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Interparticle interactions and structure in nonideal solutions of human serum albumin studied by small-angle neutron scattering and Monte Carlo simulation

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Abstract

Moderately or highly concentrated nonideal solutions of macromolecules are very important systems e.g. in biology and in many technical processes. In this work we have used the small-angle neutron scattering technique (SANS) to study the interactions and interparticle structure in solutions of human serum albumin (HSA) up to a concentration of 0.26 g/cm^3 in 1.08 M NaCl. In order to obtain a model for the interactions we have combined the SANS data with results obtained by Monte Carlo simulations where we calculate the structure factor $S(Q)$ and the pair correlation function $g(r)$. The advantage of using the Monte Carlo method is that completely general models for the particle shape and the interactions can be considered. It is found that the SANS data can be explained by a model where the shape of the HSA molecule is approximated by an ellipsoid of revolution with semiaxes $a = 6.8$ nm and $b = c = 1.9$ nm. The interaction potential between the HSA molecules consists of two parts: (a) the molecules are hard and impenetrable and (b) the molecules are surrounded by a spherically symmetric repulsive potential which can be expressed by an empirical formula containing the HSA concentration and the centre–centre distances as the only variables.

Keywords: Nonideal solutions; Small-angle scattering; Monte Carlo simulation; Interparticle interaction; Hard ellipsoids of revolution; Human serum albumin

1. Introduction

Moderately or highly concentrated solutions of colloidal particles are very complicated systems to understand both from the thermodynamic and structural point of view. This is mainly due to the fact that the nonideality caused by the interparticle interaction potential arising from the surface charge, as well as from the ionic properties of the solvent, is very difficult to deal with theoretically. For instance, even in the simplest case with spherical particles and electrostatic interactions due to a uniform charge distribution on the particle surface, there are today only approximative methods available. In the more general case with nonspherical particles and a nonuniform charge distribution the difficulties are formidable. Apart from interactions between the electric double layers we also have to consider other types of interactions, like for instance van

Abbreviations: HSA, human serum albumin; SANS, small-angle neutron scattering; SAXS, small-angle X-ray scattering; MCS, Monte Carlo step

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der Waals forces and different types of solvent effects [1], which further complicates the situation. All these interactions, and also the fact that the particles have nonzero volumes, are the main sources to the nonideality of the solution. Strictly speaking, the solution is ideal only at the limit of infinite dilution although in some cases, at low concentrations, the deviations from ideality can be neglected.

Since most real systems, like biological fluids, solutions in technical processes, surfactants, lubricants, emulsifiers, etc., deviate strongly from ideality, it is desirable to develop both experimental and theoretical methods for the exploration of the forces between colloidal particles. In fact, very many technical processes, e.g. paint industry, paper manufacture, food production, ore flotation, water purification and cosmetics are based on methods by which the forces between the particles are altered in order to obtain new properties of the system.

Especially in biochemistry the questions of macromolecular 'crowding' [2] and interactions are very important in order to understand the behavior of highly nonideal biological media. Studies on biological macromolecules are normally performed either on dilute solutions, or on crystalline phases. Neither of these extreme conditions does, however, represent the true conditions in living systems, although the water content in protein crystals can be quite large. For instance, the concentration of the dissolved components in blood plasma is about 9% (by weight) and the corresponding intracellular value is as high as 17–35% [3]. In such systems we can expect the conditions to be quite different from those in a dilute solution, or in the crystalline phase. As an example, the thermodynamic activity of hemoglobin increases dramatically with the concentration; at the in vivo concentration of hemoglobin within the erythrocytes (about 35%) the activity is approximately 100 times its concentration [4], which clearly demonstrates the errors introduced when neglecting the activity factors.

It has been demonstrated that small-angle scattering of X-rays (SAXS) and neutrons (SANS) are very useful methods in order to study the interactions in solutions of colloidal particles [5–10]. In traditional SAXS and SANS studies the methods have been used to obtain the scattering profiles that represent the intraparticle scattering density correlation, by extrapolating to zero concentration, or by collecting the data at a very low concentration. In practice, *inter*particle correlations always contribute to the scattering; the effect increases with increasing concentration. The interparticle scattering contains information concerning the interactions between the particles. Since the distances involved are relatively large, the interparticle scattering predominantly dominates at rather low scattering angles.

Of the theoretical methods available to model the interactions between colloidal particles in order to calculate the interparticle structure factor $S(Q)$, and consequently also to be in a position to analyze the experimental SAXS and SANS data, most are based on the assumption of a hard-sphere-type of interactions [11,12]. In some cases an additional potential of interaction has been added in order to compensate for electrical and other types of interactions. The classical DLVO theory [13] has been used for this purpose by several authors [14].

In this work we have used the SANS method in order to investigate solutions of human serum albumin (HSA) in the concentration range from 0.00477 to 0.259 g/cm^3 in D_2O solutions containing 1.08 M NaCl. The reason for using such a high concentration of NaCl is that we want to diminish the range of the electrical interactions as far as possible. HSA was chosen as a model substance because it is readily available in pure form and its use in several physico-chemical studies has resulted in considerable knowledge of its properties [15].

The SANS investigation has been combined with results obtained by Monte Carlo simulations where we calculate average values of the interparticle structure factor $S(Q)$ and the pair correlation function $g(r)$. The advantage of using Monte Carlo simulation instead of one of the above mentioned methods [11,12], is that completely general models for the particle shape and interactions can be considered and we are not restricted to the spherical hard core model. The only problem is to formulate the model in mathematical terms and to implement the algorithm into the computer.

By comparing the Monte Carlo and the SANS results it was found that the latter can be explained by a model of interaction consisting of a hard ellipsoid of revolution with the same dimensions as those of the HSA molecule, plus a spherically symmetric repulsive potential. Knowledge of the interparticle interactions opens the possibility to calculate and predict other ther-

modynamic and structural properties for these highly nonideal solutions.

2. Materials and SANS measurements

The samples used in this investigation were made by dissolving commercial HSA (Sigma, product A-8763) in 1.08 M NaCl in D_2O. Heavy water was used as a solvent in order to increase the signal/noise ratio. According to the manufacturer's specification this HSA product is essentially globulin free, but it is not defatted; defatting is reported to decrease the stability of HSA and to increase the risk for formation of dimers [15]. The water content of the lyophilized HSA powder was determined to 6% by drying over P_2O_5 in vacuo until constant weight and by measuring the optical absorbance of the solutions at 279 nm [15]. In order to check for dimers and multimers the molecular mass was calculated from the SANS data by extrapolating the intensity at zero angle to infinite dilution [16]. In these calculations we used the partial specific volume 0.734 cm^3/g calculated from the amino acid composition, a value which is within the errors of the experimental one, 0.733 cm^3/g [15]. The neutron scattering density was calculated from the composition of HSA [15], assuming that all hydrogen atoms bound to nitrogen and oxygen exchange in proportion to the deuterium/hydrogen ratio in the solvent. The experimental molecular mass obtained, 69.0 kDa, is slightly larger than the value 67.1 kDa reported in the literature [15]. Considering that this is a nondefatted product it, however, indicates that the dimer content is very low. When mixing the solutions 99.9% D_2O was used. The water content of the HSA powder, as well as the labile hydrogens of the HSA, results in a varying D_2O content of the solvents from 93.8%, for the highest HSA concentration, to 99.8% for the lowest concentration used. In order to equilibrate the hydrogen/deuterium exchange, at least 24 h elapsed before the SANS measurements were performed.

The SANS data were collected by using the facility at Risø National Laboratory, Denmark, set at sample-to-detector distances equal to 2.50 m and 6.00 m and a neutron wavelength $\lambda = 0.35$ nm ($\Delta\lambda/\lambda = 16\%$, full width at half maximum). The data were corrected for background and detector non-uniformities, and transformed to the radial form, exactly as described previously [17]. The temperature of the 0.2 cm quartz cuvettes used as sample containers was kept at 21.0°C.

3. The Monte Carlo simulations

The model system for the simulations was constructed by defining a basic cubic cell of side length L and volume V containing N molecules. The periodic boundary condition [18,19] was used in order to avoid surface effects. That is, the basic cell is surrounded on all sides by images of itself repeated throughout the three-dimensional space. The particle number density $\rho = N/V$ is conserved because when a particle moves out across one face of the basic cell an image of that particle moves in through the opposite face.

The interaction potential between the molecules consists of two parts. First, it is assumed that the molecules are hard and impenetrable. The HSA molecule is modeled as a hard ellipsoid of revolution with the semiaxes $a = 6.8$ nm and $b = c = 1.9$ nm. This model was obtained as a result of a traditional iterative shape analysis process [20], where different types of three-axial bodies were fitted to the SANS data extrapolated to zero concentration.

The second part of the interaction potential is based on the assumption that the molecules are surrounded by a repulsive potential. Several different approaches for the interaction potential have been considered (see discussion). It, however, followed that a spherical potential function of the form

$$U(r) = A \exp(-Br)/r \qquad (1)$$

is sufficient to explain the experimental SANS data. Here A and B are constants, r the centre–centre distances between the molecules and the potential $U(r)$ is expressed as the dimensionless ratio to k_BT.

Starting configurations were generated by filling the basic cell with ellipsoids of revolution in nonoverlapping random positions and with random orientations. Check for overlapping was made by using the criterion of Vieillard-Baron [21]. The number of particles N placed in the cell was chosen in order to give the particle number density ρ corresponding to the HSA samples used in the SANS experiments. The simulation was then started and at each Monte Carlo step (MCS) a randomly chosen particle was moved by random displacements along each of the three directions parallel

with the cell edges. Consecutive orientational displacements were generated by rotating the a axis of the ellipsoids the randomly chosen angles θ_i and φ_i, defined as the colatitude and the longitude, respectively, in a spherical polar coordinate system having the polar axis (Cartesian X axis) along the a axis of the ellipsoid; the coordinate systems are defined as in [22] except that the Cartesian Z, X and Y [22] are renamed as X, Y and Z, respectively, in this work. Successive applications of the matrix [23]

$$A_i = \begin{pmatrix} \cos\theta_i & -\sin\theta_i & 0 \\ \sin\theta_i \cos\varphi_i & \cos\theta_i \cos\varphi_i & -\sin\varphi_i \\ \sin\theta_i \sin\varphi_i & \cos\theta_i \sin\varphi_i & \cos\varphi_i \end{pmatrix} \quad (2)$$

was used in order to transform the local Cartesian coordinates of the ith orientation to the $(i-1)$st coordinate frame. Next it was checked whether the particle under consideration arrived in an overlapping position [21]; if overlap was detected the move was rejected and a new MCS was started. In case of no overlap the change in energy ΔE was calculated by summing up the contributions from all the molecules in the basic cell (Eq. (1)), also considering their interactions with the molecules in the neighboring cells. Acceptance or rejection of the step was then made according to the classical Metropolis Monte Carlo criterion [18]. For each particle in the cell record was kept regarding the position as well as regarding the elements of the rotation matrix and it was updated after each accepted MCS.

The maximum sizes of the random displacements were adjusted so that roughly half of the MCSs were accepted. In the more concentrated solutions it was impossible to fulfill an acceptance ratio of 50%. In these cases the step sizes were chosen to have as high acceptance ratio as possible. In all cases it was checked that the result was independent of the maximum displacement sizes.

A considerable decrease in computing time during the overlap and energy calculations was obtained by dividing the basic cells into subcells of side length l; only the molecules within the 26 subcells surrounding the subcell containing the molecule under consideration were taken into accounts. The length l, which also defines the cutoff distance of interaction, was chosen large enough to produce results which were independent of l.

In order to equilibrate the system and also to avoid 'jammed' configurations [24] the simulations were always started with at least 10^5 MCSs without collecting any data. Averages of the structure factor $S(Q)$ and the pair correlation function $g(r)$ were then calculated by sampling over the basic cell at every from 10^3 up to 10^5 MCSs, depending on the number of molecules in the cell.

The structure factor $S(Q)$ was calculated from

$$S(Q) = 1 + \frac{2}{N} \sum_{k=1}^{N-1} \sum_{i=k+1}^{N} \frac{\sin(Qr_{ki})}{Qr_{ki}}, \quad (3)$$

where r_{ki} is the centre–centre distance between molecules k and i. The SANS intensity was obtained from [25].

$$I(Q) = \langle F^2(Q) \rangle + \langle F(Q) \rangle^2 \times \{ S(Q) - 1 \} \quad (4)$$

where $\langle\ \rangle$ indicates averaging and F is the structure factor for the HSA molecule modeled as an ellipsoid of revolution with semiaxes $a = 6.8$ nm and $b = c = 1.9$ nm. $Q = (4\pi \sin\xi)/\lambda$, where ξ is half the scattering angle and λ the neutron wavelength. To facilitate the computations we have separated the calculation of $\langle F \rangle^2$ from the averaging of $S(Q)$ [25]. The errors introduced by this approximation are not visible in Fig. 1. Finally, the calculated intensities were smeared with the appropriate wavelength distributions, in order to make them directly comparable with the experimental SANS values.

The pair correlation function $g(r)$ was obtained by recording the total number of center–center distances falling between $r - \frac{1}{2}\Delta r$ and $r + \frac{1}{2}\Delta r$. Finally, in order to correct for the finite size of the cell, the result was divided with the distance distribution function for a cube [26] of side length L, integrated over the above interval in r.

The main difficulty with computer simulation is that only relatively small systems can be studied within reasonable computer time. Especially the structure factor $S(Q)$ will be affected by the volume scattering at small Q. Different values of the cell size L and have therefore been used in order to check that the results are independent of L. The maximum value of L used was equal to 250 nm and the maximum number of particles N was equal to 2050.

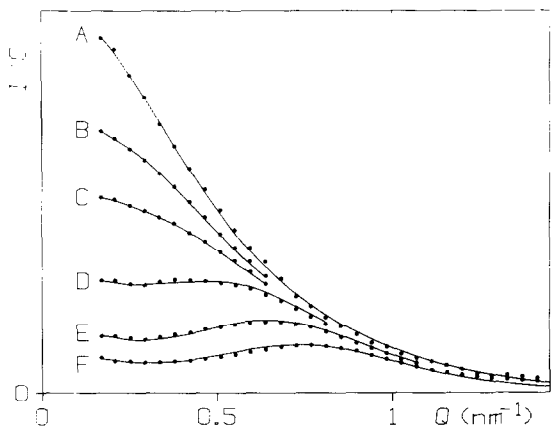

Fig. 1. Comparison between results obtained by SANS and by Monte Carlo simulation. The points are the experimental SANS intensities (normalized with the concentration C) obtained in 1.08 M NaCl. The HSA concentrations of samples A–F are given in Table 1. The full-drawn curves represent the intensities calculated from Eq. (4). The structure factor $S(Q)$ was obtained by Monte Carlo simulation where the HSA molecules are assumed to be hard and impenetrable ellipsoids of revolution surrounded by a repulsive potential according to Eq. (5). The intensities have been smeared with the neutron wavelength distribution in order to make them directly comparable with the experimental SANS data. For clarity of drawing some of the points for samples B–E at larger Q values have been omitted. These data, however, fall symmetrically between samples A and F.

4. Results and discussion

The composition of the samples used in the present investigation, together with the particle number density ρ and the packing volume fraction $\eta = \frac{4}{3}\pi\rho ab^2$, are given in Table 1. From Fig. 1 it follows that there is a good agreement between the results obtained by SANS and by Monte Carlo simulation. In the simulations the shape of the HSA molecule is approximated by an ellipsoid of revolution with semiaxes $a = 6.8$ nm and $b = c = 1.9$ nm. The potential of interaction between the molecules consists of two parts, first it is assumed that the ellipsoids of revolution are hard and impenetrable and, second it is assumed that the molecules are surrounded by a spherically symmetric repulsive potential according to Eq. (1). It was found that the experimental SANS data can be explained by a model where we keep the value of B in Eq. (1) constant equal to 1.0 nm^{-1}. The value of A (Eq. (1)), on the other hand, is proportional to the HSA concentration: $A = 38000C$ nm, where C is the HSA concentration in g/cm^3. Thus, the spherically symmetric potential according to Eq. (1) can be written

$$U(r) = 38000C \exp(-r)/r \qquad (5)$$

where r is given in nm.

Several other models for the potential $U(r)$ have been tested. The model described above is, however, the simplest one we have found which explains the experimental SANS data. For instance, it should be mentioned that a hard ellipsoid of revolution alone is not sufficient to describe the interactions; simulations where we assume $U(r) \equiv 0$ failed to explain the SANS data and it is necessary to include the additional potential, Eq. (5). In some simulations we have also modified the hard ellipsoid of revolution model by assuming that the dimensions of interaction are larger than those of the HSA molecules, that is we assume that the molecules are surrounded by an impenetrable shell. This model, however, also failed to explain the SANS data.

In a third approach we have used an interaction potential $U(\zeta)$ which, instead of being spherically symmetric, depends upon the shortest distance ζ between two surface points of two molecules. These calculations are, however, very time-consuming due to the difficulty of obtaining ζ; in our calculations ζ was calculated by an iterative procedure. This approach did not, however, give any further improvement in the fit between the SANS and the Monte Carlo results.

Table 1
Composition of the samples used in the present investigation, C is the HSA concentration, ρ the number of molecules per unit volume and η the volume fraction occupied by HSA. All samples contained 1.08 M NaCl

Sample	C (g cm^{-3})	$\rho \times 10^4$ (nm^{-3}) [a]	η [b]
A	0.00477	0.428	0.00440
B	0.0288	2.58	0.0265
C	0.0577	5.18	0.0532
D	0.116	10.4	0.107
E	0.191	17.1	0.176
F	0.259	23.2	0.239

[a] Based on M_r(HSA) = 67120 [15].
[b] The shape of the HSA molecule is approximated as an ellipsoid of revolution with semiaxes $a = 6.8$ nm and $b = c = 1.9$ nm.

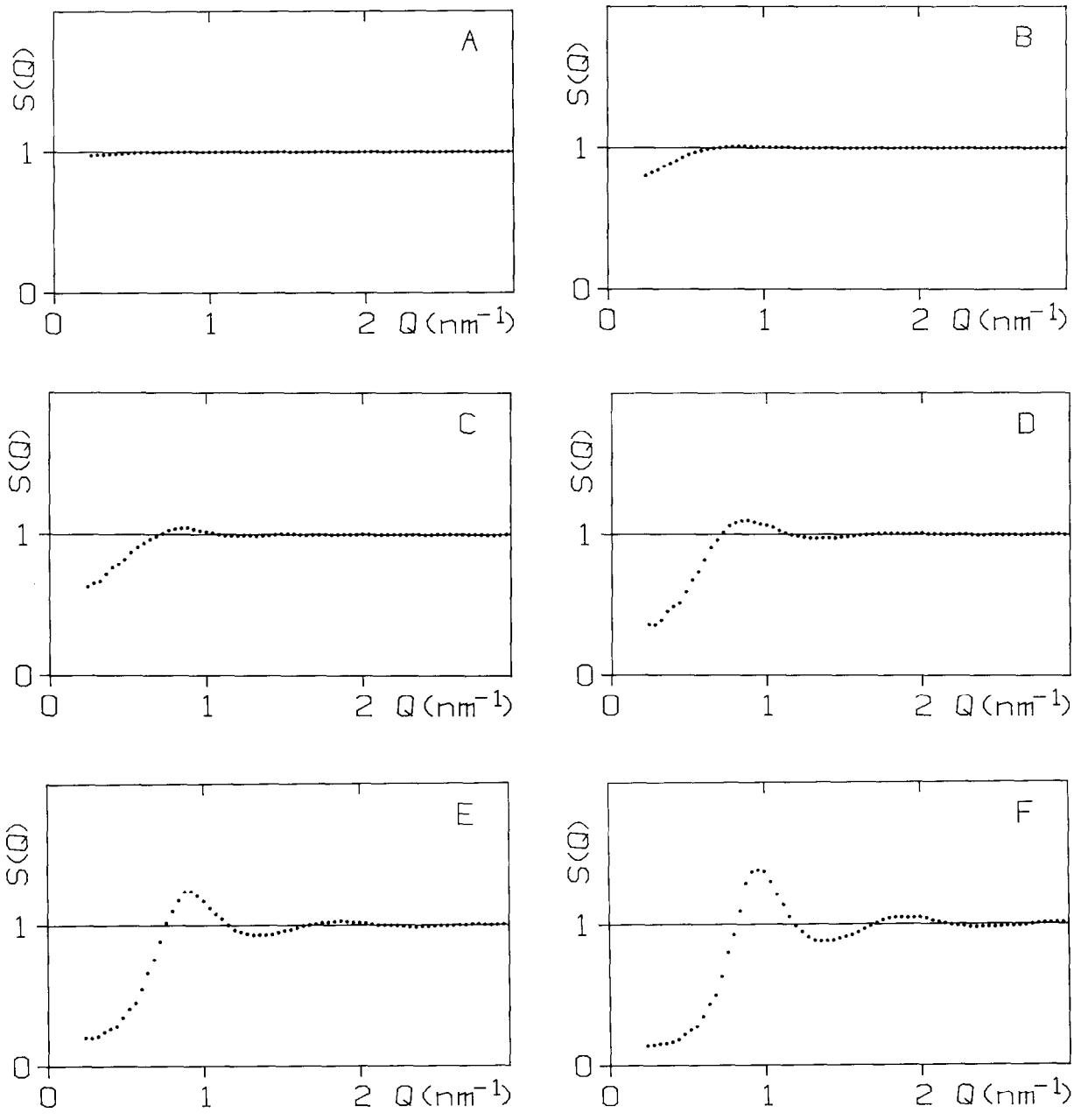

Fig. 2. The structure factor $S(Q)$ obtained from the Monte Carlo simulations for the best fitting interaction model described in the text. The composition of samples A–F follows from Table 1.

Eq. (1) is of the same mathematical structure as the equation describing the interaction between the electric double-layers of two spheres [13]. In this study the equation has, however, been used quite empirically and no conclusions can be drawn from the values of the constants A and B regarding the nature and distribution of the forces between the molecules. The interactions between the nonspherical HSA molecules can be

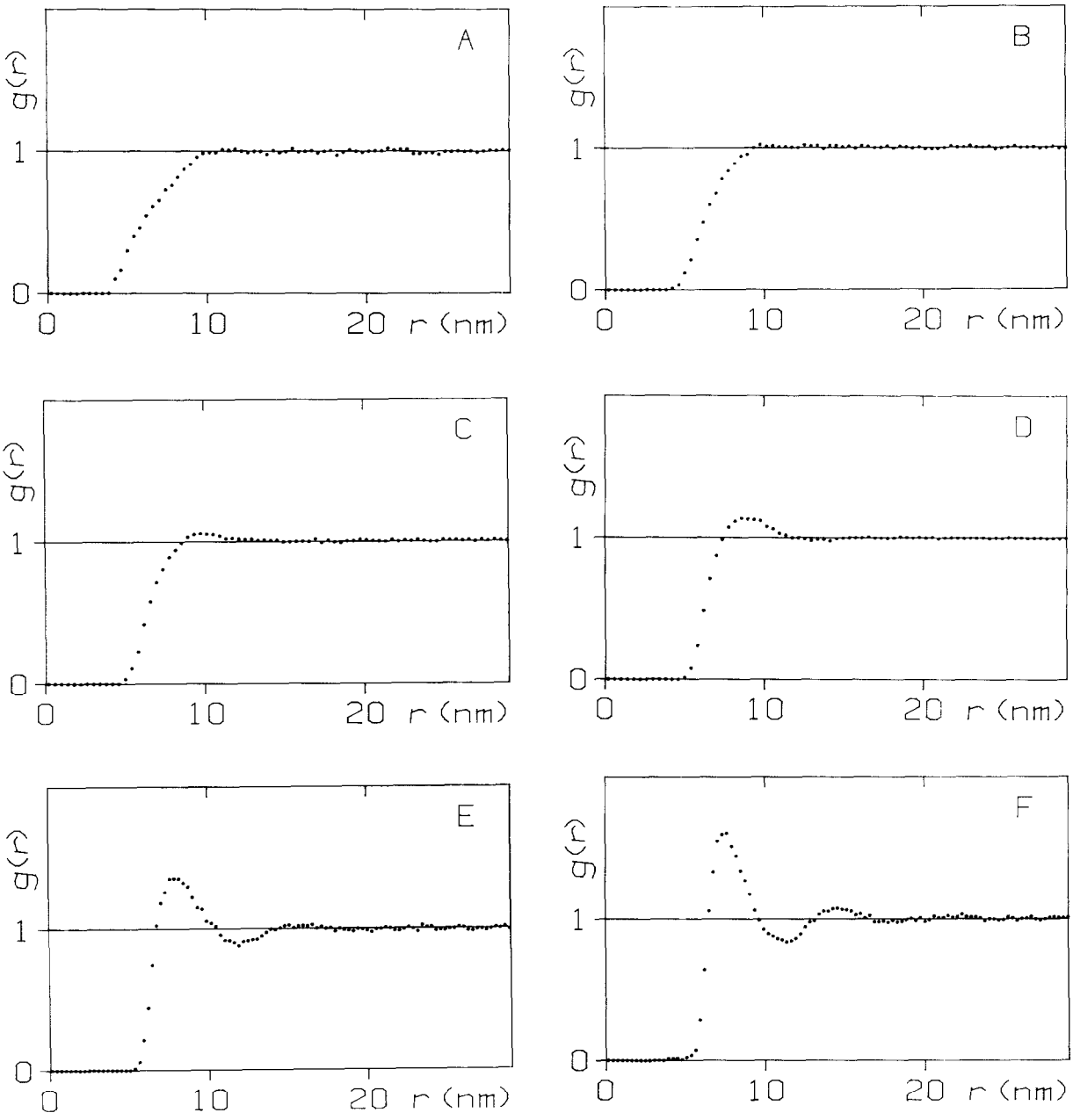

Fig. 3. The pair correlation function $g(r)$ obtained from the Monte Carlo simulations for the best fitting interaction model described in the text. The composition of samples A–F follows from Table 1.

expected to follow very complicated functions where the distribution of charges on the particle surface and the shape and relative orientations of the molecules must be considered.

The structure factor $S(Q)$ and pair correlation function $g(r)$ obtained by the Monte Carlo simulations corresponding to the compositions of the samples used in the SANS measurements, and the best fitting inter-

action model described above, are shown in Figs. 2 and 3. In principle these functions are correlated by Fourier transforms and attempts have been made to calculate $S(Q)$ from $g(r)$ and vice versa. These numerical Fourier transforms are however very unstable and it is preferable to calculate the individual functions as described above. Fourier transforms have been used only as a rough check, especially of $S(Q)$ at low values of Q.

Fig. 2 clearly demonstrates that, in spite of the high NaCl concentration (1.08 M), the structure factor $S(Q)$ deviates from the value one already at relatively low HSA concentrations. The influence of interparticle scattering in SAXS and SANS studies should therefore always be considered. From Fig. 3 it follows that there is a substantial ordering in the HSA solutions already at relatively low concentrations. As expected the first maximum of $g(r)$ is moved towards smaller values of r at increasing HSA concentration.

In an investigation of highly concentrated solutions we also must ask whether there is a tendency for aggregation. This is a complicated question which needs further investigations. First we have to define exactly what is meant by the word aggregation and what is the difference between aggregation and interaction. In this study we can only say that there is no indication of an *irreversible* aggregation. This conclusion is based on two arguments: (a) the whole data set, recorded for all HSA concentrations, can be explained by a simple few parameter model and (b) dilution of the highly concentrated solutions used for the SANS measurements gives a molecular mass, and also shape and dimensions, close to those of monomeric HSA.

Finally we also want to stress the great potential possibilities of Monte Carlo simulations in the study of nonideal solutions of macromolecules. The main advantage of the method is that any type of model for the particle shape and interactions can be considered.

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