

Investigation of electrical charge distribution on human erythrocytes by mutual adsorption of hydrosols by Ivan Yip-Keung Choi

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Chemical Engineering
Montana State University
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Abstract:
Rouleaux formation of human red blood cells is usually found in high molecular weight protein media when the blood flow rate is low. This formation may be due to electrostatic attraction between positively-charged sites on the surfaces of the red blood cells and the negatively-charged proteins. In order to test this assumption, acetaldehyde-fixed red blood cells were stained, while in suspension, with two electrical forms each of colloidal silver iodide and colloidal iron oxide. One was the positively charged colloid which could be mutually adsorbed on the negative sites of the surface of the red cells; the other was the negatively charged form which might be adsorbed on the positive sites on the surface of the red cells.

Transmission electron microscopy and scanning electron microscopy were the tools used to investigate the red blood cells. Results shown by electron micrographs support the hypothesis that the negative charge on the red cells is distributed over the entire surface, whereas the positive charge is not uniformly distributed but is found to be stronger on the side of the red blood cells.

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Date May 27, 1970

INVESTIGATION OF ELECTRICAL CHARGE DISTRIBUTION ON HUMAN ERYTHROCYTES BY MUTUAL ADSORPTION OF HYDROSOLS

by

IVAN YIP-KEUNG CHOI

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree

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ABSTRACT

Rouleaux formation of human red blood cells is usually found in high molecular weight protein media when the blood flow rate is low. This formation may be due to electrostatic attraction between positively-charged sites on the surfaces of the red blood cells and the negatively-charged proteins. In order to test this assumption, acetaldehyde-fixed red blood cells were stained, while in suspension, with two electrical forms each of colloidal silver iodide and colloidal iron oxide. One was the positively charged colloid which could be mutually adsorbed on the negative sites of the surface of the red cells; the other was the negatively charged form which might be adsorbed on the positive sites on the surface of the red cells.

Transmission electron microscopy and scanning electron microscopy were the tools used to investigate the red blood cells. Results shown by electron micrographs support the hypothesis that the negative charge on the red cells is distributed over the entire surface, whereas the positive charge is not uniformly distributed but is found to be stronger on the side of the red blood cells.

INTRODUCTION

A human red blood cell, or erythrocyte, is a cell consisting of a plasma membrane and containing a hemoglobin solution with none of the complicating features associated with the presence of mitrochondria and other subcellular components. Its undisturbed shape is that of a biconcave disc with a 8.1 micron diameter, 2.3 micron maximum thickness, and a 1-micron thickness in the center. The erythrocyte membrane is composed of an incomplete listing of various components such as simple and complex lipids, proteins with or without enzymatic activity, glycoproteins, metal ions, etc.; its structure is still not fully known (1).

Lipids play an important role in structure and function as far as membrane biochemistry is concerned. Recent estimates of the thickness of the human erythrocyte membrane place it in the range of 150-300 Å (2). Various electron microscopic investigations have suggested that 75 Å is occupied by lipids, perhaps in the form of a semicontinuous leaflet (3). This general picture is supported by data on the quantity and distribution of lipid in the erythrocyte. The lipid in the red blood cell is located in the peripheral region (4).

Under physiological conditions of pH and ionic strength, only anionic groups are detectable on the surface of the erythrocyte by electrophoretic methods (5). It has been demonstrated by different

methods that the negative charge on the red cell surface can be ascribed almost entirely to the carboxylic group of neuraminic acid since neuraminidase-treated red blood cells show a great reduction of electric mobility in an electrical field (6, 7, 8). Also, the colloidal iron staining method shows that positively charged colloidal iron particles do not attach to just specialized areas on the surface of red blood cells, since a layer of the iron particles attach uniformly over the entire surface of the cells (9).

From the fact that red blood cells carry a net negative charge, one would automatically think that red blood cells should repel each other in a physiological suspension. But microscopic investigation shows that one can find rouleaux formation by erythrocytes--aggregation of red cells in the form of a roll of coins--when the blood flow rate is reduced to a low level (10, 11). This aggregation causes the suspension-stability of non-flowing blood to be low. One may argue that surface tension plays an important role when two particles in a suspension come close enough to each other so that the interfaces between each one of them and the fluid will join to one continuous interface and thus will press the particles together.

Some workers observed that high molecular-weight proteins such as fibrinogen, or high molecular weight dextran, strongly pro-

moted rouleaux formation (12). Electron microscopy shows the fibrinogen molecule to be about 500 Å long with a central and two terminal balls, each about 65 Å in diameter. The red cell membrane consists of contiguous craters having diameters in the same range of size (13). It was therefore suggested that the fibrinogen molecule was adsorbed "end on" into the crater-like sites by one of its terminal balls, leaving the other terminal ball projecting outward for subsequent engagement with an adsorbing site on the membrane of a neighboring cell (13).

However, it is known that both red blood cells and proteins carry a net negative charge on their surfaces. Some workers suggested that calcium ions were possibly involved in red blood cell adhesion, serving as bridges between red blood cell and fibrinogen; some workers even suggested calcium ions bridged between two red blood cells (14, 15, 16). To investigate these suggestions, experiments were done to show that the effective binding energy for calcium ions to the carboxylic groups present in the cell peripheral region was very low (17, 18). Other experiments showed that rouleaux formation still took place even in erythrocyte suspensions treated with disodium EDTA, which chelates the free calcium ions in physiological fibrinogen suspensions (19).

One possible mechanism of rouleaux formation is that some as yet unidentified sites on the surface of red blood cells carry positive charges, and these sites attract one end of the fibrinogen molecule by electrostatic force; the other end of the protein molecule is free to be bonded on a neighboring red blood cell.

The purpose of this thesis is to search for these positively charged sites on the surface of red blood cells. Red blood cells obtained by venipuncture are washed with isotonic saline and fixed with 2% acetaldehyde solution. These acetaldehyde-treated red blood cells retain their electrokinetic behavior in the pH range where the untreated erythrocytes are stable (20) and in non-isotonic solutions in which untreated erythrocytes are not stable. The increase in stability of acetaldehyde-treated red cells is thus utilized in the studies of positive sites involving inorganic hydrosols which require non-physiological conditions.

Hydrosols such as silver iodide and ferric oxide prepared in both positively and negatively charged forms are suspended with acetaldehyde-treated erythrocytes. The reasons for this procedure are: (1) Negatively charged colloid particles will adhere to the sites where positive charge exists. (2) Positively charged colloid particles will adhere to all parts on the surface of an erythrocyte which are negatively charged. By means of scanning electron micro-

scopy, one can easily find out the possible location of positive sites on the surface of red blood cells by seeing where the colloidal particles adhere to the red cell surface.

The preference of using scanning electron microscopy to that of transmission electron microscopy is because the scanning electron microscope affords a unique way to look at the external surfaces of red blood cells without any disturbance to the surfaces by the thin sectioning method or replication technique necessary for the transmission electron microscopy (21). Scanning electron microscopy also gives advantages including convenience of sample preparation, large depth of field, an impression of three dimensions, large specimen area, and wide magnification range.

EXPERIMENTAL PROCEDURES

A. Preparation of Monodispersed Silver Iodide

A monodispersed sol may be defined as one in which all particles have exactly the same size and shape. The theory of monodispersed sol production was proposed by La Mer and his co-workers (22, 23). Their theory can be explained as follows: Consider a reaction which continuously generates molecules of a dispersed phase; for example, the proposed mechanism involving the AgI-KI complex:

$$AgI_3^{2-} \rightleftharpoons AgI_2^- + I^-$$

$$AgI_2^- \rightleftharpoons AgI + I^-$$

The continuous dissociation of AgI_3^{2-} to AgI by continuously adding water to the complex system will continuously generate AgI. The concentration of AgI increases steadily, passes the point of saturation A as shown in Figure 1, and reaches a point B at which point the rate of self-nucleation becomes appreciable; as D is approached the rate of nucleation rises very rapidly. However, if nucleation is sudden and occurs in an outburst manner, and the rate of production of new AgI molecules is slow, the region of nucleation (II in Figure 2) is restricted in time and no new nuclei are formed after the initial outburst. The nuclei produced will grow uniformly by a diffusion process (region III in Figure 2) and a sol of monodispersed particles is obtained.

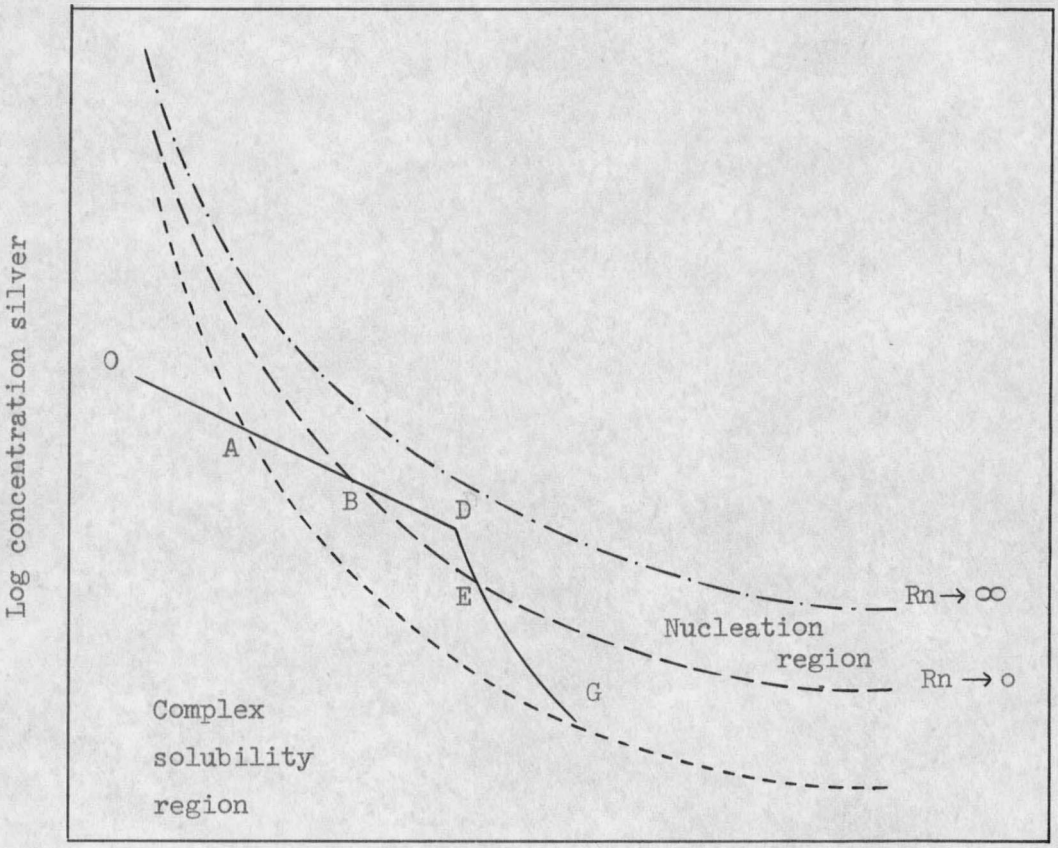


Figure 1. Schematic representation, following La Mer, of conditions for the preparation of monodispersed silver halide sols: .-.-.-. critical limiting supersaturation curve, — — — critical supersaturation curve, ------ solubility curve. Rn = rate of nucleation.

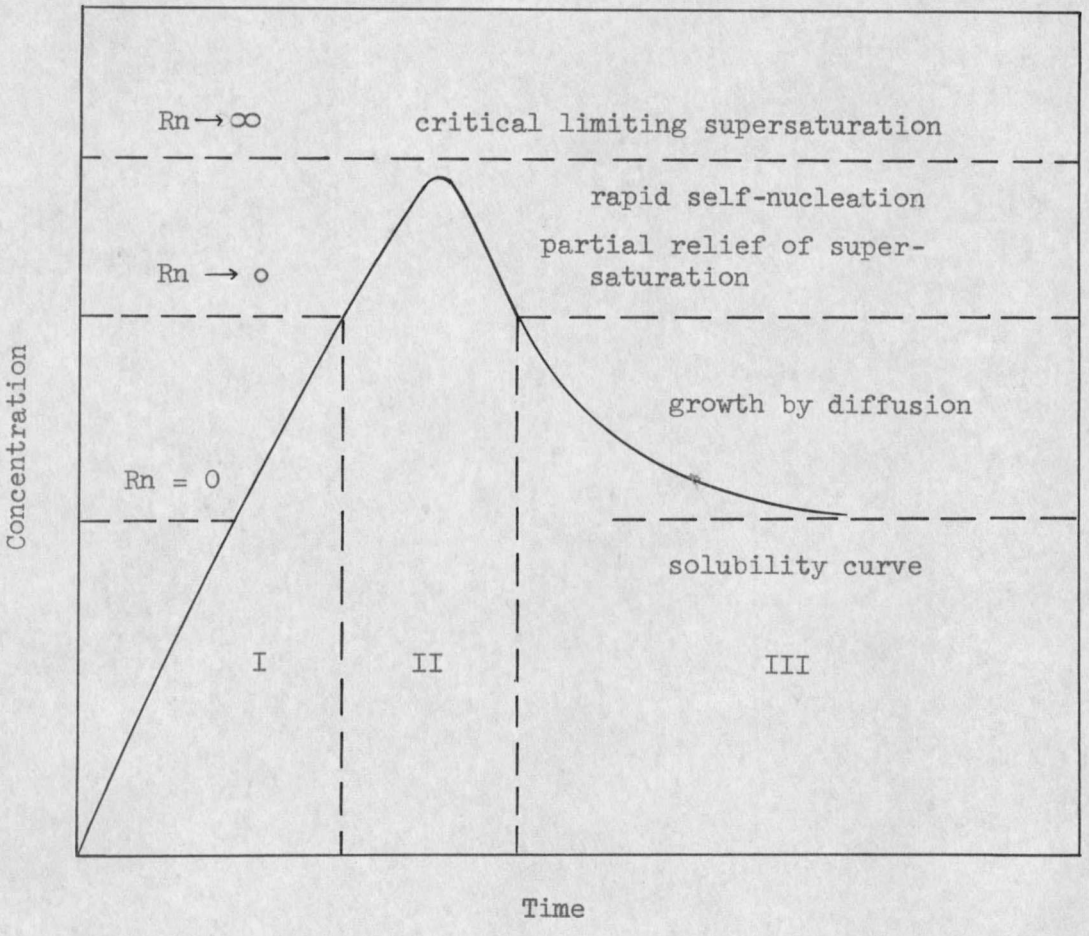


Figure 2. Schematic representation of the concentration of molecularly dissolved silver halide before and after nucleation as a function of time.

Since the size of an erythrocyte is about 8.1 microns in diameter, the sites of the hypothesized positive charges will probably be relatively small. Therefore, it is advisable to prepare silver iodide particles in a small size (0.1 μ - 0.25 μ) so that a more definite position of positive sites can be observed under electron microscopic investigation.

The purpose of preparing both negatively and positively charged AgI colloids is twofold: (1) Try to see if there are particular sites on red blood cells to which negatively charged AgI will attach; (2) Try to observe if there are particular sites that the positively charged AgI particles will not attach to. As mentioned before, it is the purpose of this thesis to locate the positive sites of the erythrocyte surface even though only a net negative charge is usually found.

B. Preparation of Negatively Charged Silver Iodide Colloid (24)

1. Preparation of Monodispersed AgI Seed Sols. The principle of the method is to precipitate silver iodide under such conditions that spontaneous nucleation is avoided, thereby forcing all the precipitate to form on a pre-determined number of seeds which have been artificially provided.

By diluting a 0.2 M KI complex solution to one hundred times its volume with distilled water, a stable silver iodide seed sol is obtained which has a concentration of about 10^8 seeds per milliliter.

2. Preparation of Complex Solution. According to the work done by Edwards, Evans, and La Mer (24), 0.05 MAgI-KI complex is needed to produce silver iodide particles with a size of 0.17 ± 0.02 μ. A complex solution is prepared from an original complex solution of 0.8 M concentration with respect to KI (and saturated with AgI, as illustrated in Table I). A 0.05 M complex solution is prepared by a stepwise dilution procedure, such as that given in Tables II, III, IV, and V.

Table I

Preparation of 0.8 M KI Complex Solution

Initial solution	6.64 M (1.76 g) AgI dissolved in 40 ml 4M KI
1st addition	60 ml of solution containing 40 ml 4M KI
2nd addition	200 ml of solution containing 40 ml 4M KI
3rd addition	300 ml of solution containing 40 ml 4M KI
4th addition*	400 ml of solution containing 40 ml 4M KI

*Only after the fourth addition is the solubility of AgI exceeded.

Table II

Preparation of 0.4 M KI Complex Solution from 0.8 M KI Complex

Initial solution	125 ml 0.8 M complex
1st addition	125 ml 0.8 M KI solution
2nd addition	250 ml 0.4 M KI solution
3rd addition	500 ml 0.2 M KI solution

Table III

Preparation of 0.2 M KI Complex Solution from 0.4 M KI Complex

Initial solution	125 ml 0.4 M complex
1st addition	125 ml 0.4 M KI solution
2nd addition	250 ml 0.2 M KI solution
3rd addition	500 ml 0.1 M KI solution

Table IV

Preparation of 0.1 M KI Complex Solution from 0.2 M Complex

Initial solution	125 ml 0.2 M complex
1st addition	125 ml 0.2 M KI solution
2nd addition	250 ml 0.1 M KI solution
3rd addition	500 ml 0.05 M KI solution

Table V

Preparation of 0.05 M KI Complex Solution from 0.1 M Complex

Initial solution	125 ml 0.1 M complex
1st addition	125 ml 0.1 M KI solution
2nd addition	250 ml 0.05 M KI solution
3rd addition	500 ml 0.025 M KI solution

3. Mixing Technique. Monodispersed sol is prepared by mixing the complex solution with seed sol. It was shown that the dilution ratio should not exceed 2.0 for a 0.05 M complex solution (Table VI, extracted from Edwards' paper, shows the appropriate dilution ratios and nominal sizes for various complex solutions). One minute after mixing, the colloidal solution is further diluted with 8 volumes of 0.01 M KI solution in order to prevent further coagulation of AgI colloid particles.

Table VI

Preparation and Properties of Monodispersed Sols

Complex solution	Seed solution	Nominal size (μ)
1 vol 0.4 M	0.1 vol (Seed ÷ 100)	3.5 ± 0.4
1 vol 0.2 M	0.5 vol (Seed ÷ 20)	0.75 ± 0.1
1 vol 0.05 M	1.0 vol (Seed ÷ 2)	0.17 ± 0.02

* One minute after mixing, sol is diluted with 8 vol of .01 M KI

C. Preparation of Positively Charged AgI Colloid (25)

1. Preparation of Complex Solution. A complex solution which is 3×10^{-5} M with respect to silver iodide and 0.1 M with respect to silver nitrate is prepared by stepwise dilution of an original complex solution of 0.4 M in $AgNO_3$ (and saturated with AgI, as illustrated in Table VII). The method of dilution is shown in Table VIII.

Table VII

Preparation of 0.4 M Complex Solution

Initial solution	0.1123 g AgI dissolved in 40 ml 2 M $AgNO_3$
1st addition	60 ml solution containing 40 ml 2 M $AgNO_3$
2nd addition	200 ml solution containing 40 ml 2 M $AgNO_3$
3rd addition	300 ml solution containing 40 ml 2 M $AgNO_3$
4th addition	400 ml solution containing 40 ml 2 M $AgNO_3$

Table VIII

Preparation of 0.2 M Complex Solution

Initial solution	125 ml 0.4 M complex
1st addition	125 ml 0.4 M $AgNO_3$ solution
2nd addition	250 ml 0.2 M $AgNO_3$ solution
3rd addition	500 ml 0.1 M $AgNO_3$ solution

Table IX

Preparation of 0.1 M Complex Solution

Initial solution	125 ml 0.2 M complex solution
1st addition	125 ml 0.2 M $AgNO_3$ solution
2nd addition	250 ml 0.1 M $AgNO_3$ solution
3rd addition	500 ml 0.05 M $AgNO_3$ solution

2. Mixing Technique. The degree of monodispersity of colloids and the particle size depend upon the volumes and concentrations of the complex solution and water used, and upon the rate of addition of water and the method of stirring employed. These factors have to be controlled carefully in order to obtain reproducible results.

Fifty milliliters of the 0.2 M complex solution are diluted rapidly, with stirring, to 200 ml with distilled water. After addition of the water, the stirrer is switched off and the sol is left to stand for one minute. Then 800 ml of distilled water is added immediately in order to stop particle growth and to prevent coagulation of particles.

D. Preparation of Positive and Negative Colloidal Iron Oxide (26)

Positively charged colloidal iron oxide has been used extensively as electron stain for the acidic groups on the surfaces of

cells (27, 28). The negatively charged form of colloid obtained by recharging the positive ferric oxide sol with ferrocyanide ions had a staining behavior which was not fully known (29). But recent experiments have confirmed that this negative stain could be used to identify positive ionogenic groups (26).

1. Procedure of the Preparation of Positive Ferric Oxide Sol. Fifty milliliters of 0.5 M $FeCl_3$ (33.75 g of $FeCl_3 \cdot 6H_2O$ in 250 ml aqueous solution) is added fairly rapidly (dropwise, but almost in a stream) to 600 ml of boiling distilled water. The resulting iron sol is dialyzed against 10 volumes of distilled water for 5 or 6 days, with two changes of water each day. To adjust its concentration, iron is determined by reduction of ferric irons to ferrous irons with stannous chloride (after acidification) and titration with potassium dichromate, using sodium diphenylamine sulfonate as an indicator (30). Water is added to the iron sol to obtain a final concentration of 1.2 g of Fe per liter. One hundred milliliters of the sol, the pH of which varies between 5 to 6, are mixed with 100 ml of 0.0012 N HCl, and the resulting mixture with an approximate pH of 3.0 is labeled as positive ferric oxide sol-stock solution.

The positive stain is prepared by mixing together the following: stock solution of the positive colloids, 10 ml; glacial

acetic acid, 10 ml; and distilled water, 20 ml. This solution gives a pH of approximately 1.8 and contains 0.15 g of Fe per liter.

2. Preparation of the Negative Ferric Oxide Sol. The negative ferric oxide sol is prepared by slowly pouring 100 ml of the stock solution of the positive colloid into a beaker containing 100 ml of 8 mM potassium ferrocyanide, previously dissolved in boiled water, with constant swirling of the beaker. The relative proportions of the two reagents are those found by Hazel and Ayres (29) to result in the largest stable negative charge on the Fe_2O_3 colloid. This is labeled negative ferric iron oxide sol, stock solution.

To prepare the stain, 20 ml of this stock solution are mixed with 20 ml of boiled distilled water. The pH of this mixture is approximately 6.0.

E. Electron Microscopic Investigation of Colloid Size and Charge

1. Electrostatic Charge Determination of Hydrosols and Ionogenic Properties of Kaolin. The very fine particles of kaolin, as shown in Figure 3, are really hexagonal platelets. Each platelet is a stack of aluminosilicate leaflets, each leaflet being only a few angstroms thick. The platelets have about a ten-to-one ratio of length to thickness. Under an aqueous condition where the pH is in the range of 2 to 7, the basal planes of a kaolin particle adsorb cations whereas the edges adsorb anions.

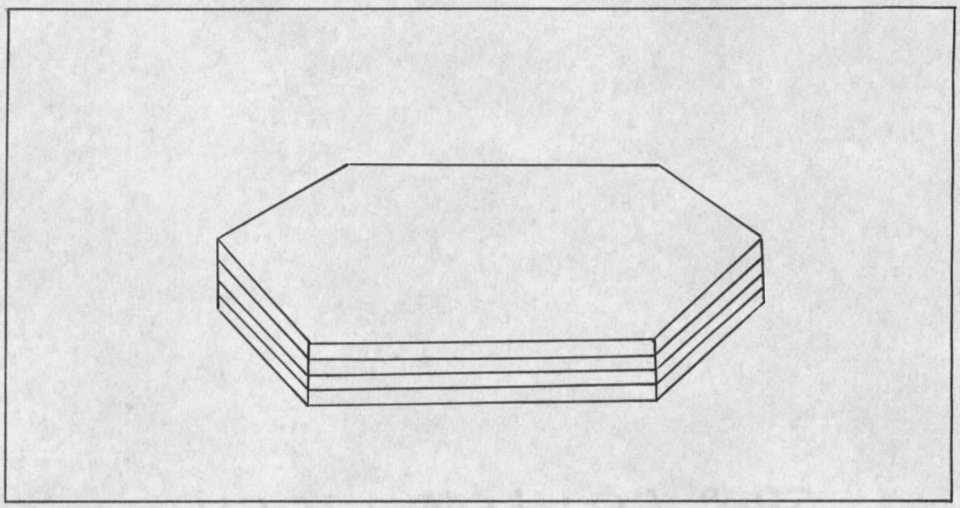


Figure 3. Schematic Representation of a Kaolin Platelet.

To understand these properties, one has to look at the crystal structure of the particle leaflets which consists of two forms. One is an hexagonal net of oxygen atoms bonded to silicon, and the other is an hexagonal net of oxygen atoms and hydroxyl groups bonded to aluminum. These are shown in Figure 4. This gives the general chemical formula for kaolin as $Al_2O_3 \cdot 2SiO_2 \cdot 2H_2O$.

The silicon-oxygen, aluminum-hydroxyl group bonds form octahedra, in order to satisfy the valence requirements of the cations. Planar projections of these arrays are shown in Figure 5. Each lattice is considered to be a layer, so it would be supposed that there would be extensive hydrogen-bonding between the hydroxyl groups in

the aluminum lattice and the oxygen atoms in the silicon lattice. Indeed, it turns out that hydrogen bonding is the major force holding the layers together to form a platelet.

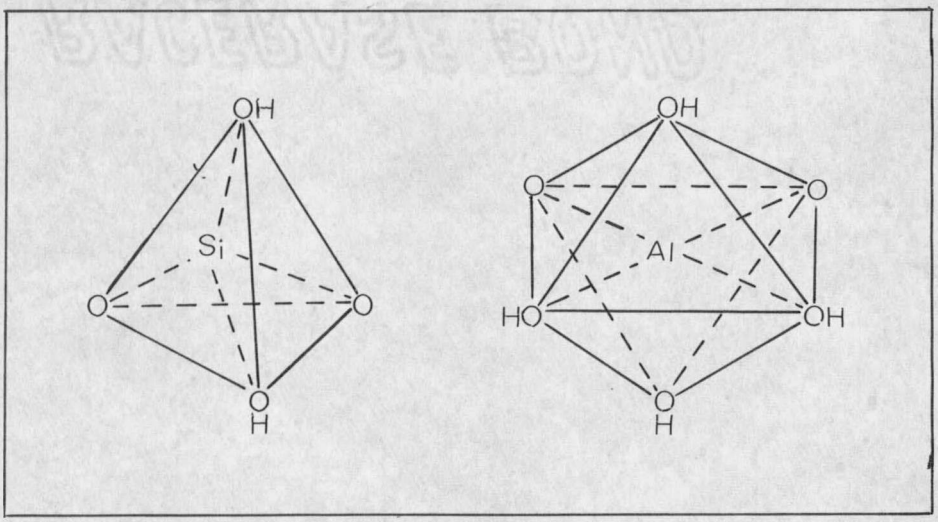


Figure 4. An Si-O tetrahedron and an Al-OH,O octahedron of kaolin crystal.

In an ionic solution-clay system, the basal planes of a kaolin particle exhibit a net negative charge, which will adsorb cations from the suspension medium. There are two reasons for this adsorption behavior (31): (1) Some of the aluminum and silicon atoms may be interchanged or missing, which means that there are negative valences on the neighboring oxygen and hydroxyl groups which are not satisfied; (2) there is a small quantity of foreign cations (Mg, Ca,

etc.) within the mineral which occupy the positions of Al^{+3} or Si^{+4} but do not supply the positive charge. In either case, the imperfect crystal lattice will attempt to absorb free cations from water or ionic solution to satisfy these valences.

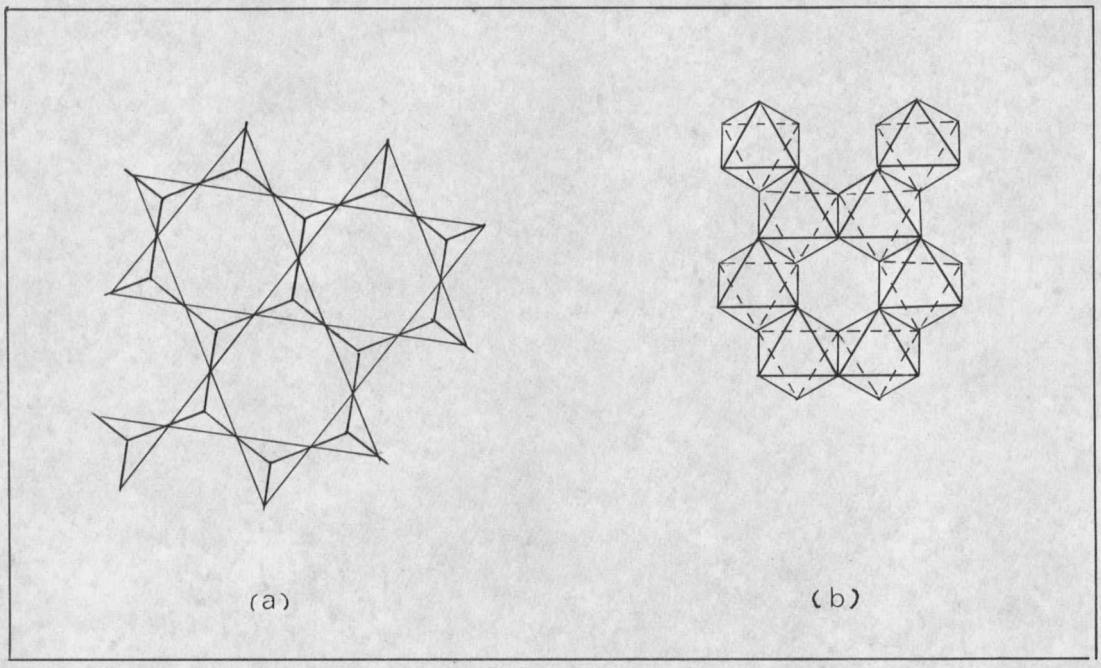


Figure 5. Lattice of tetrahedra (a) and octahedra (b) in planar projection of kaolin crystal.

However, in acid medium the edges of a clay particle exhibit a net positive charge. This can be explained, as shown in Figure 6, by the fact that hydrogen ions are attracted both to the exposed-edge oxygen atoms and the hydroxyl groups. As the pH is

adjusted to about 7, it is found that hydrogen ions only attach themselves to oxygen atoms on the edge, so that the net charge is zero.

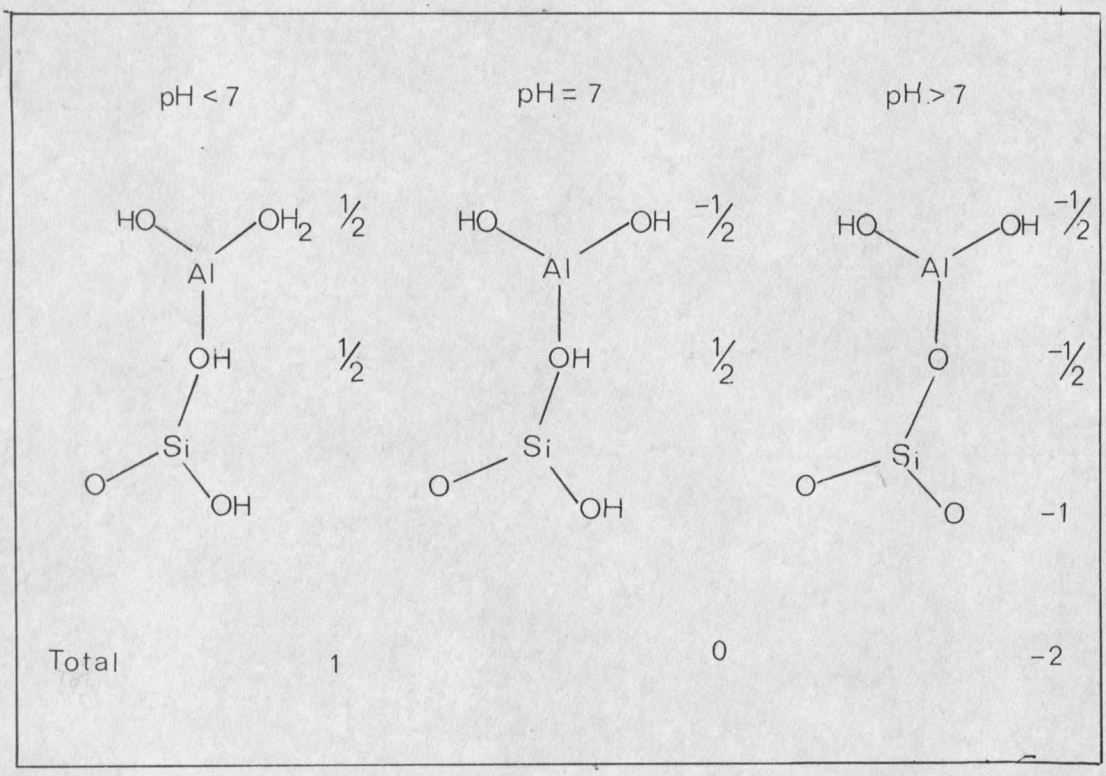


Figure 6. Effect of pH on edge charge of kaolin.

By taking advantage of the ionogenic properties of kaolin particles, one will be able to investigate the actual charge of a hydrosol system by suspending the sol with kaolin particles at a favorable pH range, and then examining the system with an electron

microscope. If a hydrosol is negatively charged, one will likely find that the sol particles attach themselves on the edges of the kaolin particles, whereas positively charged sols will be found on the basal planes of the kaolin particles. The method for preparing a sample is as follows: After a hydrosol is prepared and dialyzed, kaolin particles (concentration is 1 g per 500 ml distilled water) are suspended with the hydrosol system (14 drops of kaolin solution to 200 ml of hydrosol). The suspension is mixed by stirring for one hour, followed by centrifugation to concentrate the colloids which are then sprayed onto a formvar-coated grid (copper grid) by means of an atomizer. The grid is washed carefully with distilled water to get rid of excessive electrolytes which may form debris on the grid after the water is vaporized. The grid is then shadow casted, with palladium, at an angle of 30°. At this stage, the grid, with colloidal samples on it, is ready for electron microscopic examination.

2. Size Determination of Hydrosols. After a hydrosol system is prepared, a sample is sprayed on a formvar-coated grid which is then washed carefully with distilled water to get rid of excessive electrolytes. By taking electronmicrographs of the hydrosol particles, one can find out the actual size of the particles by measuring the dimensions of the particles in the electronmicrograph and converting this measurement into the actual particle size.

After the size and charge of hydrosols are confirmed, one is ready to investigate the charge distribution of the red blood cells. However, red blood cells must be hardened before this investigation takes place. There are two reasons for fixing the red blood cells: (1) Red blood cells hemolyze in nonisotonic solutions, such as the solutions in which hydrosols are made. (2) Hydrosols coagulate rapidly in isotonic or physiological mediums such as blood plasma or isotonic saline. These two problems can be overcome by fixation of red blood cells in 2% acetaldehyde solution, since acetaldehyde-treated red blood cells are stable in nonisotonic solution or even in distilled water (20). Also, the electrokinetic properties of acetaldehyde-treated red blood cells are the same as the non-treated cells (20).

F. Methods of Fixation of Red Blood Cells

1. Preparation of Isotonic Saline Solution. Use 34.95 g of NaCl, 0.101 g of $NaHCO_3$, and 0.134 g of disodium EDTA to make 4 liters of solution with distilled water. The concentration is about 0.145 M, 3×10^{-4} M, and 1×10^{-4} M with NaCl, $NaHCO_3$, and Na_2EDTA, respectively. This final solution is designated as "saline solution". It has an osmotic pressure of about a 300 milliosmol if checked with a freezing point depression osmometer.

2. Preparation of 2% Acetaldehyde Solution. According to the method given by Vogel (32), 4 drops of 70% H_2SO_4 are added to 200 ml of paraldehyde and the mixture is heated slowly. A fraction of about 120 ml, boiling at 21° to 25°C, is collected and redistilled to give a graction boiling at 21° to 22°C. Fifty-two ml of the acetaldehyde are diluted to 2000 ml with 0.145 M NaCl saline solution, followed by the addition of 2000 ml of 0.0667 M phosphate buffer (64 ml of 0.2 M KH_2PO_4 and 270 ml of 0.2 M Na_2HPO_4 diluted to 1 liter with distilled water). This final solution is designated as "fixing solution"; its osmotic pressure is about 440 milliosmol and the pH is about 7.4.

3. Preparation of Red Blood Cell Suspensions. Blood is obtained by venipuncture from a donor, and collected by standard blood bank procedure in ACD anti-coagulant solution. Red cells, obtained by centrifugation of the blood, are washed 4 times with the saline solution using about 50 volumes of saline solution per volume of red blood cells which are packed by centrifuging for 20 minutes. The final washing is done by using a 49:1 volume ratio of 0.145 M NaCl : 0.145 M $NaHCO_3$ at a pH of 7.4. The red cells obtained after the final wash are termed standard washed red cells.

4. Treatment of Standard Washed Red Cells with Acetaldehyde. One volume of packed standard washed red blood cells is mixed with 20 volumes of the fixing solution for 24 hours. Then the supernatant

is replaced by 20 volumes of fresh fixing solution. This final mixture is stored for 20 days at room temperature. The red blood cells are then examined under a light-microscope to see if the red cells retain their biconcave shape.

G. Preparation of Samples for Scanning Electronmicroscopy

After both positively and negatively charged forms of silver iodide and ferric oxide colloidal particle suspensions are prepared, hardened red blood cells which have been washed with distilled water are added to the colloidal suspensions immediately. Very slow stirring is applied to the mixtures for 30 minutes in order to give a better chance of collision between colloidal particles and red blood cells. Then the mixtures are centrifuged under low gravitation force for five minutes and then the supernatants are decanted.

The concentrated mixtures are then transferred by means of an eyedropper to the circular polished surfaces of solid brass cylinders which have the dimensions of 1 cm in diameter and 1 cm in height.

The red blood cells settle rapidly onto the surfaces of the brass cylinders, leaving the upper part of the mixtures with the lighter particles and the solution. By using a piece of absorbing paper, absorb half of the liquid away and then replace the liquid volume with distilled water. This is a good way to remove some of the

free colloidal particles without disturbing the red blood cells settled on the surface of the brass cylinders. Repeat this procedure two to three times.

The samples are then air dried for five hours, followed by washing with distilled water sprayed gently on the surface of the brass cylinders. The samples are sent to Applied Space Products Company at Palo Alto, California for the taking of scanning electron-micrographs.

RESULTS

All the results are in the form of photographs. Mutual adsorption between kaolin particles and hydrosols are shown in transmission electronmicrographs, whereas red blood cells and hydrosols are shown in scanning electronmicrographs. The photographs are presented in the following order:

A. Kaolin Particles

The kaolin particles used in this project are Hydrite 10, purchased from the Georgia Kaolin Company of New Jersey. The size distribution of the kaolin is from 0.5 μ to 3.0 μ. Figure 7 shows the shape and size of the pure kaolin particles.

B. Positively Charged Silver Iodide Particles

Positively charged silver iodide colloidal particles are prepared from silver excess complex solution as described in the "Procedure" section. As mentioned before, the mixing procedure is very critical and duplicate results are difficult to obtain. The best representation of an electronmicrograph is shown in Figure 8. The shape of the particles is spherical with an average diameter of about 0.12 μ, which is similar to that of the particles made by Ottewill and Woodbridge (25).

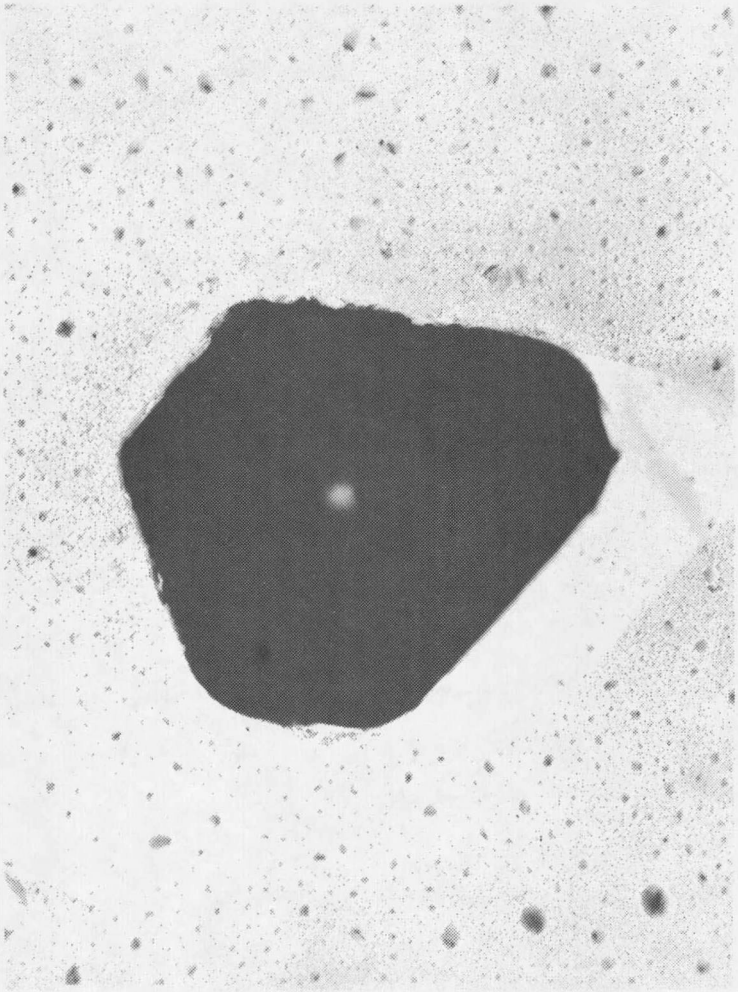


Figure 7. Transmission electronmicrograph of a kaolin platelet.

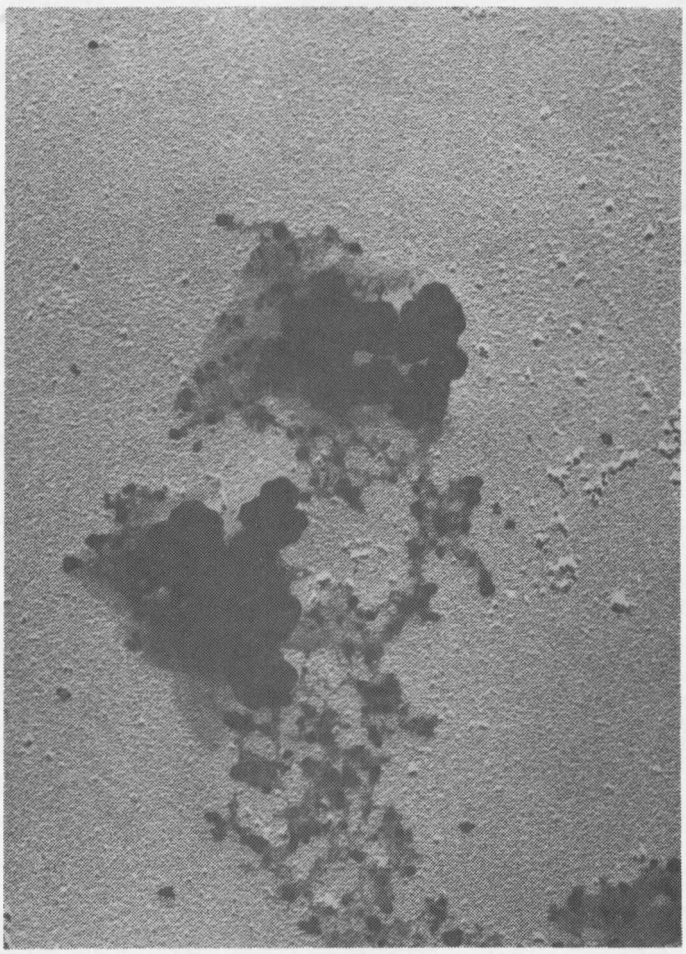


Figure 8. Transmission electronmicrograph of positively charged silver iodide particles.

C. Negatively Charged Silver Iodide Particles

Negatively charged silver iodide colloidal particles are prepared from iodide excess complex solution. The particles are similar to those made by La Mer (24), and they are shown in Figure 9. The particles are in octahedral shape with an average size of about 0.4 microns.

D. Mutual Adsorption between Kaolin Particles and Negative Silver Iodide

Transmission electronmicrographs are only presented for the work of mutual adsorption between negative silver iodide particles and kaolin particles. This is done because positively charged silver iodide particles are adsorbed only on the faces of kaolin platelets and it is therefore impossible to see these colloidal particles under the transmission electro-microscope. Figure 10 shows mutual adsorption between negative silver iodide particles with a group of kaolin particles, and this confirms the negative charge of these colloidal particles.

E. Mutual Adsorption between Kaolin Particles and Negatively Charged Ferric Oxide

Since the negatively charged ferric oxide particles are very small in size, it is difficult to see them under low magnification.



Figure 9. Transmission electronmicrograph of negatively charged silver iodide particles.



Figure 10. Mutual adsorption between kaolin particles and negatively charged silver iodide particles.

However, Figure 11 shows some very small particles adsorbed on the edges of a group of kaolin particles, and this confirms the negative charge of these ferric oxide particles.

F. Acetaldehyde-Treated Red Blood Cells

After being treated for 20 days in 2% acetaldehyde solution, the red blood cells are stable even in distilled water. It is interesting to find that some of the red cells maintain their biconcave shapes whereas some exhibit a cup shape. However, either shape can be used with the work in this paper. Figure 12 shows the real shapes of red blood cells as seen under the scanning electronmicroscope.

G. Mutual Adsorption between Red Blood Cells and Positively Charged Ferric Oxide

The scanning electronmicrograph, Figure 13, shows that the surfaces of red blood cells are almost uniformly covered with positively charged ferric oxide. This result, similar to that published in Marikovsky's paper, is reasonable due to the fact that the surface of a red cell carries a net negative charge. One can also find coagulation of ferric oxide particles as mentioned by Gasic (26).

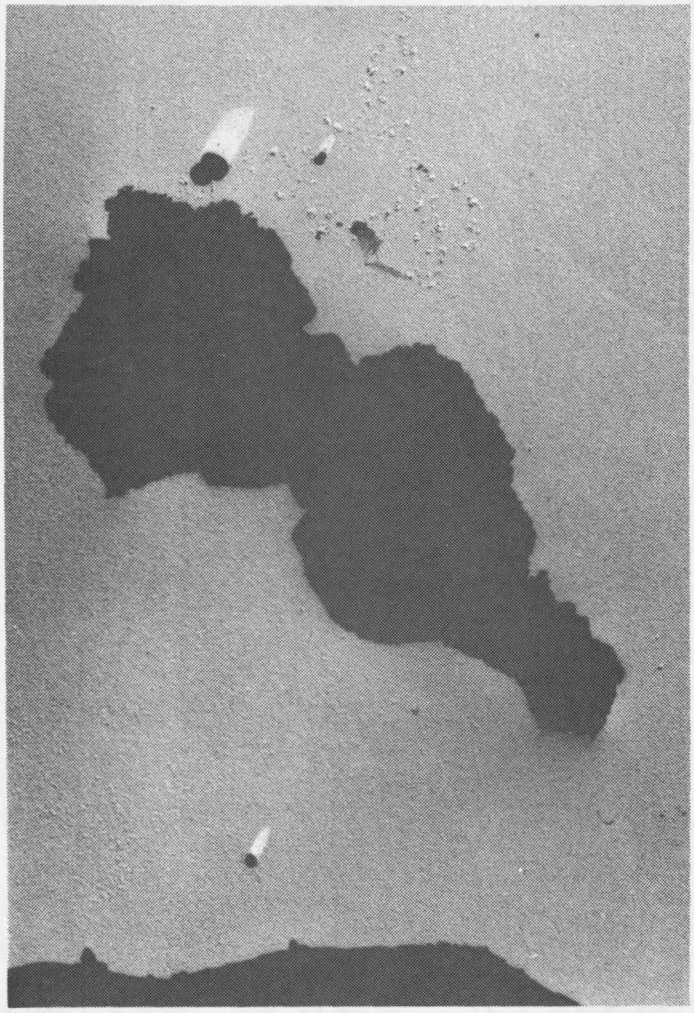


Figure 11. Mutual adsorption between kaolin particles and negatively charged ferric oxide particles.

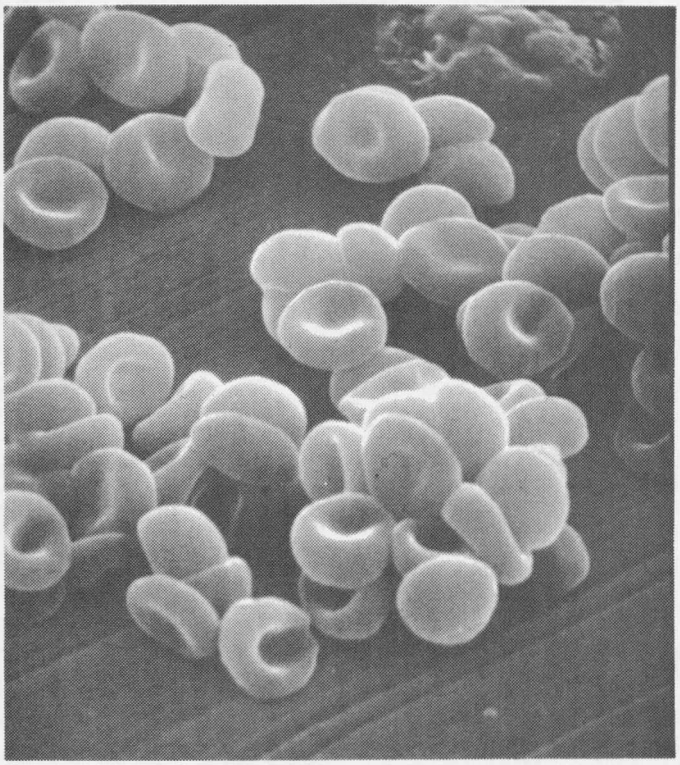


Figure 12. Pure red blood cells under scanning electron microscope.

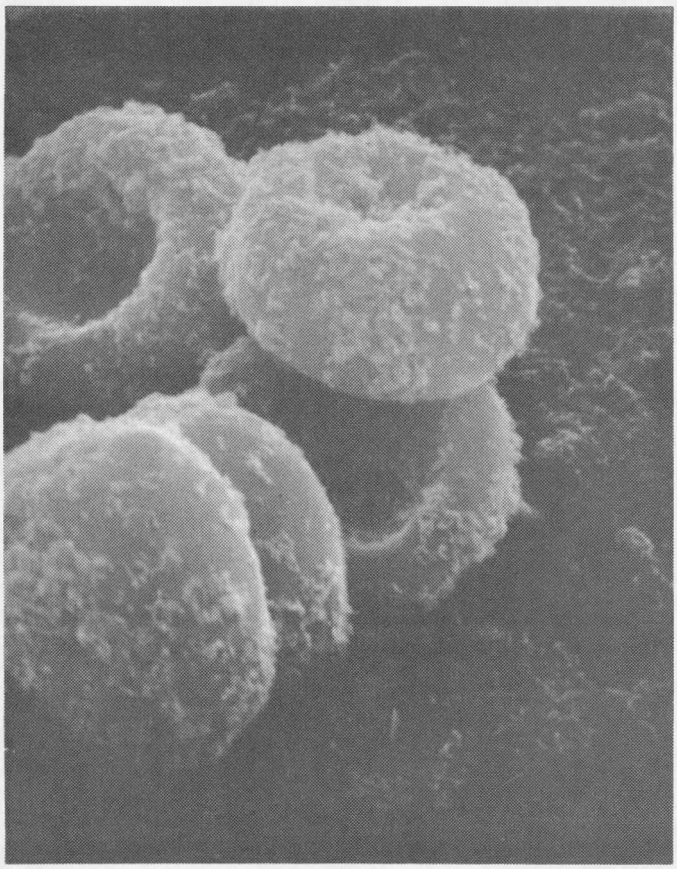


Figure 13. Mutual adsorption between red blood cells and positively charged ferric oxide.

H. Mutual Adsorption between Red Blood Cells and Negatively Charged Ferric Oxide

It is interesting to find, as in Figure 14, that the red cells are entirely covered with negatively charged ferric oxide.

I. Mutual Adsorption between Red Blood Cells and Negatively Charged Silver Iodide

From Figures 15 and 16, scanning electronmicrographs show that there are more silver iodide particles attached to the sides of the red blood cells than in the concaved centers. One can also observe the shape and size of the silver iodide particles. These results are contrary to that shown in Figure 14, which shows that there is no particular region on the surfaces of red blood cells to which the negative ferric oxide particles are attached.

J. Mutual Adsorption between Red Blood Cells and Positively Charged Silver Iodide

The silver iodide particles adsorbed on the surfaces of red blood cells are found to be different in shape from that reported in the literature (25). However, one can find the positive silver iodide particles are adsorbed evenly onto the surfaces of red cells, as shown in Figure 17.

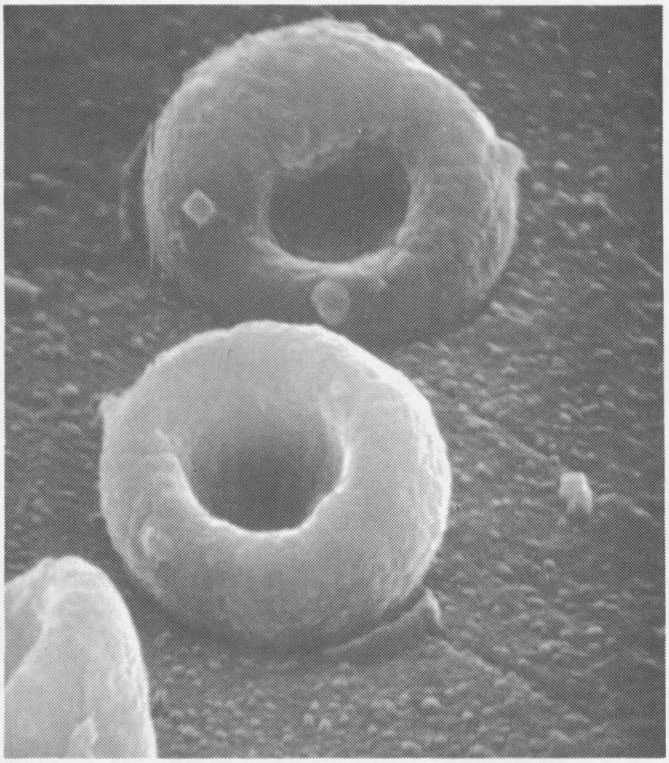


Figure 14. Scanning electronmicrograph of mutual adsorption between red blood cells and negatively charged ferric oxide particles.

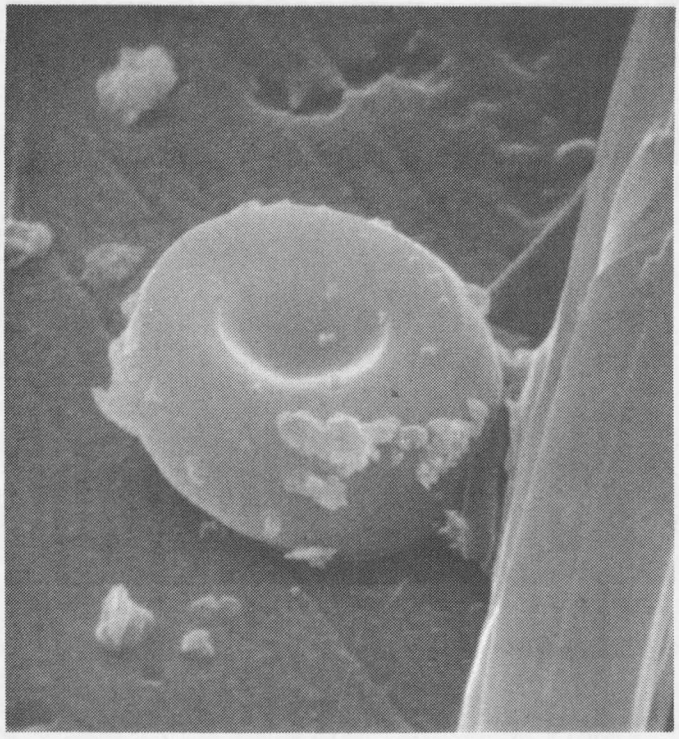


Figure 15. Mutual adsorption between a red blood cell and negatively charged silver iodide.

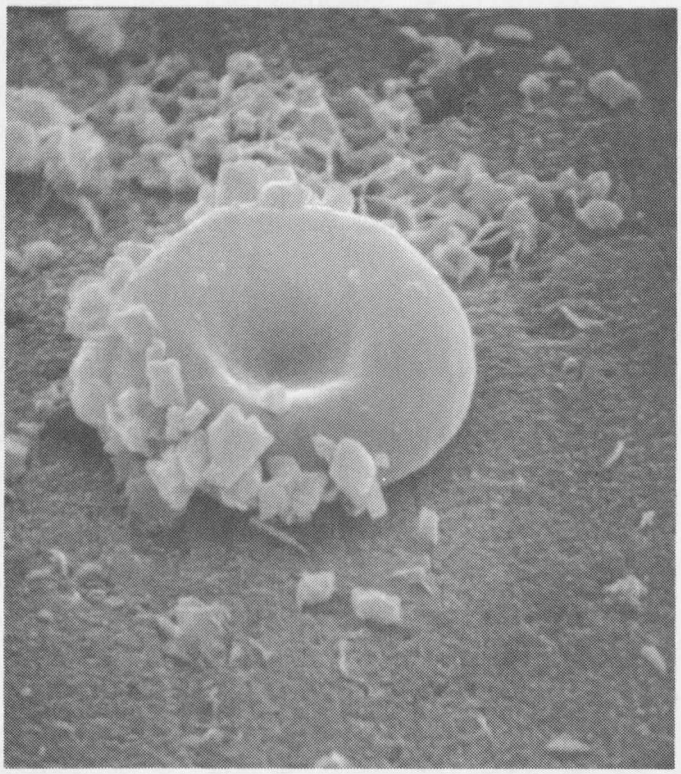


Figure 16. Mutual adsorption between a red blood cell and negatively charged silver iodide particles.

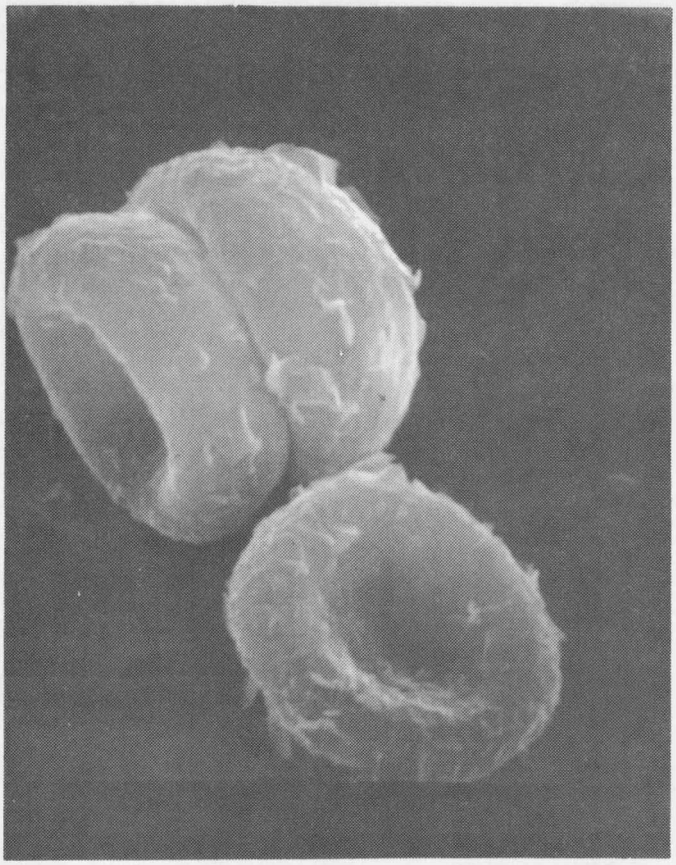


Figure 17. Mutual adsorption between red blood cells and positively charged silver iodide particles.

CONCLUSION

With the evidence from the figures shown in the "Results" section, the author would like to suggest the following conclusions:

1. Acetaldehyde-treated red blood cells are stable in a distilled water medium, so that the investigation of surface charge properties is possible in that medium.

2. Negatively charged silver iodide colloid prepared according to Edwards' method (24) is reproducible. The size of the particles can be controlled by changing the concentration of the complex solutions. The negative charge of the particles has been verified.

3. Both positively and negatively charged ferric oxide give good mutual adsorption with red blood cells.

4. The surface of a red blood cell undoubtedly carries a net negative charge, and the charge is dispersed more or less uniformly all over the surface.

5. As for a positive charge distribution, it is very difficult to say definitely where the positive sites are. But photographs show that the positive charge is stronger on the side of a red blood cell than on the rest of the surface regions.

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