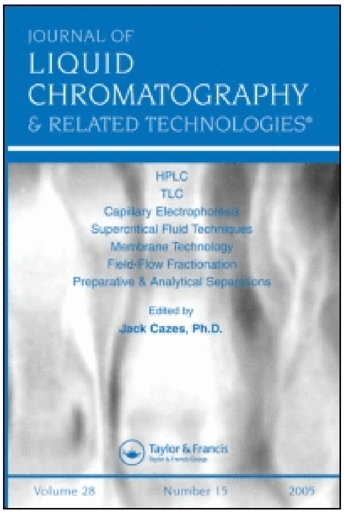

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COLLOIDAL INTERACTIONS STUDIED BY SEDIMENTATION FIELD-FLOW FRACTIONATION

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ABSTRACT

Sedimentation Field-Flow Fractionation (SdFFF) is a relatively new technique for the separation and characterization of monodisperse or polydisperse colloidal materials and macromolecules. In the present work, with the aid of SdFFF, the interactions between the polydisperse, irregular Hydroxyapatite (HAP) particles were studied. The stability of HAP, which is of paramount importance in its applications, is dependent upon the total potential energy of interaction between the Hydroxyapatite particles. The latter, which is the sum of the attraction potential energy and that of repulsion, depends on particle size, the Hamaker constant, the surface potential, and the Debye-Hückel reciprocal distance, which is immediately related to the ionic strength of carrier solution.

The larger the repulsion and the lower the attraction caused by increasing the particle's surface potential, and decreasing the effective Hamaker constant, or the ionic strength of the carrier solution, results in a higher stability. Variation of the ionic strength of the carrier solution by adding different amounts of electrolytes led to the size variation of HAP particles and to the determination of their critical aggregation concentration.

From the variation of the number average particle diameter of HAP with time, for different salt concentrations, the rate constants for the bimolecular process of aggregation were determined.

Comparison of the experimental rate constants found by SdFFF with those determined theoretically gives invaluable information about the aggregation mechanism.

INTRODUCTION

A colloidal dispersion is never thermodynamically stable. The total free energy of a dispersed system can always be lowered by reduction of the interfacial area. This reduction can occur by coalescence, or for solid dispersed in liquids, by aggregation, which is a common phenomenon for both natural and industrial colloids.[1] The high degree of stability, which is frequently observed in colloidal systems, is a kinetic phenomenon in that the rate of aggregation of such systems may be practically zero. Thus, in studies of the colloidal state, the kinetics of aggregation are of paramount importance.[2] Although the kinetics of aggregation can be described easily by a bimolecular equation, it is not an easy thing to do experimentally. One technique for doing this is, literally, to count the particles microscopically. In addition to particle size limitations, this is an extraordinarily tedious procedure. Light scattering is particularly well suited to kinetic studies since, in principle, experimental turbidities can be interpreted in terms of the number and size of the scattering particles.[2]

In the present work, sedimentation field-flow fractionation is used for the kinetic study of hydroxyapatite particles' aggregation. Normal SdFFF, which is a subtechnique of field-flow fractionation (FFF), obeys the basic principles originally formulated by Giddings et al.[3] and is capable of analysing particles up to 1 μm diameter. When the effective particle size exceeds ca. 1 μm (specifically when the particle radius becomes larger than the mean thickness of the steady-state particle layer), the steric limit of FFF (SFFF) is approached.[4] Unlike normal FFF, where smaller particles elute before larger ones, SFFF has an elution order with larger particles eluting first.

In normal SdFFF, where the interactions between the colloidal particles and the channel wall are absent, the diameter of a spherical particle, d_i, (for non-spherical particles d_i is the Stokes diameter, which is the diameter of a sphere of equal diffusivity), is given by the relation:[4]

$$d_i = \left(\frac{36kT}{\pi G w \Delta\rho V^0} \right)^{1/3} V_R^{1/3} \qquad (1)$$

where k is the Boltzmann constant, T is the absolute temperature, G is the centrifugal acceleration, w is the channel thickness, $\Delta\rho$ is the difference in density between the particle and carrier liquid, V^{o} is the retention volume of non-retained material, equal to the channel void volume, and V_R is the experimental retention volume.

For polydisperse samples, such as that of HAP under study, the number, d_N, and weight, d_w, average particle diameters can be determined from the measured d_i values, by the known procedure described elsewhere.[5]

While, in normal SdFFF, the separation parameter is the particle diameter, in potential barrier SdFFF (PBSdFFF), developed recently in our Laboratory,[6-9] the separation parameter should be the particle diameter or the surface potential, the Hamaker constant, and the ionic strength of the carrier solution. This provides a flexibility in varying the interaction energy between the particles and the FFF channel wall by adjusting one of the above parameters. Experimentally, this should be done by changing the surface tension, which is immediately related to the Hamaker constant (by adding a surfactant to the suspending medium), or the particle's surface potential (by varying the solution pH), and the ionic strength of the carrier solution (by adding an indifferent electrolyte).

Although the interactions between colloidal particles and solid surfaces have been studied in detail by FFF,[6, 7, 10, 11] little work has been performed for the interactions between colloidal particles themselves yielding colloidal aggregates.[12] The purposes of the present investigation were (i) to study the aggregation of hydroxyapatite particles in the presence of various electrolytes

Figure 1. Transmission electron micrograph of hydroxyapatite (HAP) particles (88,000×).

by SdFFF, (ii) to determine the rate constants for the bimolecular process of HAP particles' aggregation, and (iii) to investigate possible aggregation mechanisms describing the experimental data. The selection of HAP as a model sample to study the aggregation process by FFF, is due to the fact that it is often considered a prototype of dental enamel mineral.

While numerous studies have been carried out to investigate the growth and/or dissolution kinetics of HAP, not only to simulate the formation process of bone and tooth but also to create more bioactive materials,[13] as far as we know, there is no work on the aggregation of HAP and, consequently, on its stability.

EXPERIMENTAL

Materials

Polydisperse, irregular colloidal particles of hydroxyapatite (HAP) [$Ca_5(PO_4)_3OH$], provided by Prof. Koutsoukos, with number average diameter

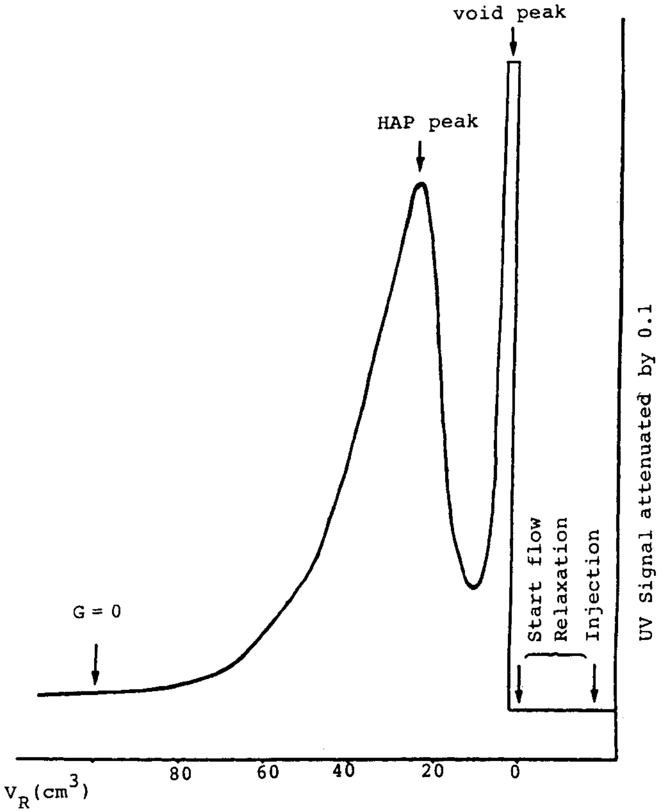

Figure 2. Fractogram of HAP particles obtained by SdFFF. Carrier : 0.5% v/v FL-70 + 0.02% w/w NaN_3; sample = 100 il; flow-rate = 140 $cm^3 h^{-1}$; relaxation time = 10 min.

d_N = (0.261 ± 0.046) μm, were used as sample. The morphology of the HAP particles was studied by means of a Phillips CM-20 transmission electron microscope at 200 kV (cf. Figure 1). The carrier was triply distilled water containing 0.5% (v/v) of a low-foaming, low-alkalinity, phosphate-, chromate-, and silicate-free detergent FL-70 and 0.02% (w/w) sodium azide as bacteriocide.

The electrolytes added to this carrier solution to adjust its ionic strength were: (i) Potassium nitrate from Riedel-De Haen A.G., (ii) Potassium sulphate from Merck A.G., and (iii) Barium nitrate from Riedel-De Haen A.G.

Apparatus and Procedure

The dimensions of the SdFFF system, which has been described in detail elsewhere,[6] were 38.4 cm × 2.35 cm × 0.0197 cm with a channel void volume of 1.68 cm^3, measured by the elution of the non-retained peak of sodium benzoate. The analysis was performed with a Gilson Minipuls 2 peristaltic pump coupled with a Gilson model 111 UV detector operated at 254 nm and a Goerz model RE 541 recorder.

The electrophoretic mobilities of the HAP particles were measured in a microelectrophoresis apparatus (Rank, Mark II) by using a four-electrode capillary cell.

For the kinetic study of the HAP particles' aggregation, 100 μL of 3 mg/cm^3 HAP suspension, which was kept thermostated at 25° C while it was continuously agitated, were injected quickly at various time intervals into the SdFFF column, and then analysed by the known procedure described elsewhere.[5] In order to study the influence of the solution ionic strength on the HAP particle's size, 100 μL of 3 mg/cm^3 sample were injected directly into the SdFFF column using as carrier the detergent FL-70 with various amounts of electrolytes. In both studies, the flow rate (140 $\text{cm}^3\ \text{h}^{-1}$), the field strength (350 rpm), and the relaxation time (10 min) were kept constant during the analysis time. The obtained fractograms had the form of Figure 2.

RESULTS AND DISCUSSION

Theory

The total potential energy of interaction between two colloidal particles, U_{tot}, is given by the sum of the energy of interaction (repulsion) of the double layers, U_R, and the energy of interaction (attraction) of the particles themselves due to van der Waals forces, U_A. Consequently:

$$U_{tot} = U_R + U_A \qquad (2)$$

For identical spherical particles U_R and U_A are defined as follows: [2,14]

$$U_R = \frac{\varepsilon r \psi_0^2}{2} \ln\left[1 + \exp(-\kappa H)\right] \quad (\kappa r >> 1) \qquad (3)$$

$$U_R = \frac{\varepsilon r^2 \psi_o^2}{R} \exp(-\kappa H) \qquad (\kappa r << 1) \tag{4}$$

$$U_A = -\frac{A_{212} r}{12H} \tag{5}$$

where ε is the dielectric constant of the dispersing liquid, r is the radius of the particle, ψ_0 is the particle's surface potential, κ is the reciprocal double-layer thickness, R is the distance of the centers of the two particles, A_{212} is the effective Hamaker constant of two particles of type 2 separated by the medium of type 1 and H is the nearest distance between the surfaces of the particles (=R-2r). The A_{212} and κ parameters are given by the expressions:[2, 14]

$$A_{212} = \left(A_{11}^{1/2} - A_{22}^{1/2}\right)^2 \tag{6}$$

$$\kappa = \left(\frac{4\pi e^2 N_A}{1000\varepsilon kT} \sum_i c_i z_i^2\right)^{1/2} = \left(\frac{8\pi e^2 N_A I}{1000\varepsilon kT}\right)^{1/2} \tag{7}$$

where A_{11} and A_{22} are the Hamaker constants for the particles and the suspending medium, respectively, *e* is the electronic charge, c_i is the bulk solution concentration of ionic species i, z_i is its charge and I is the ionic strength of the suspending medium.

Equations (2-7) show that the total potential energy of interaction between two colloidal spherical particles depends on the surface potential of the particles, the effective Hamaker constant, and the ionic strength of the suspending medium. Of the various quantities which affect the total interaction potential energy, none is as accessible to empirical adjustment as *κ*. This quantity depends on both the concentration and valence of the indifferent electrolyte as shown by Equation (7). It has long been known that the addition of an indifferent electrolyte can cause a colloid to undergo aggregation. Furthermore, it is known that for a particular salt a fairly sharply defined concentration is needed to induce aggregation. This concentration may be called the critical aggregation concentration (CAC). The actual concentration of electrolyte at the CAC depends on: (1) the time allowed to elapse before the evaluation is made, (2) the uniformity or,more likely, the polydispersity of the sample, (3) the potential at the surface , (4) the value of Hamaker constant, and (5) the valence of the electrolyte ion of opposite charge to the colloid. The

CAC value for a particular electrolyte is essentially determined by the valence of the counterion, regardless of the nature of the ion with the same charge as the surface (the Schulze-Hardy rule).

The rate of diffusion-controlled aggregation of spherical particles in a disperse system as a result of collisions in the absence of any energy barrier to aggregate, v_r, is given by the von Smoluchowski equation:[2, 14]

$$v_r = -k_r N_o^2 \tag{8}$$

where k_r is effectively a second-order rate constant for diffusion-controlled rapid aggregation, and N_o is the initial number of particles per unit volume.

In the presence of an energy barrier to aggregate, an expression analogous to Equation (8) is applied for the rate v_s:

$$\upsilon_s = -k_s N_o^2 \tag{9}$$

where k_s is the rate constant of slow aggregation in the presence of an energy barrier. The stability ratio, W, of a dispersion is defined as the ratio of the rate constants for aggregation in the absence, k_r, and presence, k_s, of an energy barrier, respectively:

$$W = \frac{k_r}{k_s} \tag{10}$$

Both rapid and slow aggregation, as Equations (8) and (9) show, are described by a bimolecular kinetic equation:[2]

$$\frac{1}{N_i} = \frac{1}{N_o} + k_{app} t_i \tag{11}$$

where N_i is the total number of particles per unit volume at time t_i, N_o is the initial number of the same particles and k_{app} is the apparent rate constant for the aggregation process. Thus, the most reliable way to evaluate a rate constant for aggregation is to measure the number of independent kinetic units per unit volume, N_i, or whichever other parameter which is related to N_i, as a function of time. The time of the experiment is measured from the addition of indifferent electrolyte to the colloid. In the present work, the number average particle diameters were measured by the sedimentation field-flow fractionation technique as a function of time. Therefore, Equation (11) must be converted to another one in which the particle diameter is immediately related to time. Considering that d_o and d_i are the diameters for spherical particles at times t_o (initial time) and t_i, respectively, the corresponding volumes of the particles V_o and V_i will be given by the relationships:

$$V_o = \frac{4}{3}\pi\left(\frac{d_o}{2}\right)^3 \qquad (12)$$

$$V_i = \frac{1}{6}\pi d_i^3 \qquad (13)$$

For polydisperse samples, as it is the case here, the d_o and d_i in Equations (12) and (13) are replaced by the d_{N_o} and d_{N_i} ,which are the measured number average diameters at times t=0 and t_i, respectively. Taking into consideration that, every time the sample volume is kept constant, the combination of Equations (11), (12) and (13) yields:

$$d_{N_i}^3 = d_{N_o}^3 + d_{N_o}^3 N_o k_{app.} t_i \qquad (14)$$

Equation (14) shows that a plot of $d_{N_i}^3$ vs. t_i should be linear with an intercept equal to $d_{N_o}^3$ and a slope equal to $d_{N_o}^3 N_o k_{app.}$, from which the apparent rate constant for aggregation, k_{app}, can be calculated. The N_o value should be determined from the ratio of the total volume of the injected solid HAP sample, V_{tot}, to the volume of the particle, V_o, which can be computed from Equation (12), using as d_o the d_{N_o} value found from the intercept or measured by SdFFF at time t=0 ($N_o=V_{tot.}/V_o$). The V_{tot} value can be computed from the relation:

$$V_{tot} = V_i c_i / \rho_i \qquad (15)$$

where V_i is the injected volume of the suspension, c_i is the sample concentration and ρ_i is the density of the solid sample.

Results

The variation of number average diameter, d_N, for the hydroxyapatite particles with the electrolytes' KNO_3, K_2SO_4 and $Ba(NO_3)_2$ concentration, c, is shown in Figures 3, 4 and 5, respectively. In the first two cases, d_N increases

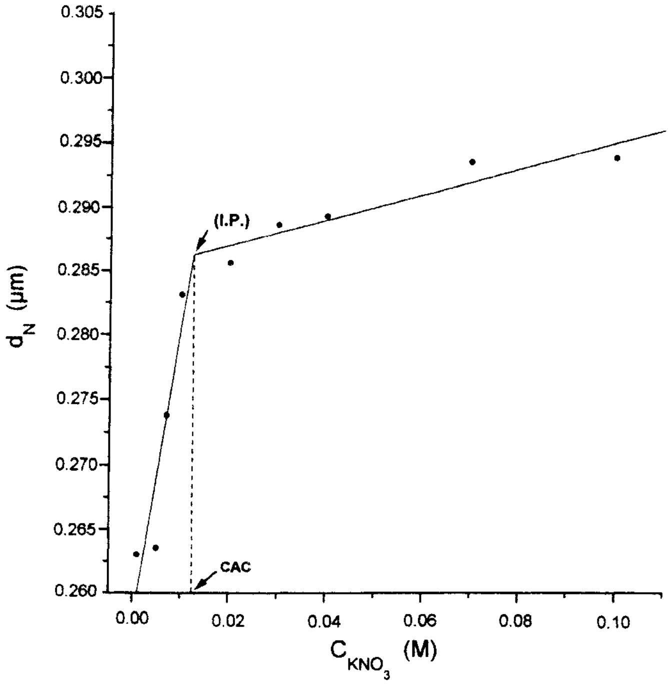

Figure 3. Variation of number average diameter, d_N, for the HAP's particles with the electrolyte KNO_3 concentration, c_{KNO_3}. CAC : Critical aggregation concentration; I.P.: Intersection point of the two straight lines.

with the electrolyte concentration until the critical aggregation concentration is reached, at which point the d_N values remain approximately constant. The starting point of the maximum number average diameter corresponds to the electrolyte concentration called CAC. There is an uncertainty about the exact localization of this starting point and, thus, a relatively large error is introduced in the measurement of the CAC values. The latter can be conveniently and more accurately determined from the intersection points (I.P.) of the two straight lines that can be drawn in Figures 3 and 4.

The obtained CAC values for the electrolytes KNO_3 and K_2SO_4 are 1.27×10^{-2}M and 0.54×10^{-2}M, respectively. These CAC values, according to Schulze-Hardy rule mentioned earlier, had to be about the same, as the HAP sample is a negative colloid at the working pH range. The deviation of the CAC_{KNO_3} and $CAC_{K_2SO_4}$ values, contrary to the theoretical predictions,

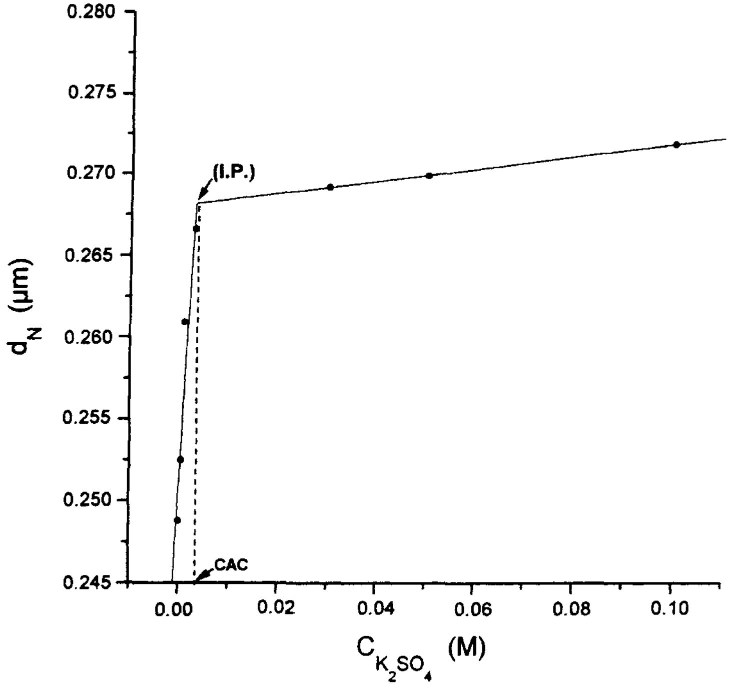

Figure 4. Variation of number average diameter for the HAP's particles with the electrolyte K_2SO_4 concentration.

would be attributed either to the increased polydispersity in K_2SO_4 than in KNO_3, which was verified by TEM pictures or to particle's conformation, although previous work[15] has shown that particle shape has little or no effect on SdFFF retention until the aspect ratio becomes quite large (>50-100). The aspect ratio in the HAP's particles, as the TEM pictures have shown, ranges between 4 and 20, thus making possible the use of this sample for studying the aggregation process by SdFFF. Theory predicts that the CAC value varies inversely with the sixth power of the valence, z, of the counterion (K^+, Ba^{++}) in solution:[2]

$$CAC \propto z^{-6} \tag{16}$$

From the theoretical ratio of CAC for KNO_3 and $Ba(NO_3)_2$, which is $1^{-6} : 2^{-6} = 64$, one can determine the $CAC_{Ba(NO_3)_2}$ ($\approx 2 \times 10^{-4}$ M) using as CAC_{KNO_3} that found by SdFFF. This explains the fact why the variation of

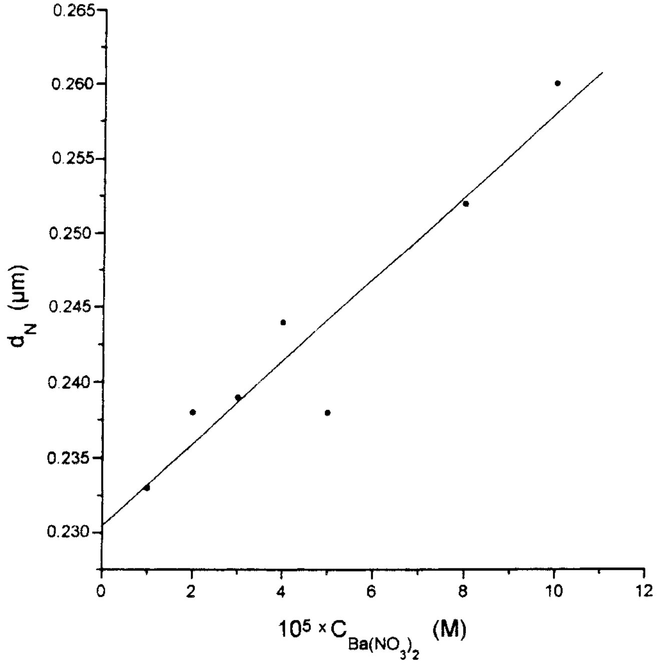

Figure 5. Variation of number average diameter for the HAP's particles with the electrolyte $Ba(NO_3)_2$ concentration.

the d_N values for the HAP particles with the electrolyte $Ba(NO_3)_2$ concentration, shown in Figure 5, is approximately linear in the working concentration range $\left(1x10^{-5}M \langle C_{Ba(NO_3)_2} \langle 1x10^{-4}M\right)$, as the intersection point of the two straight lines encountered in Figures 3 and 4 corresponds to 2 × 10^{-4}M $Ba(NO_3)_2$. Thus the plateau of the d_N values is out of the working concentration range, as at concentrations of $Ba(NO_3)_2$ higher than 1 × 10^{-4}M the detergent FL-70 is no longer soluble in water, therefore making impossible the analysis of HAP particles by SdFFF in that particular concentration range.

For the determination of the apparent rate constant, k_{app}, for the HAP particles' aggregation, as it was mentioned earlier, the plots of $d^3_{N_i}$ vs. t_i at various electrolyte concentrations are necessary [cf. Figures 6 and 7]. The found k_{app} values for the aggregation of the HAP particles in the presence of two electrolytes are compiled in Table 1. It is possible to make a calculation

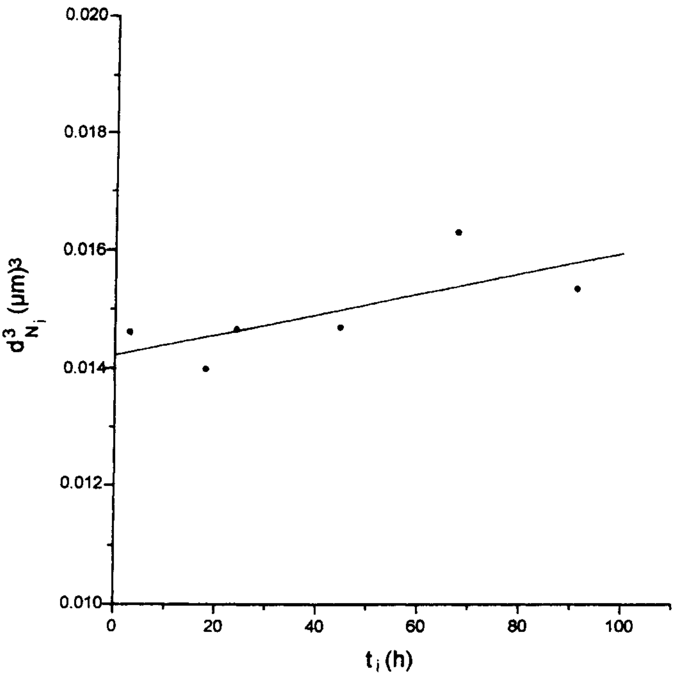

Figure 6. Plot of Equation (14) for the aggregation of HAP particles in the presence of 1×10^{-3}M KNO_3.

which shows whether the values of k_{app} are determined solely by the rate at which two HAP particles can diffuse up to each other (diffusion control), or whether the rate of reaction is limited by other slower processes.

The expected relationship between the rate constant for bimolecular collision (k_1) of HAP's particles and on their diffusion coefficient in the medium, D, was determined many years ago by Smoluchowski[16], whose treatment for spherical particles gives:

$$k_1 = 8nrD \tag{17}$$

$$k_{-1} = \frac{6D}{r^2} \tag{18}$$

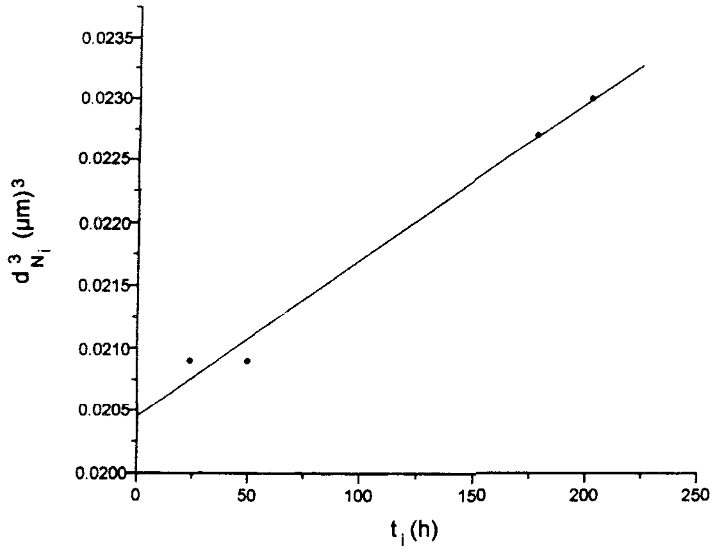

Figure 7. Plot of Equation (14) for the aggregation of HAP particles in the presence of 5×10^{-5}M $Ba(NO_3)_2$.

where k_{-1} is the rate constant for the dissociation of the formed aggregates. Combination of Equations (17) and (18) with the Stokes-Einstein relationship yields:

$$k_1 = \frac{8kT}{3n} \mathrm{cm}^3\ \mathrm{s}^{-1} \qquad (19)$$

where n is the viscosity of the medium. For water at 25°C, using n = 0.97 cp we calculate from Equation (19) a k_1 value of 1.1×10^{-11} $\mathrm{cm}^3\mathrm{s}^{-1}$. This value is about ten orders of magnitude greater than the value of k_{app} actually measured. Thus, aggregation rates are slower than those expected if the process was simply diffusion controlled when electrostatic repulsion is absent. Probably, extra repulsive hydration forces,[17] occuring at close approach of particles, are involved in the observed slow processes. The foregoing discussion indicates that the minimal mechanism for the aggregation reaction of HAP particles would be:

$$\text{Particle 1} + \text{Particle 2} \underset{k_{-1}}{\overset{k_1}{\Leftrightarrow}} \text{Intermediate complex} \xrightarrow{k_2} \text{Stable aggregate} \qquad (20)$$

Table 1

Apparent Rate Constants for Slow Aggregation, k_{app}, Stability Factors, *W*, and Rate Constants for the Rate-Determining Step, k_2, of HAP Particles' Aggregation, and Fractions of the Total Number of Collisions Which are Effective in Producing Stable HAP's Aggregates, ξ, Determined by SdFFF

Electrolyte:	**1×10^{-3} M KNO_3**	**5×10^{-5} M $Ba(NO_3)_2$**
$k_{app}(cm^3s^{-1})$	2.5×10^{-21}	1.8×10^{-21}
$k_1(cm^3s^{-1})$	1.1×10^{-11}	1.1×10^{-11}
$W=k_1/k_{app}$	4.4×10^{9}	6.1×10^{9}
$k_2(s^{-1})$	2.7×10^{-7}	2.0×10^{-7}
ζ	2.3×10^{-10}	1.7×10^{-10}

Theoretical rate constants for rapid aggregation, k_1, were calculated by Equation (19).

where k_1 and k_{-1} are the rate constants determined by Equations (17) and (18), respectively, and k_2 is the rate constant for the process representing the rate-determining step in the aggregation reaction. Since k_{app}, describing the overall process is smaller than the calculated k_1 value, there must be rapid equilibration of the individual particles and their intermediate complexes followed by the slower step of irreversible aggregation.

Under these conditions, the value of k_{app} is given by:

$$k_{app} = \left(\frac{k_1}{k_{-1}}\right) k_2 \tag{21}$$

Straightforward calculation, using Equations (17) and (18) gives $k_1 = 1.1\times10^{-11}$ cm^3 s^{-1} and $k_{-1} = 1.2\times10^{3}$ s^{-1} for the HAP's particles having $d_N = 0.261$ μm.

Thus, $k_1/k_{-1} = 9.2\times10^{-15}$ and the k_2 parameter takes the values 2.7×10^{-7} s^{-1} (in the presence of 1×10^{-3} M KNO_3), and 2.0×10^{-7} s^{-1} [in the presence of 5×10^{-5} M $Ba(NO_3)_2$]. The fraction of the total number of collisions which are effective in producing a stable aggregate is thus only $\zeta=k_2/(k_{-1}+k_2)=2.3\times10^{-10}$ (in the presence of 1×10^{-3}M KNO_3) and $\zeta = 1.7\times10^{-10}$ [in the presence of 5×10^{-5} M $Ba(NO_3)_2$].

A second treatment of the experimental results, taking into consideration that k_{app} and k_1 are identical with k_s and k_r, respectively, defined by Equations (8) and (9), leads to the stability factor, W, of HAP's particles. The found values (cf. Table 1) are too high, indicating that the HAP's particles are very stable, even in the presence of significant quantities of electrolytes.

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