Electrophoretic Studies of a Synthetic Rubber-Like Lastex.

Hulen Brown Williams

*Louisiana State University and Agricultural & Mechanical College*

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ELECTROPHORETIC STUDIES OF A SYNTHETIC RUBBER-LIKE LATEX

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy

in

The Department of Chemistry

by

Hulen Brown Williams
M. S., Louisiana State University, 1943
August, 1948
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ABSTRACT

A synthetic rubber latex-ovalbumin system was investigated as a less complex counterpart of naturally occurring latices which are stabilized by protein. The synthetic latex used was a butadiene-styrene copolymer. Experiments were performed to determine the amount of protein adsorbed per square meter of latex surface as a function of the protein concentration and the pH of the suspension. The quantity of protein adsorbed was estimated by a turbidimetric method in which sulfosalicylic acid was used as a coagulant. The turbidity of the suspension which resulted upon the addition of this reagent was determined by means of a photoelectric colorimeter and a standard transmission curve.

A method was devised by which the ovalbumin adsorbed by the latex could be determined without a separation of the latex (with its adsorbed protein) from the protein remaining in the solution at equilibrium. The calculations required that certain assumptions be made regarding the behavior of the latex-albumin system. These assumptions were: (a) dilution of latex-albumin suspensions did not cause desorption of protein, or, if desorption did result, it occurred so slowly that the amount desorbed in the time required to obtain the colorimeter reading on suspensions
of latex was negligible; (b) there was no desorption or further adsorption of albumin on latex caused by the addition of the protein coagulant; (c) the transmittancy of latex with coagulated albumin on its surface was the same as that of latex with uncoagulated albumin on its surface, and neither the number nor size of particles was altered; and (d) the presence of sulfosalicylic acid did not affect the transmittancy of latex in the absence of albumin.

The validity of postulates (a) and (d) was demonstrated experimentally. The behavior of the system with regard to (b) and (c) above could not be proved by specific experimentation, but the reproducibility of the data obtained and the type of adsorption curves which resulted established their validity.

Electrophoretic mobilities of the latex particles were determined as a function of pH in the absence of ovalbumin and in the presence of various quantities of the protein. The buffered suspensions were of pH values 3.01, 3.94, 4.60, 5.01, 6.08, and 6.98; all were at an ionic strength of 0.02.

The "S" shape of the adsorption curves was interpreted as representing orientation in the outer layers of adsorbed protein in such a way that the major axis of an ellipsoidal protein molecule was perpendicular to the adsorbing surface.

For a given concentration of ovalbumin and a definite amount of surface, the greatest quantity of protein was adsorbed at pH 3.01. Under the same conditions, considerably less protein was
adsorbed at pH values 3.94, 5.01, and 6.08. Values of the amount adsorbed at pH 4.60, and 6.98 were intermediate between highest and lowest but comparable with each other.

The amount of ovalbumin which when adsorbed would produce constant electrophoretic mobility of the latex particles was determined by comparing the mobility of the particles at various protein concentrations with the amount of the protein adsorbed at the same concentration. The smallest quantity of protein was required at pH 3.01 and the largest at pH 6.98.

For a given amount of latex surface the quantity of ovalbumin required to produce constant mobility of the latex particle was found to vary with the pH of the suspension. A theory was presented to account for the observed facts in terms of "active centers" (-OH groups) on the surface of the latex particles. The number of such active centers was considered to decrease with decreasing pH. An apparent increase in the quantity of protein required to produce active center coverage over that predicted by the theory was noted in the vicinity of the isoelectric point of the protein. The explanation of this increase is thought to lie in the peculiar behavior of a protein in the region of its isoelectric point, such behavior being characterized by lowering of interfacial tension, a decrease in all forces of repulsion between particles, and minimum stability of the protein molecules as particles of a disperse phase.
The isoelectric point of ovalbumin adsorbed on the synthetic latex particles was found to be pH 4.82. Argument was presented that this shift from pH 4.55 for the dissolved protein does not require denaturation of the protein on adsorption.
INTRODUCTION

With the turn of the century and the development of the automotive industry, rubber and rubber technology grew in industrial importance. Since raw rubber latex is colloidal in nature, both the theoretical and practical aspects of the stabilization of these colloidal suspensions became increasingly important as their use expanded.

As in all colloidal suspensions, the hysteresis of the rubber latex is very important and affects to considerable extent the character, qualities, and properties of the final product into which it ultimately finds its way. Latices undergo an entire series of chemical changes from the time they are taken from the plants until they have been processed into finished materials, usually by means of vulcanization and heat treatment.

It is definitely known that natural rubber contains protein materials which play an important part in the coagulation and polymerization processes. It is reasonable to suppose that the surface of a native latex particle should be composed for the most part of those surface active materials which exist in the vacuole of the living plant cell. The stability of native latex systems may then be closely associated with the surface activity and ampholytic character of the protein molecule.
The objective of this investigation has been to develop a simplified latex-protein system, certain properties of which might be measured. The interpretation of the data obtained would then contribute to better understanding of this particular field of colloidal phenomena. In as much as synthetic rubber-like materials are in large part replacing natural rubber, there is even greater value in undertaking such work. The basic research incorporated herein is a study of the styrene-butadiene copolymer with ovalbumin solutions.
the natural rubber latex protein.

When and how the particles aggregated in the presence of water (1) carried out the first electrocoagulation investigation of having

Heapy et al. (2) made the suggestion that the surface of the latex particles were at least partially covered by protein together with the enzymes within the surface layer which the surface proteins. Another way to make the immunological statement that the interactions possible

The process of the aggregation of the particles most natural rubber latexes

point of the mixture of protein in the sample. Many well known point of the mixture of protein in the sample. Some well known particles in a certain heparin latex was treated with the surface particles. This theory has been verified partially substantiation by the rubber particles. The theory has been verified substantiation for natural rubber latex. The theory has been verified the role of surfactants for natural rubber latex. The theory has been verified through the

Matured Rubber Latex System

recently.

absorption at surfaces, and (d) the size and shape of the latex particles.

(2) Synthesis of latex particles, (3) Aggregation of latex particles, (4) Aggregation of surfactant on natural rubber latex. In keeping with the latex. The problem of absorption of surfactant on a synthetic latex.

HISTORICAL APPROACH TO THE PROBLEM
Mayer (37) has compared the electrophoretic mobilities of latices from various species. With the exception of the poinsettias he found great similarity in behavior among related species, and within the same species the same mobility of the latex was obtained regardless of source of seed or environmental conditions. Mayer also reported that experiments performed at different dilutions did not disclose any differences so long as fresh latex was used. He commented further that ground glass particles coated with ovalbumin showed the effect of dilution of the suspension by variation in the mobilities of individual particles when the protein concentration was reduced below the amount necessary to cover completely the surface of the glass particles.

Boe and Ewart (48) made a study by the moving boundary method of the differences in electrophoretic behavior of the protein components in the serum of latex which has been treated with ammonia (preserved thus) and of the fresh latex (unpreserved) from *Hvea brasiliensis*. Seven electrophoretically separable components were found in the fresh unpreserved latex serum, but with preserved latex this number was reduced to two resolvable components and an over-all increase in the negative charge on the particle had resulted. These investigators observed an alteration in charge, caused by the addition of ammonia, which increased the stability of the colloidal system.

The surface composition of latex particles of *Hvea, Castilleoa*, and *Cryptostegia* were studied microelectrophoretically by Seifriz (49).
Although the surface charge on *Hveva* and *Castilllos* and their iso-
electric points (determined in the field immediately after the latex
was drawn) indicated protein coverings, the feeble charge on
*Cryptostegia* was indicative of a surface that was primarily non-protein
over the entire pH range studied. This investigator called attention
to the apparently non-ionizable and non-protein substance which makes
up the greater part of the surface of *Cryptostegia* latex and emphasised
the fact that the rubber hydrocarbon itself may make up a greater
portion of the surface of the latex particles from certain species
of plants.

Studies which were interpreted to give the relative amounts
of protein and "resin", a lipide, on the hydrocarbon surface at
various pH values were made by Kemp and Twiss (39). These workers
derived the following expression for \( \sigma \), the surface charge density
of the latex globule:

\[
\sigma = \sigma_p f_p + \sigma_f f_f + \sigma_r f_r
\]

where \( f_p \), \( f_f \), and \( f_r \) represent the fractions of surface covered by
the protein, the resin, and the rubber hydrocarbon itself respectively;
and where \( \sigma_p \), \( \sigma_f \), and \( \sigma_r \) are the corresponding surface charge
densities.

It had been shown previously by Kemp (28) that the relation
between the electrophoretic mobility of a particle and its surface
charge density could be satisfactorily expressed by the equation of
Henry (25):
\[ \mu = \frac{\sigma}{\eta} \frac{ka}{1 + ka} f(ka) \]

where \( \mu \) denotes the electrophoretic mobility; \( \sigma \), the surface charge density; \( \eta \), the coefficient of viscosity; \( a \), the radius; \( k \), kappa from the Debye-Hückel equation for multiply charged particles; and \( f(ka) \), a function of \( ka \). Henry has shown that if \( ka > 100 \), which it is for microscopically visible particles, then \( f(ka) = 3/2 \) and the above equation reduces to

\[ \mu = \frac{3}{2} \frac{\sigma}{\eta}. \]

If \( \eta \) is constant, \( \mu \) should be directly proportional to the surface charge densities and will be given by

\[ \mu = \mu_P f_P + \mu_R f_R + \mu_R^* f_R^* \]

which upon rearrangement and elimination of \( f_R^* \) reduces to

\[ (\mu - \mu_R^*) = (\mu_P - \mu_R^*) f_P + (\mu_P - \mu_R^*) f_P \]

where \( \mu \) corresponds to the respective electrophoretic mobilities.

These mobilities were determined separately by isolating the protein mixture, the hydrocarbon, and the resin. By calculations based on the assumptions and equations given, Kemp and Twiss were able to determine the fraction of the latex particle covered at differing pH values and in latex suspensions of various dilutions. They showed further that the mixture of isolated proteins when adsorbed on silica particles was isoelectric at the same pH as the original latex particles. Their measurement of mobility was made with a micro-cell of the capillary tube type and their absolute values,
therefore, are subject to question. These workers maintained that the solubilities of the rubber resin and the protein increased with increasing pH; consequently in highly diluted latex, the rubber particles might be quite uncovered. Curves were shown for the relationship between the fraction of the globule surface covered with protein and the pH of the serum at various dilutions.

It should be noted that Kemp and Tauss in their original assumptions define a condition wherein the particle is considered completely covered with protein when the mobility of the latex particles reaches that of the protein.

The remainder of the electrophoretic data in the literature is primarily concerned with electrodeposition of the particles to give homogeneous films at an electrode. Hauser and Bender (24) have reviewed this aspect of electrophoresis in connection with natural latex: "--- a great number of publications have shown that the surface layer of the individual latex particle of Hyeen latex is mainly composed of adsorbed protein". Flint (3) has made a study of the factors which affect the electrophoretic deposition of rubber with respect to the speed of deposition and the structure of the deposit.

**Synthetic Latex Systems**

The great majority of synthetic latices are prepared by the process of emulsion polymerization (23). At the termination of the polymerization the latex particles are coated to a greater or lesser
degree with the emulsifier which is in most cases an ammonium or
potassium soap which acts as stabilizer. Other ingredients may be
added after the polymerization to increase the stability of the
system. Certain of these are: commercial detergents, alkyl resins,
and gelatin — in each case, a surface active agent.

The literature is practically void of any data on the
electrophoresis of synthetic latices interpreted in a theoretical
way. Most studies reported stem from industrial research wherein
the investigators have been concerned with electrodeposition of
the charged latex particles. Adequate directions for the elec-
trodeposition of Buna latices (4) and polychloroprene latices (50)
have been reported. Livingston (35) has investigated the creaming
of neoprene latex. Creaming is the term used to describe the con-
centrating of latex by separation of the emulsion into two layers,
the lower one containing a redispersible concentrate of latex. He
found that this separation could be accomplished by mixing the
latex with certain hydrophilic colloids, by electric fields
(electrodecntation) or by cooling. Neoprene latex particles were
reported to be negatively charged particles of 0.12 microns diameter
in water containing dispersing agents and stabilizers (40). These
dispersions coagulated slowly at low pH values or temperatures above
16° C. Stirring produced local coagulation. The report stated that
alcohol, acetone, acids, positively charged dispersion, polyvalent
metal salts, electric current, filtering or drying would produce
coagulation.

Gamin (19) has presented "a review and discussion with special attention to the historical aspects, the mechanism of electrophoresis, the electrophoresis of the latex, the mechanisms of deposition, the technique of electrophoretic deposition of rubber, industrial applications of the electrophoresis of rubber, and the electrophoretic deposition of synthetic resins".

Thiokol latex has been described as being minute spherical particles of Thiokol synthetic rubber dispersed in water (57). A comparison of Thiokol with natural rubber latex showed the Thiokol latex particles to be larger and to have no electrical charge. Since Thiokol particles are heavier than natural rubber granules, they settle out quite rapidly leaving a clear supernatant water layer; the precipitated material is very stable.

In Volume 4 of High Polymers (5) methods have been outlined for determining the sign of the charge on particles and for testing the homogeneity of suspensions by means of electrophoresis.

**Albumin Adsorption at Surfaces**

The proteins which occur in the serum and on the surface of natural rubber latices may be classified in the general solubility group called the albumins. Ovalbumin, although an animal protein, is easy to prepare in a relatively pure state, is isoelectric at pH 4.55, and possesses water solubility characteristics similar to the proteins obtained from natural rubber latex serum.
Freundlich and Abramson (18) investigated the mobility of glass particles as influenced by changing concentrations of ovalbumin in suspensions of ionic strength 0.02 and pH 4.68. They observed that the mobility of the particles was no longer a function of albumin concentration after the latter had reached $10^{-4}$ g per liter. They assumed that the albumin was completely adsorbed to the surface at this concentration and at all lesser concentrations of albumin. These workers calculated the surface area required for the adsorption of $10^{-4}$ g of ovalbumin from the molecular weight obtained by Svedberg (57) and accepted the latter's suggestion that the ovalbumin molecule was of cubic form and of 3.9 millimicrons on an edge. Abramson and Freundlich found a discrepancy between the total surface afforded by the glass particles and the area required for adsorption of $10^{-4}$ g of ovalbumin. The available surface appeared to be covered with albumin at one tenth of that which they calculated should have been required to cover the surface. On the basis of this observation these workers proposed a flattened ovalbumin molecule with an area on one face of approximately 100 square millimicrons.

Moyer (38) repeated the experiment of Abramson and Freundlich and found that the glass particles were exceedingly variable in their mobility when the concentration of ovalbumin was reduced below that value considered to be necessary to coat the particle completely. Loeb (36) observed that ovalbumin adsorbed on collodion produced a
The concentration was found on the observation that the content worked deep in the sample and that of the material was converted. After this, the concentration was found on the assumption that the concentration was done experimentally. In results, they were able to remove all the excessive equipment and quantify the ethylene oxide for each apparatus completely. The reaction on a reactor was performed after the required stoichiometry and concentration of oxides. As shown in the previous experiments, the reaction was performed at pH 6.0 and above. However, the concentration was not achieved at pH 5.0. Expectation of pH 4.05 and below during the reaction was expected to be. At the moment that the parameters were all set, several reactions were performed with ethylene, diethyl, and methyl. They obtained good yields. Part of the ethylene oxide was removed in the ethylene equipment by the ethylene equipment. However, the reaction was performed in the presence of the equipment. After the reaction was done, a new section was performed in the equipment. However, the equipment was determined. This may have been due to a decreased
was decreased from 4.6 to 3.0, that is, the spatial requirements of
the ovalbumin molecule appeared to be about 3 to 4 times greater at
pH 3.0 than at pH 4.6. In the case of concentrated solutions, it
was proposed that 3 or 4 adsorption layers might be formed as the
albumin was adsorbed, and in the region of 50% coverage of the sur-
fase, that the surface began to change rapidly from hydrophilic to
hydrophobic in nature. They maintained that, in general, the charge,
molecular size, and general behavior of the adsorbed protein were
like the corresponding properties of the dissolved molecule of ov-
albumin. The small size of the ovalbumin molecule in the region of
the isoelectric point was regarded as indicative of a compact
dissociated structure as compared with a swollen, hydrated structure
at lower pH values. Albumin analyses were made by the Kjeldahl
method. The amount of protein adsorbed was determined by analyzing
the filtrate after the glass particles had been removed on a Jena
glass filter.

Hitchcock (27) investigated protein films on collodion mem-

branes. The adsorption of ovalbumin was found to follow a Langmuir

isotherm. He determined the amount of albumin adsorbed by weighing
a membrane before and after the adsorption process; before the second
weighing the membranes were washed in water and dried for 1 hour at
100° C. His report presented a plot of the weight of albumin on
a given surface of membrane as a function of the pH at constant
protein concentration with respect to the body of the solution. The
curve was maximal in the vicinity of pH 5.0 and dropped off rapidly on either side. Hitchcock observed that an increasing salt concentration increased the amount of protein adsorbed at a given pH value. A further observation was that with gelatin films, the greater the permeability of the membrane, the greater was the amount of protein adsorbed, adsorption occurring preferentially at the site of the pores.

Dow (13) has extended the work of Hitchcock by further study of the adsorption of ovalbumin on collodion membranes. His results are consistent for the most part with those just presented, but some extension of the work showed unexpected results. An increase in salt concentration from 0 to 0.6 molar NaCl caused a sharp increase in the amount of albumin adsorbed above pH 5.0 but had little effect on lower values. The curves which this worker has shown were defined by too few points and gross extrapolations were required to fit the data to a Langmuir adsorption equation at the lower values of albumin concentration. The curves became stepwise at higher concentrations of protein. His analytical method was based on the index of refraction of a solution of ovalbumin as a function of concentration of the protein. Dow is not in agreement with Lindau and Rhodius that the size of the albumin molecule changes appreciably with pH. He points out that changes in the chemical forces which are in effect between the protein and the membrane could result in differences in effective area occupied by a molecule of ovalbumin at differing pH.
values.

**Size and Shape of the Ovalbumin Molecule**

Certain controversies regarding the change in size and shape of the ovalbumin molecule as a function of pH of the suspension have already been reviewed above. The spatial configuration and effective size of the ovalbumin molecule have particular significance with respect to the work reported here. The literature has been surveyed in this connection.

Svedberg and Nichols (53) reported a molecular weight for salt-free ovalbumin of 34,400. This determination was made by means of the ultracentrifuge. From the specific sedimentation velocity of the particles, the application of Stokes' law, and the assumption of a spherical particle, Svedberg calculated the radius of the ovalbumin molecule to be 2.18 millimicrons. Again assuming spherical shape and by applying Einstein's diffusion law in connection with the diffusion constant, he calculated a particle radius of 2.23 millimicrons.

It appears that Devaux (12) was the first to spread a protein on water and study the properties of such a spread film. For an "Albumin" he found a thickness of 30 to 80 Angstroms. Guastalla (22) was the first to calculate the molecular weights of proteins by application of the ideal gas laws to the behavior of their spread films at low pressures. By this technique the force per cm acting against a surface film was multiplied by the area of the film and
plotted against the area of the film. The value of force times area, extrapolated to zero force, should give the molecular weight of the protein upon solution of the equation

\[
FA = \frac{w}{N} \cdot \frac{RT}{M}
\]

where \( w \) is the weight of the protein in the film, \( N \) is the molecular weight of the protein, \( R \) is the gas law constant, \( T \) is the Absolute temperature, \( F \) is the force in dynes per cm, and \( A \) is the area of the film in square cm. Guastalla studied ovalbumin, among other proteins, by this technique. He reported a concentration of 0.7 mg of ovalbumin per square meter of surface at 25° C. and at a tension of 0.1 dyne per cm; at 1.0 dyne per cm he found a concentration of 0.83 mg of protein per square meter of surface. His calculations yielded a value of 40,000 for the molecular weight of the protein.

The limiting area in square meters per mg of ovalbumin measured over water and over dilute salt solutions as a surface film of protein, has been reported by different investigators (54, 44, 41, 31, 17, 9) and varies from 0.62 to 1.10 at pH values differing not more than 0.1 unit from 4.8. Corresponding values have been reported for denatured ovalbumin at its isoelectric point (33, 10, 9): 0.99, 0.98, and 1.84. Neurath and Bull (43) criticized these values on the basis that they were obtained by extrapolation of the linear portion of a force-area curve which was the equivalent of extrapolation of
the curve in a high pressure region. These workers stated that the area per mg of the spread protein was constant over a pH range from 4.0 to 5.5 but that at pH values below 3.9, the area increased with decreasing pH. On the alkaline side of pH 5.5 the film area decreased with increasing pH.

Corter (21) found the area per mg of protein to be a maximum at pH 4.8 and to decrease sharply on either side of this value. Later work revealed that a time effect was involved, since equal areas were obtained at all pH values when sufficient time was allowed before making measurements (59).

To summarize the above, it would appear that ovalbumin when adsorbed in a monomolecular film on water or on an aqueous salt solution has an area of approximately 0.9 square meters per mg. The values for the molecular weight of ovalbumin in the film as reported by Bull (9) lie between 40,000 and 44,000. This would correspond to an effective area of about 65.7 square millimicrons occupied by an ovalbumin molecule lying flat on the surface.

Cohn (11) obtained a radius of 22 Angstroms for the ovalbumin molecule, calculated as a sphere from time of relaxation measurements of the protein molecules in an alternating electrical field.

De Netty (2), from a study of surface tension, calculated that the ovalbumin molecule in a monolayer had dimensions 30.8 x 30.8 x 41.7 Angstroms.

A number of data have been obtained by the use of diffusion
measurements. Gerin (20) considered the egg albumin molecule to behave as if it were an elongated ellipsoid of revolution the volume of which corresponded to that of a sphere of radius 23.6 Angstroms. If considered as a cylinder, the molecular volume had a radius of 25.5 Angstroms. Tiselius and Svensson (56) observed a radius of 27.5 Angstroms for the ovalbumin molecule whereas calculations based on dry weight gave a value of 23.6 Angstroms. They attributed the difference to hydration. Neurath (42) also assumed ovalbumin to be an ellipsoid of revolution with \( b \) and \( a \) respectively the semi-major and semi-minor axes. The values of \( a \) and \( b \) were determined from diffusion constants from the equation

\[
D = \frac{kT}{6\pi\eta r}
\]

where \( D \) is the specific diffusion rate or diffusion constant, \( k \) is the Boltzmann constant, \( T \) is the absolute temperature, \( \eta \) is the coefficient of viscosity of the medium, and \( r \) is the radius of the particle.
MATERIALS AND METHODS

The preparation of ovalbumin: In order to obtain the ov-albumin, the whites of eggs (two dozen, day-old) were treated according to the method of La Rosa (32). After precipitation of the globulins by addition of an equal volume of saturated ammonium sulfate to the whites of the eggs and removal of the precipitate by centrifuging, the albumin was precipitated by addition of acetic acid. When well crystallized, the albumin was collected by centrifuging, dissolved in a minimum quantity of distilled water, and reprecipitated by the addition of a measured quantity of saturated ammonium sulfate solution. The albumin was purified in this way twice and the final precipitate redissolved in distilled water. This solution was dialyzed under toluene for five days against running tap water and for three days against distilled water. Dialysis was considered completed when the water showed no test for sulfate ion upon the addition of barium chloride solution. The dialyzed solution (about 200 ml) was then divided into four equal portions, each part was frozen, and the water removed by vacuum sublimation. The yield was 12.3 grams of a white, crystalline product, 96% water soluble.

The latex samples: The synthetic rubber latex used in this investigation was supplied by the Physical and Chemical Research
Laboratories of the Firestone Tire and Rubber Company. This latex, Butaprene X-1401, is a water dispersion of the copolymer of butadiene and styrene, and was supplied entirely free of any stabilizer.

**Determination of the shape, number, and size of the latex particles:** It became necessary during the course of this work to ascertain the number, size, and shape of the latex particles. Under a magnification of 500X the particles appeared reasonably spherical and of fairly uniform size. Because of the considerable amount of Brownian movement and the relatively great depth of the available counting chambers, it became necessary to devise a procedure, other than the more common ones, in order to determine the number of particles per unit volume of suspension. By means of a Kahn pipette, 0.1 ml of a latex-water suspension to which a few mgms of albumin had been added was transferred to the surface of a clean glass slide. With a platinum wire this droplet was spread as accurately as possible over an area of 1 square cm. A total of 320 ocular micrometer squares were counted and an average of 6.25 particles per square resulted in a calculation of $7.1 \times 10^{10}$ particles per 50 ml of the suspension under investigation. By means of a calibrated ocular micrometer, the average diameter of the particles was estimated by direct measurement of the immobile particles on the microscope slide. From fifty consecutive readings, the mean diameter of the particles was estimated to be 1.5 ± 0.1 microns where 0.1 micron was the mean deviation. The surface area of the average particle ($\pi D^2$) was then 7.0 square
microns.

The electrophoresis cell: The Abramson cell (1) was used in this investigation and is illustrated in Figure 1. This cell has the advantage of being all glass and is supported so that it will rest flat on the microscope stage. The depth of the cell was determined by first calibrating the fine adjustment on the microscope. This calibration was accomplished by measuring the average thickness of a glass slide with a caliper micrometer and then determining the number of scale divisions through which the fine adjustment knob had to be turned in order to focus on the top and then the underside of the slide. For this work the microscope was fitted with a Zeiss water-immersion 40X objective having a working distance of 1.92 mm. Following the calibration of the fine adjustment of the microscope (1 division = 4 x 10^{-3} mm), the electrophoresis cell was placed on the stage and its depth determined by the procedure just described. The average depth of the cell was measured as 0.50 mm. The cross sectional area of the cell chamber was determined by first directly measuring the width of the cell with a ruler and then multiplying the value obtained by the depth. The average obtained from 40 determinations of the cross-sectional area of the cell was 5.5 square mm. A 20X Zeiss ocular fitted with a cross-hatch micrometer was used in the microscope. The ocular micrometer was calibrated by comparing the distance between lines with a known distance on the stage micrometer.
at the total magnification value of 800X. The distance between adjacent lines in the ocular micrometer was determined to be $1.38 \times 10^{-2}$ mm.

The electrical system required in conjunction with the operation of the cell is shown schematically in Figure 1. The electrodes were of copper wire (3/16" in diameter) in contact with saturated copper sulfate solution in the electrode vessels. The cell proper was separated from the electrode vessels by plaster of Paris plugs which prevented the copper sulfate from diffusing into the suspension under investigation for a period of several hours. Three B batteries (A in Figure 1) connected in series supplied a constant potential difference of 135 volts which was dropped across 20,000 ohms. This total resistance, external to the cell, was obtained by a 10,000 ohm fixed wire wound resistor (B) in series with a 10,000 ohm variable resistor of the radio potentiometer type (C). The double pole double throw switch (D) was wired as a reversing switch so as to change the polarity of the cell electrodes. A multimeter (E) with varying ranges of 0 - 1.0, 10.0, and 100.0 milliamperes was used as the current-measuring instrument.

Conductivity measurements: Conductivities of the suspensions under investigation were determined by means of a conductivity bridge so constructed that by using 0.01 normal potassium chloride of known conductivity at a given temperature, the cell constant could be set
into the instrument and the specific conductivity of the test sus-
pension read directly. The electrodes were of platinum with a plat-
inum black surface. Specific conductivity measurements could be
reproduced within ± 0.01 x 10^-3 reciprocal ohms with the apparatus
described.

**Determination of the electrophoretic mobility of the particles:** The mobility, \( \mu \), of a colloidal particle is usually
defined as its velocity in microns per second when under a poten-
tial gradient of one volt per cm. That is,

\[
\mu = \frac{V}{X}
\]

where \( V \) is expressed in microns per second and \( X \), the field strength,
is determined by the following equation:

\[
X = \frac{I}{kA}
\]

where \( I \) is the current in amperes, \( k \) is the specific conductivity
of the solution, and \( A \) is the cross-sectional area of the cell
chamber.

In practice the current was so adjusted that the particles
would travel some arbitrary distance in a convenient time interval.
For a given set of readings, the current once set was maintained
constant and the time for the particle to move a desired distance
was determined with a stopwatch. Ten determinations were made on
as many different particles moving in one direction; then the
current was reversed, and ten readings taken in the opposite direction.
In all cases, the time required for the particle to move a given
distance was considered to be the average of the time values obtained for both directions. Agreement was excellent.

The velocity of a particle in viscous flow is a function of the viscosity of the medium. Most mobility data in the literature are reported at 25°C. To be consistent with this convention, each mobility value was multiplied by a factor of the viscosity of water at t°C (observed) divided by the viscosity of water at 25°C. The viscosity of water at the temperature of the experiment was obtained from standard tables. All necessary data for calculation of the mobilities were then available for substitution into the following equation:

\[ \mu = (1.38 \times 10^{-2} \times 5.5) \frac{d k \eta}{t I \eta 25^0 C} \times 10 \]

where \( \mu \) is the mobility in microns per second per volt per cm; 
\( d \) is the number of divisions over which the particle was clocked; 
\( 1.38 \times 10^{-2} \) is the width of one division of the cross-hatch micrometer in mm; \( k \) is the specific conductivity in reciprocal ohms; \( \eta \) is the viscosity of water; \( t \) is the average time in seconds for the particle to travel across \( d \) divisions; and \( I \) is the current in amperes.

**Buffer solutions:** The pH of the buffer solutions and latex suspensions was determined using Beckman pH meter. Electrodes were saturated calomel and glass.

During the course of this investigation buffer solutions of
pH 3.01, 3.94, 4.68, 5.01, 6.08, and 6.98 were prepared. Relative amounts of the components to be dissolved per liter of solution were calculated in advance with the limitations imposed that the resulting buffer solutions should have a predetermined pH at an ionic strength of 0.02. The calculations were based on simple ionic equilibria and assumed activities to equal the concentrations of the respective ions. The pH values recorded were measured after the preparation of the solutions. The maximum deviation from the desired value was at pH 6.08 where pH 6.0 had been the value sought.

**Albumin Analyses:** Ovalbumin concentrations were determined by a standard method which is based upon the turbidity developed in an albumin solution when sulfosalicylic acid is added. Exton (15) has developed this turbidimetric procedure to a high degree of precision and has maintained an accuracy of ± 2% over the concentration range which could be covered by most photoelectric colorimeters. The instrument used in this study was the Fisher Electrophotometer, A.C. model, equipped with a filter for transmitting light of wavelength 525 millimicrons.

The standard curve was a plot of transmittancy vs. ovalbumin concentration and was obtained in the following way. A weighed amount of ovalbumin was dissolved in a suitable amount of distilled water. This solution was filtered and the dry weight of ovalbumin determined by evaporation of 10 ml aliquots of the filtrate to constant weight in an oven at 80° C. The maximum deviation among
triplicate samples was 0.4 mg which was a deviation of 3% from the average of three values. Serial dilutions were made on the stock solutions as prepared above. A 5 ml aliquot of each solution in turn was pipetted into a 25 ml volumetric flask, 5 ml of the sulfosalicylic acid solution (50 g sulfosalicylic acid and 10 g C. P. sodium sulfate crystals in distilled water, diluted to 1000 ml, and filtered) was added and the suspension allowed to stand exactly five minutes. At the end of this time, 15 ml of distilled water was added to the suspension in the flask. This 25 ml volume of suspension was transferred to the absorption cell of the photoelectric colorimeter and the transmittancy determined. Distilled water was used as a blank for setting the instrument at a transmittancy of 100.

The standard curve was first established for ovalbumin in distilled water but was checked for its dependence on ionic strength and the pH of the solution. It was found that the standard curve was independent of these variables for ionic strengths of 0.01, 0.02, and 0.03, and at certain pH values ranging from 3 to 7.

**Determination of amount of albumin adsorbed on a synthetic latex:** A method was required which would permit direct analysis of the amount adsorbed or an indirect method whereby the albumin remaining in solution after adsorption could be determined. In the second case, the amount of albumin on the surface of the latex could then be calculated by difference. Several possible methods which had been considered practical were applied without successful results. It was concluded that the analysis for the amount of albumin adsorbed would
have to be based on the implications of the two following observations. First, the latex particles impart a turbidity to their suspensions, the degree of which is a function of the number of particles present. Secondly, a water-clear solution of albumin when treated with excess sulfosalicylic acid reaches a degree of turbidity which is a function of the albumin concentration. In view of these observations the following method was developed for determining the amount of albumin adsorbed without separation of the albumin solution from the latex particles.

Fifty ml solutions of varying concentrations of albumin were prepared of the desired pH and ionic strength. To each of the solutions, in 125 ml Erlenmeyer flasks, was added 0.5 ml of the desired latex suspension which had been prepared by diluting the stock latex with distilled water. The contents of the flasks were then carefully mixed and allowed to stand for two hours. After this period of time, 5 ml of each suspension in turn was pipetted into a 25 ml volumetric flask and made up to volume with distilled water. This volume of suspension was poured into an absorption cell and its transmittancy determined by means of the photoelectric colorimeter. The turbidity thus measured was that due to the latex alone, no coagulant having been added to the protein-latex suspension. A second 5 ml portion of each solution was then pipetted into a 25 ml volumetric flask and an excess (5 ml) of sulfosalicylic acid coagulant added. After this suspension had
stood exactly 5 minutes, 15 ml of distilled water was added and
the transmittancy determined. In both cases the suspensions were
compared with an equal volume of distilled water in a matched
absorption cell.

For each dilution these two transmittancy values along with
the original albumin concentration of the solution being measured
constituted the data needed for determining the amount of albumin
adsorbed on the latex. Both transmittancy values were converted
to the corresponding equivalents of albumin by reading from the
standard curve. Since the second transmission reading described
above represented turbidity caused by latex and coagulated
unadsorbed albumin, the adsorbed albumin could be calculated from
an equation of the following form:

\[ \text{Mgs albumin adsorbed} = \left( \text{Latex correction} + \text{Mgs albumin in original soln} \right) - \text{Mgs albumin in adsorbed soln} \]

\( \text{(Mgs albumin equivalence of transmittance of latex + coagulated albumin)} \)

If the calculations given above are valid, the following
restrictions must then apply to the system:

(a) dilution of latex-albumin suspensions did not cause desorption
of protein, or if desorption did result upon dilution it occurred so
slowly that the amount desorbed in the time required to obtain the
colorimeter reading on suspensions of latex (about one minute) was
negligible;

(b) there was no desorption or further adsorption of albumin on
latex caused by the addition of the protein coagulant. Further adsorption was unlikely, but it was considered highly probable that the adsorbed albumin might be caused to desorb by the sulfosalicylic acid:

(c) the transmittance of latex with coagulated albumin on its surface was the same as that of latex with uncoagulated albumin on its surface, and neither the number nor size of the particles was altered;

(d) the presence of sulfosalicylic acid did not affect the transmittance of latex in the absence of albumin.

The validity of postulated (a) and (d) was demonstrated experimentally. There was no change in the transmission of the latex-albumin suspensions caused by dilution even though the transmittance readings were made 30 minutes after dilution. The presence of sulfosalicylic acid coagulant did not alter the transmittance of the latex in the absence of albumin. The behavior of the system with regard to (b) and (c) above could not be proved by specific experimentation, but the reproducibility of the data obtained and the type of adsorption curves which resulted established their validity.
ABRAMSON ELECTROPHORESIS CELL WITH ELECTRODES AND SUPPORTING FRAME.

WIRING DIAGRAM

LEGEND
A -- EMF SOURCE
B -- 10,000 OHM FIXED RESISTOR
C -- 10,000 OHM VARIABLE RESISTOR
D -- DPDT REVERSING SWITCH
E -- MULTIRANGE AMMETER

FIGURE 1
In the study of the behavior of a synthetic latex in the presence of ovalbumin, the following areas were investigated:

(a) the effect of increasing latex concentration on the amount of protein adsorbed at various protein concentrations;

(b) the effect of pH on the amount of protein adsorbed at various protein concentrations; and

(c) the effect of increasing protein concentrations on the electrophoretic mobility of the latex particles at various pH values.

The data obtained from these investigations were treated according to such procedures as would indicate their significance in the latex-protein system.

A Typical Set of Calculations

The amount of ovalbumin adsorbed on a given weight of latex was determined at different albumin concentrations by the method presented in the previous chapter. As an example of a characteristic set of calculations, Table 1 gives the data obtained when working with a latex concentration of 0.162 g per 100 ml and at a pH of 6.98. All determinations were made at an ionic strength of 0.02. The values tabulated in columns A, B, and D were experimentally determined for each series. The values in columns C and E were read
<table>
<thead>
<tr>
<th>Albumin Conc. of Original Solution (mg/100 ml)</th>
<th>% Transmission Latex Only</th>
<th>Equivalent Albumin Conc. of Latex (mg/100 ml)</th>
<th>% Transmission Latex + Coag. Albumin</th>
<th>Equivalent Albumin Conc. of Latex + Coag. Albumin (mg/100 ml)</th>
<th>Total Albumin Conc. if None is Adsorbed (mg/100 ml)</th>
<th>Apparent Amount Albumin Adsorbed (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>E</td>
<td>F</td>
<td>G</td>
</tr>
<tr>
<td>6.1</td>
<td>48.4</td>
<td>47.5</td>
<td>46.4</td>
<td>50.0</td>
<td>53.6</td>
<td>3.6</td>
</tr>
<tr>
<td>12.3</td>
<td>48.0</td>
<td>47.9</td>
<td>43.1</td>
<td>54.3</td>
<td>60.2</td>
<td>5.9</td>
</tr>
<tr>
<td>24.5</td>
<td>47.4</td>
<td>48.6</td>
<td>36.7</td>
<td>64.0</td>
<td>73.1</td>
<td>9.1</td>
</tr>
<tr>
<td>36.8</td>
<td>47.1</td>
<td>49.0</td>
<td>31.0</td>
<td>74.2</td>
<td>85.8</td>
<td>11.6</td>
</tr>
<tr>
<td>49.0</td>
<td>47.1</td>
<td>49.0</td>
<td>25.6</td>
<td>86.0</td>
<td>98.0</td>
<td>12.0</td>
</tr>
<tr>
<td>61.3</td>
<td>46.9</td>
<td>49.2</td>
<td>21.9</td>
<td>96.0</td>
<td>110.5</td>
<td>14.5</td>
</tr>
<tr>
<td>73.5</td>
<td>46.5</td>
<td>49.8</td>
<td>18.5</td>
<td>104.5</td>
<td>123.3</td>
<td>18.8</td>
</tr>
</tbody>
</table>
directly from the standard curve for the determination of ovalbumin by the turbidimetric method. The quantities in columns $F$ and $G$ were calculated from the other data shown and by a method previously given.

**Effect of Increasing Latex Concentration**

Figure 2 is a graphical presentation of the amount of albumin adsorbed, corresponding to the values given in columns $A$ and $G$ of Table I, for ovalbumin on different quantities of latex at pH 6.98. The weights of latex were 0.045, 0.090, and 0.162 g per 100 ml of suspension. These curves demonstrate that the procedure employed in this adsorption study yielded consistent and reasonable results for the quantity of protein adsorbed both as a function of the protein concentration and of the available space for adsorption.

**Effect of pH on Adsorption**

In order to investigate the amount of protein adsorbed on the surface of the latex particles as a function of the concentration of albumin at different pH values of the suspension, data were obtained, corresponding to those in Table I, for pH values of 3.01, 3.94, 4.60, 5.01, 6.08, and 6.98. These results are illustrated in Figures 3 and 4. Each curve showing the amount of protein adsorbed as a function of the concentration of the latter definitely extends beyond that amount necessary to form a monolayer and is further characterized by a rapid increase in the amount adsorbed with high concentrations of protein. These curves are similar to certain
ADSORPTION OF ALBUMIN ON LATEX AT DIFFERENT LATEX CONCENTRATIONS.

pH 6.98

FIGURE 2

CONCENTRATION OF ALBUMIN (MG. PER 100 ML.)

ALBUMIN ADSORBED (MG.)

LATEX CONC.= 0.162 G. 100ML.
LATEX CONC.= 0.09 G 100ML.
LATEX CONC.= 0.045 G. 100ML.
AMOUNT OF ALBUMIN ADSORBED ON LATEX VS. ALBUMIN CONCENTRATION AT VARIOUS pH VALUES.

LATEX CONCENTRATION = 0.09 G. PER 100 ML.

FIGURE 3
AMOUNT OF ALBUMIN ADSORBED ON LATEX VS. ALBUMIN CONCENTRATION AT VARIOUS pH VALUES.

LATEX CONCENTRATION = 0.09 G. PER 100 ML.
"S" shaped adsorption isotherms which have been reported for the adsorption of vapore on silica gel (30), titania gel (26), iron oxide gel (46), and similar porous materials. Foster (16) believes that multilayer adsorption explains satisfactorily the portion of the "S" isothermals in the low pressure region (corresponding to the regions of low concentration of protein in this study), but that the upper part of the curve is influenced by capillary condensation of the vapor being adsorbed. This latter statement would mean the actual liquification of the vapor and a filling of the pores by the liquid through capillary action. A physical analogy has been sought for the adsorption study reported here. It is possible that the portion of the curves in the region of high protein concentration may correspond to a solubility curve of the protein, but unless the presence of the latex exerts anomalous effects on the solubility of the ovalbumin such a viewpoint seems to be without basis. A more likely explanation is that the protein molecules which are adsorbed at higher protein concentrations are oriented at the solid-liquid interface in such a manner that the effective area per adsorbed molecule is lessened. In such a case more molecules of protein could come under the influence of the forces tending to cause adsorption and at the same time more could be adsorbed per unit area.

Neurath (42) has shown that the ovalbumin molecule is an ellipsoid of revolution with a ratio of major axis to minor axis
of 3 to 1. It seems reasonable to consider that the first few layers which are adsorbed are constituted of protein molecules lying flat on the surface of the latex. The forces attracting more molecules to the surface would probably decrease as multilayers form, but counteracting this would be the mass action effects of the protein in the solution. If now more protein can adsorb, with its major axis perpendicular to the surface, a sharp increase in amount adsorbed is not unreasonable.

When certain of the data shown in Figures 3 and 4 are plotted in a different way (Figure 5), it can be seen that for a given albumin concentration the quantity of albumin adsorbed per square meter of surface of the latex particles is dependent on the pH. Each curve in the family of curves shown represents a constant protein concentration. Their "W" shape is of considerable interest. Over the pH range investigated and at all concentrations of albumin, the weight of protein adsorbed per square meter of latex surface showed a maximum at pH 3.01, passed through a minimum with increasing pH until a sharp rise occurred in the neighborhood of the isoelectric point, reached another minimum above the isoelectric point and climbed upward again at pH 6.98.

The work of Hitchcock (27) has been reviewed by this writer and attention called to his finding of maximum adsorption of albumin on collodion membranes at pH 5.0. His work may be criticized on the basis that he produced variation in pH by merely adding base or acid to a distilled water solution of the protein, and it is inconceivable
MG. ALBUMIN ADSORBED PER SQUARE METER SURFACE AREA VS. pH AT VARIOUS ALBUMIN CONCENTRATIONS.

- 10 MG. ALBUMIN PER 100 ML.
- 20 MG. ALBUMIN PER 100 ML.
- 30 MG. ALBUMIN PER 100 ML.
- 40 MG. ALBUMIN PER 100 ML.
- 50 MG. ALBUMIN PER 100 ML.
- 60 MG. ALBUMIN PER 100 ML.

FIGURE 5
that by this method the ionic strength of the solution could have remained constant. It is known that the charge on the protein molecule varies with the ionic strength (51), and because of this change in charge the amount of albumin adsorbed should be affected. Dew (13) also showed that the curve which related the amount of ovalbumin adsorbed on collodion membranes to the pH of the suspension was discontinuous. He observed a maximum at pH 5.0 in the absence of salt, but in the presence of the latter, the amount of protein adsorbed increased abruptly at higher pH values. Lindau and Rhodius (34) found that the amount of albumin adsorbed on ground glass particles was a maximum at pH 4.8. These workers assumed a tightly packed dehydrated structure in the region of the isoelectric point with a loose water-saturated structure at higher and lower pH values. Neurath and Bull (43) believe that the action of electrolytes on the adsorption of protein to solid surfaces is electrostatic in nature and that the ions of the electrolyte decrease the mutual repulsion forces between the protein molecules. The result of such decreased forces might be that the surface area per protein molecule would be lessened.

Another factor, no less important than others mentioned, must be considered. There is always a tendency on the part of a system to decrease its surface energy. In a colloidal system such a tendency is usually referred to in terms of the capillary activity of the disperse phase. The surface energy of a system will be decreased if there
is a lessening of surface tension (interfacial tension in this instance) or if there is a decrease in total surface area. Certain results obtained by Bull (10) are of interest in this connection. He determined the effect of the pH on the surface tension of ovalbumin solutions and found the curves to be maximal at pH 3.2 and pH 8.0 with a broad minimum from pH 5.0 to 5.5. The value of 5.0 is reported to be the isoelectric point of surface denatured ovalbumin (1). The minimum in the surface tension curve in the region of the isoelectric point is consistent with the results shown here if it may be assumed that there is associated with the lowering of the surface tension a corresponding lowering of the interfacial tension at the solution-later boundary. The "W" curves in Figure 4 would indicate that the change in the interfacial tension with pH is inadequate in itself to explain the observed amount of protein adsorbed at high and low pH values.

It would seem appropriate at this point to summarize those factors which should play an important role in the adsorption of proteins to solid surfaces and which thereby would affect the stability of heterogeneous protein systems. The amount of protein adsorbed should depend upon:

(a) the relative forces of repulsion or attraction between protein molecules,

(b) the relative forces of repulsion or attraction between protein molecules and the surface of the adsorbing agent,
(c) the change in surface energy of the system which would result as a consequence of adsorption, and

(d) the effective size and shape of the protein molecule which is believed to vary with the extent of hydration and certainly with its degree of denaturation.

Effect of pH and Concentration on Electrophoretic Mobility

Other data which are pertinent to explanation of the "W" curves and which also require elucidation are shown in the electrophoretic mobility curves of Figure 6. These curves have been plotted in terms of the electrophoretic mobility of the latex particles versus the cube root of the ovalbumin concentration as a convenient set of coordinates whereby the mobility curves at all pH values investigated might be shown on the same scale. The points at which these curves have a slope of zero are considered to correspond to the formation of a monolayer of protein adsorbed on the surface of the particle (1, 29, 34); further, there is good evidence that even if multiple layers form, the surface charge density of the particle is not altered appreciably from that of the monolayer.

By proper combination of the data for the amount of albumin adsorbed as a function of the protein concentration (Figures 3 and 4), with the mobility of the latex particles as a function of the protein concentration (Figure 6), the electrophoretic mobility which corresponds to a given amount of protein adsorbed may be determined at each pH investigated. Figure 7 shows a plot of \( \mu_0 - \mu \), i.e., the change
MOBILITIES OF LATEX PARTICLES VS. $\sqrt[3]{\text{CONC.}}$ OF ALBUMIN AT VARIOUS pH VALUES

LATEX CONCENTRATION = 0.09 G. PER 100 ML.

FIGURE 6
CHANGE IN $\mu$ OF LATEX PARTICLE DUE TO ADSORBED PROTEIN
VS. LOGARITHM OF MG. OVALBUMIN ADSORBED.  pH 6.98

FIGURE 7
in the mobility of the latex particle from its mobility in the absence of protein to its measured mobility at some concentration of protein, versus the logarithm of the measured amount of protein adsorbed at that concentration. The treatment shown is for pH 6.98 and a total latex surface of 1.25 square meters. The curve shows an abrupt change in slope at the point where \((\bar{M}_0 - M)\) becomes constant. This point should represent the amount of protein adsorbed to form a monolayer on the available surface at the particular pH. A similar treatment was carried out for five of the pH values investigated and the results are expressed in terms of mg of ovalbumin per square meter of surface area of the latex particles.

The following results were obtained:

<table>
<thead>
<tr>
<th>pH</th>
<th>mg ovalbumin in monolayer per square meter of latex surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.01</td>
<td>undetermined</td>
</tr>
<tr>
<td>3.94</td>
<td>4.7</td>
</tr>
<tr>
<td>4.60</td>
<td>6.8</td>
</tr>
<tr>
<td>5.01</td>
<td>3.3</td>
</tr>
<tr>
<td>6.08</td>
<td>3.6</td>
</tr>
<tr>
<td>6.98</td>
<td>7.6</td>
</tr>
</tbody>
</table>

At pH 3.01 the amount of adsorbed albumin required to produce constant mobility of the latex particles was below that which could be determined by the analytical method employed. From the mobility curves at this pH (Figure 6), it can be seen that the concentration required to cause leveling of the curve is approximately 1.3 mg per
100 ml of suspension. Even if one considers that all this protein is adsorbed, the weight of ovalbumin on the surface of the latex could not exceed 1.8 mg per square meter.

It is of interest to compare the amount of protein required to form a monolayer on the surface of the latex with that reported by Svedberg (52) in terms of the spatial requirements for the ovalbumin molecule. From his value for the specific volume of the protein (0.79 cc per g) and accepting his statement that the molecule is spherical it can be calculated that 2,870 square cm are required per mg of adsorbed protein, or 3.5 mg per square meter. Svedberg worked in the absence of salt at pH 4.9. The value of 3.5 mg per square meter is to be compared with the amount reported from this work at pH 5.01 which was also 3.5 mg per square meter of surface. Lindau and Rhodius (34) obtained a value of 3.55 mg of ovalbumin per square meter of surface using quartz particles as the substrate. At pH 3.0 they report that less than one third of this amount was required. This is in fair agreement with the "less than 1.8 mg" reported in the present work for the adsorption on a synthetic latex at pH 3.01.

Other valuable information may be obtained from Figure 3. The four curves show the electrophoretic mobilities of the latex particles at different concentrations of ovalbumin, plotted against the pH of the suspension. The lowest curve is for the latex in the absence of protein, the three upper curves represent concentrations of 0.1, 1.0, and 10.0 mg of albumin respectively. There is a
MOBILITIES OF LATEX PARTICLES VS. pH AT DIFFERENT CONCENTRATIONS OF ALBUMIN.

LATEX CONC. = 0.09 G./100 ML.
IONIC STRENGTH = 0.02

FIGURE 8
strong shift in the equilibria between dissolved and adsorbed protein in the direction of the latter as the pH is decreased. The latex particles themselves were not isoelectric at any pH investigated; however, they coagulated in the buffer of pH 2, so it is presumed that this pH is near to their isoelectric point. The latex particles were negatively charged at all pH values for which mobilities could be obtained. Since the protein was positively or negatively charged depending upon the pH of the medium, it can be seen that the electrical properties of the particles, both latex and protein, play an important part in the adsorption phenomenon.

It would seem at this point that two considerations become paramount:

(a) the amount of protein which imparts constant mobility to the latex particles is that required to form a close-packed, continuous protein film over a given amount of latex surface and is a function of the pH of the medium, or

(b) this same amount of protein is that which, when adsorbed, with the resultant electrical changes and decrease in surface energy, will form the most stable system. This amount may or may not be equal to that required spatially for physical coverage of the surface.

This investigator believes the observed facts can be adequately explained by means of a theory of "active centers" on the surface of the latex particle when it is suspended in the buffer solution. The origin of the negative charge on the particle is probably due to the selective adsorption of hydroxyl ions. This assumption is consistent
with the observed decrease in magnitude of the negativity of the particle with decreasing pH, which might represent the adsorption of hydrogen ions or the desorption of hydroxyl ions. The theory which is presented requires a definition of a monolayer in terms of adsorption at all active centers and which would not necessarily correspond to the total available "space" on the surface of the adsorbing agent.

At pH 6.98 there exists a great number of active centers as shown by the high negative charge on the latex particle. It is presumed that the linkage between the albumin molecule and the latex particle will be through interaction of the $\text{NH}_3^+$ groups of the protein and the hydroxyl ions on the surface of the latex. At high pH values, relatively few $\text{NH}_3^+$ groups, in comparison to $\text{COOH}^-$, exist on the albumin molecule. The protein is then negatively charged but to a lesser extent than the surface of the latex particles. When the surface charge is only due to adsorbed protein, then each active center on the latex surface has combined with an $\text{NH}_3^+$ group and the charge no longer changes with an increase in the amount of albumin adsorbed.

As the pH of the suspension is decreased, the number of active centers on the surface of the latex is less; also there are a greater number of $\text{NH}_3^+$ groups per protein molecule. The combined effects of these conditions is to require a smaller amount of protein to produce active center coverage at pH 6.08. It would appear that the amount of protein required to cover the active centers on the latex at pH 5.01
should continue to show a decrease to be consistent with the theory presented thus far. The ovalbumin is, however, rapidly approaching its isoelectric point between pH 6.08 and 5.01 (Figure 9). Under these conditions the stability of the protein molecule as a disperse particle is rapidly decreasing and the repulsive forces between albumin particles as well as between albumin particles and the latex surfaces are decreasing. The solubility of the protein is likewise approaching a minimum. These factors combine to offset those tending to produce a decrease in the amount of protein adsorbed, so that the expected decrease is not observed. At a pH of 4.60 a sharp increase in the amount of protein adsorbed to form the monolayer is indicated. This increase in the amount adsorbed is consistent with the reasoning already advanced. This worker believes that the true maximum should occur at a pH of 4.55 which is the isoelectric point of dissolved albumin (Figure 9), (55). At pH 4.55, with previous considerations specifically in mind, it may be said that all repulsive forces should be a minimum, the forces affecting the energy of the system should now be acting under optimum conditions, and —since the protein molecule may be essentially dehydrated at its isoelectric point— its size may be a minimum.

At lower pH values the number of active centers (adsorbed hydroxyl ions) on the latex particles is decreased. There has been a reversal of charge on the protein molecule due to the suppression of the carboxyl group ionization, and an attendant increase in the number of NH₃⁺ groups per molecule. The reduction in the amount of albumin required for active center coverage of the latex surface upon
Figure 9

Mobility of albumin covered latex particle (microns per second per volt cm)

- Surface denatured ovalbumin (Moyer)
- Ovalbumin adsorbed on a synthetic latex
- Dissolved ovalbumin (Tiselius)
departure from the isoelectric region is consistent with the theory which has been presented. At pH 3.01 less protein was required to produce active center coverage than at any other pH investigated.

A final word may now be said concerning the "W" curves of Figure 5. It is apparent that the number of milligrams of protein adsorbed on a unit area of the latex surface is a function of the pH and of the amount of protein in solution. Since all values of the amount adsorbed (Figure 5) are above those required to form a monolayer, whatever the mechanism and bonding forces may be, the result is a multilayer adsorption phenomenon which may not be expressed adequately in terms of a simple Langmuir or Freundlich isotherm.

In a natural or synthetic rubber latex system which has been stabilised by protein it is obvious that those changes in pH which will affect the properties of the system as a colloidal dispersion lie within a range that is characteristic of the particular protein. This pH range is within rather definite limits on either side of the isoelectric point of the protein.

**Denaturation and the Isoelectric Point**

Proteins are thought to change their shape from a globular to an unfolded linear form upon denaturation. In this process a different number and kind (-OH) groups are exposed to the surface. It is presumed that this change causes an increase in the positive charge on the surface and a consequent increase in the pH of the isoelectric point for the denatured protein.
When ovalbumin is adsorbed at an interface the isoelectric point is 4.82 (38). Tiselius (55) has shown that the isoelectric pH of dissolved ovalbumin is 4.55 while Moyer (1) found the isoelectric point of surface denatured ovalbumin to be pH 5.05. This shift to an intermediate pH value for the isoelectric pH of the adsorbed protein has been interpreted to mean that the protein has been at least partially denatured in the process of adsorption. In the opinion of this investigator a change in isoelectric point would be a consequence of denaturation, but it is not necessary that denaturation of the protein must accompany a shift in the isoelectric point.

The reasoning in support of the above statement is as follows: at the isoelectric point the protein contains an equal number of positive and negative groups and is, on the whole, electrically neutral. If, however, it is adsorbed through certain of its positive groups to a negative center on a foreign surface, the residual negative groups on the protein become active and will adsorb hydrogen ions from the solution. The result is that the pH must be increased to effect an isoelectric condition of the protein. Denaturation, then, does not necessarily accompany a shift in the isoelectric point of the protein.

An interesting study in this connection would be the adsorption of a protein on a positively charged latex. The theory of the shift in the isoelectric point as presented would predict a shift to a lower pH value.
A synthetic rubber latex-ovalbumin system was investigated as a less complex counterpart of naturally occurring rubber latex systems which are stabilised by protein.

The quantity of ovalbumin adsorbed on a given amount of latex and the electrophoretic mobility of the latex particles have been determined as functions of the protein concentration. A method has been devised by which the amount of protein adsorbed on the latex particles could be determined without a separation of the latex (with its adsorbed protein) from the protein remaining in solution at equilibrium. This has been effected through a new adaptation of a common analytical method (turbidimetry) and has produced results which are consistent with those obtained by other investigators.

The results indicate:

1. Adsorption of ovalbumin on the latex surface was in excess of the quantity required to produce coverage of the surface over most of the protein concentration range which was investigated;
2. "S" shaped adsorption isothermals which may be interpreted in terms of specific orientation of the ovalbumin molecule, were obtained;
3. The quantity of ovalbumin required to produce constant mobility,
when adsorbed to the same number of latex particles, was a function of the pH of the suspension, and a theory of active centers (\(-\text{OH}^-\) ions) on the surface of the latex has been presented as a reasonable explanation of this phenomenon.

(4) A shift in isoelectric point from that of the dissolved ovalbumin (pH 4.55) to pH 4.82 for the adsorbed protein was observed, and an argument was presented that such a shift does not require denaturation of the protein on adsorption.

Certain results of the study are pertinent to natural rubber. Kemp and Twiss (29), as a result of their electrophoretic study of Hevea latex, maintained that the solubility of the rubber protein increased with increasing pH and consequently in highly diluted latex the rubber particles might be completely void of protein. The work reported here has indicated that such might not be the case but that since both latex particles and protein are negative at high pH values less change in electrophoretic mobility would be imparted to the latex particle for a constant amount of protein adsorbed.

In a natural or synthetic rubber latex which has been stabilized by protein it is obvious those changes in pH which will affect the properties of the system as a colloidal dispersion lie within a range that is characteristic of the particular protein and within rather definite limits on either side of its isoelectric point.
If the quantity of protein which is adsorbed to the surface of the latex particles affects the properties of the resulting rubber product, then the pH of the suspension from which the latex is to be separated and dried should be carefully adjusted because it is a factor in controlling the amount of protein in the rubber before final electroplating or vulcanization.
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Hulen Brown Williams was born at Lauratown, Arkansas, on October 8, 1920. His elementary education was begun in the elementary school of Vilonia, Arkansas, and completed at Hoxie, Arkansas. He attended junior high school at Fort Smith, Arkansas, and was graduated from senior high school at Paragould, Arkansas, in May, 1937.

The following September he entered Hendrix College, Conway, Arkansas, from which institution he was graduated with a Bachelor of Arts Degree in June, 1941.

In September, 1941, he entered Louisiana State University with an appointment as graduate assistant in the Department of Chemistry. He received his Master's Degree in June, 1943, and became instructor of chemistry the following August.

From June, 1944, until April, 1946, he was on military leave of absence in the service of the United States Naval Reserve. Following his return, he has been engaged in work leading to the Degree of Doctor of Philosophy in chemistry.
EXAMINATION AND THESIS REPORT

Candidate: HULEN BROWN WILLIAMS

Major Field: CHEMISTRY

Title of Thesis: ELECTROPHORETIC STUDIES OF A SYNTHETIC RUBBER-LIKE LATEX

Approved:

[Signatures]

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

[Signatures]

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