Fractal Aggregates Induced by Antigen–Antibody Interaction

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We report measurements on the aggregation processes in a colloidal suspension of polystyrene particles covered with F(ab′)2 (immunoglobulin IgG fragment) in the presence of CRP antigen (C-reactive protein) performed by static and dynamic light scattering. To study the cluster morphology of aggregates, the fractal dimension is obtained from the dependence of the scattered intensity on the scattering wavenumber. The fractal dimension values so determined were higher than those predicted by irreversible aggregation simulation models and then closer to those obtained by reversible clustering models.

Introduction

The process of aggregation or agglutination of small particles to form larger clusters and the structures that result are important in many areas of science such as physics (dendritic growth),¹ chemistry (aggregation of colloids, formation of gels),² polymer physics (kinetics of polymerization),³ medicine (growth of tumors),⁴ and meteorology and ecology (cloud formation, coagulation of smoke particles).⁵–⁶

The clusters formed during an aggregation are characterized by their tenuous, chainlike structure. Virtually all of our knowledge of the growth of these structures has come from computer simulations⁶–¹¹ which have suggested that the resultant structures exhibit scale invariance and can be well described as fractal.¹² This particle clustering may occur by a nonequilibrium process (irreversible aggregation) or by an equilibrium clustering formation (reversible aggregation).

Two representative and very useful models of particle aggregation which have received considerable attention are the Eden¹³,¹⁴ and the diffusion-limited aggregation models.⁹,¹⁵ These models describe irreversible growth processes in terms of rate-limiting steps. In the Eden model (RLCA) the growth is limited by the reaction kinetics at the interface of the growing clusters and only a small fraction of particle collisions leads to permanent contact. This model produces compact clusters of different morphology.

In the diffusion-limited aggregation model (DLCA), the growth is controlled by diffusion and every collision between particles results in the formation of a permanent contact. This model generates tenuous treelike structures with fractal branching and deep fiords between the branches. Computer simulations of the DLCA and RLCA models yield fractal dimensions of 1.78 and 2.0 in three dimensions, respectively.¹⁰,¹⁶,¹⁷

In contrast to the wealth of recent theoretical work, experimental studies that can test the validity and applicability of the modern theories have been rather sparse. Some of these have demonstrated the existence of both aggregation regimes for silica,¹⁸ gold,¹⁹ and polystyrene²⁰,²¹ colloids. However, as Dudowicz et al.²²,²³ suggest, the presumption of irreversibility in theoretical discussions of particle clustering often conflicts with observations indicating that the clustering is reversible. For example, it has been found that in a rapid aggregation process, aggregate structures with an initially lower fractal dimension of 1.75 can restructure after a certain time to more compact clusters with a higher fractal dimension of 2.1²⁴,²⁵ or 2.4.²⁶ The contact of aggregates in this case is considered to be reversible so that they can loosen and reform repeatedly after the first collision. Shih, Aksay,

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and Kikuchi27 (SAK) have built a reversible growth model in which the equilibrium clustering transition is possible. Accordingly, the fractal dimension would increase with a decrease in the absolute magnitude of the interparticle attractive energy. Recently, different authors have reported that particles covered with surfactant show fractal dimension ranging from 1.75 to 2.7, depending on the surfactant coating level.28,29 All these results give evidence that the fractal dimension of aggregates is not strongly universal and their structure depends on the interparticle interaction. When interparticle interactions are weak, the particle clustering is reversible, and a dynamical equilibrium occurs. This way, clusters so formed are compact.

Polystyrene latex has been used as a carrier for antigen–antibody reaction in agglutination tests where, for example, the presence of visible agglomerates determines the presence of the cross-linking antigen. These spheres bearing attached proteins on their surfaces are of great significance in immunological and clinical studies.30,31 It is generally accepted that the antibody-coated latex suspended in a buffer solution becomes unstable when the antigen present in the solution at a given concentration creates a link between two particles. On the basis of this principle, the adsorption of antibody molecules onto latex as an application for diagnostic test systems was first reported by Singer and Plotz.32 This immunoassay is one of the most commonly used techniques in clinical chemistry. Specific knowledge about the kinetics of the antigen–antibody reaction at the solid–liquid interface is useful for optimizing a solid-phase assay technique. To our knowledge, there are not studies in the literature about the internal structure of clusters of antibody-coated particles agglutinate by antigen, which nevertheless represents an important class of colloids.

This paper investigates the mechanism of the antibody-coated latex destabilization due to antibody–antigen interaction. Static and dynamic light scattering measurements were used to study growth kinetics and cluster morphology, respectively. In this way the time evolution of the number-average mean cluster size and the fractal dimension were obtained.

**Experimental Section**

**Sample Preparation.** All chemicals used were of analytical grade quality. Water was purified by reverse osmosis, followed by percolation through charcoal and a mixed bed of ion-exchange resins. In protein experiments, pH was controlled using acetate buffer at pH 5.2 (ionic strength 2 mM).

The sulfonate polystyrene latex used was synthesized by surfactant-free emulsion polymerization. Latex was deaggregated by serum replacement until the electrical conductivity of the liquid was below 2 μS/cm. The particle-size distribution (PSD) of the latex was ascertained by transmission electron microscopy (TEM) using a Philips EM300 microscope on representative samples of more than 500 particles (automatically analyzed with the software, AQ Systems, Spain). The diameter of the polystyrene beads was 151 ± 4 nm and the polydispersity index was 1.054, which indicates monodispersity. TEM was also used for direct visualization of the aggregates.

C reactive protein (CRP), anti-CRP F(ab)? fragments, and monomeric bovine serum albumin (BSA) were kindly donated by Biokit S.A. (Spain). F(ab)? fragments were obtained by pepsin digestion of IgG and purified by gel filtration followed by Protein A chromatography to remove undigested IgG. Purity was checked by SDS–PAGE, and the molecular weight was found to be 102 kDa. No IgG contamination was detected. The isoelectric points (pI) of the F(ab)? preparations, determined by isoelectric focusing, were in the range 4.7–6.0.

Lyophilized monomeric bovine serum albumin (M-BSA), separated from the oligomers by exclusion chromatography, was dialyzed before use. All proteins were stored at –30 °C.

F(ab)? was attached to the latex particles by incubating the latex (0.4 m2) and protein solution in acetate buffer (pH 5.2) at 35 °C and 5 h. The sample was then centrifuged and resuspended in deionized water. The amount of protein attached to the latex particles was determined from the difference in protein concentration before and after adsorption by spectrophotometry at 280 nm. The protein coverage of the latex–F(ab)? complex used in this work is 2.0 mg/m2. Monomeric bovine serum albumin was used to cover free surface of the sensitized particles to avoid nonspecific agglutination.

**Static Light Scattering Measurements.** Two different static light scattering experiments were performed. One of them to obtain quantitative structural information, such as the fractal dimension D of the aggregates, and the other one to calculate coagulation rate constants. In the first set of experiments we measure the angle dependence of the scattered intensity I(q), where q is the scattering wavenumber, this related to the scattering angle θ by q = (4π/λ)sin(θ/2), with λ the refractive index of the solvent and θ the wavelength of light in vacuo. If cluster–cluster interactions are negligible and the intensity scattered by a cluster of mass M is expressed as I1(q), the total intensity scattered by the distribution N(M) of a cluster of mass M is given by

\[
I(q) = \sum_{M} N(M) I_1(q)
\]

(1)

The expression of I1(q) depends on the qRg value, where Rg is the radius of gyration of an aggregate. I1(q) can be expressed as M^2S(qRg), where S(qRg), called the structure factor, has a complicated form that depends on the type of aggregation kinetics.33,34

The total intensity is then given by

\[
I(q) \propto \sum M^2S(qR_g)
\]

(2)

It can be shown for qRg >> 1 that the structure factor satisfies the following expression35,36

\[
S(qR_g) = (qR_g)^{-1}
\]

(3)

where D is the fractal dimension of the aggregates.

Finally, since the measured intensity I(q) is proportional to the structure factor S(q), we have the next asymptotic behavior36,37

\[
I(q) \propto S(q) \propto q^D
\]

(4)

over the range Rg^1 \sim q < Rg^D, where Rg is the radius of the particles. For higher q values, the length scale corresponds to individual spheres and the intensity is related to the particle form factor. In the low-q region, topological length correlations between clusters could be studied. Thus, the fractal dimension D of the aggregates can be obtained by plotting I(q) versus q on a double-logarithmic scale. The negative of the slope in the power-law region gives the fractal dimension D.


The measurements of the scattered intensity as a function of the scattering angle \( \theta \) (scattering wavevector \( q \)) were carried out with the use of a computer-controlled goniometer (Malvern 4700c system) and an argon laser operating at a wavelength of 488 nm (150 mW). The collected intensity data were corrected in order to take into account the variation of the scattering volume with the scattering angle. All experiments were performed at the constant temperature of 25 °C in an optical thermostat.

To ensure the validity conditions of eq 4, the angular range 15° ≤ \( \theta \) ≤ 50° was chosen in our experiment (0.3 ≤ \( qR_0 \) ≤ 1). For scattering angles higher than 50°, eq 4 is not satisfied for our system. The intensity measurements are repeated at different times in order to reach the asymptotic behavior. The duration of each run is determined by the time required to observe a straight line in the log-log plot of \( I(q) \). It varies from a few hours to a day. Great care was taken to avoid dust contamination. The sample cell was thoroughly rinsed with distilled, filtered water in a dust-free chamber where the solution was prepared and the cell filled.

Agglutination was initiated by rapid mixing of a stable dispersion with antigen (CRP) solution. The mixture was immediately transferred into the optical cell for light scattering measurements; less than 1 min elapsed between the mixing and the beginning of the intensity measurements. We have worked with a particle concentration of ca. 2\( \times 10^{10} \) parts/mL.

On the other hand, the rate constant of agglutination of the antibody-coated particles was measured using the low-angle light scattering technique developed by Lips and Willis, where the total scattering intensity for a dispersion of identical primary particles with a time varying distribution sizes is

\[
\frac{I(t)}{I(0)} = 1 + 2k_0n_0t 
\]

(5)

where \( I(0) \) is the initial intensity of light scattered at angle \( \theta \), \( n_0 \) the number of primary particles, and \( k_0 \) the rate constant. The scattered light intensity at low angles increases linearly with time, and then an absolute coagulation rate can be obtained from the slope if the number of primary particles is known.

**Dynamic Light Scattering Measurements.** A useful magnitude is the number-average mean cluster size \( \langle n \rangle \). A consequence of the fractal morphology is that the number-average mean cluster size in an aggregate of mean hydrodynamic radius \( (R_h) \) scales as39,40

\[
\langle n \rangle = \left( \frac{R_h}{R_0} \right)^{\lambda} 
\]

(6)

where \( R_0 \) is the monomer radius.

The number-average mean cluster size increases with time describing a power law in time with the exponent related to parameter \( \lambda \),

\[
\lim_{t \to \infty} \langle n \rangle \propto t^{\lambda(1-\lambda)} 
\]

(7)

where \( \lambda \) is the van Dongen and Ernst homogeneity exponent.41

These authors introduced a classification scheme for homogeneous kernels, based on the relative probabilities of large clusters sticking to large clusters and small clusters sticking to large clusters. Different growth kinetics and size distributions are obtained depending on which of these unions dominates. \( \lambda \) describes the tendency of a large cluster to join with another large cluster and determines the overall rate of aggregation. It should take the value 0 for diffusion limited cluster aggregation (DLCA) and 1 for reaction limited cluster aggregation (RLCA). It has to be pointed out that these two universal regimes should be considered as the two limiting cases of a more complex range of possibilities. The work on pure DLCA and RLCA has been so influential as to often generate the incorrect impression that all the aggregation processes should fall into either one of these two modes. In fact some experimental works have recently shown the existence of intermediate regimes between DLCA and RLCA.42-45

The agglutination kinetics of antibody-coated particles has been studied using dynamic light scattering (photon correlation spectroscopy, PCS). This technique employs the laser beam to probe a small volume of particle suspension. As they undergo Brownian motion, interference with scattered light produces a fluctuation in the intensity with time at the detector. This temporal fluctuation contains information on the motion of particles and may be analyzed by means of a correlator that yields, in real time, the autocorrelation function. The nonexponential behavior of this function must be analyzed by the method of cumulants. The first cumulant is used to obtain the effective translational diffusion coefficient, and the apparent hydrodynamic radius of the aggregates is obtained from the Stokes–Einstein relation.

Photon correlation measurements were made at a scattering angle of 60° with a 256-channel photon correlator (Malvern 4700c system). Prior to static and dynamic light scattering experiments, a fresh suspension of antibody-coated particles was sonicated for 30 min to break up any initial clusters.

**Agglutination Tests.** The immunoreactivity of the sensitized latex coated with aCRP – F(ab')2 was measured by nephelometric monitoring of the immunoadsorption reaction with human CRP for 10 min (typical clínical run time). This immunoresponse is quantified by the intensity increment in scattered light. We have used a noncommercial apparatus with a 10 mW HeNe laser (\( \lambda \) = 632.8 nm) and a rectangular flow cell with 2 mm path length.

Scattered light is monitored at an angle of 20°.

The injection into the nephelometer cell was performed by simultaneous stopped flow mixing of two dispersions: one made up of 1100 \( \mu \)L reaction buffer and 50 \( \mu \)L CRP at different concentrations, and the other of 1100 \( \mu \)L reaction buffer and 50 \( \mu \)L sensitized latex. The final particle concentration was ca. 2.1 \( \times 10^{10} \) particles/mL.

The experiments were performed in phosphate-buffered saline (PBS–BSA) containing 5 mM phosphate, 0.17 M NaCl, 1 mg/mL NaN3 used as a preservative, and 1 mg/mL M-BSA to cover patches of latex surface free of aCRP – F(ab')2 in order to avoid bridging coagulation of the complexes. The pH was adjusted to 8 with NaOH.

**Results and Discussion**

**Immunoreactivity.** Figure 1 shows the immunoreactivity (scattered light intensity increment, left axis) of the antibody-coated particles as a function of CRP concentration (left axis). As can be seen, the response is dependent on antigen concentration and the shape of the curve coincides with that of the precipitine curve proposed by Heidelberger and Kendall.46 Such response can be explained considering that an antigen molecule acts as a bridge to coagulate two sensitized particles. Before the maximum is reached (antibody excess region), it is easy to understand why the immunological response increases as CRP concentration does. At higher antigen concentrations (antigen excess region) the system seems to lose reactivity. It may be due to the blocking of the antibody active sites by antigens, and thus the bridging process is unfavored.

In the right axis of Figure 1 is shown the rate agglutination constant calculated from eq 5. As can be
seen, the maximum agglutination rate, $k_{\alpha}^{\text{max}} = 6.2 \times 10^{-14}$ cm$^3$/s, is reached for the maximum immunoreactivity. At both sides of the maximum agglutination rate the kinetics constant decreases with antigen concentration as the immunoprecipitation curve proposed by Heidelberger.

Very low aggregation rates are generally observed for colloidal systems undergoing irreversible reaction-limited processes, whereas fast rates are observed for irreversible diffusion-controlled processes. The obtained $k_{\alpha}^{\text{max}}$ value is 100-fold lower than the Smoluchowski rate constant for irreversible diffusion-controlled aggregation (DLCA regime). This slower agglutination kinetics may be due to different factors, for example, the important steric restriction needed to provoke particle agglutination; i.e., the agglutination only occurs when an antigen molecule of one particle interacts with an antibody molecule of another particle. The effectiveness of collisions between particles leading to agglutination is very low. This effectiveness should depend on the antibody coated level and on the antigen concentration. The ligand concentration (antigen) strongly determines the kinetics and the mode of the aggregation process. In fact, on both sides of the optimum dosage leading to fast aggregation the rate of the process strongly decreases. In these regions a high percentage of particulate collisions is inefficient toward sticking.

According to a previously published model, if we use the nephelometer to monitor the immunoagglutination, the initial slope of the variation of scattered light with time is given by

$$v_0 = (dI/dt)_0 = (C/4)((N_0n)^2 - (A + K^{-1} - \Delta n^{1/2}))^2$$

where $K$ is the chemical equilibrium constant, $N_0$ is the initial concentration of latex particles, $A$ is the initial concentration of antigen in the bulk solution, $n$ is the total number of immunologically active antibody sites per particle, and

$$\Delta = (N_0n + A + K^{-1})^2 - 4AN_0n$$

and

$C = 2k^*i(Q,0)n^{-2} \sin(Qd_0)/Qd_0$ (10)

is a proportionality constant for every experiment. $Q$ is the modulus of the scattering vector, $i(Q,0)$ is the initial intensity, and $d_0$ is the distance between the centers of monomers forming a dimer. Thus, $v_0$ depends on three fitting parameters, namely $N_0n$, $K^{-1}$, and $C$.

Figure 2 shows the value of $v_0$ obtained as a function of CRP concentration for the antibody-coated particles. The application of the kinetic model is represented by the curve in the figure. The three fitting parameters $P_1 = C/4$, $P_2 = N_0n$, and $P_3 = K^{-1}$ are $2.1 \times 10^{-24}$, $2.1 \times 10^{12}$, and $4.3 \times 10^{11}$ molecules$^{-1}$ mL. From the $P_2$ parameter, the percentage of $F(ab')_2$ active sites can be calculated. In our case a value of 3.1% has been obtained. This extremely low value suggests that upon adsorption most of the antibody molecules lose their ability to bind antigens, probably as a consequence of unfavorable orientation and/or steric hindrance of the antigen binding sites at the sorbent surface, reducing antigen accessibility. The low percentage of active antibody molecules should be responsible of the slow agglutination kinetics measured.

On the other hand, the aggregation process could develop so extremely slowly due to the cluster fragmentation. Different computer simulations indicate that reversibility leads to a slowing down of the aggregation kinetics. Weaker aggregates exist where the individual bonds between colloidal particles are of the order of $-10 \times K_{S}T$. The relative weakens of the bonds may allow consolidation of the structure during and after aggregation. External fields such as gravity, convection, or Brownian (thermal) motion move the structure during aggregation and fragmentation may occur.

Let us consider the nature of the attractive interactions between the antibody-coated particles in the presence of antigen. There are two principal interactions involved. The first one is the short-range van der Waals attraction which depends on the Hamaker constant of the particles, characteristic of each material. The Hamaker constant is

\[ H = A_i^p - A_i^m - A_i^s \]

\[ H = A_i^p - A_i^m - A_i^s \]
low for an antibody-coated latex particle. On the other hand, the van der Waals energies for rough particles (as antibody-coated ones) are lower than those for smooth ones. This reduction in the van der Waals energy, which is predominant at the closest approach distance, provokes the existence of relative weakness of the bonds between particles.

The second interaction is the specific antigen–antibody binding connected with the bridge formation between carrier particles. In general, antigen–antibody interaction is very weak and reversible.

Fragmentation studies have been performed in order to obtain information about the structural stability of the aggregates. In a typical fragmentation experiment, equal volumes of antibody-coated particles and antigen solution are mixed in a test tube at time zero. The average diameter of the aggregates is determined as a function of time by photon correlation spectroscopy. At a certain time of the experiment, the cell is taken out of the device, shaken for 20 s, and then inserted again.

Figure 3 shows the time evolution of the average diameter before and after agitation for the antibody-coated particle aggregates in the presence of 110 ng/mL CRP. It can be observed that fragmentation of aggregates occurs after agitation. Similar fragmentation was observed for all the antigen concentrations above and below the maximum dosage. It suggests a weaker interaction between antibody-coated particles in the aggregate. It is normal for equilibrium clustering that a breakdown of the structure occurs under mechanical agitation. The aggregation could be reversible and the net coagulation rate will be reduced.

Cluster Morphology. Static light scattering has been also used to assess the internal structure of aggregates by measuring the fractal dimension, D. According to eq 4, a logarithmic representation of the measured scattered intensity I(q) as a function of q will be a straight line with slope -D.

In Figure 4 we report some typical intensity profiles log I(q) versus log q collected at various times from the beginning of the agglutination process for antibody-coated particles induced by antibody–antigen interaction in the presence of 500 ng/mL CRP. This plot allows an easy verification of the validity of the scaling laws of colloidal aggregation. At the earlier stages one can notice the typical behavior with the rolloff corner at q = 1/R_G and the asymptotic power-law decay q^D at larger q. One can also notice at later times that the asymptotic behavior is displayed over the entire q range accessible to the instrument. When the clusters are large enough, i.e., at sufficiently long aggregation time, the final fractal structure is fully developed and the scattered intensity function contains information about this structure in the q region corresponding to the characteristic structure length. The duration of each run is determined by the time required to observed straight lines in the log plot of I(q). It varies from a few hours to a day.

In Figure 5 the fractal dimensions obtained as a function of antigen concentration are plotted. To interpret these numbers physically, it is useful to recall that if the particles aggregate in a linear array the dimension would be close
to unity, whereas if they formed spherical aggregates the dimension would approach 3. The growth of aggregates at all antigen concentrations studied followed the same power law behavior depicted in Figure 4.

As can be seen the obtained fractal dimensions of the aggregating clusters are for all the CRP concentrations higher than the expected values for the DLCA and RLCA regimes (the computed fractal dimensions are 1.78 and 2.0, respectively).

The cluster structures formed in the immunoagglutination of antibody-coated particles are more compact than those proposed by computer simulation of irreversible aggregation regimes. This fact is supported by direct visualization of the aggregate clusters growth under two different antigen concentrations with TEM. As can be seen in parts a (256 ng/mL CRP) and b (919 ng/mL CRP) of Figure 6, the aggregate structures are very compact changing from more branched (256 ng/mL CRP) to dense (919 ng/mL). These results are qualitatively in agreement with the fractal dimension obtained by static light scattering experiments (Figure 5).

The computed simulations of the DLCA and RLCA models assume irreversible aggregation with an infinite interparticle attraction. Biological molecules exhibit markedly different aggregation behavior, producing clusters with considerably higher D. It is worth noting that a value of 2.5 has been determined by experiments for human immunoglobulin aggregation. These observations may be understood if equilibrium clustering transitions are assumed (a low finite attraction energy allowing particle rearrangements). Compact aggregates result from the existence of combined aggregation and fragmentation processes followed by internal reorganization. If this fact is taken into account, as in the reversible growth model developed by Shih, Aksay and Kikuchi, the fractal dimension values obtained are higher than those computed with the irreversible models, in accordance with our experimental results.

The growth of fractal aggregates is divided into two successive mechanistic steps: (i) a kinetic one for the diffusional approach of particles or aggregates, so as to be able to undergo collision, and (ii) a subsequent step to allow the aggregates so formed to pack more closely once they have come into contact, i.e., a restructuring process. When the interactions between particles into the cluster are weak, as in the antibody–antigen case, particle clustering is reversible and a dynamical equilibrium occurs.

The final fractal dimension should depend on the antigen concentration which controls the linking points between different particles into the cluster. This influence of CRP concentration on fractal dimension is more important for the antigen excess region than for the antibody excess region. Stoll et al. have reported that for the highest antigen concentration the aggregation process is reversible due to cluster fragmentation. For the lowest antigen concentration the aggregation process is in principle irreversible and becomes reversible with time.

**Aggregation Kinetics.** From the mean hydrodynamic radius and the measured fractal dimension was determined the number-average mean cluster-size, \( \langle n \rangle \), according to Eq 6. Figure 7 shows the results for the antibody.

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coated particles aggregated at 110 and 25 ng/mL of CRP.

For 110 ng/mL it may be observed that the number-
average mean cluster-size scales at long times describing
a power law in time, as predicted by theory (eq 7). The
slope is related to \( \lambda \). The same power law was obtained
for 48, 256, 500, and 919 ng/mL of CRP. The \( \langle n \rangle \) curves
already scale for very small clusters, and so eq 7 is suitable
for describing the aggregation process for almost the entire
process; despite this prediction it is strictly valid for very
long times. The same behavior was observed in other
works.\(^{41,58}\)

On the other hand, at a CRP concentration of 25 ng/mL
no power law was found, and therefore, the measurement
of \( \lambda \) was not possible. After a certain time the mean
hydrodynamic radius of aggregates slowly decreases. This
result suggests that equilibrium clustering is being
observed.

Figure 8 shows the homogeneity exponent \( \lambda \) as a function
of antigen concentration. As can be clearly seen, the
aggregation mechanism is different for the antibody excess
region and the antigen excess region. In the first region
positive values of \( \lambda \) have been obtained whereas in the
second region negative values appear.

Conclusions

We have extensively investigated the kinetics and
fractal morphology of aggregating polystyrene latex
particles covered by F(ab')\(_2\) antibodies in the presence
of CRP antigen by means of dynamic and static light
scattering.

Static light scattering was used to assess the internal
structure of aggregates by measuring the cluster fractal
dimension. It was found that the fractal dimensions
obtained for antibody-coated particles are higher than the
values predicted by the normal models of irreversible
aggregation. These results were supported by TEM
observations of clusters. Our observations suggest that
equilibrium clustering occurs and some rearrangement
of particles is possible as a function of the antigen
concentration. As a consequence, the fractal dimension
should be higher than the computed values where ir-
reversible aggregation in an infinite interparticle attraction
is assumed.

We found that antibody-coated particle aggregates are
delicate, showing substantial fragmentation effects by
agitating the sample. This fragmentation is probably due
to the weakness of the antibody–antigen interaction
between particles, being the particle clustering an equi-
librium phenomenon.

Aggregation kinetics study by dynamic light scattering
shows that different kinetics mechanisms exist as a
function of antigen concentration, characterized by the
homogeneity exponent \( \lambda \).

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