasing q, and the binodal lines cross at a low ϕ_c. For computational reasons the simulations only go up to $q = 3.2$ and we expect better quantitative agreement for larger q since ϕ_c^{crit} decreases so that the second-virial theory should become more accurate. In the $q \to \infty$ limit, this theory yields $\phi_c^{\text{crit}} \to 1/\hat{b}_{cp} \sim 1/(3q^2)$, and $\phi_p^{\text{crit}} = q^3/\hat{b}_{cp} \sim q/3$. Note that in the same limit, the penetrable sphere or Asakura-Oosawa model [28] scales somewhat differently: $\phi_c^{\text{crit}} \to 1/q^3$ and $\phi_p^{\text{crit}} \to 1$. Sear [7] already pointed out the $\phi_c^{\text{crit}} \to 0$ behavior using a slightly different prescription for the free volume than we employ here.

In the IP case, f_{cp} is more difficult to estimate, even for a second cross-virial theory. The $R_c \ll \xi$ limit is given by Eq. (1) with the prefactors from RG theory. For $R_c \gtrsim \xi$ we have previously shown that $F_c^{(1)}$ is given by $F_c^{(1)} = \frac{4}{3}\pi R_c^3 \Pi + 6\pi R_c^2 \gamma_s$ [12], where the polymer osmotic pressure $\Pi \sim \xi^{-3}$ is well known [10]. However, since Eq. (1) is essentially a surface (depletion layer) contribution, we use a simple approximate second cross-virial term $f_{cp} = \rho_c[\beta \Pi(\rho)\frac{4}{3}\pi R_c^3 + 4.39\phi_p q^{-1.3}]$, which reduces to the correct form in both the $R_c \ll \xi$ and the $R_c \gg \xi$ limit. (Obviously this approach, which resembles the more schematic theory of Sear [16], could be improved.) As

with our treatment of NIP, we take the effect of the colloid excluded-volume into account by computing the polymer densities in the free volume fraction $1 - \phi_c$ (see Ref. [29] for a complimentary approach). The theoretical binodals were calculated using accurate expressions for ξ and Π [12] and are compared with the simulation results in Fig. 2, in the R_c/ξ versus ϕ_c plane. The qualitative agreement suggests that we can also use this theory to estimate the effect of a poorer solvent on the binodals. Following Ref. [15], we alter the scaling of ξ to $\xi \sim \phi_p^{-\delta/3}$ so that $\Pi \sim \xi^{-3} \sim \rho_p \phi_p^{\delta-1}$. Interestingly, Fig. 2 shows that using $\delta \sim 1.5$ instead of the appropriate exponent for polymers in a good solvent ($\delta \sim 2.3$), does not result in important differences in the binodals. Of course, the differences will appear larger in the (ϕ_c, ϕ_p) plane due to the different scaling of ξ. One must keep in mind, however, that these predictions follow from a simple scaling theory and qualitatively different behavior may emerge when one approaches the θ point (where $\delta = 1$).

To illustrate the many-body nature of the depletion interaction we estimated the phase behavior by approximating the system by colloids interacting via pairwise effective potentials. We computed the effective pair interaction $v(r)$ between two colloids in a bath of IP's, by $\beta v(r) = -\ln g(r)$ for $\rho_c \to 0$. The colloid radial distribution function $g(r)$ was estimated by measuring the insertion probability of a HS at a distance r from a second fixed HS in a SAW polymer solution using the above MC techniques. Results for a single size-ratio $q = 7.78$ as a function of ϕ_p are shown in Fig. 3. Several features are similar to the colloid limit [30]: the range shortens and
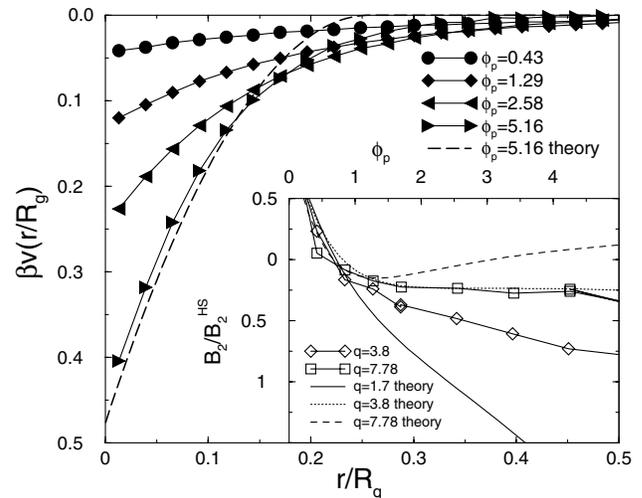
FIG. 3. Effective colloid-colloid pair potentials induced by interacting polymers for $q = 7.78$. Theoretical lines from Ref. [30]. Inset: Reduced second osmotic virial coefficient $B_2^* = B_2/[(16/3)\pi R_c^3]$ as a function polymer densities for several size ratios.

the well depth increases with increasing ϕ_p. Interestingly, our simple depletion potential [30], derived for the colloidal limit, also works semiquantitatively in this regime. A good measure for the attractive strength of effective pair potentials is given by the second osmotic virial coefficient [27], shown in Fig. 3. The saturation of B_2^* for larger q is an interesting qualitative feature: apparently the shortening of the range compensates the deepening of the attraction, so that the total cohesion does not increase with increasing ϕ_p, something also found in RG [11] and integral equation calculations [19]. For pairwise interacting systems, phase separation typically sets in when $B_2^* \lesssim -1.5$ [31]. Here, the saturation of B_2^* suggests that for large q the pair interactions do not provide enough cohesion to explain the phase separation. We arrive at the same conclusions with simple mean-field theories [27], which should be relatively trustworthy given the long range of the pair potentials. Obviously, for $q \gg 1$ a pair-level description is not sufficient, and many-body interactions must be invoked.

For the NIP, one might also expect many-body interactions to be important for large q. A good approximation to the pair potentials exists [23,30], from which the second-virial coefficients at the calculated critical points follow: $B_2^*(q = 1.03) \approx -13.1$; $B_2^*(q = 1.45) \approx -16.4$; $B_2^*(q = 2.05) \approx -22.7$. Even though the actual critical ϕ_c's are very low, so that a second-virial description might be thought to be sufficient, the analysis above shows that for NIP the pair interactions provide too much cohesion, *opposite* to the IP case. Clearly, many-body interactions must also be invoked to describe the phase behavior correctly, as suggested by other authors [7,8,19,23,32].

In conclusion, we have shown by computer simulations that a mixture of polymers and nonadsorbing HS colloids shows extensive immiscibility in the protein limit, where the polymer-colloid size-ratio $q \gg 1$. For IP the phase behavior is dictated by a universal binodal in the semidilute regime. For NIP, the colloid packing fraction tends to zero for increasing polymer length. In contrast to the better studied colloid limit, pair interactions are not sufficient to rationalize the phase behavior. We hope that future experiments on HS colloids with nonadsorbing polymer will test these predictions. Future work might include extensions to nonspherical particles, poor solvents, and adsorbing systems.

[1] A. M. Kulkarni *et al.*, Phys. Rev. Lett. **83**, 4554 (1999); J. Chem. Phys. **113**, 9863 (2000).
[2] S. Tanaka and M. Ataka, J. Chem. Phys. **117**, 3504 (2002).
[3] S. D. Durbin and G. Feher, Annu. Rev. Phys. Chem. **47**, 171 (1996).
[4] S. B. Zimmerman and A. P. Minton, Annu. Rev. Biophys. Biomol. Struct. **22**, 27 (1993).
[5] S. Asakura and F. Oosawa, J. Chem. Phys. **22**, 1255 (1954).
[6] See W. C. K. Poon, J. Phys. Condens. Matter **14**, R859 (2002) for a recent review.
[7] R. P. Sear, Phys. Rev. Lett. **86**, 4696 (2001).
[8] T. Odijk, J. Chem. Phys. **106**, 3402 (1997).
[9] P. G. de Gennes, C. R. Acad. Sci., Paris B **288**, 359 (1979).
[10] P. G de Gennes, *Scaling Concepts in Polymer Physics* (Cornell University Press, Ithaca, New York, 1979).
[11] E. Eisenriegler *et al.*, Phys. Rev. E **54**, 1134 (1996); E. Eisenriegler, J. Chem. Phys. **113**, 5091 (2000).
[12] A. A. Louis, P. G. Bolhuis, J. P. Hansen, and E. J. Meijer, J. Chem. Phys. **116**, 10 547 (2002).
[13] R. Tuinier *et al.*, Langmuir **16**, 1497 (2000).
[14] For a review, see, e.g., J.-L. Doublier *et al.*, Curr. Opin. Colloid Interface Sci. **5**, 202 (2000).
[15] S. Wang *et al.*, Biomacromolecules **2**, 1080 (2001).
[16] R. P. Sear, Phys. Rev. E **56**, 4463 (1997).
[17] R. P. Sear, cond-mat/0206320.
[18] H. M. Schaink and J. M. Smit, J. Chem. Phys. **107**, 1004 (1997).
[19] M. Fuchs and K. S. Schweizer, Europhys. Lett. **51**, 621 (2000); J. Phys. Condens. Matter **14**, R239 (2002).
[20] A. A. Louis *et al.*, Phys. Rev. Lett. **85**, 2522 (2000); P. G. Bolhuis *et al.*, J. Chem. Phys. **114**, 4296 (2001).
[21] P. G. Bolhuis *et al.*, Phys. Rev. Lett. **89**, 128302 (2002).
[22] D. Frenkel, J. Phys. Condens. Matter **2**, SA265 (1990).
[23] E. J. Meijer and D. Frenkel, Phys. Rev. Lett. **67**, 1110 (1991); J. Chem. Phys. **100**, 6873 (1994).
[24] D. Frenkel and B. Smit, *Understanding Molecular Simulations* (Academic Press, San Diego, 2002), 2nd ed.
[25] Q. Yan and J. J. de Pablo, J. Chem. Phys. **113**, 1276 (2000).
[26] A. M. Ferrenberg and R. H. Swendsen, Phys. Rev. Lett. **61**, 2635 (1987).
[27] J. P. Hansen and I. R. McDonald, *Theory of Simple Liquids* (Academic Press, London, 1986), 2nd ed.
[28] H. N. W. Lekkerkerker *et al.*, Europhys. Lett. **20**, 559 (1992).
[29] D. G. A. L. Aarts *et al.*, J. Phys. Condens. Matter **14**, 7551 (2002).
[30] A. A. Louis *et al.*, J. Chem. Phys. **117**, 1893 (2002).
[31] G. A. Vliegenthart and H. N. W. Lekkerkerker, J. Chem. Phys. **112**, 5364 (2000).
[32] A. P. Gast, C. K. Hall, and W. B. Russel, J. Colloid Interface Sci. **96**, 251 (1983).