Supplemental Declaration of Neil P. Desai Pursuant to 37 C.F.R § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Madam:

I, Neil P. Desai, declare as follows:

1. This declaration is in addition and supplemental to the 37 C.F.R. §1.132 declaration ("the Previous Declaration") previously submitted to the Patent Office on January 27, 2012.

2. I have reviewed the Office Action dated May 2, 2013. I understand that claims in the above-captioned patent application remain rejected as being obvious over one of Abraxis’ earlier patents, U.S. Pat. No. 5,439,686 ("Desai"), for which I am also a named inventor, in view of U.S. Pat. No. 5,407,683 ("Shively"). In this supplemental declaration, I provide more information about the data presented in the Previous Declaration as well as the cited reference Desai.
3. In the Previous Declaration, I presented, in part, experimental data showing the advantageous properties of the nanoparticle formulations recited in the claims of the above-captioned patent application ("the '479 application"). The experiment compared the physical stability of two pharmaceutical formulations (Composition 1 and Composition 2) containing nanoparticles comprising a solid core of paclitaxel and an albumin coating at a paclitaxel concentration of 5 mg/ml.

4. As discussed in the Previous Declaration, upon storage at 40 °C for 24 hours, there was a distinctly visible sediment layer at the bottom of the vials containing Lot 1 and Lot 2 of Composition 2 indicating instability of Composition 2. Exhibit 1; See also Exhibit 3 of the Previous Declaration. Such sedimentation was not observed in the vial containing Composition 1. Microscopic observation of the formulations stored at 40 °C for 24 hours at 400x magnification revealed large particles in Composition 2 indicating particle growth and aggregation, which were not observed in Composition 1. Exhibit 2; See also Exhibit 4 of the Previous Declaration.

5. Further, as discussed in the Previous Declaration, upon storage at 40°C for 24 hours, the weight mean diameter of the nanoparticles in Composition 1 remained unchanged. In Composition 2, by contrast, the weight mean diameter of the nanoparticles increased significantly upon storage demonstrating instability of Composition 2. Exhibit 3; See also Table 1 of the Previous Declaration.

6. The table below summarizes and provides additional particle size characteristics of the two different formulations tested in the experiment.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Weight Mean Diameter, nm</th>
<th>95% Weight Distribution (D95), nm</th>
<th>99% Weight Distribution (D99), nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composition 1</td>
<td>140</td>
<td>240</td>
<td>282</td>
</tr>
<tr>
<td>Composition 2, Lot 1</td>
<td>245</td>
<td>500</td>
<td>633</td>
</tr>
<tr>
<td>Composition 2, Lot 2</td>
<td>228</td>
<td>496</td>
<td>638</td>
</tr>
</tbody>
</table>

1 Storage at 40 °C for 24 hours is equivalent to storage at room temperature for at least three days.
2 Particle size was determined by disc centrifugation method immediately after reconstitution of the formulations at about 5 mg/ml. Size may differ slightly when using a different measurement method such as dynamic light scattering.
The 95% and 99% weight distribution in the table above provides the size in nanometers below which 95% and 99% by weight of the particles lie, respectively. For example, in Composition 1, 95% of the particles in the formulation have a particle size below 240 nm. In Composition 2, 95% of the particles in the formulation have a particle size below 500 (Lot 1) and 496 nm (Lot 2). In Composition 1, there was no detectable percentage of nanoparticles that have a size above 400 nm, with 99% of the particles lying below 282 nm. In Composition 2, by contrast, at least 10% of the nanoparticles in the formulation had a particle size that was above 400 nm, with 99% of the particles lying below 633 (Lot 1) and 638 nm (Lot 2).

7. The diagram below further illustrates the 99% weight distribution of the two different formulations.

8. Notably, both Composition 1 and Composition 2 are albumin-coated paclitaxel nanoparticle formulations having a particle size below 1000 nm, yet they behave differently in stability assays. Composition 1, which contains no detectable percentage of nanoparticles that have a size above 400 nm, was shown to be stable at paclitaxel concentration of 5 mg/ml. By contrast, Composition 2, which contains nanoparticles slightly greater than 400 nm, was unstable at the same paclitaxel concentration under the same conditions. This result was unexpected.

9. As discussed in the Previous Declaration, physical stability is a key consideration for ensuring safety and efficacy of nanoparticle drug products. The tendency of nanoparticles to precipitate and/or increase in size (for example by aggregation) increases as the drug concentration increases. For example, an increase in drug concentration in a nanoparticle formulation can result in
an increase in particle concentration, namely, the number of particles per unit volume. An increase in particle concentration in turn would increase the frequency of collision of the particles and thus increase the tendency of the particles to aggregate and become unstable. This is demonstrated in Burns et al., Langmuir 1997, 13, 6413-6420 (Exhibit 4), for example, which examined particle aggregation in various formulations having different particle concentrations. The authors concluded that “[a]s the particle concentration is increased, the aggregate growth is more rapid, most likely due to the increased collision frequency.” See also Kallay et al., J. Colloid and Interface Science 253, 70-76 (2002) (Exhibit 5) at page 75 (“the aggregation rate is proportional to the square of the particle concentration....”).

10. The estimated particle concentration for Composition 1 discussed above, namely, the albumin-coated solid paclitaxel nanoparticle formulation having a particle size less than 400 nm at paclitaxel concentration of 5 mg/ml, is about 8.0 x 10^{13}/ml.\(^3\) The stability of such a formulation at 5 mg/ml or higher was unexpected based on the high particle concentration.

11. The stability of albumin-coated paclitaxel nanoparticle formulation having particle size less than 400 nm is in stark contrast with that of a different non-albumin based paclitaxel nanoparticle formulation having particle size less than 400 nm. In a study conducted to compare the physicochemical characteristics and stability of two different commercially-approved nanoparticle formulations of paclitaxel, namely, Abraxane® (an albumin-coated solid paclitaxel nanoparticle formulation having particle size less than 400 nm, similar to Composition 1 described above) and Genexol-PM ® (a non-albumin polymeric-micelle formulation of paclitaxel having particle size less than 400 nm), only Abraxane® was shown to be stable at 40 °C over 24 hours at paclitaxel concentration of 5 mg/ml while the Genexol-PM ® formulation showed excessive precipitation under these conditions. Ron et al., 99th AACR Annual Meeting Abstract, No. 5622 (Exhibit 6). This study further illustrates the difficulty and challenge in obtaining paclitaxel nanoparticle formulations having

\(^3\) The particle concentration is estimated with the assumption that the average particle size of the particles in the formulation is about 140 nm and the particle density is about 1165 kg/m\(^3\).
particle size less than 400 nm that are stable at paclitaxel concentration of 5 mg/ml or higher, and the unexpected stability of the claimed albumin-coated solid nanoparticle formulation.

12. Thus, albumin-coated paclitaxel nanoparticle formulations having particle size less than 400 nm were stable at 5 mg/ml. This is in stark contrast with an albumin-coated paclitaxel nanoparticle formulation which contains particles slightly greater than 400 nm, and a non-albumin based paclitaxel nanoparticle formulation having particle size less than 400 nm, both shown to be unstable under the same conditions at paclitaxel concentration of 5 mg/ml. These results demonstrate the advantageous and unexpected stability of the albumin-coated paclitaxel nanoparticle formulation recited in the claims of the '479 application, especially in view of the high particle concentration in such a formulation and the well-known principle that the aggregation rate of nanoparticles is proportional to the square of the particle concentration.

13. The Examiner cites Desai as allegedly teaching a stable albumin-coated nanoparticle formulation. As discussed in the Previous Declaration, Example 5 of Desai, which the Examiner relies on as teaching stability of albumin-coated nanoparticle formulations, refers to the stability of polymeric shells containing buoyant soybean oil with density less than water. No drug was present within the polymeric shell. The stability of the "drugless" oil-containing polymeric shells discussed in Example 5 of Desai thus provides no suggestion that a nanoparticle formulation comprising a solid core of paclitaxel and an albumin coating would be stable at paclitaxel concentration of between 5-15 mg/ml. Furthermore, as discussed in the Previous Declaration, an increase in loading of paclitaxel within the polymeric shells as taught in Example 4 of Desai would be expected to increase the particle size and/or density of the particles, which in turn could increase the tendency of the particles to precipitate.

14. Although a separate example in Desai, Example 9, teaches preparation of polymeric shells containing a solid core of pharmaceutically active agent such as paclitaxel, there is no information about the concentration of the paclitaxel in such polymeric shell formulation. Nor is there any indication that the particles in such polymeric shell formulation are smaller than 400 nm.
Example 9 teaches that “these polymeric shells are examined under a microscope to reveal opaque cores....” The fact that the polymeric shells were viewable under a microscope to reveal opaque cores indicates that a substantial portion of the particles in the formulation taught in Example 9 were larger than 400 nm. Thus, these formulations taught in Desai differ from the formulation recited in claims of the ‘479 application in at least two aspects: paclitaxel concentration and particle size.

15. To arrive at the claimed formulation from Desai’s nanoparticle formulations, one would at least need to: 1) substantially decrease the size of the particles in the formulation to less than 400 nm; and 2) substantially increase the paclitaxel concentration to 5-15 mg/ml. Desai provides no teaching on how to obtain a nanoparticle formulation having a particle size of less than 400 nm. Nor would one expect that an albumin-coated solid nanoparticle formulation having a particle size of less than 400 nm and paclitaxel concentration of 5-15 mg/ml would have been stable. Specifically, according to Example 5 of Desai, the particle concentrations of the formulations reported therein is about 7-9 x 10^{10} per ml. See Table 1 at Column 13 of Desai. The estimated particle concentration of the albumin-coated paclitaxel nanoparticle formulation having a particle size less than 400 nm at paclitaxel concentration of 5 mg/ml, on the other hand, is about 8 x 10^{13}/ml. This is 1000 fold higher than those reported in Desai. Since the aggregation rate of nanoparticles is proportional to the square of the particle concentration, one would not have expected that the albumin-coated paclitaxel nanoparticle formulation having a particle size less than 400 nm at paclitaxel concentration of 5 mg/ml, whose particle concentration is at least 1000 fold higher than those reported in Desai, would be stable.

16. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.
11/1/2013

Date

Neil P. Desai
Exhibit 1
Composition 2, Lot 1, 24 hrs at 40°C, 400x

Composition 2, Lot 2, 24 hrs at 40°C, 400x

Composition 1, 24 hrs at 40°C, 400x
Exhibit 3
<table>
<thead>
<tr>
<th>Sample</th>
<th>Storage Condition</th>
<th>Weight Mean Diameter, nm&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Composition 1</strong></td>
<td>0 time</td>
<td>136.9</td>
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<tr>
<td></td>
<td>24 hours at 40°C</td>
<td>135.2</td>
</tr>
<tr>
<td><strong>Composition 2, Lot 1</strong></td>
<td>0 time</td>
<td>244.5</td>
</tr>
<tr>
<td></td>
<td>24 hours at 40°C</td>
<td>1159.5</td>
</tr>
<tr>
<td><strong>Composition 2, Lot 2</strong></td>
<td>0 time</td>
<td>228.0</td>
</tr>
<tr>
<td></td>
<td>24 hours at 40°C</td>
<td>561.5</td>
</tr>
</tbody>
</table>

<sup>1</sup> Size determined by disc centrifugation method.
Exhibit 4
A Light Scattering Study of the Fractal Aggregation Behavior of a Model Colloidal System

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Departments of Chemistry and Chemical Engineering, The University of Newcastle, University Drive, Callaghan, NSW 2308, Australia

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The mass fractal dimension of aggregates of colloidal polystyrene latex particles was measured using small-angle static light scattering over a range of electrolyte and particle concentrations. The measured fractal dimensions ranged from 1.78 to 2.20, which are in good agreement with the predicted values of the diffusion-limited cluster–cluster aggregation and reaction-limited cluster–cluster aggregation regimes, respectively. It was found that increasing the salt concentration had the effect of reducing the fractal dimension, indicating a more open aggregate structure. Two regimes of behavior were observed for the fractal dimension as a function of particle concentration. At high salt levels (>1 M KNO₃) the particle concentration was seen to have little or no effect, while at low salt levels (<1 M KNO₃) an increase in concentration led to a decrease in the fractal dimension. This was attributed to a reduction in the time available for reconfiguring of particles in the aggregates due to an increased particle collision frequency. The particle aggregation rate was also found to increase at higher electrolyte levels and with larger particle concentrations.

Introduction

Aggregation of colloidal particles is of fundamental interest to many academic and industrial researchers. A typical aqueous colloidal dispersion consists of particles whose stability is controlled by the presence of some surface charge. The suppression of this stabilizing effect is easily achieved by the addition of moderate amounts of an inert electrolyte. Until recently, the structures formed upon aggregation of these colloidal dispersions were not easy to define in a mathematical sense. The advent of fractal mathematics has resulted in a renaissance in this field of study. The interest is greatly motivated by the fact that fractal growth phenomena are closely related to many processes of practical importance. For instance, the structure of particle aggregates has important implications for problems relating to air and water pollution control.1

Mandelbrot2 was the first to recognize that many common structures possess a rather special kind of complexity. He gave them the term fractal to express that they can be characterized by a noninteger (fractal) dimensionality. With the development of research in this direction, the list of examples of fractals has become extensive and includes structures ranging in size from microscopic aggregates to clusters of galaxies. Fractal morphology has already been demonstrated for a range of particle aggregates including those of silica, gold, latex, clay, polymer, shale, coal, and soot, among others.

Computer simulations have played a major role in understanding the structure of these aggregates. This is because it is difficult to devise real experiments that can isolate an aggregation mechanism as precisely as do computer simulations. Hence, much of the knowledge about aggregation and fractal growth has been derived from computer modeling. Simulation studies have shown that the mass fractal dimension, dₚ, of particle aggregates is a function of the spatial dimension alone and is essentially independent of the details of the simulation. And the resulting fractal dimensions are largely dependent upon the particle sticking efficiency, that is, the potential energy barrier to aggregation.

For a Brownian particle, when it follows a random walk between two points, it covers a large space in between. There is a high probability that as the particle moves from an exterior point to a point deep inside a cluster, it will intercept an arm of the cluster. Therefore, the growing arms will screen the interior of the cluster from the incoming particles. As a consequence, a particle is more likely to stick near the outside of the cluster than to penetrate near the center, resulting in a very open structure. In many real systems involving diffusion-limited growth, a large number of diffusing clusters will exist at any given time, and these can grow by sticking to each other as well as from single particle addition. It became clear that cluster–cluster aggregates have very open structures with dₚ = 1.75-1.80.34 The low fractal dimensions of this diffusion-limited cluster–cluster aggregation (DLCA) model reflect the loose, open appearance of the aggregates formed.

In this simple model only aggregation processes in which the rate-limiting step is diffusion have been considered. It is also possible for the aggregation rate to be limited by the probability that particles will stick upon contact. If the sticking probability (or collision efficiency) is very small, the clusters will need to collide many times before they stick, and this will have the net effect of allowing the diffusing clusters to penetrate further into each other before sticking. The factor responsible for the low sticking probability is usually an electric double-layer repulsion. This type of aggregation is commonly referred to as reaction-limited cluster–cluster aggregation (RLCA). Simulations5 show an increase in the fractal dimension over the diffusion-limited case with dₚ = 2.1 (i.e., denser aggregates), confirming that the effect of screening is somewhat reduced.

The first experiments that explicitly investigated the fractal nature of aggregates were reported in 1979 by

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† Department of Chemistry.
‡ Department of Chemical Engineering.
Forrest and Witten. In these experiments metallic oxide smoke particles were deposited onto transmission electron microscopy (TEM) substrates and their fractal dimensions determined using image analysis techniques to compute the pair correlation function and the mean density as a function of size. Values in the range 1.85–1.90 were found for "d_f." The primary difficulty in these experiments was the three-dimensional clusters were simply projected onto the two-dimensional TEM substrates. Nonetheless, these experiments were the first to demonstrate the essential fractal nature of particle aggregates.

Schaefer et al. avoided the inherent limitations of the sample preparation required by microscopy methods by using light and X-ray scattering to measure the fractal dimension of colloidal silica aggregates in situ. Since their introduction, a wide variety of colloidal systems have been studied using these scattering techniques. These range from silica to polystyrene latex, and even carbon black. Within the published fractal studies nearly all have considered the aggregation of colloidal particles in the presence of electrolyte, though studying a wide range of parameters. Some include the effect of varying the electrolyte concentration, temperature, particle size, and the mean density as a function of the fractal structure of the aggregates.

In this study, an extensive investigation into the effect of particle concentration, over a wide range of salt concentrations, on the mass fractal dimensions of aggregates of polystyrene latex particles has been conducted using small-angle static light scattering. The changes in average aggregate size distribution and the scattering exponent were monitored over time to gain additional insight into the aggregation processes.

**Theoretical Background**

**Fractals and Static Light Scattering.** A fractal is an object that is considered to be self-similar, meaning that the structure of the object is invariant to a change of scale. In the general theory of fractals, the fractal dimension corresponds to the degree of irregularity or the space-filling capacity of an object. For a mass (or volume) fractal aggregate its mass, m(R), is proportional to its radius, R, raised to power "d_f" i.e.,

\[ m(R) \propto R^{d_f} \]  

Here "d_f" is called the mass fractal dimension and is not limited to integer values, unlike ordinary mass-size relationships. This fractal dimension can further be used to characterize changes in mass density of the aggregate, \( \rho(R) \), observed within a sphere of radius R centered at some point in the aggregate:

\[ \rho(R) \propto R^{d_f - 3} \]  

where 1 \( \leq d_f \leq 3 \) for an object in 3-dimensional Euclidean space. An unrestricted aggregate such as a coalesced sphere will have a "d_f" of 3. Aggregates with an open configuration of particles are characterized by smaller fractal dimensions.

Static light scattering has been extensively used in the study of aggregating and aggregated colloidal systems. In a static light scattering experiment a beam of light is directed onto a sample and the scattered intensity is recorded as a function of time. The intensity is related to the size distribution of the aggregates, and the angular dependence of the scattered light can provide information about the shape and orientation of the aggregates.
measured as a function of the magnitude of the scattering vector, Q, with

\[ Q = 4\pi n_0 \sin(\theta/2) A_0 \]

(3)

where \( n_0 \) is the refractive index of the dispersion medium, \( \theta \) is the scattering angle, and \( A_0 \) is the in vacuo wavelength of the incident light.

If the individual particles in a fractal aggregate are monodisperse and within the Rayleigh–Gans–Debye regime, the scattered intensity \( I(Q) \) from such an aggregate can be written as\(^{34}\)

\[ I(Q) = k_0 P(Q) S(Q) \]

(4)

In the above expression \( k_0 \) is a scattering constant. \( P(Q) \) is the single particle form factor and is related to the shape of the primary particle. \( S(Q) \) is the interparticle structure factor, which represents the correlations between different primary particles within an aggregate, assuming there are no correlations between the aggregates themselves. Thus, it describes the spatial arrangement of the particles in an aggregate.

At large \( Qs \) or \( Q \gg 1 \), where \( r_0 \) is the radius of the primary particle, \( S(Q) \) is approximately equal to 1. The scattered intensity is then dominated by the single particle form factor and only the scattering due to individual particles is seen. At \( Qs \) small compared to \( 1/r_0 \) but large compared to \( 1R \) (i.e., \( 1R < Q < 1/r_0 \)), \( P(Q) \approx 1 \) and \( S(Q) \) reduces to\(^{34}\)

\[ S(Q) = Q^{-d_t} \]

(5)

Hence, provided that \( R \) is much larger than \( r_0 \), eq 4 takes the form of the well-known power-law scattering, i.e.,

\[ I(Q) \propto S(Q) \propto Q^{-d_t} \]

(6)

It is eq 6 that was used in estimating \( d_t \) in this work. In the remainder of this paper, any reference to a fractal dimension will necessarily imply a mass fractal dimension.

**Potential Energy Calculations.** The total potential energy interaction between particles in a dispersion can be obtained by summation of the repulsive and attractive interaction energies. Healy et al.\(^{35,36}\) derived an expression for the repulsive interaction energy between two particles. Assuming low surface potentials, spherical particles of equal size, electric double layers that are thin compared to the particle size (\( \kappa r_0 > 1 \)), and small electric double-layer overlap, the repulsive interaction energy, \( V_R \), is given by

\[ V_R = 2\varepsilon \kappa e \psi_0^2 \exp(-\kappa H) \]

(7)

where \( \varepsilon \) is the permittivity, \( \psi_0 \) is the particle surface potential, and \( H \) is the separation distance between particles. The inverse Debye length, \( \kappa \), is related to the concentration of a symmetrical electrolyte, \( c \), by

\[ \kappa^2 = 2(\varepsilon c)^2 N_A e^2 (kT) \]

(3)

where \( z \) is the ion charge, \( N_A \) is Avogadro's constant, and \( kT \) is the thermal energy. It is customary to refer to \( 1/k \) as the thickness of the diffuse double layer. It is important to note that apart from fundamental constants, \( \kappa \) depends only on the temperature, concentration of electrolyte, and the ion charge. For a 1–1 type electrolyte, the value of \( \kappa \) (in \( \text{nm}^{-1} \)) in water at \( 25^\circ C \) is 3.23\(^{11,12} \). Clearly, any change in the ionic strength of a colloidal dispersion will significantly influence the energy of interaction of the particles.

The forces of attraction between neutral particles are London–van der Waals dispersion forces. Hamaker\(^{37}\) derived the following expression for the attractive interaction energy, \( V_A \), between two spherical particles of radius \( r_0 \). At a small interparticle separation, i.e., \( H \ll r_0 \), \( V_A \) is given by

\[ V_A = -\frac{A_0}{12H} \]

(9)

where \( A \) is the Hamaker constant. Hence, the total potential energy of interaction between two particles in an aqueous dispersion is obtained by summing the electric double layer and van der Waals energies, i.e.,

\[ V_T = V_R + V_A \]

(10)

The repulsive energy is approximately an exponential function of the distance between the particles with a range of the order of the thickness of the double layer (\( 1/k \)), and the van der Waals attractive energy decreases as an inverse power of the distance of the particles. Consequently, van der Waals attraction will predominate at small and at large distances. At intermediate distances double-layer repulsion usually predominates. However, this largely depends on the actual values of the two forces and hence on the electrolyte concentration in solution.\(^{38}\)

**Experimental Section**

**Materials.** Styrene monomer (Aldrich) was first washed with 10% NaOH (3 × 30 cm) followed by Millipore water (3 × 200 cm\(^3\)) and then vacuum distilled at 50 °C. The ammonium persulfate initiator (Aldrich) and all other reagents employed were of analytical grade and were used without further purification. Millipore water was used in all preparations. A four-necked, 2L reaction vessel was used and an overhead stirrer with variable speed control used to maintain constant stirring. The thermostated water bath was able to control the temperature to within ±0.5 °C, and the dialysis tubing was well boiled in Millipore water three times before use.

**Latex Preparation.** The polystyrene latexes were prepared by the surfactant-free emulsion polymerization method of Goodwin et al.\(^{39}\) using ammonium persulfate as the initiator. A typical emulsifier-free preparation was carried out in the following way. The required amount of water (800 cm\(^3\)) was added to the reaction vessel and the stirrer fitted to one outlet. Typically, the stirrer was adjusted to a distance of about 1–2 cm from the bottom of the flask. A water-cooled reflux condenser was added to a second outlet and the flask immersed in the thermostated bath. Nitrogen was bubbled through the water in the reaction vessel to remove oxygen from the system using a third inlet to the flask. The gas flow was continued throughout the reaction, but the flow rate was kept low to minimize evaporation. The stirrer was adjusted to the required speed (200 rpm) and after stirring for 30 min with nitrogen passing through the system, styrene (100 g) was added via the fourth outlet. The system was then left to equilibrate for another 30 min to allow the temperature (80°C) to attain equilibrium and to saturate the aqueous phase with styrene.

The initiator (0.55 g) was dissolved in 50 cm\(^3\) of water, and the solution was preheated to the reaction temperature and then added to the reaction vessel. A typical reaction time of 24 h was used. At the end of the reaction time, the vessel was removed

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**Light Scattering Study of a Model Colloidal System**

from the bath and allowed to cool for about 30 min. The latex was then decanted through a filter packed with glass wool to remove any unreacted monomer and any condensate formed. The latex was then dialyzed against Millipore water in order to remove residual monomer, ammonium persulfate, and any other ionic species left in the reaction mixture. The dialysis was considered complete when the conductance of the dialysate became close to that of the Millipore water after 24 h.

**Latex Characterization.** The size and polydispersity of the latex particles were determined using transmission electron microscopy (JEOL JEM 1200 EXII electron microscope). Micrographs of a number of representative regions of the sample were taken to obtain images of at least 300 particles. A commercial sizing software package (Optimas 5.1a) was then used to calculate the average particle diameter, and the relative standard deviation was used to indicate the polydispersity of the particles. A TEM image of the polystyrene latex spheres is given in Figure 1, clearly indicating the particle size regularity. The average diameter of the polystyrene latex particles was found to be 330 ± 10 nm.

Electrophoretic mobilities of the latex particles were measured using a Brookhaven Zetaprobe apparatus. The effect of pH and KNO₃ concentration on the particle mobility was examined. The analysis was performed at 25 °C, using a particle concentration of 10% w/v. Determination of the stability region of the polystyrene latex particles in KNO₃ was carried out using an optical method that relies on the sedimentation rate of the aggregated dispersion. This involved measurement of the percent transmittance of the supernatant for latex dispersions controlled to various salt concentrations. The transmittance was measured at 540 nm using a Hitachi spectrophotometer after the dispersions were left undisturbed for 24 h. The results were then plotted against the concentration of KNO₃ and the critical coagulation concentration (c.c.c) was determined as the inflection point of the curve.

**Fractal Studies.** Small-angle static light scattering was used here to study the structure of the latex particle aggregates. The instrument used was a Malvern Mastersizer S, which has a 633 nm He–Ne laser as the light source. The Mastersizer simultaneously measures scattered intensities at a range of scattering angles. It also provides a direct measure of the average size distribution of the material in the scattering cell. Aggregation was achieved by adding the required amount of polystyrene latex particles to a solution of KNO₃ of known concentration at pH 6 and at room temperature. Directly after mixing, the sample was gravity fed into the light scattering cell. The log I(Q) vs log Q plot was then used to determine the scattering exponent (cf, eq 6). Typical experimental data from which the scattering exponents were calculated are illustrated in Figure 2. The change in the scattering exponents and the average of the size distribution of the scattering material were recorded with time. Fractal dimension values for the aggregates were determined by taking the absolute value of the limiting scattering exponent in the plateau region (vide infra Figure 8).

**Results and Discussion**

The electrophoretic mobility of the polystyrene latex particles was measured at three different KNO₃ concentrations and across a broad range of pH values. The resultant data are illustrated in Figure 3. These mobility curves indicate that as the pH was increased, there was a significant increase in the ionization of the surface functional groups. Above pH 6 the curves begin to flatten out, suggesting that the surface ionization is complete in this region. As expected, there was a reduction in electrophoretic mobility of the particles on increasing the electrolyte concentration.

Generally, the measurement of sedimentation rate is a useful means for aggregation studies in colloid science. The transition between stability and coagulation occurs over a small electrolyte concentration, and the critical coagulation concentration (c.c.c) can be determined quite sharply. To obtain quantitative information on the stability of the latex dispersion, the percent transmittance (at 540 nm) of the supernatant of each dispersion was measured after 24 h and plotted against the concentration of KNO₃ in each solution. The supernatant of each sample was carefully pipetted to prevent disturbance of the sedimentation boundary. Results obtained for this system are shown in Figure 4.

Literature sources offer conflicting definitions for the
while the lower limit was selected such that the concentration of latex dispersion (at KNQ) which slow aggregation is not expected to occur. However, Hunter et al. describe the critical coagulation concentration as the concentration at which slow coagulation gives way to rapid coagulation. This is determined experimentally as the concentration where the settling material leaves behind it an essentially clear supernatant. On the basis of this definition, the concentration of latex particles is expected to be closer to 0.7 M KNQ. It seems more likely that the concentration of 0.28 M KNQ defines the region of stability of the latex dispersion; i.e., below 0.28 M the dispersion is stable, while above this concentration the dispersion will be essentially unstable, although the coagulation kinetics may not be rapid. In contrast, 0.7 M KNQ corresponds to the concentration at which rapid coagulation begins.

The fractal dimensions of the polystyrene latex particle aggregates formed in the presence of KNQ and measured by small-angle static light scattering are summarized in Table 1. The upper limit of the initial particle concentration was chosen to avoid the effects of multiple scattering, while the lower limit was selected such that background noise would not have a significant effect. The electrolyte concentrations used in the experiments ranged from 0.4 to 1.5 M, which allowed both regimes of aggregation (DLCA and RLCA) to be observed. This also allowed observation of the behavior of the fractal dimension at intermediate salt concentrations. The fractal dimensions were found to range from 1.78 to 2.23 which correspond well to known literature values of 1.8 and 2.1 for diffusion-limited cluster-cluster aggregation and reaction-limited cluster-cluster aggregation, respectively. To illustrate more clearly the observed trends with particle concentration and ionic strength, the fractal dimensions are plotted in Figure 5.

Clearly, the structure of aggregates formed from the colloidal particles differs according to the prevailing conditions within the aggregating system. At any given particle concentration, more tightly packed compact structures were formed at low salt concentrations, while more open structures were formed at higher salt concentrations. At intermediate salt concentrations, the fractal dimensions were found to be intermediate to the theoretical values predicted by the DLCA and RLCA models. As explained later in this section, strictly speaking, these intermediate values should be referred to as "effective" fractal dimensions.

According to the DLVO theory of colloidal stability, the aggregation behavior of aqueous colloids is determined by the energy barrier on the interaction potential energy curve between two colloidal particles, which in turn is controlled by the salt concentration in the medium. The primary effect of salt on a charge-stabilized aqueous dispersion is to suppress the extent of the electric double layer (eq 8). This leads to a reduction in the repulsive potential energy (eq 7). Since $V_h$ is largely unaffected and is typically attractive, the system becomes more unstable. The estimated potential energy curves for the polystyrene latex particles in water at 25 °C are plotted in Figure 6. A value of $1 \times 10^{-20}$ J was taken for the Hamaker constant. The surface potentials used in the calculations ranged from $-30$ to $-35$ mV, which were estimated from the experimental mobility data (Figure 3).

Observation of these potential energy diagrams indicates two regimes of behavior. At salt concentrations less than about 0.7 M, some form of a potential energy barrier exists, even when the curve is essentially negative over all particle separations. Hence, for salt levels between 0.3 and 0.7 M the potential energy curves indicate that:

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Critical coagulation concentration. Some authors have determined the ccc of a latex dispersion by extrapolation of the middle section of the data shown in Figure 4 back to the abscissa. From Figure 4, the value obtained in this way for our polystyrene latex particles at pH 6 is 0.28 M KNQ. However, Hunter describes the critical coagulation concentration as the "electrolyte concentration at which slow coagulation gives way to rapid coagulation". This is determined experimentally as the concentration where the settling material leaves behind it an essentially clear supernatant. On the basis of this definition, the ccc of our latex particles is expected to be closer to 0.7 M KNQ. It seems more likely that the concentration of 0.28 M KNQ defines the region of stability of the latex dispersion; i.e., below 0.28 M the dispersion is stable, while above this concentration the dispersion will be essentially unstable, although the coagulation kinetics may not be rapid. In contrast, 0.7 M KNQ corresponds to the concentration at which rapid coagulation begins.

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the particles can initially come to rest in a weak secondary minimum from which deflocculation is relatively easy. If the colliding particles have sufficient thermal energy, they can surmount the barrier and enter the deep primary minimum from which particle escape is virtually impossible. At greater than about 0.7 M salt, no barrier is present and all collisions must result in an irreversible aggregation. Such potential energy curves should be compared with the observed transition from dense to loose aggregates over a range of concentrations from 0.7 to 1.0 M salt. Thus, the absence of any barrier to coagulation in a potential energy curve would indicate the presence of a significant barrier leads to a higher fractal dimension and the RLCA regime.

The fractal results reported here are consistent with those by Zhou and Chu, who used a combination of static and dynamic light scattering measurements to study the fractal aggregates of polystyrene spheres induced by the addition of salt (0.2-1.5 M NaCl) at a constant pH of 3.2. Fractal dimensions ranging from 1.72 (DLCA) to 2.15 (RLCA) were found. Intermediate values for $d_f$ were also obtained at intermediate salt concentrations. They attributed this to the restructuring of the ramified colloidal aggregates, which they suggest may occur in the presence of a finite interparticle attraction energy. Hence, although a different univalent salt was used at a much higher pH, the trends observed for the fractal dimension of the latex aggregates are similar to ours.

In contrast, some of the reported observations of the aggregation behavior of polystyrene latex particles do not agree with the above findings. Boile et al. investigated the aggregation of polystyrene particles in a univalent salt (NaCl) over a wide concentration range. They found a value of 1.75 for the fractal dimension of aggregates in the fast aggregation regime when the salt concentration was high. In low salt conditions a different kinetic regime was observed, hence a different fractal dimension was expected. However, the growth of the aggregates was found to proceed with a fractal dimension ($d_f = 1.75$) equal to that found for the DLCA regime. Asnaghi et al. also found for low salt concentrations that $d_f$ was at first larger than 2 and therefore close to the typical RLCA fractal dimension but then gradually reverted to lower values not far from those typical of DLCA. They suggested that the aggregation crossed from reaction-limited to diffusion-limited because as the average cluster mass grows, diffusion becomes the rate-limiting process. However, they did note that decreasing the concentration of salt resulted in higher fractal dimensions that were more stable in time.

In this study, aggregate structures were also found to become less compact as the latex particle concentration was increased (cf. Figure 6). Very little has been reported on the effects of particle concentration on the aggregate structure of colloidal systems. Carpineti et al. have studied the aggregation of polystyrene spheres at a fixed salt concentration ($30 \text{mM} \ \text{NaCl}$) in the fast aggregation regime and varying particle concentration from $1 \times 10^9$ to $5 \times 10^{10}$ particles/cm$^3$. The data showed that at larger concentrations they consistently found $d_f = 1.61 \pm 0.02$, but as the concentration was decreased, $d_f$ grew to $1.83 \pm 0.02$. In contrast, Martin et al. have provided experimental evidence for an increase in the fractal dimension with an increase in the concentration of aggregating silica particles, in the rapid aggregation regime.

In our study, three particle concentrations were examined: $1.06 \times 10^9$, $1.85 \times 10^9$, and $3.70 \times 10^9$ particles/cm$^3$, with each being investigated over a wide range of salt concentrations. Our results exhibited trends similar to those of Carpineti et al. on increasing the particle concentration at a fixed level of electrolyte; that is, the fractal dimension was observed to increase with a decrease in the number of initial particles in the aggregating system. However, the particle concentration had its most pronounced effect on the aggregate structure at low ionic strengths, i.e., in the slow aggregation regime.

Our work reveals that there is a noticeably larger variation in the fractal dimension with particle concentration at lower salt concentrations. At high levels of salt, where there is no potential energy barrier to aggregation, the fractal dimension does not vary significantly with changes in the particle concentration. This can be attributed to the fact that all cluster (or particle) collisions would result in permanent contact; hence reconfiguration of clusters would be effectively prevented. In contrast, under slow growth conditions, clusters (or particles) could undergo a number of collisions before they became permanently locked into position, since they may initially only be in a weak secondary minimum. Thus, particles attached to the edge of an aggregate will have the ability to "roll around" as the structure develops as they attempt to find their minimum energy state. The ability of such a particle to sample a number of possible conformations within an aggregate will depend on the probability of another particle colliding with it in a given time, which may effectively lock it in position. This will, of course, be influenced by the frequency of particle collisions within the aggregating system. This suggests that if the primary particle concentration is reduced, the frequency of collisions is also reduced and hence there will be more time for the particles to restructure and form a denser aggregate. Consequently, the fractal dimension will be higher at lower particle concentrations, as our results indicate.

The rate at which a dispersion will coagulate depends on the frequency with which particles encounter each other and the probability that the energy barrier to coagulation can be overcome when collisions take place. Figure 7 illustrates our findings on the dependence of the relative rate of aggregate growth on the electrolyte concentration and hence the potential energy barrier to aggregation. In excess electrolyte, the aggregate grows rapidly and to a comparatively large size. This growth
shows a power-law behavior and is, therefore, associated with diffusion-limited cluster-cluster aggregation. Furthermore, since every particle collision results in a permanent contact, the number of single particles present becomes rapidly reduced. This, in combination with the decreasing probability of cluster-cluster aggregation due to the reduced mobility of these clusters, results in an eventual plateau of the aggregate size. The introduction of a repulsive energy barrier by reducing the concentration of electrolyte reduces the size of the aggregates and causes the aggregation kinetics to be slowed. The reduction in the sticking probability of the particles also produces a linear growth in the size of the aggregates over the time scale examined, where singletons are still readily available to attach to the existing aggregates. The common exponential growth behavior of reaction-limited cluster-cluster aggregation cannot readily be identified in this study due to the relatively short time scale of the observation.

An interesting situation arises when the latex dispersion is aggregated at intermediate electrolyte concentrations. The aggregate size appears to grow in more of a stepwise fashion, which suggests a transition from a slow aggregation to rapid aggregation at this level of electrolyte. This stepwise growth, although not clearly visible at 0.0020% w/w particles, appears to begin around 0.7 M KNO₃. Therefore, at the intermediate salt levels our data indicate that there was a crossover behavior from RLCA to DLCA as the aggregation progressed. According to the scale-invariant definition of fractal aggregates, the previously reported fractal dimensions at these intermediate salt concentrations should, in a strict sense, be termed "effective" fractal dimensions, as already suggested in the literature. In all, these results indicate that due to the low sticking probability of the particles at low levels of electrolyte the growth of the aggregates is slow but results in consistently higher fractal dimensions. In contrast, fast aggregation observed under high salt conditions gives larger aggregate sizes but less dense structures, observations consistent with a high probability of particle sticking. Furthermore, the growth of the aggregates is dependent on the number of particles present in the aggregating system. As the particle concentration is increased, the aggregate growth is more rapid, most likely due to the increased collision frequency. The fractal dimension of the aggregates is observed to decrease under such conditions, with this effect being more pronounced at the low salt concentrations.

In this study the change in the slope of the scattering curves (i.e., the scattering exponent) was also monitored with time and the results are illustrated in Figure 8. The results indicate that in excess electrolyte the scattering exponent is constant over time, while at low electrolyte levels a growth in the scattering exponent was observed. The absolute value of the scattering exponent will represent the mass fractal dimension of the aggregated particles, provided that the aggregate size is much larger than that of the primary particles. In these experiments it was noted that the limiting scattering exponents corresponded to sufficiently large aggregates, and thus, a fractal dimension could be assigned at this point. As a result, the scattering exponents obtained in the initial stages of the aggregation, with particular concern to the low-salt conditions, do not correspond to a fractal dimension for the aggregating system. Nevertheless, the change in the scattering exponent with time does provide a measure of the relative rate of aggregate growth and reaction kinetics for various aggregating conditions. It was found that the lower the salt concentration used in the aggregation, the longer the time required to achieve a constant scattering exponent, hence slower reaction kinetics. Similarly, longer times were required for the aggregates to become scale invariant when the particle concentration was lowered.

On the basis of the results given in Figure 8, it appears that the transition from slow to fast aggregation can be readily observed. If rapid aggregation is assumed to be the region in which the scattering exponent is constant over time, then the critical coagulation concentration can be readily determined. It appears that this change occurs between 0.70 and 0.85 M KNO₃. This is consistent with the results in Figure 7, where the above concentrations of electrolyte correspond to a stepwise growth in aggregate size. These results also compare favorably with experimental data obtained from turbidity measurements [Figure 4] using the definition provided by Hunter. Hence, fractal measurements appear to also provide a
barrier sensitive methylation occurs at the point when the maximum in the energy becomes entirely negative.

Figure 8 Variation of the scattering exponent with time at different particle concentrations: (a) 0.070%; (b) 0.003%; (c) 0.0020% w/w. The corresponding concentrations of electrolyte are (a) 0.4 M, (c) 0.5 M, (d) 0.6 M, (e) 0.7 M, (f) 0.85 M, (g) 1.0 M, (h) 1.25 M, and (a) 1.5 M KNO₃. For the particle concentration of 0.0020% w/w, 0.4 M KNO₃ has been excluded for clarity.

Comparing this critical coagulation concentration to the potential energy curves in Figure 6 should also be of interest. Textbook definitions of the ccc imply that it occurs at the point when the maximum in the energy barrier to coagulation is reduced to zero (i.e., $V_1 = 0$) and the curve becomes entirely negative. From Figure 6 this would correspond to ca. 0.4 M salt. However, the fractal dimension and size data given above appear to offer conclusive evidence for a ccc at about 0.7 M salt, which corresponds to the disappearance of the peak in the potential energy curve. This condition may, therefore, offer a more reliable estimate of the true ccc.

Conclusions
Small-angle static light scattering offers an effective tool for the determination of the fractal dimension of aggregates of colloidal particles. The fractal dimension of clusters formed by diffusion-limited processes (i.e., fast aggregation) has been found both by experiment and via computer simulations to be in the range 1.75 - 1.80. This is in good agreement with our results, which were obtained under conditions of high electrolyte concentrations. Under these conditions, any electrostatic energy barrier is completely removed and clusters stick irreversibly whenever they come into contact. Furthermore, this resulted in rapid growth in the aggregate size and essentially no change in the scattering exponent could be observed with time.

Under low salt conditions, the fractal dimensions of the latex particle aggregates were found to range from 2.09 to 2.20. This is also in good agreement with literature findings of 2.1 - 2.2 for reaction-limited aggregation. Under these conditions, a significant electrostatic energy barrier exists, introducing a finite probability of the particles sticking upon contact. Although the rate of aggregation is noticeably slower, the conditions are favorable for the production of more compact structures. Furthermore, this reduction in the aggregation rate meant that changes in the scattering exponent could be monitored with time. The results indicated a gradual growth in the scattering exponent, coming to an eventual plateau where a fractal dimension could be assigned to the aggregate structures. As the concentration of electrolyte was lowered, more time was required to reach a constant scattering exponent. In the region between these two aggregation limits, intermediate "effective" fractal dimensions were observed.

The initial latex particle concentration was seen to have a significant effect on the fractal dimension of the resultant aggregates at low salt levels. Under these conditions, lower fractal dimensions were observed on increasing the number of initial particles in the aggregating system. This was thought to be the result of an increased collision frequency at higher particle concentrations, which acts to lock particles in a given conformation, restricting their rearrangement. This is accentuated at lower salt concentrations where the aggregation process is slowed due to electrostatic repulsion of the particles. At high salt concentrations, the aggregation process is rapid and hence we see little effect on the fractal dimension with particle concentration. The rate of aggregation was also observed to change with the particle concentration, as expected.

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Stability of Nanodispersions: A Model for Kinetics of Aggregation of Nanoparticles

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In the course of aggregation of very small colloid particles (nanoparticles) the overlap of the diffuse layers is practically complete, so that one cannot apply the common DLVO theory. Since nanoparticles are small compared to the extent of the diffuse layer, the process is considered in the same way as for two interacting ions. Therefore, the Brønsted concept based on the Transition State Theory was applied. The charge of interacting nanoparticles was calculated by means of the Surface Complexation Model and decrease of effective charge of particles was also taken into account. Numerical simulations were performed using the parameters for hematite and rutile colloid systems. The effect of pH and electrolyte concentration on the stability coefficient of nanosystems was found to be more pronounced but similar to that for regular colloidal systems. The effect markedly depends on the nature of the solid which is characterized by equilibrium constants of surface reactions responsible for surface charge, i.e., by the point of zero charge, while the specificity of counterions is described by their association affinity, i.e., by surface association equilibrium constants. The most pronounced is the particle size effect. It was shown that extremely small particles cannot be stabilized by an electrostatic repulsion barrier. Additionally, at the same mass concentration, nanoparticles aggregate more rapidly than ordinary colloidal particles due to their higher number concentration.

Key Words: stability of nanodispersions.

INTRODUCTION

It is commonly accepted that the stability of colloidal systems is, in most cases, the result of an extremely slow aggregation process. The main reason for such a slow aggregation process is a high electrostatic energy barrier, and in some cases a protective layer of adsorbed chains. The theory of Colloid Stability considers collision frequency and efficiency (1, 2). Collision frequency was theoretically solved by Smoluchowski (3), while the basis for evaluation of the collision efficiency was given by Fuchs (4). In order to use the Fuchs theory one should know the interaction energy as a function of the distance between interacting particles. The effect of dispersion forces was solved by Hamaker (5), Bradly (6), and de Boer (7), while electrostatic repulsion could be evaluated on the basis of the Derjaguin, Landau, Vervey, Overbeek (DLVO) theory (1, 8). Recently, more sophisticated models were elaborated (9–14). In most of the cases the theory of Colloid Stability explains the experimental data, especially if the correct values of the electrostatic surface potentials, as obtained from the Surface Complexation model (15–19), are used (20–22). However, small particles, with sizes below 10 nm (called nanoparticles), generally do not show electrostatic stabilization. According to de Gennes (23), the reason for the instability of nanocolloidal systems might be in their low charge (surface charge density times surface area). In some cases stable systems of nanoparticles could be prepared (24, 25) but no kinetic measurements were published.

In this paper we analyze the theoretical aspect of the kinetics of aggregation of nanoparticles based on the Brønsted theory (26, 27), which was developed for the salt effect on the kinetics of ionic reactions (primary salt effect). The reason for such a choice lies in the fact that the classical DLVO approach cannot be used for nanoparticles: nanoparticles are small with respect to the thickness of the electrical diffuse layer, so that in the course of the collision of two nanoparticles a complete overlap of two diffuse layers takes place. Let us consider extension of the diffuse layer. According to the Gouy-Chapman theory, depending on the ionic strength and surface potential, the latter is reduced to 10% of its original value at a distance of 2 to 2.5 reciprocal ionic strength. This means that at the ionic strength of 10−2 mol dm−3 the diffuse layer is extended up to 6 nm from the surface. As shown on Fig. 1, in such a case overlap of diffuse layers of two nanoparticles is practically complete. In the case of ordinary colloid particles the overlap is partial so that the DLVO theory is applicable.

A nanoparticle surrounded by a diffuse layer is similar to an ion situated in the center of an ionic cloud. In the course of collision two nanoparticles in contact have a common diffuse layer or "ionic cloud." Therefore, interaction of nanoparticles could be considered in a manner similar to that for two interacting ions, and consequently described by the Brønsted theory. This theory considers the "transition state" or "activated complex" which is a pair of two interacting ions with a common ionic cloud. The
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FIG. 1. Overlap of electrical interfacial layers for two ordinary colloid particles \( r = 30 \) nm and for two nanoparticles \( r = 3 \) nm.

Equilibration of the transition state is fast, while the transformation of the transition state into product(s) is slow, and thus the rate determining step.

THEORY

Introduction of the Brønsted Concept to Kinetics of Aggregation of Nanoparticles

The quantitative interpretation of kinetics of aggregation of nanoparticles will follow the Brønsted concept (26, 27). It will be based on the Transition State theory using the activity coefficients as given by the Debye-Hückel limiting law.

Aggregation of two charged nanoparticles \( A^{z_A} \) and \( B^{z_B} \) could be represented by

\[
A^{z_A} + B^{z_B} \rightarrow AB^{z_A+z_B},
\]

where \( z \) denotes the charge number. The rate of aggregation \( v \) is proportional to the product of concentrations of interacting particles \([A^{z_A}][B^{z_B}]\)

\[
v = k[A^{z_A}][B^{z_B}],
\]

where \( k \) is the rate constant (coefficient) of aggregation.

According to the Brønsted concept, in the course of aggregation two charged nanoparticles undergo reversible formation of the transition state with charge number being equal to the sum of the charges of interacting species. The transition state \( AB^{z_A+z_B} \) undergoes the next step (binding) which is slow and is therefore the rate determining step

\[
A^{z_A} + B^{z_B} \leftrightarrow AB^{z_A+z_B} \rightarrow AB^{z_{AB}}.
\]

Note that equilibration of the interface may result in a change of the total charge of the doublet. In such a case \( z_A + z_B \neq z_{AB} \). Since the equilibration of the first step is fast, and the second process is slow, the overall rate of reaction \( v \) is proportional to the concentration of the transition state

\[
v = k'[AB^{z_A+z_B}],
\]

where \( k' \) is the rate constant (coefficient) of the second process. Equilibration of the first step is fast so that one calculates the concentration of the transition state \([AB^{z_A+z_B}]\) from the relevant equilibrium constant \( K^\# \) taking into account the activity coefficients \( y \) of reactants and of the transition state

\[
K^\# = \frac{y(AB^{z_A+z_B})[AB^{z_A+z_B}]}{y(A^{z_A})y(B^{z_B})[A^{z_A}][B^{z_B}]}.
\]

The equilibrium constant \( K^\# \) is defined in terms of activities, and consequently its value does not depend on the ionic strength; i.e., it corresponds to infinite dilution. Equations [4] and [5] result in

\[
v = k'K^\# \frac{y(A^{z_A})y(B^{z_B})}{y(AB^{z_A+z_B})}[A^{z_A}][B^{z_B}].
\]

According to the above equation, the overall rate constant, as defined by Eq. [2], is given by

\[
k = k'K^\# \frac{y(A^{z_A})y(B^{z_B})}{y(AB^{z_A+z_B})}.
\]

It is clear that the overall rate constant \( k \) depends on the ionic strength of the medium through activity coefficients. Activity coefficients could be obtained from the Debye-Hückel equation derived for ionic solutions. The same equation is assumed to be applicable for extremely small particles of charge number \( z \).

\[
\log y_i = -\frac{z_i^2}{L}A_{DH}I_1^{1/2} \left( 1 + abI_1^{1/2} \right).
\]

The ionic strength \( I_1 \) for 1 : 1 electrolytes is equal to their concentration. The Debye-Hückel constant \( A_{DH} \) depends on the electric permittivity of the medium \( \varepsilon (=\varepsilon_0\varepsilon_r) \)

\[
A_{DH} = \frac{2^{1/2}}{8\pi L \ln 10} \left( \frac{F^2}{\varepsilon RT} \right)^{3/2},
\]

where \( L \) is the Avogadro constant and \( R, T, \) and \( F \) have their usual meaning. (For aqueous solutions at 25°C: \( A_{DH} = 0.509 \) mol\(^{-1/2}\) dm\(^{3/2}\).) Coefficient \( b \) in Eq. [8] is equal to

\[
b = \left( \frac{2F^2}{\varepsilon RT} \right)^{1/2},
\]

while parameter \( a \) is the distance of closest approach of the interacting charges, which is in the case of nanoparticles related
to their radius. By introducing Eq. [8] into Eq. [7] one obtains

$$\log k = \log k' + \log K^+ + \frac{2z_A z_B A_{DH} I_c^{1/2}}{1 + ab I_c^{1/2}}.$$  \[11\]

Equation [11] suggests that the plot of the experimental log $k$ value as the function of $I_c^{1/2}/(1 + ab I_c^{1/2})$ should be linear with the slope of $2z_A z_B A_{DH}$ which is true if charges of interacting species do not depend on the ionic strength. However, as it will be shown later, the charge of a colloidal particle decreases with ionic strength due to association of counterions with surface charged groups.

**Estimation of the Equilibrium Constant of the Transition State Formation**

To analyze the effect of repulsion between two charged particles on the equilibrium constant $K^+$ we shall split the Gibbs energy of the transition state formation $\Delta \Psi G^0$ into electrostatic term, $\Delta \Psi G_{el}^0$, and the rest, which we shall call the chemical term, $\Delta \Psi G_{ch}^0$. The latter includes van der Waals dispersion attraction

$$-RT \ln K^+ = -RT \ln (K_{ch}^+ K_{el}^+) = \Delta \Psi G^0 = \Delta \Psi G_{el}^0 + \Delta \Psi G_{ch}^0,$$  \[12\]

where $\Delta \Psi G_{el}^0 = -RT \ln K_{el}^+$ and $\Delta \Psi G_{el}^0 = -RT \ln K_{ch}^+$.

As noted before, the equilibrium constant $K^+$ is based on the activities of the interacting species and its definition (Eq. [5]) considers the corrections for the nonideality. It corresponds to the zero-ionic strength so that the value of $K_{el}^+$ could be obtained considering simple Coulombic interactions between two nanoparticles. Accordingly, the (molar) electrostatic energy between particles $A^{z_A}$ and $B^{z_B}$ of radii $r_A$ and $r_B$ in the medium of the permittivity $\varepsilon$ is

$$\Delta \Psi G_{el}^0 = \frac{z_A z_B F^2}{4\pi \varepsilon L (r_A + r_B)},$$  \[13\]

where $r_A + r_B$ is the center to center distance between interacting particles in close contact. In the case of two identical particles ($r_A = r_B = r$ and $z_A = z_B = z$)

$$\Delta \Psi G_{el}^0 = \frac{z^2 F^2}{8\pi \varepsilon L r}.$$  \[14\]

This approach, based on the Coulomb law, could be tested by the Hogg-Healy-Fuerstenau (HHF) theory (9). For two equal spheres of the same surface potential $\varphi$, separated by surface to surface distance $x$, the electrostatic interaction energy, expressed on the molar scale, is equal to

$$\Delta \Psi G_{HHF}^0 = 2\pi \varepsilon L r \varphi^2 \ln [1 + \exp(-\kappa x)],$$  \[15\]

where $\kappa$ is the Debye-Hückel reciprocal thickness of the electrical interfacial layer

$$\kappa = \left(\frac{2 L c F^2}{\varepsilon RT}\right)^{1/2}.$$  \[16\]

At the zero-ionic strength ($l_c \to 0$) the surface potential $\varphi$ of a sphere of radius $r$ and the charge number $z$ is

$$\varphi = \frac{ze}{4\pi \varepsilon r}.$$  \[17\]

(Note that $\varphi$ potential is in fact the electrostatic potential at the onset of diffuse layer.) Under such a condition the diffuse layer extends to infinity ($\kappa \to 0$), so that for zero separation ($x \to 0$) Eq. [15] reads

$$\Delta \Psi G_{HHF}^0 = \frac{z^2 F^2}{8\pi \varepsilon L r} \ln 2.$$  \[18\]

The comparison of Eq. [18] with Eq. [14] shows that HHF theory results in ~30% lower value of energy than the Coulomb law. This discrepancy is not essential for the purpose of this study, so that in further analysis we shall use the Coulomb expression.

By introducing Eqs. [12] and [13] into Eq. [11] for the rate constant of aggregation of nanoparticles $A^{z_A}$ and $B^{z_B}$, one obtains

$$\log k = \log k' + \log K^+ - \frac{z_A z_B F^2}{4\pi \varepsilon L (r_A + r_B)} + \frac{2z_A z_B A_{DH} I_c^{1/2}}{1 + ab I_c^{1/2}}.$$  \[19\]

or in another form

$$\log k = \log k_0 - z_A z_B \left(\frac{B}{r_A + r_B} - 2A_{DH} I_c^{1/2} \frac{I_c^{1/2}}{1 + ab I_c^{1/2}}\right),$$  \[20\]

where

$$B = \frac{F^2 \ln 10}{4\pi \varepsilon L RT}.$$  \[21\]

and

$$k_0 = k' K^+. $$  \[22\]

At high ionic strength the counterion association is so pronounced that the effective charge number of nanoparticles approaches to zero. In such a case the electrostatic repulsion diminishes and the aggregation is controlled by the diffusion ($k = k_{diff}$), as described by the Smoluchowski theory. Accordingly,

$$k_0 = k_{diff}.$$  \[23\]
The stability coefficient (reciprocal of the collision efficiency), commonly defined as $W = \frac{k_{\text{diff}}}{k}$, is then equal to
\[
\log W = \log \frac{k_0}{k} = z_A z_B \left( \frac{B}{r_A + r_B} - \frac{2A_{\text{DH}}}{L_c^{1/2}} \right). \tag{24}
\]
In the case of aggregation of identical nanoparticles the above equation is reduced to
\[
\log W = \log \frac{k_0}{k} = 2z^2 \left( \frac{B}{r} - \frac{A_{\text{DH}}}{L_c^{1/2}} \right). \tag{25}
\]

Evaluation of the Charge Number

For a given electrolyte concentration, the stability coefficient of the nanodispersion could be obtained by Eq. [24] (or by Eq. [25], in the case of uniform particles), once the charge number of particles is known. The surface potential (as used in the theory of Colloid Stability) and charge number are determined by the ionic equilibrium at the solid/liquid interface which will be considered here for metal oxide particles dispersed in aqueous electrolyte solutions. The Surface Complexation model (2-pK concept) considers (15-22) amphotheric surface =MOH groups, developed by the hydration of metal oxide surfaces, that could be protonated (p) or deprotonated (d)

\[
\begin{align*}
&M\text{OH} + H^+ \rightarrow =\text{MOH}_2^+; \\
&K_p = \exp(F\phi_0/RT) \frac{\Gamma(\text{MOH}_2^+)}{a(H^+)\Gamma(\text{MOH})} \tag{26} \\
&M\text{OH} \rightarrow =\text{MO}^- + H^+; \\
&K_d = \exp(-F\phi_0/RT) \frac{\Gamma(\text{MO}^-)a(H^+)}{\Gamma(\text{MO})}. \tag{27}
\end{align*}
\]

$K_p$ and $K_d$ are equilibrium constants of protonation and deprotonation, respectively, $\phi_0$ is the potential of the 0-plane affecting the state of charged surface groups MOH$_2^+$ and MO$^-$, $\Gamma$ is the surface concentration (amount per surface area), and $a$ is activity in the bulk of solution.

Charged surface groups bind counterions, anions A$^-$ (surface equilibrium constant $K_a$), and cations C$^+$ (surface equilibrium constant $K_c$)

\[
\begin{align*}
&M\text{OH}_2^+ + A^- \rightarrow =\text{MOH}_2^+ \cdot A^-; \\
&K_a = \exp(-F\phi_\beta/RT) \frac{\Gamma(\text{MOH}_2^+ \cdot A^-)}{a(A^-)\Gamma(\text{MOH}_2^+)} \tag{28} \\
&M\text{O}^- + C^+ \rightarrow =\text{MO}^- \cdot C^+; \\
&K_c = \exp(F\phi_\beta/RT) \frac{\Gamma(\text{MO}^- \cdot C^+)}{a(C^+)\Gamma(\text{MO}^-)}. \tag{29}
\end{align*}
\]

where $\phi_\beta$ is the potential of $\beta$-plane affecting the state of associated counterions.

From the d-plane (onset of diffuse layer, potential $\phi_d$), ions are distributed according to the Gouy-Chapman theory. The total concentration of surface sites $\Gamma_{\text{tot}}$ is equal to
\[
\Gamma_{\text{tot}} = \Gamma(\text{MOH}) + \Gamma(\text{MOH}_2^+) + \Gamma(\text{MO}^-) + \Gamma(\text{MO}^- \cdot C^+) + \Gamma(\text{MOH}_2^+ \cdot A^-). \tag{30}
\]

Surface charge densities in the 0- and $\beta$-planes are
\[
\begin{align*}
\sigma_0 &= F(\Gamma(\text{MOH}_2^+) + \Gamma(\text{MOH}_2^+ \cdot A^-)) \\
&\quad - \Gamma(\text{MO}^-) - \Gamma(\text{MO}^- \cdot C^+) \tag{31} \\
\sigma_\beta &= F(\Gamma(\text{MO}^- \cdot C^+) - \Gamma(\text{MOH}_2^+ \cdot A^-)). \tag{32}
\end{align*}
\]

The net surface charge density $\sigma_s$ corresponding to the charge fixed to the surface is opposite in sign to that in the diffuse layer $\sigma_d$
\[
\sigma_s = -\sigma_d = \sigma_0 + \sigma_\beta = F(\Gamma(\text{MOH}_2^+) - \Gamma(\text{MO}^-)). \tag{33}
\]

The relations between surface potentials, within the fixed part of electrical interfacial layer (EIL), are based on the constant capacitance concept
\[
C_1 = \frac{\sigma_0}{\phi_0 - \phi_\beta}; \quad C_2 = \frac{\sigma_s}{\phi_\beta - \phi_d}, \tag{34}
\]

where $C_1$ and $C_2$ are capacities of the so-called inner and outer layer, respectively. The general model of EIL could be simplified (19) by introducing $\phi_\beta = \phi_d$, which corresponds to $C_2 \rightarrow \infty$.

The equilibrium in the diffuse layer is described by the Gouy-Chapman theory.

For planar surfaces (relatively large particles)
\[
\sigma_s = -\sigma_d = -\frac{2RT\kappa}{F} \sinh(-F\phi_d/2RT) \tag{35}
\]
and for small spherical particles (nanoparticles)
\[
\sigma_s = \frac{\varepsilon \phi_d}{r} \left( 1 - \frac{r}{r + k^{-1}} \right)^{-1}. \tag{36}
\]

Once the system is characterized, the Surface Complexation model enables calculation of the colloid particle charge number under given conditions. This means that one should know equilibrium constants of surface reactions, capacitances of inner and outer layers, and total density of surface sites. By an iteration procedure one obtains the net surface charge density $\sigma_s$ (defined by Eq. [33]) from which the particle charge number is
\[
z = 4r^2\pi \sigma_s/e. \tag{37}
\]
Numerical Simulation and Discussion

The above theory, developed for kinetics of aggregation of nanoparticles (nanocoagulation), will be demonstrated on a few examples. Two systems (hematite and rutile) under different conditions will be examined. The values of equilibrium parameters, used in calculation of the particle charge number, were obtained by interpretation of adsorption and electrokinetic data for ordinary colloid particles (21, 22). It was assumed that these parameters approximately describe the properties of corresponding nanosystems. In the evaluation the Gouy-Chapmen equation for spherical interfacial layer, Eq. [36], was used. Once the charge number was obtained, the stability coefficient was calculated via Eq. [25].

Figure 2 demonstrates the effect of electrolyte concentration on the stability of hematite nanodispersions containing particles of $r = 3$ nm. It is obvious that the stability of the system decreases rapidly with electrolyte addition. At pH 4, particles are positively charged so that association of anions with the surface charged groups takes place. Nitrate ions were found to aggregate the system more effectively with respect to the chloride ions, which is due to lower values of the surface association equilibrium constant of the latter counterions. The effect of electrolyte concentration is explicitly included in Eq. [25] through ionic strength. However, particle charge number also depends on the electrolyte concentration due to counterion association so that both effects result in a decrease of stability at higher electrolyte concentrations. Figure 3 demonstrates the effect of the activity of potential determining H$^+$ ions. At lower pH values particles are more positively charged, the system is more stable, and higher electrolyte concentration is necessary for aggregation. The effect of particle size on the stability of the system is dramatic. As shown in Fig. 4, systems with smaller particles are more stable at lower pH values and require higher electrolyte concentrations.

![Figure 2](image1)

**FIG. 2.** Effect of electrolytes on the stability of hematite aqueous nanodispersion ($r = 3$ nm) at $T = 298$ K and pH 4, as obtained by Eq. [25]. The charge number was calculated by the Surface Complexation model (Eqs. [26]–[39]) using parameters obtained (21) with hematite colloid dispersion ($r = 60$ nm): $\Gamma_{\text{sat}} = 1.5 \times 10^{-5}$ mol m$^{-2}$; $K_p = 5 \times 10^5$; $K_d = 1.5 \times 10^{-11}$; $pH_{\text{pzc}} = 7.6$; $K(\text{NO}_3^-) = 1410$; $K(\text{Cl}^-) = 525$; $C_1(\text{NO}_3^-) = 1.88$ Fm$^{-2}$; $C_1(\text{Cl}^-) = 1.81$ Fm$^{-2}$; $C_2 = \infty$; $\varepsilon = \varepsilon_f / \varepsilon_0 = 78.54$.

![Figure 3](image2)

**FIG. 3.** Effect of pH on the stability of hematite aqueous nanodispersion ($r = 3$ nm) in the presence of potassium nitrate at $T = 298$ K. The parameters used in calculations are the same as in Fig. 2.

![Figure 4](image3)

**FIG. 4.** Effect particle size on the stability of hematite aqueous nanodispersions in the presence of potassium nitrate at pH 4 and $T = 298$ K. The parameters used in calculations are the same as in Fig. 2.
The absolute values of surface potentials are approximately the same. However, due to the different values of the equilibrium parameters, rutile was found to be significantly less stable than hematite.

It may be concluded that application of the Brønsted concept to the stability of nanodispersions shows that the presence of electrolytes may completely reduce the stability, and that the electrolytes with counterions exhibiting higher affinity are more effective for association at the interface. Lower surface potential (pH closer to the point of zero charge) slightly reduces the stability. In the above analysis the parameters used for calculations may differ from the reality since they were obtained from measurements with relatively large colloid particles; however, the general behavior of nanosystems may be still explained. The comparison between hematite and rutile showed how sensitive the stability of nanosystems is on the inherent characteristics of the solid. Different equilibrium parameters result in a very pronounced difference in the stability. However, the major characteristics of the system are the particle size; smaller particles are significantly less stable. The interpretation based on the Brønsted theory is more close to the reality if the particles are very small.

In this paper we have introduced the Brønsted concept for interpretation of the kinetics of aggregation of nanoparticles. Both DLVO and the Brønsted approach result in reduced stability at high electrolyte concentrations, but according to the latter approach the stability coefficient does not directly depend on the Hamaker constant governing dispersion attraction. The presented concept suggests that dispersion forces are cause for binding of particles but do not affect the kinetics of aggregation of nanoparticles. In considering the stability of nanosystems one should also take into account the effect of particle concentration. Let us compare two systems with the same mass concentration but different in particle size. One system is an ordinary colloidal system (r = 30 nm), while the second one is a nanosystem (r = 3 nm). The difference in particle size by a factor 10 results in 1000 times higher concentration of nanoparticles. Since the aggregation rate is proportional to the square of the particle concentration the collision frequency in the nanosystem will be a million times higher. Thus, the aggregation rate will be a million times higher while the aggregation half-time will be reduced by a factor of 1000. Accordingly, one may conclude that nanoparticles can hardly form a stable dispersion without additives, (e.g., surface-active agents) because their aggregation is fast due to both low stability coefficient and high particle number concentration.

REFERENCES

Comparison of physicochemical characteristics and stability of three novel formulations of paclitaxel: Abraxane, Nanoxel, and Genexol PM

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Background: Abraxane (Abraxis BioScience, Inc., Los Angeles, CA, approved in USA and Canada), Nanoxel (Dabur Pharma, H.P., India, approved in India), and Genexol PM (Samyang Pharmaceuticals, Seoul, Korea, approved in Korea) are 3 commercially approved, novel formulations of paclitaxel. Abraxane consists of albumin-bound injectable nanoparticles of paclitaxel, while Genexol and Nanoxel (utilizing cosolvents) are polymeric-micelle formulations. Abraxane and Genexol are lyophilized products approved for 25°C ± 2°C storage, while Nanoxel is a liquid formulation approved for 2-8°C storage. This study investigated the physicochemical characteristics and short-term stability of the 3 products under recommended clinical use conditions and under accelerated conditions.

Methods: The drugs were reconstituted and prepared per the instructions provided in the respective package inserts. Abraxane and Genexol were reconstituted using the recommended saline diluent, while Nanoxel was mixed and diluted in 10% dextrose. Each drug was reconstituted to 0.7 mg/mL and 5 mg/mL. Physical stability was monitored both visually and microscopically; particle size was measured and monitored over time at room temperature (RT, measured to be 23°C) and 40°C using photon correlation spectroscopy (PCS) (Zetasizer 3000, Malvern, UK). Chemical purity was measured by reduced reversed-phase HPLC (Shimadzu Scientific Instruments, MD).

Results: Following reconstitution, Abraxane was determined to be stable both physically and chemically...
Comparison of physicochemical characteristics and stability of three novel formulations of paclitaxel: Abraxane, Nanoxel, and Genexol.

at RT and 40°C over 24 hrs, with no evidence of nanoparticle size growth at either 0.7 mg/mL or 5 mg/mL. While reconstituted Genexol was stable at RT over 24 hrs, micelle instability resulting in precipitation of paclitaxel in the form of large needle-like crystals for both 0.7 mg/mL and 5 mg/mL formulations was seen between 2 to 4 hrs at 40°C. These observations were confirmed using orthogonal techniques, visual assessment from photomicrographs, and PCS particle size measurement. For Nanoxel at 0.7 mg/mL, a minor, but consistent, increase in particle size was observed at RT over 24 hrs. However, significant aggregation, particle-size growth, and crystallization were seen within 4 hrs at 40°C. HPLC data comparing pre- and post-filtration confirmed that the crystal formation for both Nanoxel and Genexol resulted from paclitaxel precipitation and aggregation. In addition, analytical results showed that Nanoxel had slightly lower paclitaxel purity as compared to either Abraxane or Genexol. Conclusions: Nanoparticle albumin-bound paclitaxel, Abraxane, showed excellent physicochemical stability as compared to the micellar formulations, Nanoxel and Genexol. Particle size growth and crystal formation were readily apparent in Nanoxel and Genexol, especially in the short term under accelerated conditions.