Structural coefficients in aggregates of protein-coated colloidal particles

M. Tirado-Miranda a,*, M.A. Rodríguez-Valverde b, A. Schmitt a, J. Callejas-Fernández a, A. Fernández-Barbero c

a Department of Applied Physics, University of Granada, Campus de Fuentenueva s/n, E-18071 Granada, Spain
b Emulsion Research Group, EUROVIA-PROBISA, E-28320 Pinto, Madrid, Spain
c Department of Applied Physics, University of Almería, E-04120 Almería, Spain

Available online 8 September 2005

Abstract

The structural coefficients of bare and protein-coated polystyrene particles aggregating at different electrolyte concentrations were determined by light scattering techniques. The time dependence of the average diffusion coefficient could be fitted considering a simple form for the aggregation kernel. The fits allowed the structural coefficient to be determined. This parameter was then used to obtain the surface-to-surface separation between the monomers contained within the clusters. The results show that this distance is directly related to the size of the adsorbed protein molecules.

© 2005 Elsevier B.V. All rights reserved.

Keywords: Structural coefficient; Cluster structure; Colloidal aggregation; Light scattering; BSA

1. Introduction

The structural and dynamical properties of colloidal aggregates play an important role in fields such as polymer and colloid science, food industries and biophysics, among others. The structure of the formed clusters is strongly related to the mechanism and kinetics of aggregation. For example, relatively open branched clusters grow under pure diffusion limited growth conditions while reaction limited aggregation processes give rise to more compact structures. Although colloidal aggregates are of very complex nature, the description of their internal structure can be significantly simplified using fractal geometry [1]. The fractal dimension, \( d_f \), links the number of primary particles per cluster, \( n \), to the aggregate radius of gyration, \( R_g \), according to the relationship:

\[
n = k_0 \left( \frac{R_g}{R_0} \right)^{d_f}
\]

where \( R_0 \) is the monomer radius and \( k_0 \) is the structural coefficient. The latter parameter is often neglected and simply set to one. It is, however, an important factor for a complete quantitative characterization of fractal aggregates. In fact, clusters with identical \( d_f \), \( R_g \) and \( R_0 \) contain less primary particles when they have a smaller structural coefficient. A larger distance should then exist among the monomeric particles contained within the clusters. Consequently, the structural coefficient \( k_0 \) must be related to that distance. Oh and Sorensen [2] expressed this dependency as \( k_0 = k_0^{(1)} \delta^{d_f} \), where \( \delta = 2R_0/l \) is the monomer overlap, \( l \) the center-to-center distance of two adjacent monomers and \( k_0^{(1)} \) is the structural coefficient for particles that are in direct surface-to-surface contact. The above mentioned relationship shows clearly that \( k_0 \) increases with increasing monomer overlap. This agrees with simulations and experiments from stereoviews of three-dimensional (3D) aggregates for which high \( k_0 \) values were found [3]. The monomer overlap \( \delta \) may also be expressed as:

\[
\delta = \frac{2R_0}{R_0 - S}
\]

where \( S \) is the surface-to-surface distance of two adjacent monomers [4]. In this relationship, \( S \) is positive for separated particles and negative for overlapping monomers. In

* Corresponding author.
E-mail address: miraldo@ugr.es (M. Tirado-Miranda).
previous work, we studied surfactant-covered particles aggregating under diffusive conditions and the obtained values of about twice the thickness of the adsorbed surfactant layer [4]. Information about the structural properties of aggregates can be obtained in many different ways. Scattering methods are probably the most frequently employed techniques for this purpose. They have been widely used to characterize the fractal nature of clusters and to study the underlying aggregation kinetics [5–7]. The fractal dimension of colloidal clusters is usually determined by means of static light scattering. The structural coefficient, however, is quite difficult to determine experimentally. The aggregation kinetics is normally expressed in terms of an aggregation kernel, i.e., a set of kinetic rate constants for all possible cluster–cluster reactions. Since most aggregation kernels are homogeneous functions of the cluster size, they may be classified by the well-known van Dongen–Ernst homogeneity exponent λ [8].

In this work, the structural coefficient for base and protein-covered particles aggregating at different electrolyte conditions will be determined. The mean diffusion coefficient will be employed as experimental parameter for monitoring the aggregation kinetics. The fractal dimension of the formed aggregates and the homogeneity coefficient λ will be determined by means of static light scattering (SLS) and dynamic light scattering (DLS). The results will then be used to calculate the separation between adjacent monomers contained in the clusters and to estimate the adsorbed protein layer thickness. A thorough discussion of the obtained results will show that the structural coefficient and the homogeneity exponent provide useful additional information on the cluster structure and aggregation kinetics that could not be obtained otherwise.

The paper is structured as follows: the first section is this introduction. The theoretical background is resumed briefly in Section 2. The following section details the materials and methods used for the experiments. The results are presented and discussed in Section 4. Finally, the conclusions are tackled in Section 5.

2. Theoretical background

Information on the cluster structure is frequently obtained by means of SLS. The light intensity, I(q), scattered by a system with a given cluster-size distribution, Nn, is given by [6]:

\[ I(q) = \sum_{n=1}^{N} N_n q^2 S(q R_n) P(q R_0) \]  (3)

where \( n \) is the number of monomer particles per cluster, \( S(q R_n) \) the aggregate structure factor, \( P(q R_0) \) the monomer form factor, \( R_0 \) the monomer radius, \( R_n \) the aggregate radius of gyration and \( q \) is the modulus of the scattering vector. For elastic light scattering, \( q \) is given by \( q \approx (4\pi/\lambda) \sin(\theta/2) \), where \( \lambda \) is the wavelength of the incident beam in the surrounding medium and \( \theta \) is the scattering angle with respect to the incident beam. The aggregate structure factor \( S(q R_n) \) accounts for the spatial distribution of individual particles within the clusters. For \( R_n \gg q^{-1} \gg R_0 \), Eq. (3) may be expressed as [5]:

\[ I(q) \sim (q R_n)^{-d} \]  (4)

if one assumes that the monomers are sufficiently small so that their form factor \( P(q R_0) \) becomes unity. Eq. (4) allows the cluster fractal dimension to be determined directly by means of SLS measurements in the adequate \( q \) range.

The aggregation kinetics is usually studied by means of DLS. On the one hand, the electric field autocorrelation function for a polydisperse system of diffusing particles is modelled theoretical as [6]:

\[ g(\tau) = \frac{1}{I(q)} \sum_{n=1}^{N} N_n (t/2) S(q R_n) P(q R_0) \exp(-\Gamma_n \tau) \]  (5)

where the decay rate, \( \Gamma_n \), for an \( n \)-size cluster is linked to its translational, \( D_n \), and rotational, \( D_n^{rot} \), diffusion coefficients according to [9]:

\[ \Gamma_n = D_n q^2 + 6 D_n^{rot} \]  (6)

Assuming that \( D_n q^2 \gg 6 D_n^{rot} \) for all cluster sizes [10], the following expression may be obtained for the logarithmic derivative of \( g(\tau) \) in the limit \( \tau \to 0 \):

\[ \frac{d}{d\tau} \ln g(\tau) \bigg|_{\tau=0} = -\sum_{n=1}^{N} \frac{N_n (t/2) S(q R_n) D_n q^2}{\sum_{n=1}^{N} N_n (t/2) S(q R_n)} \]  (7)

On the other hand, the experimentally obtained autocorrelation functions are usually fitted according to the expression [11]:

\[ g(\tau) = A \exp \left( -\Gamma_\delta \tau + \frac{1}{2} \Gamma_\delta \tau^2 + \cdots \right) \]  (8)

which is known as cumulant analysis. Here, \( A \) is a constant, \( \Gamma_\delta \) the average decay rate and \( \Gamma_\delta \) is related to the degree of polydispersity. Evidently, the limit \( \tau \to 0 \) of the logarithmic derivative of \( g(\tau) \) becomes:

\[ \frac{d}{d\tau} \ln g(\tau) \bigg|_{\tau=0} = -\Gamma_\delta \]  (9)

Combining Eqs. (7) and (9) yields finally [6]:

\[ \left( D(q, t) \right) = \frac{\Gamma(q)}{q^2} = \frac{\sum_{n=1}^{N} N_n (t/2) S(q R_n) D_n}{\sum_{n=1}^{N} N_n (t/2) S(q R_n)} \]  (10)

where the finite character of the aggregation processes has been considered introducing a cut-off size, \( n_c \). This cut-off size corresponds to the largest aggregates present in the system. Evidently, \( n_c \) rises as the clusters grow. Eq. (10) means that the average diffusion coefficient, obtained from experiments as \( \Gamma(q)/q^2 \), may be calculated theoretically as the average of the diffusion coefficients, \( D_n \), of individual aggregates weighted by the corresponding scattering intensity and cluster mass distribution at any given time.
Assuming the aggregates to be fractal objects, the average translational diffusion coefficient, \(D_n\), of aggregates of size \(n\) may be expressed as a function of the number average mean cluster size, \(\langle n \rangle\), as [2,12]: \(D_n = Bn^{d-1/d} \) (11) where \(B = D_kk_0^{1/d}\) is a parameter [2] that depends on the cluster fractal dimension, \(d\), the structural coefficient, \(k_0\), and the diffusion coefficient of free monomeric particles, \(D_k\).

The cluster structure factor, \(S(qR_0)\), is related to the mean scattered light intensity according to Eq. (3). Several functional forms for \(S(qR_0)\) can be found in the literature [13,14]. The difficulty lies in obtaining an expression that remains valid for the whole range \(qR_0 > 1\). Lin et al. [15] calculated the aggregate structure factor directly from computer generated clusters obtained under diffusion and reaction limited conditions. They parameterized their result by fitting the polynomial expression:

\[
S(qR_0) = \left(1 + \sum_{i=1}^{n} C_i(qR_0)^{d_i/2n}\right)^{-d_i/2n} \] (12)

For diffusion limited cluster aggregation (DLCA) aggregates, they obtained \(n=4\), \(C_1 = 2m/3d_c\). \(C_2 = 2.50, C_1 = -1.52, C_4 = 1.02\) and \(d_1 = 1.8\). For reaction limited cluster aggregation (RLCA) aggregates the best fit yielded \(n = 4, C_1 = 2m/3d_c, C_4 = 2.1\), \(C_1 = -2.58, C_4 = 0.95\) and \(d_1 = 2.1\). Since the experimental cluster fractal dimensions generally differ from 1.8 and 2.1, the structure factors may be approximated by interpolating the values given by both polynomials. This structure factor form provides a good description of aggregates of finite size. Furthermore, Eq. (12) is in good agreement with experimental \(I(q)\) curves in the range \(qR_0 \leq 1 < qR_0\) and has the expected long-time asymptotic power-law form:

\[
S(qR_0) \sim (qR_0)^{-d_0/2}\] (13)

The time evolution of the cluster-size distribution, \(N_c(t)\), arising in an aggregating system may be obtained solving Smoluchowski’s rate equation [16]:

\[
\frac{dN_c}{dt} = \frac{1}{\eta} \sum_{j=1}^{j \neq i} k_{ij}N_iN_j - N_c \sum_{n=1}^{\infty} k_{0n}N_i \] (14)

This equation is valid for dilute systems when fragmentation does not occur. The aggregation kernel, \(k_{ij}\), quantifies the rate at which two \(i\)- and \(j\)-size clusters react and form an \((i+j)\)-size cluster. \(k_{ij}\) has to be understood as an orientational and configurational average of the exact aggregation rate for two specific clusters colliding under a particular orientation. All physical information about the aggregation mechanism is contained in the kernel.

Most kernels are homogeneous functions of the cluster size \(i\) and \(j\). A classification scheme for homogeneous kernels was introduced by Van Dongen and Ernst [8]. They defined the scaling exponent, \(\lambda\), according to:

\[
k_{ij}(a,b) \sim a^\lambda k_{ij} \] (15)

where \(a\) is a large constant. The homogeneity parameter \(\lambda\) determines how the aggregation rates depend on the cluster size and so, controls the overall rate of aggregation. For \(\lambda > 1\), gelation occurs, i.e., the aggregation processes become so fast that an infinite-size cluster forms at finite time. Aggregation kernels with \(\lambda \leq 1\) show a non-gelling behaviour, i.e., an infinite-size cluster is formed only at infinite time. The homogeneity exponent is well known only for the limiting DLCA regime and to some extent also for the RLCA regime [17].

Diffusion-limited cluster aggregation is modelled by the Brownian kernel [18] but no analytical solutions are known for the Smoluchowski equation. The Brownian kernel was derived by considering solid spheres which approach due to Brownian motion and stick as soon as they come into physical contact. Its analytical form is given by:

\[
k_{ij}^{\text{Brown}} = \frac{1}{2}k_{ij}^{\text{Smol}}(1/d) + j^{1/d}(1-1/d) + j^{-1/d} \] (16)

where \(k_{ij}^{\text{Smol}} = 8\pi\eta T/3\eta\). Here, \(\eta\) is the thermal energy and \(\eta\) is the solvent viscosity [16]. For DLCA processes, cluster fractal dimensions, \(d_1\), close to 1.75 are typically found when particle rearrangements within the clusters do not take place. DLCA is the fastest possible aggregation mode in the absence of repulsive interactions. According to Eq. (15), the Brownian aggregation kernel given by Eq. (16) has \(\lambda = 0\).

Recently, Schmitt et al. proposed a kernel that is based on the Brownian kernel but also considers multiple monomer contacts in the collision area of two approaching clusters [19]. This effect should be taken into account when not every monomer-monomer collision leads to bond formation. Consequently, this kernel seems to be more appropriate for describing aggregation processes that do not take place under diffusion limited conditions. The analytical form of this kernel that is given by [19]:

\[
k_{ij}^{\text{MC}} = \frac{k_{ij}^{\text{Smol}}}{2}(1/d) + j^{1/d}(1-1/d) + j^{-1/d} \] (17)

where \(d_0\) is the fractal dimension for pure diffusion-limited aggregation. As mentioned before, \(d_0\) is approximately 1.75 \pm 0.05 when additional effects such as internal cluster rearrangement do not occur [20]. We will refer to the kernel given by Eq. (17) as MC-kernel. According to Eq. (15), this kernel has:

\[
\lambda = 6 \frac{N_c - 1}{d_0} \] (18)

As expected, the MC-kernel reduces to the Brownian kernel for pure DLCA processes where \(d_1 = d_0\).

No valid kernel for the description for aggregation processes of coated particle suspensions is known so far [21].
Therefore, we will employ the Brownian and the MC-kernel as a first approximation in this case.

3. Materials and methods

Aqueous suspensions of polystyrene microspheres were used for the aggregation experiments. The particle size and polydispersity were checked by photoncorrelation spectroscopy (PCS). The measured number average mean particle diameter was \( 99 \pm 1 \) nm and the polydispersity index (pdi) was calculated from the first and second cumulants to be \( 0.09 \pm 0.02 \). Sulphate groups, derived from the initiator molecules for the polymerization reaction, stabilize the particles electrostatically. Conductometric titrations were performed in order to determine the total surface charge. In addition, charging and discharging potentiometric titrations were carried out in order to obtain the electric net charge and its \( pH \) dependence. The surface charge density at \( pH \) 4.8 was \( (2.5 \pm 0.1) \mu \text{C/cm}^2 \). The critical coagulation concentration (ccc) of \( 0.495 \pm 0.007 \) M KCl was determined by means of nephelometry.

Bovine serum albumin (BSA) was chosen as adsorbing globular model protein. Monomeric BSA molecules have a size of approximately \( 11.6 \times 2.7 \times 2.7 \) nm. They may also form covalent dimers through the SH group containing in their polypeptidic chains. The net charge of the BSA molecules can be varied from negative to positive by a simple \( pH \) change around their isoelectric point of approximately 4.8. Moreover, the BSA molecules have a relatively low structural stability which gives rise to more stable unions and, guarantees irreversible adsorption onto the surface of the polystyrene microspheres. The protein suspensions were cleaned by dialysis against distilled and deionized water during three days until the water conductivity remained constant. Afterwards, they were filtered through a Millipore 0.2 \( \mu \text{m} \) low affinity filter. The protein concentration was determined from the absorbance peak at a wavelength of 280 nm using a Spectronic-601 Milton Roy spectrophotometer. Through electrophoresis in polyacrilamide gel with silver dyeing, the BSA sample was found to be mainly composed of BSA dimers with a trace of larger BSA aggregates.

The amount of adsorbed protein was determined from the adsorption isotherms that were measured using 5 ml of particle suspension containing a total adsorption surface of 0.4 \( \text{m}^2 \). Acetate buffers were employed in order to fix the \( pH \) to the isoelectric point of the BSA molecules at 4.8. This guarantees maximum protein adsorption [22]. The polystyrene particles were added to the protein solution and incubated during 2 h at 25 °C. The amount of non-adsorbed protein was determined from absorbance measurements after centrifugation and filtration using low affinity filters. Samples with 50\% of their surface covered by BSA molecules were selected for the aggregation experiments.

All the solutions and suspensions used in this study were prepared with pure water obtained by reverse osmosis followed by percolation through charcoal and a mixed bed of ion-exchange resins. Aggregation was started by mixing equal amounts of sample and buffered electrolyte through a Y-shaped mixing device. Samples with a degree of surface coverage of 0 and 50\% were selected and aggregated at an electrolyte concentration of 0.700 M and 0.495 M. Also for the aggregation measurements, the \( pH \) was fixed to the BSA isoelectric point at which the protein molecules do not bear a net electric charge. The initial particle concentration for all experiments was set to \( 1.6 \times 10^{-3} \text{ cm}^{-3} \). This concentration is sufficiently low to avoid the hazardous effects of multiple scattering and photomultiplier saturation. The temperature was stabilized at \( 25 \pm 1 \) °C.

The time dependence of the average diffusion coefficient was obtained using light scattering from a 4700 model of Malvern Instruments. This instrument works with a 488 nm wavelength argon laser and is able to perform sequential SLS and DLS measurements. For SLS, the scattering angle was varied from \( 10^\circ \) to \( 150^\circ \). The DLS measurements were performed at \( 60^\circ \). The mean scattered intensity showed the expected asymptotic time-independent behaviour when the final structure of the clusters was totally established. From these curves, the fractal dimensions were calculated according to Eq. (4). Considering dynamic scaling and fractal cluster morphology, the time evolution of the mean hydrodynamic aggregate radius can be expressed as [23]:

\[
R_\text{agg}(t) \propto \tau_\text{agg}^{-1}(1-\lambda)
\]

This relationship was used to determine \( \lambda \) from the experimental time evolution of the mean hydrodynamic cluster radius, once the cluster fractal dimension was known.

Using the dynamic scaling hypothesis and the constant kernel approximation for the time evolution of the cluster-size distribution, the following relation for the first cumulant of the field autocorrelation function can be obtained [24]:

\[
\Gamma_2(t) = \Gamma_2(0)(1 + t/\tau_\text{agg})^{-1/\lambda(1-\lambda)}
\]

where \( \tau_\text{agg} \equiv 2(c_0 \sigma_0^2) \) is the characteristic aggregation time. \( \tau_\text{agg} \) is expressed as a function of the initial particle concentration, \( c_0 \), and the Smoluchowski rate constant, \( k_\text{S} \). Eq. (20) allows the characteristic aggregation time, \( \tau_\text{agg} \), to be obtained once \( d_f \) and \( \lambda \) are known. \( k_\text{S} \) can then be determined from \( \tau_\text{agg} \) using the known initial particle concentration [25].

The experimental time dependence of the mean diffusion coefficient was confronted with theoretical curves calculated by means of Eq. (10). In order to obtain the cluster-size distribution \( S(qR_\text{agg}) \), the Smoluchowski equation had to be solved for a given kernel. Due to the lack of a general analytical solution, a well-established stochastic algorithm was employed for this purpose [26]. The structure factor \( S(qR_\text{agg}) \) was determined using either Eqs. (12) and (13).
4. Results and discussion

Bare and protein-coated polystyrene microspheres were coagulated under well-known conditions, i.e., under pure particle diffusion and under the presence of small repulsive coulombian interactions, for which KCl was added at concentrations of 0.700 and 0.495 M, respectively.

Fig. 1a shows the scattered intensity as a function of the scattering vector for protein-coated particles aggregated at 0.700 M KCl. The asymptotic behaviour predicted by Eq. (4) is clearly observed. From the slope, a fractal dimension \( \hat{d}_f = 2.15 \pm 0.03 \) was determined. The time evolution of the mean hydrodynamic radius is plotted in Fig. 1b. Fitting the long-time behaviour according to Eq. (19), the homogeneity exponent \( \lambda = 0.04 \pm 0.03 \) was calculated using the previously obtained value for \( \hat{d}_f \). Both values were employed in order to determine the quantity \( \left( \frac{\Gamma_a(t)}{\Gamma_a(0)} \right)^{-\hat{d}_f(1-\lambda)} \) and plot it versus time. The results are shown in Fig. 1c. The characteristic aggregation time, \( t_{agg} \), was then obtained by fitting a straight line according to Eq. (20). From the obtained value of \( t_{agg} \), the Smoluchowski rate constant, \( k_S = (5.57 \pm 0.28) \times 10^{-12} \text{ cm}^3 \text{ s}^{-1} \) was finally calculated as \( k_S = 2/\left( c_0 t_{agg} \right) \) using the known initial particle concentration.

Table 1 summarizes the obtained results. All the experiments were repeated three times in order to guarantee the validity of the results. The fitting error was calculated as the standard deviation of the three repeated aggregation experiments.

As can be seen in Table 1, the bare particles behave in the well-known salt-induced aggregation manner and \( \hat{d}_f \approx 1.75 \), \( \lambda \approx 0 \) and \( k_S \approx 6 \times 10^{-12} \text{ cm}^3 \text{ s}^{-1} \) is observed at high elec-

![Fig. 1. Protein-coated particles aggregated at pH 4.8 and 0.700 M KCl: (a) scattered intensity as a function of the scattering vector, (b) mean hydrodynamic radius as a function of time, (c) time evolution of the normalized first cumulant as a function of the scaled time. The points correspond to the experimental data. The straight lines show the linear fit.](image-url)

<table>
<thead>
<tr>
<th>Electrolyte Concentration</th>
<th>Fractal Dimension ( \hat{d}_f )</th>
<th>Homogeneity Exponent ( \lambda )</th>
<th>Smoluchowski Rate Constant ( k_S \times 10^{12} \text{ cm}^3 \text{ s}^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.700 M 0%</td>
<td>1.75 ± 0.03</td>
<td>0.018 ± 0.014</td>
<td>6.33 ± 0.18</td>
</tr>
<tr>
<td>0.700 M 50%</td>
<td>2.15 ± 0.03</td>
<td>0.044 ± 0.033</td>
<td>5.57 ± 0.28</td>
</tr>
<tr>
<td>0.495 M 0%</td>
<td>1.91 ± 0.04</td>
<td>0.114 ± 0.016</td>
<td>5.33 ± 0.16</td>
</tr>
<tr>
<td>0.495 M 50%</td>
<td>2.21 ± 0.03</td>
<td>−0.090 ± 0.018</td>
<td>5.81 ± 0.30</td>
</tr>
</tbody>
</table>
trolyte concentration. These values are usually found in the literature for the DLCA regime. At lower electrolyte concentration (0.495 M), repulsive interactions between particles become important and the fractal dimension and the homogeneity parameter λ grow while the aggregation rate decreases. This means that the sample is now aggregating in the transition region between DLCA and RLCA [27].

For the coated particles, the cluster fractal dimension behaves in a similar way, i.e., it decreases for increasing electrolyte concentration. However, higher values than for the bare particles are found. This means that more compact clusters are formed under the same experimental conditions [7]. With regard to the aggregation kinetics, some differences can be observed for the dependency of λ and λ0 on the electrolyte concentration. The homogeneity parameter λ takes negative values when the electrolyte concentration decreases and so, bigger clusters become less reactive than smaller ones. Despite of the strong difference in λ, the aggregation rates change only slightly. Moreover, the aggregation rates seem even to increase for decreasing electrolyte concentration. This shows that the adsorbed protein molecules have a stronger influence on the aggregation kinetics than what would be expected analyzing only the changes in the aggregation rate.

The time evolution of the average cluster diffusion coefficient was measured by means of DLS in order to determine the structural coefficient k0 and the corresponding surface-to-surface distance S of the monomers contained in the formed aggregates. Fig. 2 shows the obtained results for the bare particles aggregating under DLCA conditions. The data were normalized by the monomer diffusion coefficient D0 and are shown as points in the plot. The solid and dashed lines represent the best fits according to Eq. (10) using the Brownian aggregation kernel and the structure factor S(q) given by Eqs. (12) and (13), respectively. From these values, the structural coefficient k0 can be obtained according to the relationship B = D0k0/λ. In the first case, k0 = 1.002 was obtained. This implies that the surface-to-surface distance between the monomers contained in the clusters is close to zero and the particles are, as expected, just in direct contact. In the second case, however, k0 = 2.374 was found. A value larger than unity means that the polystyrene monomers are overlapping or partially interpenetrating. This is, of course, unphysical for our hard polystyrene particles. For that reason, only the polynomial form of the structure factor S(q) given by Eq. (12) will be employed in this work.

As was mentioned before, the bare particles at lower electrolyte concentration were found to aggregate in the transition region between DLCA and RLCA. Evidently, the Brownian kernel is not expected to give an adequate description for this case since it was not deduced for a sticking probability lower than unity. Hence, we fitted the data using the MC-kernel given by Eq. (17) as a possible alternative. This kernel was already shown to reproduce aggregation of bare particles much better than the Brownian kernel at all electrolyte concentrations [27]. The main feature of the MC-kernel is that it accounts also for multiple intercluster contacts between two approaching clusters that become important as soon as not every monomer-monomer contact leads to bond formation. Our experimental results also confirm that the MC-kernel fits the data much better at lower electrolyte concentrations. Nevertheless, the fit is not very satisfactory if we use the previously determined values for λ and D0. We also tried to fit the sum kernel k0 = k1(1 + f)/2 that several authors claim to provide a suitable description for the RLCA regime. Nevertheless, this kernel is characterized by λ = 1 and so, it was not surprising to obtain an even worse fit.

In order to overcome the difficulties in fitting an adequate kernel for the different aggregation processes studied in this work, we tried to use a kernel as simple but as versatile as possible. We found the following analytical kernel to be quite useful:

\[ k_{ij}(0) = \frac{k_1^{(0)}(\alpha + f')}{2} \quad \alpha \in \mathbb{R} \tag{21} \]

This kernel was employed before for describing coagulation-fragmentation processes and its applicability was confirmed by computer simulations [28]. Olivier y Sorensen established that it allows to model slow aggregation kinetics quite reasonably well [24]. From a mathematical point of view, the kernel given by Eq. (21) allows other known kernels to be obtained as relatively simple combinations of k0(0) with different exponents α. As an example, the constant and the sum kernel are directly given for α = 0 and α = 1, respectively. The Brownian kernel can be expressed as k0(0)k1(0)(1+D0/2k1(0)) and the product kernel as k0(0)k1(0)k1(0). This shows clearly the versatility of this kernel.

It should be pointed out that the kernel given by Eq. (21) is a relatively simple analytical kernel that was not derived...
for any specific aggregation process or physical situation. Hence, we can only expect it to be an approximation that might fit our data reasonably well. In order to keep things as simple as possible, we tried to use it directly for fitting all our experimental data. Doing so, the exponent $\alpha$ can be identified immediately as the homogeneity exponent $\lambda$ and the dimer formation rate constant $k_{11}$ may be approximated by $k_S$. Fig. 3 shows the obtained results using the values of $\lambda$ and $k_S$ given in Table 1. In the figure, the experimental data for the normalized average diffusion coefficient $\langle D \rangle / D_0$ are plotted as points and the solid lines represent the best fits using the kernel given by Eq. (21). As can be observed, the fitted curves agree quite well with the experimental data for both, the bare and coated particles, and for all the different electrochemical conditions of the aqueous medium. A $\chi^2$ method was used to select the most adequate fit using $B/D_0$ as fitting parameter. Table 2 summarizes the minimized $\chi^2$ and the corresponding $B/D_0$ values. The structural coefficient $k_0$ and the surface-to-surface distance $S$ values were obtained from and are also included in the table. The fitting error was calculated as the standard deviation of three repeated aggregation experiments. For the bare particles, the structural coefficient $k_0$ is very close to unity and the surface-to-surface distance between the particles contained in the aggregates is approximately zero. These expected results are in good agreement with other experiments [4,12] and simulations [29]. The obtained values are, however, significantly smaller than the values reported in [30] for carbonaceous soot aggregates formed in laminar diffusion flames. This is not surprising since overlap between the soot monomers and surface growth of the formed aggregates is possible in the latter case. For the coated particles, smaller structural coefficients were found. This means that, in this case, the distance between the surfaces of two adjacent primary particles is not vanishing. As can be seen in Table 2, the corresponding surface-to-surface separation were $S \approx 9$ nm and 6.6 nm at 0.700 and 0.495 M, respectively. This means that the presence of protein molecules on the particle surface impede the particles to get in close contact. Consequently, the found surface-to-surface distances should be related with the adsorbed protein layer thickness. It seems reasonable to assume that the BSA molecules adsorb onto a not completely covered surface in a flat configuration. Hence, the surface-to-surface distance should lie around 2.7 nm, if only one flat layer of BSA molecules were present in the space separating the particles.

Table 2

<table>
<thead>
<tr>
<th></th>
<th>B/D0</th>
<th>0.700 M</th>
<th>0.495 M</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>0.997 ± 0.003</td>
<td>0.915 ± 0.003</td>
<td>0.933 ± 0.003</td>
</tr>
<tr>
<td>50%</td>
<td>0.904 ± 0.004</td>
<td>0.904 ± 0.004</td>
<td>0.904 ± 0.004</td>
</tr>
<tr>
<td>$S_{(\text{nm})}$</td>
<td>0.18 ± 0.18</td>
<td>1.03 ± 0.04</td>
<td>1.03 ± 0.10</td>
</tr>
</tbody>
</table>

The corresponding structural coefficient $k_0$ and surface-to-surface distance $S$ are also shown.
The results obtained in this work, however, indicate that the gap between two adjacent particles contains between two and four layers of individual BSA molecules or between one and two layers of BSA dimers. To our opinion, the latter case seems to be more likely since gel electrophoresis measurements of the protein composition revealed that the BSA sample used for adsorption contained mostly BSA dimers and some larger BSA aggregates. This finding confirms that the data reported in this work for the structural coefficient are reasonable and that the analysis of $k_0$ is an adequate tool for estimating the thickness of adsorbed macromolecule layers contained in growing particle aggregates. It shows furthermore that $k_0$ is directly related to the particle packing within the formed clusters and so, contains additional information on the inner cluster structure. Hence, the fractal dimension alone is insufficient for characterizing cluster morphology completely. In our case, for example, the coated particles aggregating at 0.700 M and at 0.495 M have the similar aggregation rate $k_0$ and cluster fractal dimension $d_S$ (see Table 1). This seems to indicate that there are no significant differences in neither the aggregation kinetics nor the cluster structure. Nevertheless, the detailed analysis of the homogeneity exponent $\lambda$ and the structural coefficient $k_0$ gives clear evidence that this is not the case. The changing sign of $\lambda$ indicates a strong change in the aggregation kinetics and the varying interparticle distance $S$ shows that the formed clusters differ also in their internal structure.

5. Conclusions

The structural coefficient was determined for bare and protein-coated particles aggregating at different electrolyte concentrations. Therefore, the time dependence of the average diffusion coefficient was fitted using the measured values for the fractal dimension, the homogeneity parameter and the aggregation rate constant. A simple analytical form for the aggregation kernel had to be employed for the fits. The monomer separation within the clusters was calculated from the obtained structural coefficients. The results show that aggregated bare monomers are, as expected, in close contact. Cluster forming covered monomers, however, are separated a minimal distance that is of the order of several BSA molecule diameters. This finding agrees perfectly with the fact that polymeric BSA was used in this work and shows that analysis of the structural coefficient $k_0$ is an adequate tool for estimating the thickness of adsorbed macromolecule layers contained in growing particle aggregates.

Acknowledgements

This work was supported by the Spanish Ministerio de Ciencia y Tecnología (Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (I+D+i), projects MAT2003-08356-C04-01, MAT2003-08356-C04-04 and MAT2003-03051-C03-01) and by the European Regional Development Fund (ERDF). We also wish to express gratitude to Manuel Quesada for supplying the latex particles.

References