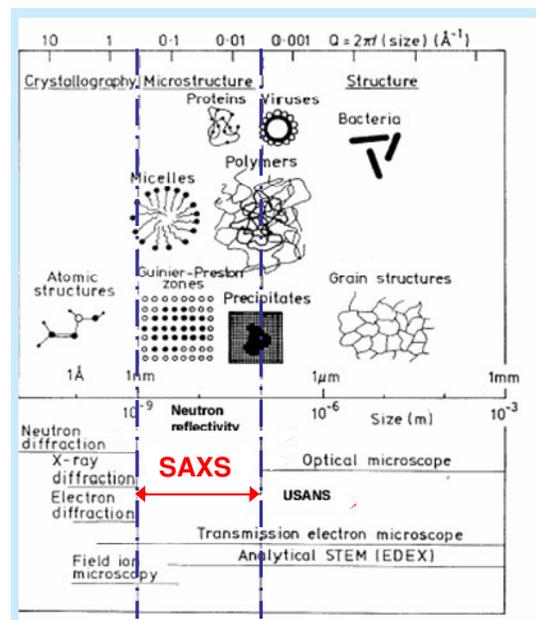


Small-Angle Scattering and Data Analysis

1. Introduction

Small-angle scattering (SAS) is a powerful technique to study structure and interactions of systems with the size on the order of 10 to 1000 Å. SAS includes light scattering (due to the large wavelength), small angle X-ray and Neutron scattering (Lindner and Zemb 2002). In each of these techniques radiation is **elastically** scattered by a sample and the analysis on the resulting scattering pattern provide the information about size, shape and correlations of the sample. Unless stated otherwise, we will focus on SAXS, SANS shares the same basic principle as SAXS, whereas some fundamental differences will be addressed. Since most of the structures in soft matter systems, such as proteins, colloid, polymers and micelles formed by amphiphilic molecules located in this order, SAS is widely used in soft condensed matter physics (FIG. 1). The systems studied by SAS can be a dispersed particulate system, nonparticulate mixtures, periodic system and so on. In this project, we are interested in a dispersed particulated system, i. e. particles (gold colloid, proteins) dispersed in a uniform matrix of a solvent, where SAS provides information (a) on the **size** and (b) on their **correlations** and thus on their **interactions**.

FIG. 1, the length scales of typical soft matter systems and corresponding techniques used in each length scale.



Scattering length and scattering length density: the local interaction of radiations with materials is characterized by a scattering length b_i , and its density $\rho(r) = \sum \rho_i(r)b_i$, with $\rho_i(r)$ being the local density of scatterers of type i . For X-rays, the photons interact with every electron in the sample, and the scattering length is the Thomson scattering length $b=0.282*10^{-14}$ m. For light scattering, the photon energy is much lower than X-rays, the photos are scattered only by the outer part of the electronic cloud of an atom and the scattering length density is proportional to the polarizability of the materials. In neutron scattering, the interaction is nuclear and the scattering length depends on the nature of the nuclei of atoms.

SAS has been widely used to study the particulate suspension system, where particles of one material are dispersed in a uniform matrix of a second material. When the concentration of the particles is low, e.g. in a dilute solution, there are no correlations between positions or orientations of particles. Under this condition, the waves scattered from different particles are incoherent among them, and the observed intensity simply becomes a sum of the individual scattering. In a dilute particulate system the matrix is assumed to be devoid of any internal structure and simply presents a uniform, homogenous background. In a real system, whether liquid or solid, there is always a fluctuation, but as long as such inhomogeneity is of a size scale much smaller than $1/q$, the effect does not manifest itself in the q range of interest here.

Radius of Gyration: Before we calculate the scattering intensity for the single particles, we introduce an important parameter for describing the size of the particles, i.e. the radius of gyration, R_g . The radius of gyration is one of the most important parameters in the field of small-angle scattering. It is defined as:

$$R_g^2 = \frac{\int \Delta\rho(r_i)r_i^2 dV_i}{\int \Delta\rho(r_i)dV_i} \quad (1)$$

As examples of the radius of gyration of particles, we have for solid sphere of radius R ,

$$R_g = \sqrt{\frac{3}{5}}R \quad (2.1)$$

For a solid ellipsoid of half axes a, b, and c

$$R_g = \frac{1}{\sqrt{5}}(a^2 + b^2 + c^2)^{1/2} \quad (2.2)$$

For a solid rod with length of L and circular cross section of radius R,

$$R_g = \frac{L^2}{12} + \frac{R^2}{2} \quad (2.3)$$

2. Form factor and the shape of proteins

The form factor $P(q)$ describes the scattering from a single particle, which strongly depends on its size and shape. As shown in FIG 2, homogeneous particles with electron density ρ_p and volume V_p , dispersed in a homogeneous matrix with electron density of ρ_0 . The scattering intensity $I(q)$ from a single particle at a scattering angle 2θ as a function of the scattering vector q , $q = 4\pi\sin\theta/\lambda$ can be expressed by:

$$I(q) \propto \frac{d\Sigma(q)}{d\Omega} = \frac{1}{V} \left| \int_V \rho(r) e^{-iqr} dr \right|^2 \quad (3)$$

where $\rho(r)$ is the electron density, V is the illuminated volume. The scattered intensity $I(q)$ is proportional to the square of the Fourier transformation of electron density. For identical particles, $\rho(r)$ is a constant and the total scattering intensity can be further expressed by:

$$I(q) = \frac{d\Sigma(q)}{d\Omega} = \frac{N}{V} (\rho_p - \rho_0)^2 V_p^2 \left| \frac{1}{V_p} \int \rho(r) e^{-iqr} dr \right|^2 \quad (4)$$

$\rho(r) = \rho_p - \rho_0$ is the difference of electron density between particle and matrix, it is normally called “scattering contrast”. The form factor $P(q)$ is defined as:

$$P(q) = \left| \frac{1}{V_p} \int \rho(r) e^{-iqr} dr \right|^2 \quad (5)$$

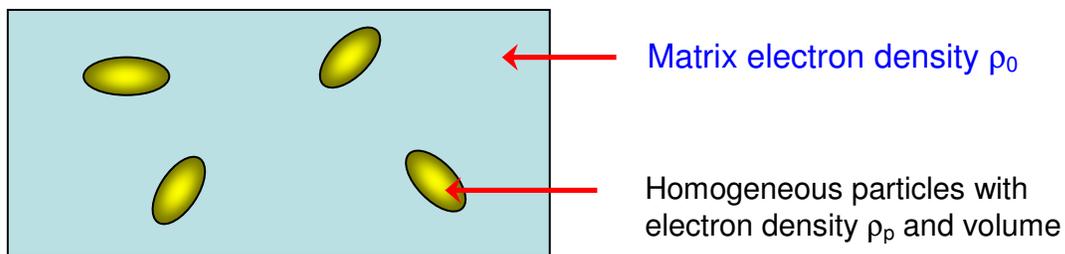


FIG. 2 Schematic drawing for “dilute” particles suspended in a homogeneous matrix.

The simplest example of form factor is sphere, which has a form factor of eq. (6) and the plot of scattering intensity as a function of qR is shown in FIG. 3. Some interesting and useful features from the plot are worth noting: first, the slope of the plot in the high qR range is equal to -4, and $qR_g < 1$ defines the Guinier region.

$$P(q) = 9 \left[\frac{\sin(qR) - (qR) \cos(qR)}{(qR)^3} \right]^2 \quad (6)$$

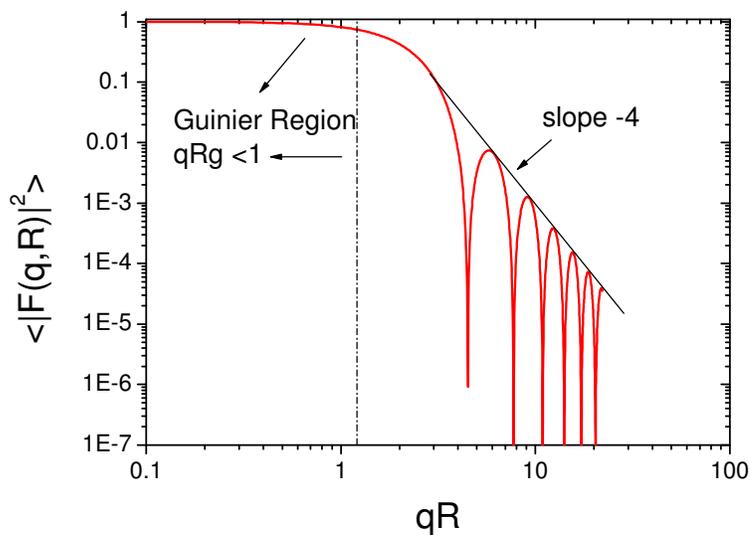


FIG 3 form factor of an ideal sphere with $R=20\text{nm}$.

More examples for the form factor of other shapes:

(a) The form factor of a monodisperse spherical particle with a core-shell structure is described in the following equation.

$$P(q) = \frac{1}{V} \left[\frac{3V_c(\rho_c - \rho_s)j_1(qr_c)}{qr_c} + \frac{3V_s(\rho_s - \rho_{solv})j_1(qr_s)}{qr_s} \right]^2 \quad (6.1)$$

Where $j_1(x) = (\sin x - x \cos x) / x^2$; $r_s = r_c + t$ and $V_i = (4\pi/3)r_i^3$

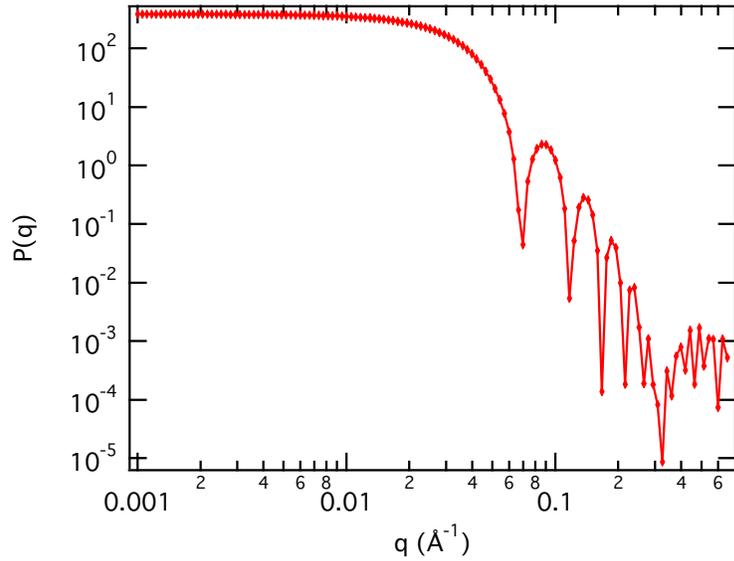
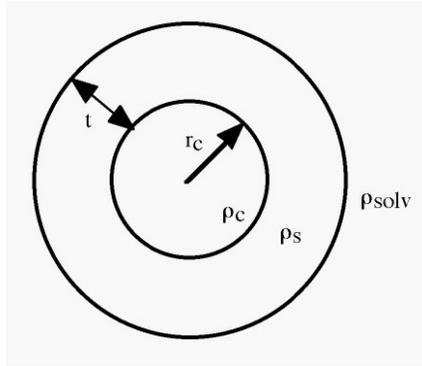


FIG 4 form factor of core-shell sphere with $r_c = 60 \text{ \AA}$, $t = 10 \text{ \AA}$

(b) Monodisperse Rigid Cylinders (a simple model for DNA)

$$P(q) = \frac{1}{V} \int_0^{\pi/2} f^2(q, \alpha) \sin \alpha \, d\alpha \quad (6.2)$$

$$f(q, \alpha) = 2(\rho_{cyl} - \rho_{solv})V_{cyl} j_0(qH \cos \alpha) \frac{J_1(qr \sin \alpha)}{qr \sin \alpha}$$

$$V_{cyl} = \pi r^2 L \text{ and } j_0(x) = \sin(x) / x$$

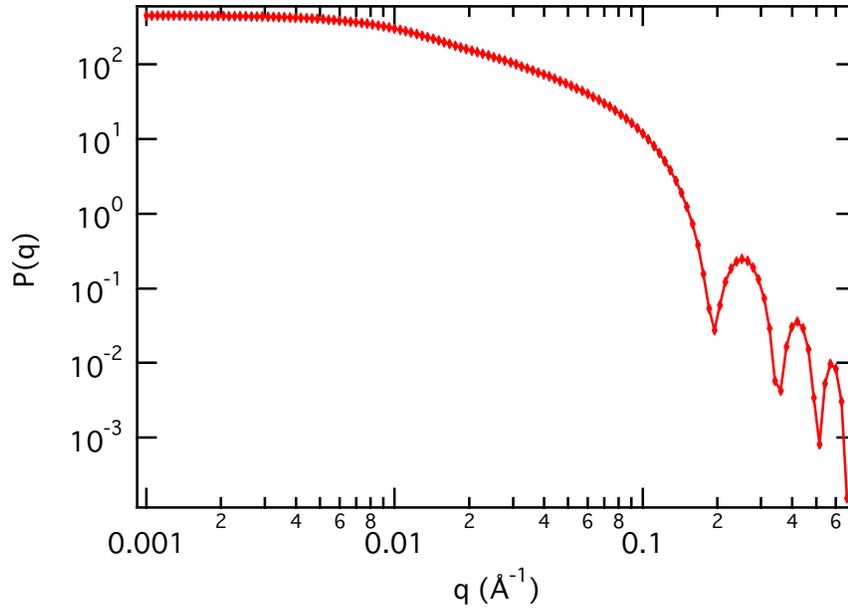
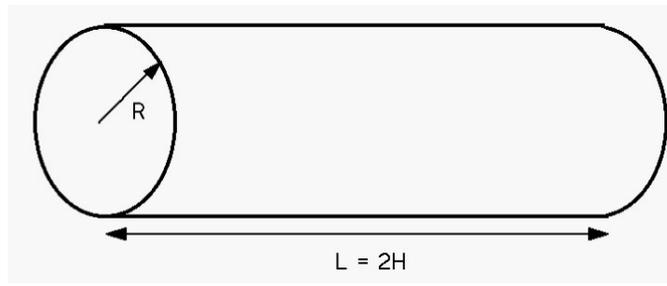


FIG 5 form factor of cylinder with $R = 20 \text{ \AA}$ and $L = 400 \text{ \AA}$

Guinier Law:

The Guinier law (Guinier, 1955) is widely used to determine the basic molecular parameters, such as radius of gyration, and molecular weight. Scattering function for monodispersed dilute solution of Eq. (3) can be expanded to Eq. (7) in the low q range ($qR_g < 1$) with the assumption that the orientation of the particles is spatially averaged and the solution is isotropic.

$$\frac{d\Sigma(q)}{d\Omega} = \frac{1}{V} \left(\int_V \rho(r) dr \right)^2 \left[1 - \frac{1}{3} q^2 R_g^2 + \dots \right] \quad (7)$$

The integration of the right part of the formula corresponds to the forward scattering intensity $I(q=0)$, and the other part in the bracket can be written in exponential form, therefore the above formula is written by the Guinier form;

$$I(q) = I(0) \exp\left(-\frac{1}{3} q^2 R_g^2\right) \quad (8)$$

In practice, the Guinier plot, i.e. the $\ln I(q)$ vs q^2 is widely used to determine the radius of gyration, R_g .

FIG.6 gives an example for protein BSA dilute solution in Guinier plot, from the slope, the R_g of BSA is determined as 27 Å.

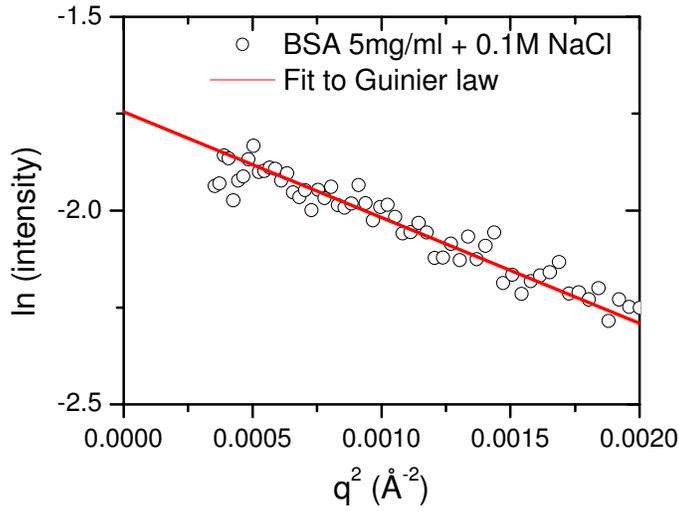


FIG 6 Guinier plot for BSA dilute solution with 0.1M NaCl.

Another important parameter which can be determined from the Guinier plot for dilute systems is the molecular weight. If the scattering intensity has been calibrated in absolute intensity, the forward intensity $I(q=0)$ corresponds to the total number of electrons in the scattered volume. $I(0)$ can be expressed as follow:

$$I(0) = \frac{d\Sigma(0)}{d\Omega} = \frac{N}{V} (\rho_p - \rho_0)^2 V_p^2 \quad (9)$$

Further, if we use the particle concentration of c (mg/mL) = $\frac{NV_p}{vV}$, and the particle

molecular weight $M_w = \frac{V_p N_A}{v}$, where N_A is Avogadro's number, v is the specific

volume of the particle, V the illuminated volume or scattering volume, and V_P the volume of the particle, we have the following expression:

$$\frac{d\Sigma(0)}{d\Omega} = \frac{cvM_w}{N_A} (\rho_p - \rho_0)^2 \quad (10)$$

With all other parameters available in practice, the molecular weight can be calculated.

Porod law:

The Porod law (Porod, 1951) describes that the slope of the plot ($\ln I(q)$ vs $\ln q$) represents the interface and fractal dimension of the scattering objects. At high q , a slope of -2 is a signature of Gaussian chain in a dilute solution, whereas a slope of -1 points to rigid rods. A slope of -4 represents a smooth interface between domains in a multiphase system (Figure 3). Slopes between -3 and -4 characterize rough interface of fractal dimension D (Schmidt, 1988). Scattering from such a rough interface drops as $1/q^{6-D}$. In the case of smooth interfaces, the scattered intensity at high q gives the following expression:

$$I(q) = b\phi(1-\phi)(2\pi \frac{S}{V} q) \quad (11)$$

Where b is the contrast factor, ϕ is the volume fraction of one of the component, S/V is the surface to volume ratio. This is a general result independent of the actual shape of the scattering particles. This result can be derived from the pair correlation function.

3. Structure factors derived from interaction potential

The above considerations are for dilute solution, where the interactions between the suspended particles are negligible. Upon further increasing the particle concentration, step by step, the particle will “feel” the existence of their neighbors as illustrated in FIG. 7. The scattered intensity from a collection of discrete particles can be written as:

$$\frac{d\Sigma(q)}{d\Omega} = \frac{1}{V} \sum_{k=1}^N \langle |F_k(q)|^2 \rangle + \frac{1}{V} \left\langle \sum_{k=1}^N \sum_{\substack{j=1 \\ j \neq k}}^N F_k(q) F_j^*(q) e^{-iq(r_k - r_j)} \right\rangle \quad (12)$$

where r_i and r_j are the centers of mass of particles k and j respectively. The scattering amplitude is a Fourier integral of the distribution of scattering length density within each

cell. For the special case of monodisperse spherical particles, $F_k(q) = F_j^*(q) = F(q)$, and Equation 12 can be factored into the form:

$$\frac{d\Sigma(q)}{d\Omega} = n \langle |F_k(q)|^2 \rangle \left\{ 1 + \left\langle \sum_{k=1}^N \sum_{\substack{j=1 \\ j \neq k}}^N e^{-iq(r_k - r_j)} \right\rangle \right\} \quad (13)$$

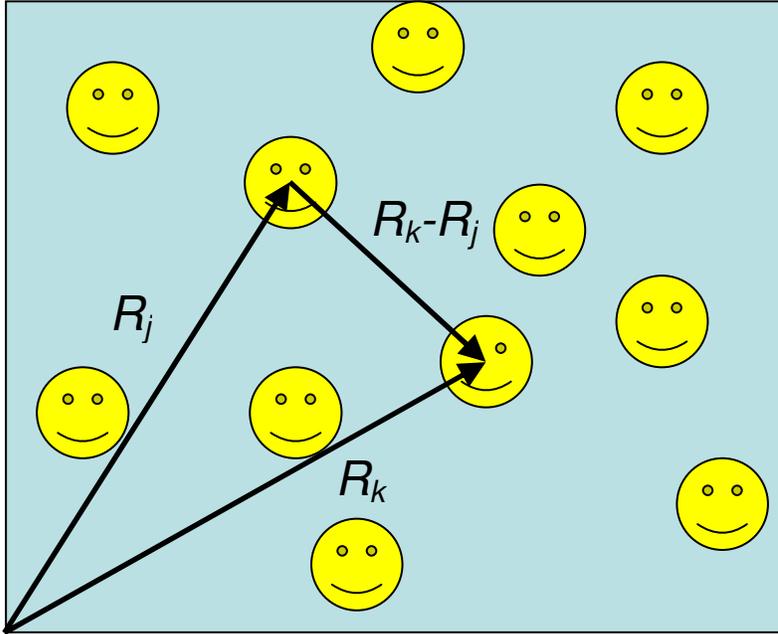


FIG.7 Schematic illustration of particle-particle interaction (correlation) in solution.

The first term on the right-hand side of Eq. (13) corresponds to the form factor of the particle, $P(q)$, and the second term in the bracket presents the distribution of the particles in the space, this distribution is of course determined by their interactions, this term is often called structure factor and denoted by $S(q)$. So, the Eq. (13) can be rewritten in:

$$\frac{d\Sigma(q)}{d\Omega} = nP(q)S(q) \quad (14)$$

where n_p is the number density of particles.

The structure factor contains all of the information about the spatial arrangement of the particles relative to an arbitrary origin, i.e. the correlations. In an ideal solution, where the protein molecules are well-separated from each other, i.e. there is no position or orientation correlation between them, $S(q) = 1$, and the total scattering is only has

contributions from the form factor $P(q)$. With increasing protein concentration, the interference effect between proteins cannot be neglected, and the structure factor becomes important in the total scattering intensity. $S(q)$ in the low q range strongly depends on the interaction potential between protein molecules. The structure factor at the origin $S(q=0)$ is equal to the normalized osmotic compressibility. With repulsive interactions, the protein molecules are uniformly distributed and $S(0)$ is lower than unity, while with attractive interactions, fluctuations dominate the particle distribution and $S(0)$ is larger than unity (Tardieu, et al 1999). Therefore, a detailed analysis of the $S(q)$ can provide information on the nature of interaction potentials. For the case of an isotropic solution, the average can be calculated around a centrally located sphere. The orientational average can be calculated as:

$$\langle S(q) \rangle = S(q) = 1 + n_p \int_0^{\infty} 4\pi r^2 [g(r) - 1] \frac{\sin qr}{qr} dr \quad (15)$$

The pair correlation function, $g(r)$, can be calculated using liquid state theory by solving the Ornstein-Zernike (OZ) equation, and thus the structure factor can be calculated (Hansen and McDonald 2006). The pair correlation function can be related to thermodynamic properties of the fluid such as pressure or compressibility. To obtain information from systems of interacting colloids, it is necessary to model the scattered intensity by calculating the form and structure factors. This is easily done for monodisperse, spherical particles. For a homogeneous, isotropic fluid of spheres, the Ornstein-Zernike equation is:

$$h(r) = g(r) - 1 = c(r) + \rho \int c(|\vec{r} - \vec{r}'|) h(\vec{r}') d\vec{r}' \quad (16)$$

The physical meaning of OZ equation is: the “total” correlation, $h(r)$, between two particles is due in part to the “direct” correlation, $c(r)$, between them but also to the “indirect” correlation propagated via increasingly large numbers of intermediate particles. With this physical picture in mind it is plausible to suppose that the range of $c(r)$ is comparable with that of the pair potential $u(r)$, and the $h(r)$ is generally much longer ranged than $u(r)$ due to the effects of indirect correlation. The structure factor $S(q)$ depends directly on $c(r)$ (compare Equations 15 and 16). Unfortunately, $c(r)$ and $h(r)$ are

both unknown functions, and the Ornstein-Zernicke equation can only be solved if there is an additional relation between them. This additional equation is an approximation, called a closure relation, which relates $h(r)$ and $c(r)$. A deeper understanding of the meaning of $c(r)$, can be obtained by diagrammatic and density functional derivative methods (Hansen and McDonald 2006).

The most popular closure relation is the Percus-Yevick closure (Percus and Yevick 1958):

$$c(r) = g(r)[1 - e^{\beta u(r)}] \quad (17)$$

which provides a good description of fluids with very short ranged interactions.

To introduce the PY closure, the OZ equation (16) is reformulated as:

$$c(r) = g(r) - \left[1 + \rho \int d\bar{r}' c(r') \{g(|\bar{r} - \bar{r}'|) - 1\} \right] = g(r) - g_{ind}(r) \quad (17a)$$

The term in brackets, $g_{ind}(r)$, describes the indirect part of the pair correlations. It is known that $g(r) = \exp[-\beta w(r)]$, $w(r)$ is the so-called potential of mean force. The function $w(r)$ is generally a more complicated object than the mere pair potential $u(r)$, since it involves the effects of indirect interactions from other particles. But in the limit $\rho \rightarrow 0$ follows that $w(r) \rightarrow u(r)$ (Nägele, 1996). Thus, one can approximate $g_{ind}(r)$ by

$$g_{ind}(r) \approx e^{-\beta[w(r)-u(r)]} \quad (17b)$$

Substitute (17b) to (17a), the PY closure is obtained.

As model potentials, the following ones have been considered:

(a) For the simplest case of hard sphere interactions:

$$U(r) = \begin{cases} \infty & r \leq \sigma \\ 0 & r > \sigma \end{cases} \quad (18)$$

$c(r)$ is identically zero for $r > \sigma$, where σ is the diameter of the particles. In this case an analytic solution for the structure factor is obtained as following (Pedersen, 1997):

$$S(q) = \frac{1}{1 + 24\eta f(\sigma q)/(\sigma q)} \quad (19)$$

In this equation, $f(x)$ is further defined as follow:

$$\begin{aligned}
f(x) &= \alpha(\sin x - x \cos x) / A^2 \\
&+ \beta(2x \sin x + (2 - x^2) \cos x - 2/x^3) \\
&+ \gamma[-x^4 \cos x + 4((3x^2 - 6) \cos x + (x^3 - 6x) \sin x + 6)] / x^5
\end{aligned} \tag{19.1}$$

And

$$\begin{aligned}
\alpha &= (1 + 2\eta)^2 / (1 - \eta)^4 \\
\beta &= -6\eta(1 + \eta/2)^2 / (1 - \eta)^2 \\
\gamma &= \eta\alpha/2
\end{aligned} \tag{19.2}$$

(b) Short ranged attractive interactions combined with the PY closure also yield analytical solutions for the structure factor. For example, the square well (SW) potential:

$$U(r) = \begin{cases} \infty & r \leq \sigma \\ -U_0 & \sigma < r < \delta\sigma \\ 0 & r > \delta\sigma \end{cases} \tag{20}$$

The range of the square well is given by δ and is typically less than 1.5.

(c) If the attractive interactions are shorter - ranged, then the sticky hard sphere potential (SHS) can be used.

$$U(r) = \begin{cases} \infty & r \leq \sigma \\ -U_0 & \sigma < r < \sigma + \Delta \\ 0 & r > \sigma + \Delta \end{cases} \tag{21}$$

This is a perturbation solution of the PY closure for the case of a narrow square well of width Δ and depth U_0 . The perturbation parameter is $\varepsilon = \Delta/(\sigma + \Delta)$, and must be less than 0.1. The SHS potential has the advantage that the phase diagram can be determined analytically.

(d) The screened Coulomb interaction is widely used to describe the interactions of macroions and charged colloids in solutions:

$$U(r) = \begin{cases} \infty & r \leq \sigma \\ \frac{z^2 e^2}{\varepsilon(1 + \kappa_D \sigma/2)^2} \frac{\exp[-\kappa_D(r - \sigma)]}{r} & r > \sigma \end{cases} \tag{22}$$

Some examples:

See our recent publication: Zhang et al. J. Phys. Chem. B. 2007, 111, 250.

References and extension reading:

1. A. Guinier; G. Fournet, "Small Angle Scattering of X-rays", John Wiley, 1955
2. O. Glatter; O. Kratky ED., "Small Angle X-ray Scattering", Academic Press, 1982.
3. R. J. Roe, "Method of X-ray and Neutron Scattering in Polymer Science", Oxford, Oxford University Press, 2000.
4. P. Lindner, Th, Zemb, Ed. "Neutrons, X-rays and Light: Scattering Methods Applied to Soft Condensed Matter", North-Holland, Elsevier, 2002.
5. SANS program developed by NIST provides a detailed description on various form factors and structure factors: <http://www.ncnr.nist.gov/programs/sans/data/index.html>
6. J. S. Pedersen, "Analysis of small-angle scattering data from colloids and polymer solutions: modeling and least squares fitting", Adv. Coll. Interface Sci., 70(1997) 171-210.
7. G. Porod, Kolloid Z. 1951, 124, 83.
8. P. W. Schmidt, Makromol. Chem., Makromol. Symp. 1988, 15, 153.
9. L. S. Ornstein, F. Zernike, Proc. Ned. Akad. Sci. 1914, 17, 793.
10. J. K. Percus, G. J. Yevick, Phys. Rev. 1958, 110, 1.
11. J. P. Hansen, I. R. McDonald, "Theory of Simple Liquids" 3rd Edition, Academic Press, Elsevier, 2006
12. G. Nägele, Phys. Rep. 1996, 272, 215.
13. A. Tardieu, A. Le Verge, M. Malfois, F. Bonneté, S. Finet, M. Riès-Kautt, L. Belloni, J. Cryst. Growth 1999, 196, 193.