The role of long-range forces in the phase behavior
of colloids and proteins

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Abstract. – The phase behavior of colloid-polymer mixtures, and of solutions of globular proteins, is often interpreted in terms of a simple model of hard spheres with short-ranged attraction. While such a model yields a qualitative understanding of the generic phase diagrams of both colloids and proteins, it fails to capture one important difference: the model predicts fluid-fluid phase separation in the metastable regime below the freezing curve. Such demixing has been observed for globular proteins, but for colloids it appears to be pre-empted by the appearance of a gel. In this paper, we study the effect of additional long-range attractions on the phase behavior of spheres with short-ranged attraction. We find that such attractions can shift the (metastable) fluid-fluid critical point out of the gel region. As this metastable critical point may be important for crystal nucleation, our results suggest that long-ranged attractive forces may play an important role in the crystallization of globular proteins. However, in colloids, where refractive index matching is often used to switch off long-ranged dispersion forces, gelation is likely to inhibit phase separation.

Introduction. – X-ray crystallography is still the standard technique to resolve the three-dimensional structure of globular proteins. But crystallography requires crystals, and protein solutions are notoriously difficult to crystallize. In order to understand the factors that favor crystallization, it is useful to gain insight into the phase behavior of the protein solution. As a first approximation, it is often sufficient to consider proteins as hard spherical bodies, interacting through a short-ranged attractive potential. In fact, Rosenbaum *et al.* [1] have shown that the crystallization curves for a number of globular protein solutions appear to coincide with those of a system of hard spheres with a rather short-ranged attractive Yukawa interaction, as studied in the simulations of Hagen and Frenkel [2]. These simulations were primarily aimed at modeling the phase behavior of polymer-colloid mixtures.

As the attractive Yukawa model is used to model both colloids and globular proteins, one should expect that the conclusions that hold for one system should be transferable to the other. This does indeed appear to be correct, as far as the equilibrium phase behavior is concerned: experimental studies of colloid-polymer mixtures [3] show that, as the range of

the attractive interaction between the colloids is shortened, the phase diagram changes in the way predicted originally by Gast *et al.* [4] and subsequently these predictions were analysed in considerable detail, both by computer simulation [5] and theoretically [6]. In particular, this analysis shows that fluid-fluid coexistence occurs only if the range of the attraction is sufficiently large compared to the "hard-core" radius of the particle (typically, more than 30%). For shorter-ranged attractions, the stable fluid-fluid transition is pre-empted by freezing. There are several experimental studies that indicate that solutions of globular proteins may exhibit the phase behavior expected for spherical particles with short-ranged attraction [7]. In the context of protein crystallization, the presence of a metastable fluid-fluid coexistence curve and, in particular, of a metastable critical point may be important, as ten Wolde and Frenkel [8] have argued that the presence of such a metastable critical point will lower the barrier for crystal nucleation.

However, experimental studies of suspensions of colloids with a short-ranged attractive interaction suggest that there is an important difference in the phase behavior of proteins and colloids: whereas a metastable fluid-fluid coexistence curve has actually been observed for several globular proteins, colloids with short-ranged attractive interactions tend to form a gel-like phase instead. Although the latter phase is metastable, it can delay [9], or even suppress, crystallization [3]. Clearly, the model of (mono-disperse) hard spheres with short-ranged attractive interactions is an oversimplification. Real proteins are non-spherical and have non-isotropic ("patchy") interactions. In contrast, while colloids may be quite spherical, they are hardly ever monodisperse. All these factors will affect the tendency to crystallize, to phase-separate and to form a gel. In the present paper, we focus on a very simple phenomenon, namely the effect of long-range forces. The reason why we focus specifically on long-range forces (rather than on poly-dispersity or particle anisometry) is that we are looking for a mechanism that can move the gelation regime well below the fluid-fluid critical point. We shall argue that long-range attractive interactions do precisely that.

Model and equations of state. – We wish to consider a system that can exhibit freezing, fluid-fluid phase separation and gelation. In our model two spherical hard particles of diameter σ experience a short-ranged attraction through a potential of the form

$$u(r) = \begin{cases} \infty \,, & r \leq \sigma \,, \\ -\epsilon \cdot (r/\sigma)^{-n} \,, & \sigma < r \,. \end{cases} \tag{1}$$

The specific form of the interaction potential has been chosen for convenience. In the case of proteins, we do not really know the detailed form of the attractive interaction (other than that it has a short-ranged component). In mixtures of "hard-core" colloids and non-adsorbing polymer, the short-ranged attraction is induced by depletion forces (see [6] and references therein). Other functional representations could have been chosen for the short-ranged potential (and have indeed been considered in studies of fluid-fluid coexistence and percolation [10]). However, the choice should become unimportant for very short-ranged attractions [11]. The fluid is then well described by Baxter's adhesive hard-sphere model of infinitely short-ranged attraction [12]. An approximate equation of state for this model is known (see the first two terms in eq. (4) below, given in [12,13]). The only parameter is the value of the second virial coefficient, usually expressed in terms of a "stickiness" parameter $1/\tau^{\mathrm{SS}}$:

$$B_2^{\mathrm{SS}} \equiv B_2^{\mathrm{HS}} \left(1 - \frac{1}{4\,\tau^{\mathrm{SS}}} \right) \,, \tag{2}$$

where $B_2^{\mathrm{HS}} = 2\pi\sigma^3/3$ is the virial coefficient of hard spheres. τ^{SS} can be thought of as a

measure of the temperature. In particular, the limit $\tau^{\mathrm{SS}} \to \infty$ corresponds to the situation where the effect of short-ranged attraction becomes negligible.

In the present case, the second virial coefficient is given by

$$B_2^{\mathrm{HS}} \, (1 - \frac{1}{4 \, \tau^{\mathrm{SS}}(t)}) = B_2^{\mathrm{HS}} - 2\pi \int_\sigma^\infty \left[e^{1/t \cdot (r/\sigma)^{-n}} - 1 \right] \, r^2 \mathrm{d}r \,. \tag{3}$$

$t \equiv k_{\mathrm{B}} T/\epsilon$ is the dimensionless temperature. In the present approach we account for the additional long-range attraction, by adding a van-der-Waals–like contribution to the equation of state:

$$\frac{p_{\mathrm{fl}} \, v_0}{k_{\mathrm{B}} T} = \eta \cdot \frac{1 + \eta + \eta^2}{(1 - \eta)^3} - \eta^2 \cdot \lambda \frac{18(2 + \eta) - \lambda^2 \eta}{36(1 - \eta)^3} - \frac{\alpha_0}{t} \cdot \eta^2 \,. \tag{4}$$

p is the pressure, v_0 the hard-sphere volume, η the volume fraction. α_0 is related to the usual van der Waals parameter a by $\alpha_0 = a/(\epsilon v_0)$, and thus measures the long-range attraction in units of ϵ. The stickiness enters through the parameter $\lambda = \lambda(\tau^{\mathrm{SS}}, \eta)$, given in ref. [12]. The first term on the right-hand side of eq. (4) describes the hard-sphere contribution to the pressure, the second term accounts for the stickiness, and the third term describes the effect of long-ranged attraction.

To describe the solid phase, we follow Daanoun *et al.* [14], estimating its entropy by a cell-theory and its energy by the mean field interaction energy between nearest neighbors at their average positions. The equation of state of the solid is most readily expressed in terms of a re-scaled distance, $s \equiv (\eta_{\mathrm{cp}}/\eta)^{1/3}$, where η_{cp} is the volume fraction at regular close packing:

$$\frac{p_{\mathrm{sol}} \cdot v_0}{k_{\mathrm{B}} T} = \eta_{\mathrm{cp}} \cdot \left[\frac{1}{s^2(s - 1)} + \frac{1}{t} \frac{z \, u'(s)}{6s^2} \right] - \frac{\alpha_0}{t} \cdot \eta^2 \,. \tag{5}$$

z denotes the number of nearest neighbors (here $z = 12$ as for the FCC lattice).

Gelation. – In the gel state the colloids form a space-spanning network. The tendency to form gels depends strongly on the range and strength of the forces acting between the particles. There appears to be no general recipe to predict whether a given system should be in the gel state. However, in the case of adhesive spheres, there is a simple analytical expression [15], that allows us to estimate the percolation curve. In a system of adhesive spheres, percolation is a necessary, but not a sufficient condition to form a gel. Hence, we can use the percolation criterion of ref. [15] to delimit the region where gelation is possible. For purely adhesive spheres, the analysis of ref. [15] indicates that cluster percolation occurs if

$$\tau^{\mathrm{SS}} \leq \frac{1 - 2\eta + 19\eta^2}{12 \, (1 - \eta)^2} \,. \tag{6}$$

One might expect that a different percolation criterion should apply if the particles have a longer-ranged attraction, in addition to the "sticky" interactions. However, long-ranged attractions should have little effect on the percolation. In fact, in the true van der Waals limit (infinitely weak, infinitely long-ranged attractions) the long-ranged attractions do not affect the structure of the fluid at all, and have therefore no effect on the percolation transition. It seems plausible that attractions with a long, but finite, range will have a small effect on percolation. Indeed, Kaneko [16] has shown that this is even true for electrostatic long-range interactions. In what follows, we shall therefore continue to use eq. (6) to delimit the regions where no percolation occurs, and where gelation can thus safely be ruled out.
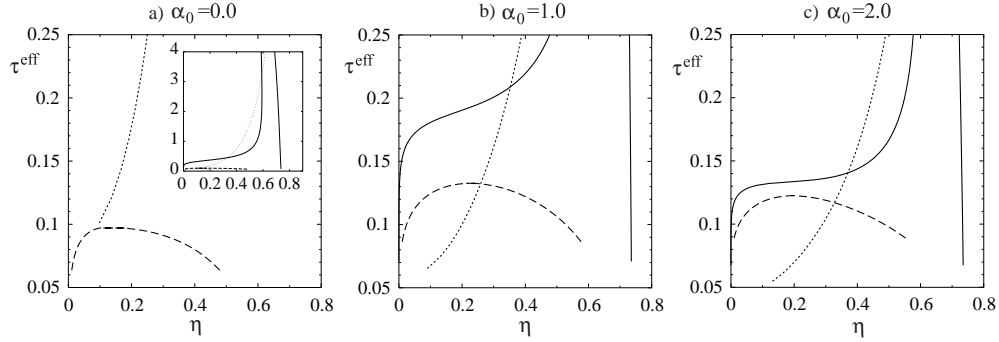
Fig. 1. – Phase diagrams in the $(\tau^{\text{eff}}, \eta)$ representation, shown for increasing van der Waals attraction. Every plot shows the fluid-fluid coexistence (broken line), the fluid-solid coexistence (solid line) as well as the percolation line (dotted line). As the long-range attraction becomes more important, the fluid-fluid critical point shifts out of the region where percolation can occur. For the solid, $n = 50$ has been used.

Results. – Using the equations of state given above, we study the effect of additional long-range attraction on the phase diagram of particles with a short-ranged attraction. We locate the phase-coexistence boundaries numerically, by imposing that the pressures and the chemical potentials of the coexisting phases be equal. In order to evaluate the effect due to the long-range attraction, we have to compare to the case of pure sticky spheres. In analogy with the stickiness parameter τ^{SS}, we define a second parameter τ^{vdW}, such that τ^{SS} and τ^{vdW} characterize long- and short-range interactions separately:

$$B_2 = B_2^{\text{HS}} \left(1 - \frac{1}{4\,\tau^{\text{SS}}} - \frac{1}{4\,\tau^{\text{vdW}}}\right). \tag{7}$$

Both parameters depend on temperature. Whereas we have simply $\tau^{\text{vdW}} = t/\alpha_0$ from eq. (4), τ^{SS} is obtained (numerically) from eq. (3) for $n = 50$. To compare the phase diagrams of different model systems, it is convenient to introduce the *effective* stickiness, defined as

$$\frac{1}{\tau^{\text{eff}}} \equiv \frac{1}{\tau^{\text{SS}}} + \frac{1}{\tau^{\text{vdW}}}. \tag{8}$$

In what follows, we shall compare phase diagrams as a function of τ^{eff}.

The main results of our analysis are presented in fig. 1. Without the van der Waals contribution, fig. 1a, τ^{eff} is identical to τ^{SS}, and we simply obtain the phase diagram of spheres with purely short-ranged attraction. The fluid-fluid critical point is metastable and lies *below* the percolation line. This implies that percolation, and presumably gelation, occurs before the system can be quenched to the metastable critical point. Hence, in such systems one should not expect to observe a fluid-fluid phase separation.

In fig. 1b, a moderate van der Waals attraction is present. Due to the long-range attraction, the percolation line now occurs for lower values of τ^{eff}, as it only depends on τ^{SS}. In contrast, the fluid-fluid coexistence is not strongly affected by the different range. It consequently shifts out of the percolation region, and it becomes possible to quench to this point without risk of gelation.

As the long-range attraction is increased further, fig. 1c, the same trends are observed: the fluid-solid line approaches the fluid-fluid critical point, which remains clearly outside the percolation region. For even stronger long-range attraction, the fluid-fluid critical point
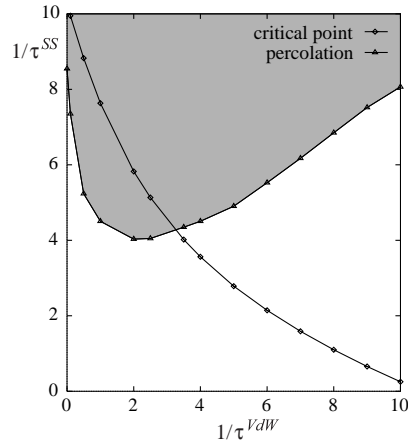
Fig. 2. – The curve connecting the diamonds delimits the region (in the upper right-hand corner of $(1/\tau^{\mathrm{SS}}, 1/\tau^{\mathrm{vdW}})$-plane) where fluid-fluid coexistence can occur. The curve thus represents the collection of the critical points. The curve connecting the triangles delimits the region (shaded) where percolation is expected to occur. The curve was constructed by plotting the value of the percolation threshold evaluated at the fluid-fluid critical density, at fixed τ^{vdW}. In the absence of long-range attraction, percolation precedes fluid-fluid demixing. However, even a moderate long-ranged attraction moves the fluid-fluid coexistence curve out of the gelation region.

becomes stable. This reflects the fact that a sufficiently strong van der Waals attraction can, of course, force the fluid to phase separate, whether short-range attractions are present or not.

This result is summarized in fig. 2. To read this figure, first consider the $1/\tau^{\mathrm{vdW}}$-axes, corresponding to long-ranged attraction only: when the attraction exceeds a certain value, fluid-fluid demixing occurs. Similarly, for purely sticky spheres demixing occurs when $1/\tau^{\mathrm{SS}} \gtrsim 10.9$, when both types of interaction are present, the critical point deviates a little from a line of constant $1/\tau^{\mathrm{eff}}$. Percolation, on the other hand, can only occur in the shaded area. The figure shows that, as the strength of the long-range attraction increases (increasing $1/\tau^{\mathrm{vdW}}$), the fluid-fluid critical point moves to a region where no gel formation is possible.

Discussion. – It is tempting to speculate that the difference in phase behavior of globular proteins and colloids may, at least partly, be due to the different role of long-ranged attractive forces. In most studies of the phase behavior of colloid-polymer mixtures, the refractive index of the solvent is matched to that of the colloidal particles to facilitate light scattering or microscopy studies of the colloidal structure. But, by refractive-index matching, the attractive dispersion forces between the colloids are effectively switched off. Hence, such suspensions are expected to behave as the model system with purely short-ranged attraction, for which the metastable fluid-fluid critical point lies well within the percolation region and fluid-fluid demixing is pre-empted. This is the state of affairs observed in the experiments of ref. [3]. In contrast, recent experiments by Hachisu [17] on colloids with strong van der Waals interactions show that, in such systems, fluid-fluid demixing does occur. Protein-protein interactions have been extensively studied both theoretically and experimentally [18,19]. However, even the most careful theoretical calculations, accounting for steric, electrostatic, dispersional and short-range interactions, yield estimates for the second virial coefficient that may differ appreciably from the experimental values. In other words, much less is known about the long-ranged attractions between globular proteins (not necessarily dispersion forces) than those acting between colloids.

As we have shown above, the presence of moderately strong long-ranged attractions would move the fluid-fluid critical point outside the regime where gelation is likely to occur. In such systems, the metastable fluid-fluid phase separation may be observed, as indeed it is in the experiments of ref. [7]. Of course, there are many other factors that play a role in phase behavior of proteins and colloids. For instance, colloids are usually slightly poly-disperse, while proteins are not. It seems likely that the main effect of poly-dispersity will be to bring down the fluid-solid coexistence curve to lower values of τ. However, the effect on the fluid-fluid phase transition and on the percolation curve is expected to be small. The same is expected for the effect of increasing the range of the "short-ranged" attraction. In contrast, anisotropic interactions (be they due to non-sphericity or surface "patchiness") are likely to increase the tendency to form gels. Clearly, the role of long-ranged attractions is only one out of many—but at least it is an effect that provides a simple explanation why metastable fluid-fluid separation is observed in globular proteins but not in colloid-polymer mixtures.

Let us finally consider the role of the metastable spinodal in crystal nucleation. In the model studied in ref. [8], crystal nucleation was facilitated by the vicinity of the metastable critical point. Moreover, in this model system, gelation did not interfere with either fluid-fluid phase separation or crystal nucleation. However, as fig. 1 shows, we should expect that in many cases gelation interferes with fluid-fluid phase separation—if not in the early stages, than at least in the later stages. Poon [20] has argued that it is the metastable fluid-fluid phase separation *itself* that leads to gelation, and thereby inhibition of crystallization. In the case of colloid-polymer mixtures, phase separation and gelation tend to occur in the same region of the phase diagram. However, we stress that it is important to identify gelation as the primary phenomenon that suppresses crystallization—fluid-fluid phase separation may also be suppressed by gelation, but it does not *cause* gelation. In fact, the simulations of ref. [8] provide a nice example of fluid-fluid phase separation without gelation: in that case the metastable fluid-fluid critical point actually *enhances* crystal nucleation.

The results of ref. [8] suggest that, above the metastable fluid-fluid coexistence curve, the early stages of crystal nucleation involve the formation of a liquid-like nucleus. If this happens, gelation can still interfere with the crystallization process at a later stage, namely through the gelation of the liquid-like pre-critical (and, below the fluid-fluid binodal, even post-critical) nuclei. This would slow down the subsequent formation of crystals. However, it is unlikely that gelation would completely immobilize the particles in small clusters—in fact, recent experiments on vitrification in thin films indicate that the effective glass-transition temperature may be lowered appreciably as the linear dimensions of the system are reduced [21].

Finally, we note that Sear [22] has pointed out one more reason why systems with short-ranged attractive interactions will not crystallize easily: in the relevant part of the phase diagram, the interfacial free energy $\gamma_{\text{solid-fluid}}$ of such systems tends to be large compared to the thermal energy. According to classical nucleation theory, the crystal-nucleation barrier is proportional to the cube power of $\gamma_{\text{solid-fluid}}$. Hence deeper quenches are needed to get appreciable nucleation rates. But, of course, the deeper the quench, the more likely it is that gelation will interfere. However, Sear's argument does not distinguish between colloids and proteins.

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REFERENCES

[1] ROSENBAUM D., ZAMORA P. C. and ZUKOSKI C. F., *Phys. Rev. Lett.*, **76** (1996) 150; GEORGE A. and WILSON W., *Acta Crystallogr. D*, **50** (1994) 361.

[2] HAGEN M. H. J. and FRENKEL D., *J. Chem. Phys.*, **101** (1994) 4093.

[3] ILETT S. M., ORROCK A., POON W. C. K. and PUSEY P. N., *Phys. Rev. E*, **51** (1995) 1344.

[4] GAST A. P., HALL C. K. and RUSSEL W. B., *J. Colloid Interface Sci.*, **96** (1983) 251.

[5] MEIJER E. J. and FRENKEL D., *Phys. Rev. Lett.*, **67** (1991) 1110.

[6] LEKKERKERKER H. N. W., POON W. C. K., PUSEY P. N., STROOBANTS A. and WARREN P. B., *Europhys. Lett.*, **20** (1992) 559.

[7] BERLAND C. R., THURSTON G. M., KONDO M., BROIDE M. L., PANDE J., OGUN O. O. and BENEDEK G. B., *Proc. Natl. Acad. Sci. USA*, **89** (1992) 1214; ASHERIE N., LOMAKIN A. and BENEDEK G. B., *Phys. Rev. Lett.*, **77** (1996) 4832.

[8] TEN WOLDE P. R. and FRENKEL D., *Science*, **277** (1997) 1975.

[9] SMITS C., VAN DUIJNEVELDT J. S., DHONT J. K. G., LEKKERKERKER H. N. W. and BRIELS W. J., *Phase Trans.*, **21** (1990) 157.

[10] XU J. and STELL G., *J. Chem. Phys.*, **89** (1988) 1101.

[11] REGNAUT C. and RAVEY J. C., *J. Chem. Phys.*, **91** (1989) 1211.

[12] BAXTER R. J., *J. Chem. Phys.*, **49** (1968) 2770.

[13] BARBOY B., *J. Chem. Phys.*, **61** (1974) 3194.

[14] DAANOUN A., TEJERO C. F. and BAUS M., *Phys. Rev. E*, **50** (1994) 2913.

[15] CHIEW Y. C. and GLANDT E. D., *J. Phys. A*, **16** (1983) 2599.

[16] KANEKO T., *Phys. Rev. E*, **53** (1996) 6134.

[17] HACHISU S., *Croatica Chem. Acta*, **71** (1998) 975.

[18] VELEV O. D., KALER E. W. and LENHOFF A. M., *Biophys. J.*, **75** (1998) 2682.

[19] See *e.g.*, NEAL B. L., KANEKO D. T. and LENHOFF A. M., *Biophys. J.*, **75** (1998) 2469.

[20] POON W. C. K., *Phys. Rev. E*, **55** (1997) 3762.

[21] JEROME B. and COMMANDEUR J., *Nature*, **386** (1997) 589.

[22] SEAR R., preprint cond-mat/9903293.