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Conference Paper

Interaction of Colloidal Particles with Surfaces of Biological Significance*

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The adhesion of colloidal gold on membranes was examined with an electron microscope with protein on the membrane, on the colloidal particles and present on both membrane and particles. The conditions for best adhesion were determined. Quantitative measurements were carried out using adhesion to the membrane to monitor diffusion, centrifugation and electrophoresis of colloidal particles. Electrophoresis in a centrifugal field was used as a null method to determine the charge on the colloidal particle.

Inorganic colloidal particles play an interesting role in biological systems. Thus colloidal gold has been used for disease diagnosis by examination of its interaction with spinal fluids.¹ It is also used for treatment of arthritis.² Radioactive colloidal gold, phosphorus and technicium on colloidal sulfur are used extensively for cancer diagnosis and treatment³⁻⁵.

With this in mind it was of interest to investigate the adhesion of monodisperse colloidal gold on surfaces covered with proteins. This investigation is an extension of work on colloidal particles carried out in the Chemistry Department of Princeton University.⁶⁻¹⁴

Conditions were found under which adhesion took place under every collision of the particle with the surface. This permitted development of new techniques for quantitative study of diffusion, centrifugation and electrophoresis. Electrophoresis carried out in the electric field was used to calculate the charge on the colloidal particle much in the way Millikan determined the charge on the electron.¹⁵

Methods were devised for uniform mounting of particulate matter for examination in the electron microscope.

EXPERIMENTAL

Standard 200 Å Colloidal Gold

100 ml of HAuCl₄ solution containing 50 mg of gold is added to 850 ml of distilled water, boiling in a two-liter flask. When the solution comes to boiling after this addition, 50 ml of 1% sodium citrate solution is added with good mechanical stirring. The solution is brought back to boiling. It undergoes a series of color changes, the yellow color disappears, a greying-blue color appears which changes into deep wine red color in the course of fifteen minutes. After thirty minutes boiling, the solution is allowed to cool.

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Excess electrolyte was removed by treating the colloidal solution with Amberlite MB-1 ion exchange resin: 100 ml solution was stirred magnetically for one-half hour with 3 ml of resin after which the resin was removed by filtration through glass wool. Longer contact time caused adsorption of gold on the resin. The solution was stabilized by adding 7.5 ml of 1% sodium citrate to 1 liter of the colloid. To prevent coagulation and minimize bacterial growth the final solution was kept in a refrigerator. The colloidal particles had an average size of 200 Å with 12% deviation from the mean. The concentration was 6×10^{11} particles per ml of solution.

Preparation of 300 Å Colloidal Gold

Before treatment of the 200 Å gold with exchange resin, 246 ml of the solution were diluted with 554 ml of water in a 2 liter round bottom flask. The solution was brought to boiling and 38 ml of HAuCl_4 (50 mg Au per liter) and 38 ml of 1% sodium citrate were added simultaneously drop by drop from two separate funnels. The solution was stirred and boiled at the same time. After the addition was complete, the solution was boiled for another half hour. It was then cooled in water and brought up to 1 liter.

The solution was de-ionized by treating each 400 ml of solution with 3 ml of Amberlite MB-1 ion exchange resin for one-half hour with magnetic stirring and filtering the resin off the glass wool. 3 ml of 1% sodium citrate solution was added to stabilize the colloid. The particle size was 300 Å again with a 12% deviation from the mean. The concentration of particles was 1.5×10^{11} per ml.

Preparation of 400 Å Colloidal Gold

Before treatment of the 300 Å gold with ion exchange resin, 422 ml was diluted to 800 ml, brought to boiling in a two-liter flask. 29 ml of HAuCl_4 solution (50 mg Au per liter) and 29 ml of 1% sodium citrate were added simultaneously drop by drop to the boiling solution. It was boiled for an additional half hour after the addition, cooled and brought up to one liter in volume. The same treatment was carried out to de-ionize and stabilize the colloidal solution.

Due precautions were taken to ensure cleanliness. The laboratory distilled water was redistilled, the glass vessels were cleaned with aqua regia and steamed for thirty minutes in live steam to remove any film of grease.

Preparation of Membrane

A dish of about 20 cm in diameter is filled about two-thirds with distilled water. A copper wire gauze of the size of a microscopic slide with a handle at right angles was loaded with a number of copper or silver electron microscope specimen grids (200 mesh). The wire gauze was then lowered carefully to the bottom of the dish. Three or four drops of 1% solution of collodion in amyl acetate were allowed to fall on the surface at the center of the dish from a dropping pipette. The solid film which formed when the solvent evaporated, was removed from the surface with a needle mounted in a holder. This procedure was repeated. The purpose was to clean the water surface. The collodion membrane was prepared by adding two drops of the 1% collodion solution. After the film formed, the wire gauze with the grids was lifted until it formed a firm contact with the film on the surface. It was then raised above the water level and placed on a filter paper to remove excess water. After drying, the film around each grid was cut with the sharp tips of tweezers. The membrane was then ready for use.

Formvar and carbon membranes were obtained from Ernest F. Fullam P. O. Box 444, Schenectady, N. Y. 12301. Gelatin was Knox Brand.

Electron Microscope Examination

Electron micrographs of particles adhering to the films were taken with the RCA type EMU-2 electron microscope. The resolution was 20–30 Å. The electronic magnification was about 25,000 diameters while the optical was six-fold.

Adhesion of Colloidal Particles to Membranes

The first set of experiments dealt with the adhesion of colloidal particles to membranes. Electron microscope grids containing the membranes were placed at the ends of four short arms attached to a copper rod, at right angles to each other and to the rod. The diameter of the short arms was the same as that of the grids. The copper rod holding the arms passed through a circular plate so that the holder could be placed in a 10 ml weighing bottle containing the colloidal solutions. The whole copper holder was gold plated. Each of the four electron microscope grids with membrane attached to them were placed in an electron microscope specimen caps and the caps with the grid were placed on each of the arms of the holder. The holder with the membranes was immersed in the protein solution for a fixed amount of time, then into two weighing bottles of distilled water for a fixed time, then for varying times into colloidal solution. One of the grids was then removed from one of the arms of the holder and the rest were immersed again into the colloidal solution for a longer time period. The removed grid was picked up with a pair of tweezers dipped into several changes of distilled water and dried. Excess of water present between the prongs of the tweezers was removed with filter paper. The other three grids were treated in the same way after being immersed in the colloidal solution for increasing times.

In another procedure, the grids with the membranes were held between the points of sharp tweezers using a paper clip. After immersion in the colloidal solution, the grids were not rinsed, but were dried with the edge of a filter paper. Any excess of solution on the membrane itself was removed with a small capillary.

Untreated Membrane

Using 200 Å gold colloid solution and formvar membrane, it was found that immersion times of 10, 20, and 60 minutes produced very few particles on the membrane (34 in 10 minutes, 39 in 60 minutes over a standard area). The membrane was relatively free of gold particles around pieces of dust which were occasionally present on the membrane. The same results were obtained with cellulose nitrate and carbon evaporated films. Furthermore, no adhesion of colloidal palladium, alumina, silica, zinc sulfide particles was observed on untreated membranes, after the washing procedure outlined above.

Protein on Membrane

Using formvar membranes, the following procedure was carried out with 200 Å gold: 3 minutes in 0.1% gelatin solution, 3 minutes in distilled water, 3 minutes in another batch of distilled water; 10—20—40—60 minutes in colloid solution, rinse in distilled water, drying with capillary and filter paper. The number of particles counted per unit area of membrane was 260 at 10 minutes, 580 at 20 minutes, 1140 at 40 minutes and 1265 after 60 minutes. The number adhering increases linearly with time through 40 minutes and then stops, possibly due to charging up of the membrane by adsorption of charged particles or desorption of the protein from membrane.

The tenacity of adhesion was studied by a washing technique. The grid was immersed in 0.1% gelatin solution for 3 minutes, washed for 12 minutes in water. It was then examined in the microscope. The washing did not affect the con-

centration of particles on the grid. A further 12 minute immersion in water did not change the concentration of adsorbed particles.

In another set of experiments the adhesion was studied on a collodion membrane using the procedure outlined above, but the time of immersion in the 200 Å colloidal gold solution and the concentration of colloidal gold were varied (Fig. 1). It is seen that for the first fifty minutes the concentration of

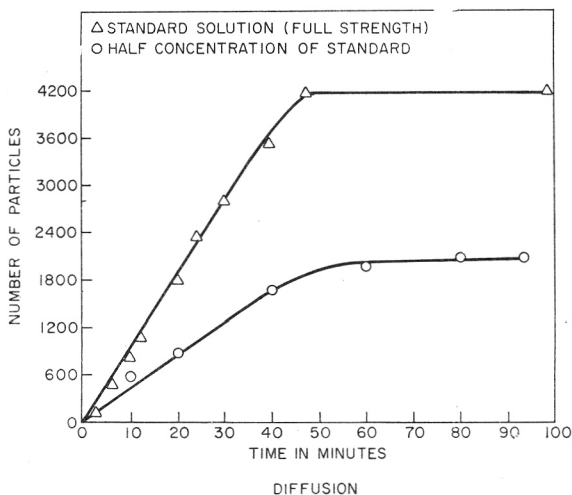


Fig. 1. Adhesion of gold particles to membrane due to diffusion.

particles on the surface increases linearly with time and the rate is proportional to the concentration in solution. The process stops at about fifty minutes, the concentration of adsorbed particles remaining constant. This is interpreted to indicate that the gelatin which was free of gold on the surface had desorbed.

A calculation of the efficiency of adhesion can be carried out applying the Sutherland-Einstein equation for the diffusion constant, *i. e.* for number of particles crossing unit area per unit time and thus a unit concentration gradient

$$D = \frac{RT}{N_A} \frac{1}{6 \eta r}$$

where N_A is Avogadro's number, r is the radius of the colloidal gold and the other symbols have their conventional significance. The velocity of the colloidal gold particle is the velocity of its Brownian motion so that the mean displacement in the X direction in time t given by

$$\bar{X}^2 = 2Dt$$

The mean velocity \bar{U} is half the mean displacement so that $\bar{U} = 1/2 \bar{X}$. The number of collisions is $1/6 NU$ per unit surface, where N is the number of particles per ml, in our case 6×10^{11} . If the adhesion efficiency is 100%, we should obtain 105 particles per minute per 5×10^{-8} cm² which we have taken as the standard area for electron microscope observation. For standard solution we obtain after 35 minute observation, 2900 particles instead of 3150 with an

adhesion efficiency of 89%. The theoretical value for a colloid solution of half the concentration in gold particles at 40 minutes is 2100 particles while we observe 1667 with 79% efficiency of adhesion.

In another experiment using 400 Å colloidal gold 7.5×10^{10} particles per ml, the theoretical adhesion in 20 minutes was 186 particles while we observed 168 with an adhesion efficiency of 90%. The membrane was then introduced in a 200 Å gold sol for 10 minutes of concentration 6×10^{11} particles per ml. The theoretical concentration was 1050, the actual count was 1068 giving an efficiency of close to 100%. There was an absence of adhesion of small particles to the larger ones, if anything there seemed to be an area free of smaller particles around the larger ones. It should be noted that in all the adhesion experiments, initially the particles were scattered as single units randomly on the surface, but as the surface fills out linear configurations of particles become apparent, the particles either touching or often spaced from one another. This phenomenon is independent of whether the membrane is cellulose nitrate, formvar or evaporated carbon.

Protein in Solution

It had been shown previously that coagulation of colloidal particles could be stopped by adding a protein to the solutions. We had used this phenomenon to study the process of fast and slow coagulation using the electron microscope.^{11,13} With this background we investigated the effects of adding 0.1 ml of 1% gelatin solution to 10 ml colloidal 200 Å gold solution, then placing an untreated formvar membrane into such a solution for varying times of 10, 20, 40, 60 minutes, drying the grids with filter paper and then examining them in the electron microscope. The amount observed per standard area was 272 in 10 min., 900 in 20 min., 1077 in 40 min. and 2351 in 60 min. Thus the efficiency of a »sticky« collision is lower than that for the gel on the membrane and none in the solution. Furthermore, the adhesion continues after forty minutes lending support to the view that in the case of gelatin on membrane, continued immersion in colloidal solution leads to the desorption of the gelatin. The decrease in the concentration of the particles on the surface may be due to a decrease in adhesion of gelatin coated particle to a membrane partially coated with gelatin present in the gold solution and not on the gold particle. Or it may be due to a lowering of the diffusion constant of the gold particle coated with gelatin layers.

The strength of adhesion was determined in a qualitative way by a washing technique. A membrane which had been immersed for forty minutes in the colloid gold sample with a concentration of 1077 particles per standard area, showed after 3 minute immersion in distilled water 51 particles, after 6 minutes 278, after 12 minutes 67, after 24 minutes 209 particles. This is in marked contrast to the resistance to washing shown by the system in which gelatin is present only on the membrane. Further experiments showed that most of the weakly adhering particles were washed off in 12 minutes.

That excess gelatin in solution over what is necessary to just coat the particles, is deleterious to adhesion is shown by the following experiment. The amount of gelatin was varied from 0.1 ml of a 0.1% solution to 1 ml in 10 ml of 200 Å colloidal gold. The immersion time was 40 minutes followed by a 24 minute wash in distilled water. Examination in the electron microscope showed that the concentration of the particles *increased* with *decrease* in

gelatin concentration. Decrease in the concentration to 0.01% gelatin again showed that 0.1 ml gelatin gave the largest concentration of particles after washing, but that clustering became noticeable.

Gelatin on Membrane and in Solution

To check the deleterious effect on adhesion of excess gelatin in both solution and on the membrane, the following procedure was followed. A gold gelatin solution was prepared by mixing 4 drops (0.13 ml) of a 0.1% gelatin solution with 10 ml of 200 Å gold sol. Four membranes on grids were immersed in the 0.1% gelatin solution for 3 minutes and then washed twice for 3 minutes in two changes of distilled water. The grids were then immersed in the combined gelatin-gold solution for 10, 20, 40 and 60 minutes. They were then washed for 3 minutes in distilled water and dried with a filter paper and capillary pipette. The concentration of particles was small with singlets and doublets present: 35 singlets and 21 doublets in 10 minutes; 142 singlets and 40 doublets in 20 minutes; 265 singlets and 56 doublets in 40 minutes, and 245 singlets and 33 doublets in 60 minutes. Although the number adhering is much less than in the absence of gelatin in solution, the number adhering to the membrane is proportional to time up to 40 minute immersion. Adlineation of particles which are separated by distances corresponding to three particle diameters was noted. Furthermore, the particles tend to group around certain blank areas on the surface of the membrane. The adhesion is weak since a 24 minute wash decreases in the 40 minute immersion sample the concentration of 265 singlets and 56 doublets to only 39 singlets.

In conclusion it was established that a thin film of adsorbed protein (gelatin, serum, albumen) made the membrane adhesive, so that every collision of a colloidal particle with the membrane produced a firm bond between the particle and the membrane. Furthermore, there seems to be an adlineation of the particle so adhering, both when the particles touch one another and also when they are separated by distances which are multiples of the size of the colloidal particles. Presence of gelatin in solution and on the membrane produce weak adhesion. The particles so adhering are easily washed off.

Centrifugation

Electron microscope specimen grid with a membrane was placed at the flat bottom of a centrifuge tube (5 mm in diameter and 25 mm long). Colloidal solution was then introduced to a height of 13 mm from the membrane. Centrifugation was carried out in an International Centrifuge (Model H7) which has eight port holes for tubes located in the centrifuge head at 45° angle to the axis of rotation. Velocities up to 18,000 rpm could be attained.

Centrifugation was first used to determine the optimum concentration of gelatin for treatment of the membrane to obtain maximum adherence of the colloidal particles to the membrane. The samples were rotated at a constant velocity of 7500 rpm for three hours with a colloidal gold concentration one-tenth of the standard. The supernatant solution was pipetted off and the membrane was washed twice with distilled water. In one set of experiments, the membrane was pretreated with a solution of gelatin and in another set the gelatin was added to the gold solution. The concentration of the gelatin was varied in both sets of experiments. The results indicate that gelatin on the membrane gives about twice as high a concentration of particles as gelatin in

solution. This result is similar to the one obtained in the diffusion experiments. The optimum concentration of gelatin for pretreatment of the membrane was 0.05 to 0.125 wt percent. On the other hand a much smaller concentration of gelatin was necessary for adhesion of particles coated with gelatin in solution, namely from 2.5×10^{-4} to 10^{-2} wt percent. (Fig. 2).

The effect of time and rate of centrifugation was examined by counting the number of particles that accumulate on a membrane (Fig. 3) coated with

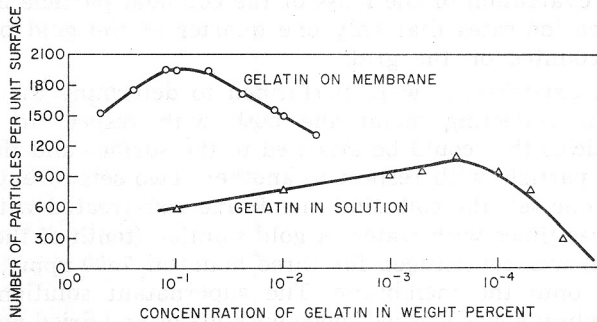


Fig. 2. Effect of protein in solution and in membrane on adhesion due to centrifugation.

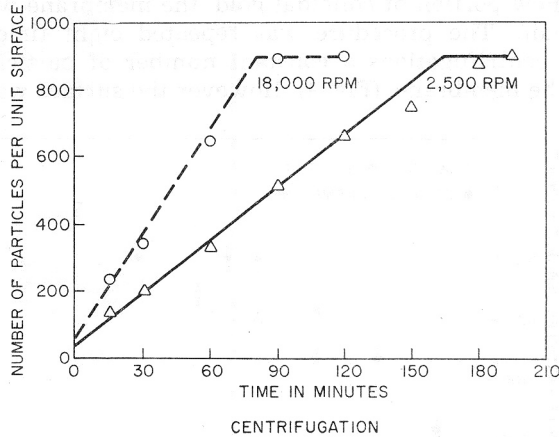


Fig. 3. Adhesion of gold particles to membrane due to centrifugation.

gelatin after a definite period of centrifugation. It is seen that the number of particles increases linearly with time and remains constant at the same value at two different speeds of centrifugation. The time to reach the constant value, *i.e.* to centrifuge the particles in the whole column of colloid is 80 minutes for 15,000 rmp and 165 minutes for the slower rate of 7,500. This is unexpected since the time should be inversely proportional to the square of the angular velocity.

$$a^2 = \frac{9 \eta \ln^{3/2} x_0}{2 \Delta d t (2 \pi n)^2}$$

where η is the viscosity of water, t is the time to just centrifuge the colloid in a column to the membrane, Δd is the difference in density of gold 19.4 and

water 1 to give a value of 18.4; n is the number of revolutions per second and a is the radius of the particle. We obtain 30 Å for the slower rate and 22 Å for higher centrifugation rate. This is three or five times smaller than the 100 Å value obtained from direct electron microscopy. However it is pointed out by Hartman that sedimentation takes place at much greater rate when the centrifuge tubes are at an angle less than 50° to the axis revolution of the centrifuge.¹⁷ Undoubtedly further refinements in the technique will permit a more precise evaluation of the mass of the colloidal particle by this method. Material balance indicates that only one quarter of the gold particles on the solution were counted on the grid.

A series of experiments were performed to determine the distribution of particles on the collecting membrane both with respect to the maximum number of particles that could be attached to the surface and the mutual relationship of one particle with respect to another. Two sets of experiments were carried out. In one set, the collodion membrane was treated with 0.1% gelatin and washed three times with water. A gold solution (tenth of the concentration of the standard) was centrifuged for three hours at 7500 rpm to drive all the particles down onto the membrane. The supernatant solution was pipetted off and the membrane was washed twice with water and dried with filter paper. It was then photographed in the electron microscope. The centrifugation was repeated with a new portion of colloidal gold, the membrane washed, dried and photographed again. The procedure was repeated eight times. It was found that after seven centrifugations a constant number of particles was attained, 1550 per $5 \mu^2$ of the membrane. (Fig. 4). However the surface was not completely

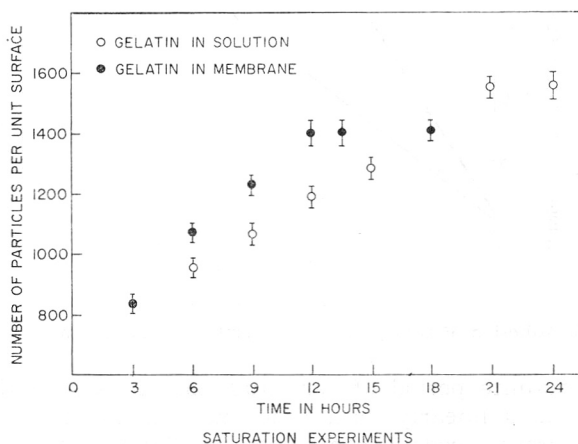


Fig. 4. Adhesion of gold particles to a membrane due to repeated centrifugation.

covered by the particles. Furthermore, the particles were strung up in a line at separations comparable to particle diameter or its multiple. (Table I).

In order to determine whether the gelatin adsorbed on collodion produced fibers on denaturation, which gave ultimately the aggregates, the above experiment was repeated with no gelatin on the membrane and 2.3×10^{-4} wt% gelatin in the gold solution. Results of cumulative centrifugation showed that a constant density of particles, 1400 per $5 \mu^2$ of the membrane surface was

TABLE I

Number of Aggregates with Given Separation between Adsorbed Particles

Separation	Gelatin on Membrane Number	Gelatin in Solution Number
200 Å	243	286
400 Å	83	80
600 Å	50	50
800 Å	22	14
100 Å	14	8

TABLE II

Number of Clusters of Different Aggregates

	Gelatin on Membrane	Gelatin in Solution
Doublets	137	161
Linear Triplet	35	32
Non-linear Triplet	23	28
Linear Quadruplet	18	17
Non-linear Quadruplet	15	—
Linear Quintriplet	11	6

reached in four centrifugations. Analysis of the surface distribution of particles is similar to that observed when the membrane was pretreated with gelatin (Tables I and II). We must conclude that the clustering is a characteristic of the particles and their long range forces rather than of the membrane. Preliminary results obtained with structureless carbon film gave similar results on clustering and adlineation. It was also shown that if gelatin was absent from the membrane and from the solution, no gold particles adhered to the membrane.

Electrophoresis

The electrophoresis cell was a 5 mm diameter, 8 mm long tube with two female standard-taper joints on each end. The copper electrodes were introduced through standard male-taper joints. The copper electrodes were of a size that permitted the placement of an electron microscope specimen cap with grid and membrane. The top electrode had a hole for venting entrapped air. The colloidium membrane on the grid was prepared by dipping in 0.1% gelatin solution for three minutes, then washing in two changes of distilled water. A standard gold colloid was diluted 2, 4, 8, 10, 12-fold. A potential of 50, 40, 20, 4.5, 3.0, and 1.5 volts was applied. The particles adhered to the membrane on the positive electrode. Clusters of particles were present at all values of the potential in the more concentrated solutions and at high potentials in the case of dilute solutions. The ten-fold diluted colloid solution at 3 volts potential difference gave the most evenly distributed particles whose concentration increased linearly with time of electrophoresis (Fig. 5). The average electrophoretic velocity for 1 volt potential gradient can be calculated to be 1.4×10^{-4} cm/sec from

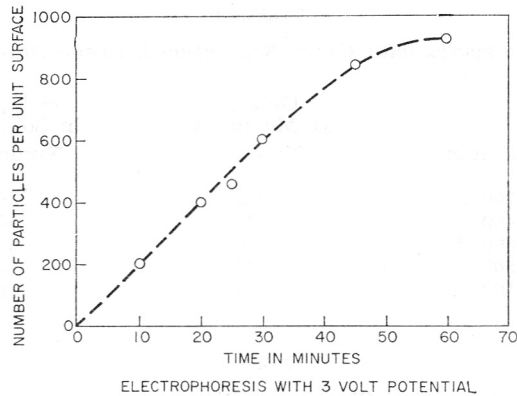


Fig. 5. Adhesion of gold particles to a membrane due to electrophoresis.

the observation that in 10 minutes 204 particles adhered to an area of $4 \mu^2$, with a concentration of 6×10^{10} particles per ml in solution at 3 volts potential drop. This compares favorably with the value of 2.16×10^{-4} found by Burton.¹⁷

Electrophoresis in a Centrifugal Field

The motion of a negatively charged colloidal gold particle in a centrifugal field can be retarded or even stopped by applying an electric field which moves the particle in the opposite direction. This is the colloidal chemical equivalent of the Millikan oil drop experiment. Proper compensation of centrifugal and electric forces should produce no motion and consequently permit the determination of the charge on the colloidal particle. The immobility of the particle in the electric centrifugal field should minimize the hydrodynamic problem associated with the identification of charge distribution around a moving particle.

The electrophoresis cell was carefully wrapped with rubber tape and placed in a centrifuge tube in such a way that both electrodes of the electrophoretic cell could be attached to a small battery and a potentiometer placed in an adjacent port hole of the centrifuge head. Four electrophoresis cells could be centrifuged in the same experiment. The centrifugation was carried out for five minutes at 5000 rpm while the electrophoresis was performed for ten minutes since it took 2.5 minutes to bring the centrifuge to 5000 rpm and the same period to slow it down. The experimental results are given in Fig. 6, where the upper portion of the graph shows the effect of a positive potential on the upper electrode in overcoming the centrifugal force and producing a deposit on the upper electrode. The lower graph shows the effect of a negative electrode in repelling particles from the lower electrode in spite of the centrifugal field. The value for the effective potential gradient which just prevents the motion of the particles at the upper electrode is 2.7 volts while at the lower electrode it is higher at 3.3 volts since the centrifugal force is greater. The »effective charge« Z on the particle can be determined by equating the electric force and the centrifugal force.

$$ZeE = \frac{4}{3} \pi a^3 \Delta \rho \cdot \omega^2 X$$

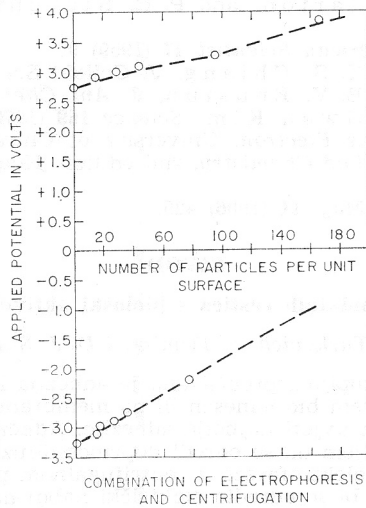


Fig. 6. Adhesion of gold particles to membrane under action of electrophoresis and centrifugation.

where Z is the number of electronic charges on the colloidal particle, e is the elementary charge, E is the electric field near the electrode, a is the radius of the particle, $\Delta\rho$ is the difference in density of gold and water, 18 , w is the angular frequency of rotation 523.6 per sec, X is the distance from the axis of rotation to the electrode, 5 cm for top and 7.0 for the lower electrodes. The value for Z obtained from the data of the upper electrode is a charge 239 while from the bottom electrode data a value of 173. We feel that this method of determining the charge avoids the difficulty present in previous electrophoretic methods which involve the motion of massive charged colloidal particles through a viscous medium defined by macroscopic quantity viscosity η . It is quite possible that diffuse electric atmosphere is distorted by the external field, but this effect can be taken care of by varying the centrifugal force and extrapolating to smaller and smaller electric field. Aside from the advantage of determining the charge on the particle, it offers an opportunity of separating particles of equal mass but different charge.

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IZVOD

Interakcija koloidalnih čestica s biološki aktivnim površinama

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Elektronskom mikroskopijom proučavana je adhezija koloidalnog zlata na membranama, s time da je protein bio nanesen ili na membranu, ili na koloidalne čestice, ili na oboje. Određivani su uvjeti najbolje adhezije. Adhezija na membranu proučavana je kvantitativno s ciljem da se odredi doprinos difuzije, centrifugiranja i elektroforeze čestica. Tehnika elektroforeze u centrifugalnom polju upotrijebljena je kao kompenzaciona metoda da bi se odredio električki naboj na koloidalnoj čestici.

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I

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