phenols. With (1) pyridine and *o*-phenylphenol: one compound, $C_5H_5N \cdot C_{12}H_{10}O$, is formed; (2) pyridine and *m*-phenylphenol: one compound, $C_5H_5N \cdot C_{12}H_{10}O$, is formed; (3) pyridine and *p*-phenylphenol: two compounds, (a) $C_5H_5N \cdot C_{12}H_{10}O$ and (b) $C_5H_5N \cdot 2C_{12}H_{10}O$, are formed; both are unstable at their melting points.

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[CONTRIBUTION FROM THE GENERAL LABORATORIES OF THE UNITED STATES RUBBER COMPANY, PASSAIC, NEW JERSEY]

# An Electrophoretic Study of the Proteins in Rubber Latex Serum

BY CHARLES P. ROE AND ROSWELL H. EWART

The proteins in rubber latex serum (*Hevea brasiliensis*) have been the subject of several researches during the past fifteen years. Some of this work has been concerned with the chemical properties of the proteins[1] and some with their electrophoretic properties.[2] Recently, the improvements introduced by Tiselius[3] in the electrophoretic technique have made it possible to increase the precision and significance of electrophoretic measurements very considerably. The work here reported is part of a program of electrophoretic research on latex proteins. This program was initiated in an attempt to obtain information which would be helpful in correlating protein behavior with the colloidal and other properties of latex.

## Experimental

**The Electrophoresis Apparatus.**—The apparatus was supplied by the Klett Manufacturing Company of New York City and was built according to specifications furnished by Dr. L. G. Longsworth of the Rockefeller Institute in New York. Details may be found in Longsworth's publications.[4] A few modifications of the apparatus are described in the following paragraphs.

**Illumination System.**—Many latex serum samples used in this work did not transmit the visible lines of the mercury spectrum very well. In some cases this was on account of slight turbidity, in others, on account of color. The transmission coefficient of such solutions was found to be much greater in the red. Consequently, the mercury arc supplied with the apparatus was replaced by a 500-watt Tungsten filament projection lamp in conjunction with a Corning no. 246 lighthouse red filter. The object of this arrangement was to produce the most uniform possible exposure of photographic plate over the entire length of the cell image when the cell contained either a turbid or colored protein solution separated from a colorless buffer solution by a boundary in the exposed part of the cell. This expedient did not in all cases yield perfect results, but it was much more generally practicable than the use of the mercury arc, and well-defined Schlieren patterns were obtained.[5] Wratten and Wainwright Contrast Thin Coated Panchromatic plates were used. They are insensitive at wave lengths greater than 6800 Å. and the red filter cuts out all light of wave length less than 5600 Å. These figures are extreme values. The range of practical intensities lies between 5800 and 6600 Å.

In using the mercury arc in cases where this was feasible, it was found advantageous to introduce a Corning yellow–yellow filter in order to filter out the violet part of the mercury spectrum. This resulted in the production of straighter base lines in the schlieren diagrams through the elimination of a small dispersion effect due to some undetermined cause in the optical system.

**The Electrodes and Source of Potential.**—The silver–silver chloride electrodes were replaced by copper electrodes dipping into a concentrated copper sulfate solution. These electrodes are perfectly reversible and are easier to prepare than the silver–silver chloride system. In addition, they require no special care or attention after they are made. The authors used no. 14 copper wire wound into a compact flat coil and soldered to a small copper tube which was cemented to a piece of glass tubing just as in the case of the silver electrodes. When an alkaline buffer solution was used, a layer of saturated sodium sulfate solution was introduced between the buffer and copper sulfate solutions in order to prevent the formation of insoluble basic copper compounds at the liquid junction.

The potential difference applied to the electrophoresis cell and electrodes was furnished by a bank of 45-volt heavy duty B batteries. Conditions were always adjusted so as to make the power consumption less than three watts in the cell and electrode system.

The field strength within the electrophoresis cell was calculated from measured values of the current passing, the specific conductance of the solution and the cross section of the cell.

**The Compensating Device for Shifting Boundaries.**—The motor-driven compensating device was replaced by a gravity feed through a one-meter U-shaped length of capillary glass tubing of 1-mm. inside diameter which was connected at one end to the closed electrode vessel and at the other end to a separatory funnel which served as a reservoir.

(1) Bishop, *Malayan Agr. J.*, **15**, 27 (1927); Bondy and Freundlich, *Rubber Age*, **44**, 377 (1938); Kemp and Straitiff, *J. Phys. Chem.*, **44**, 788 (1940).

(2) I. Kemp and Twiss, *Trans. Faraday Soc.*, **32**, 890 (1936).

(3) Tiselius, *ibid.*, **33**, 524 (1937).

(4) Longsworth, THIS JOURNAL, **61**, 529 (1939); Longsworth and MacInnes, *Chem. Rev.*, **24**, 271 (1939).

(5) Verbal communication from Prof. J. W. Williams has informed the writers that a similar expedient has also been used successfully in his laboratory.

**Ammonia Preserved Serum from Sumatra Normal Latex.**—This was a one-gallon lot which was prepared on the plantations of the U. S. Rubber Company in Sumatra by freezing fresh unpreserved normal Hevea latex. Freezing caused complete coagulation of the rubber into a coherent clot. After thawing, the rubber-free serum was expressed, preserved with 2% ammonia and shipped to America in a stoppered glass container.

**Serum from Unpreserved Sumatra Normal Latex.**—Fresh unpreserved normal Hevea latex was frozen on the plantations in Sumatra and shipped in the frozen state to America. After this material had been thawed, the rubber-free serum was expressed and refrozen as quickly as possible to prevent deterioration. Serum thus prepared and containing no chemical preservative had a light straw color and a perfectly sweet odor. Its *p*H value was 6.4 at 25°. When kept stored in glass in the frozen state at −20° it retained these characteristics over a period of months with no signs of any deterioration.

**Serum from Unpreserved Normal Florida Latex.**—This latex (Hevea) was treated exactly like that prepared in Sumatra but it originated in the Plant Introduction Garden of the United States Department of Agriculture, Coconut Grove, Florida. The *p*H value of this serum was also 6.4 at 25°.

**Direct Preparation of Latex Serum for Electrophoresis.**—All electrophoresis determinations were made in solutions which were well buffered so as to secure adequate *p*H control. Each serum sample was, therefore, dialyzed exhaustively against the appropriate buffer solution at the temperature of the Tiselius thermostat. Conductance measurements were used to determine the extent of dialysis. Conductance and *p*H measurements (glass electrode) were made at the temperature of the cold thermostat which was held at +1°.

**Concentration of Latex Serum Proteins.**—It was necessary in several cases to have a more concentrated solution of the serum proteins than that in which they naturally occur in serum from frozen latex. For this purpose dry serum solids were prepared by the technique of vacuum sublimation of rubber-free frozen serum. This technique has been used extensively in the past by workers with animal sera.[6] The latex serum solids thus obtained contained some hygroscopic material which could be largely removed by dialysis with distilled water for twenty-four hours before starting the vacuum sublimation process. Dialysis could not be carried farther than this without causing partial flocculation and denaturation of the proteins. When properly prepared, the dried serum solids showed no outward signs of deterioration and could be completely redissolved in aqueous solutions at all *p*H values at which the original serum was stable. All concentrated latex protein solutions used in this work were prepared from completely vacuum dried samples. Dialysis against buffer solutions was carried out prior to electrophoresis in the case of these concentrated solutions just as in the case of the native serum samples.

**Buffer Solutions.**—All buffer solutions were made by use of uni-univalent electrolytes at an ionic strength equal to 0.1. In the following table the acid-base combinations used to cover the various segments of the *p*H interval between 2.0 and 10.5 are listed. *p*H values were measured

| *p*H Interval | Acid | Base |
|---|---|---|
| 2 – 4 | HCl | Glycine |
| 4 – 5.5 | Acetic | NaOH |
| 5.5– 7 | Cacodylic | NaOH |
| 7 – 8.5 | HCl | Triethanolamine |
| 9 –10.5 | Glycine | NaOH |

with the glass electrode at 1°. In order to calibrate the electrode, it was assumed, following Harned and Ehlers[7] that the ionization constant of acetic acid at 0° was $10^{-4.79}$ at an ionic strength of 0.1.

## Results and Discussion

**General Remarks on the Interpretation of Schlieren Diagrams.**—The detailed theory of the schlieren scanning method of measuring refractive index gradients has been described elsewhere[4,8] It will be recalled that in electrophoretic schlieren diagrams the position and the time rate of displacement of a given peak along the axis of abscissas give, respectively, a direct index of the position and the rate of motion of the corresponding protein boundary within the cell. From this may be calculated the electrophoretic mobility by the introduction of the appropriate magnification and electric field factors. Each peak corresponds to a separate boundary. The ordinate of a schlieren diagram is proportional to the gradient of refractive index, and hence the area under the diagram included between two abscissas is proportional to the total refractive index change between the two abscissas. If the relationship between concentration and refractive index is known, then the area under a schlieren diagram, upon introduction of the proper optical factors, gives a measure of the concentration change of solute from one part of the cell to another.

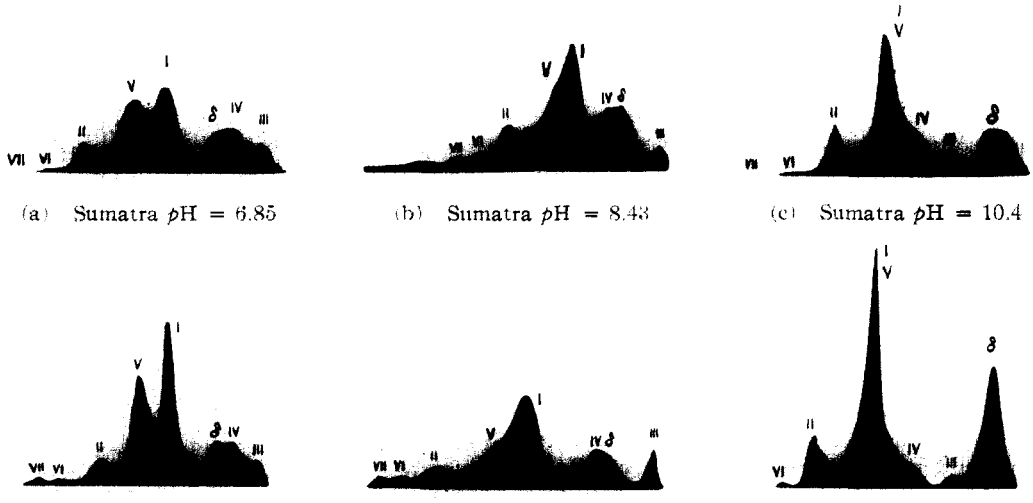
**Protein Components of Latex Serum.**—Figure 1 shows electrophoretic schlieren diagrams (rising boundaries) at three *p*H levels for each of two samples of protein from Sumatra and Florida unpreserved whole latex serum. The presence of seven resolvable components is evidenced by inspection at the *p*H values of 6.85 and 8.43. Only six components are resolvable at *p*H 10.4, since I and V have the same mobility at this point.

Complete electrophoretic schlieren diagrams for the proteins in whole latex serum can be obtained only with concentrated serum, since two of the minor components (VI and VII) are present in such small quantities that they are not detect-

(6) Mudd, Reichel, Flosdorf and Eagle, *J. Immunol.*, **26**, 341 (1934).

(7) Harned and Ehlers, THIS JOURNAL, **54**, 1350 (1932).

(8) Lamm, *Z. physik. Chem.*, **A138**, 313–331 (1928).



(a) Sumatra $p$H = 6.85 (b) Sumatra $p$H = 8.43 (c) Sumatra $p$H = 10.4

(d) Florida $p$H = 6.85 (e) Florida $p$H = 8.43 (f) Florida $p$H = 10.4

Fig. 1.—Schlieren diagrams showing comparison of Florida and Sumatra latex serum proteins, all samples concentrated 3/1.

able at their natural concentrations in serum from frozen latex.

It is not known at present how to classify these electrophoretically resolvable components in terms of usual protein nomenclature. So far as the authors are aware, the only classification which has ever been made depends upon the separation of the whole protein into the so-called glutelin, globulin and albumin fractions.[1] Probably the protein components of unpreserved latex serum can be fitted into this classification, but it will require a combination of analytical results with those of electrophoresis experiments.

**Comparison of Florida and Sumatra Latex Serum Proteins.**—The schlieren diagrams of Fig. 1 show that the Florida and Sumatra protein samples are electrophoretically very similar in most respects. The number of resolvable components is the same, and the mobilities and relative abundance of the various components are nearly the same. The mobility figures are given in Table I and are represented graphically in Fig. 2.

Inspection of the diagrams in Fig. 1 shows that the relative abundance is about the same in the sera from the two sources and also that the total protein content is not much different in the two cases. A quantitative comparison of the areas under the schlieren diagrams is impossible since through an unfortunate oversight base line photographs were not recorded in all cases. No attempt has been made to fit the correct base lines in any case, since no use is made of them. The principal difference between the Florida and Sumatra diagrams lies in the sharpness of the peaks. The sharper peaks in the Florida sample indicate a greater degree of electrophoretic homogeneity of the several components in this than in the Sumatra sample. The cause is not definitely known, but the difference probably indicates more sanitary handling and less incipient deterioration before freezing on the Florida experimental plot than on the Sumatra plantation.

TABLE I

MOBILITY *versus* $p$H VALUES FOR TOTAL SERUM PROTEINS FROM SUMATRA AND FLORIDA LATEX

| | Mobility in microns per second per volt per cm. | | | | | |
|---|---|---|---|---|---|---|
| | $p$H = 6.85 | | $p$H = 8.43 | | $p$H = 10.4[a] | |
| Component | Sumatra | Florida | Sumatra | Florida | Sumatra | Florida |
| I | −0.200 | −0.210 | −0.355 | −0.335 | −0.575 | −0.580 |
| II | − .590 | − .615 | − .690 | − .680 | − .880 | − .980 |
| III | + .255 | + .240 | + .120 | + .110 | − .230 | − .165 |
| IV | + .090 | + .120 | − .150 | − .160 | Not resolved | |
| V | − .335 | − .360 | Not resolved | | − .575 | − .580 |
| VI | − .800 | − .825 | − .850 | − .825 | −1.18 | −1.21 |
| VII | − .955 | − .915 | − .985 | − .915 | −1.39 | −1.53 |

[a] This $p$H value is actually an average figure. The Sumatra values were measured at $p$H 10.2 and the Florida values at 10.6.

The similarity of schlieren patterns obtained from latex sera with such widely different geographical origins is not surprising. Workers with human sera[9] have found that the schlieren pattern of normal human serum is nearly independent of the source of the serum and that only abnormal or pathological conditions cause large departures from the characteristic normal pattern. The results here reported on latex serum proteins are not sufficiently extensive to be accepted as proof that the geographical origin of latex from a healthy tree has no effect on the schlieren pattern of the serum proteins, but the close similarity of the patterns here presented, taken together with the findings of investigators in other related fields, makes such an assumption seem plausible. If this is true, it is a matter of some practical interest in latex technology. It would indicate that variations in normal latex from healthy trees cannot be ascribed to qualitative variations in the protein content of the serum prior to tapping. Variations traceable to the proteins must be due, then, to variations in handling conditions and preservation procedures.

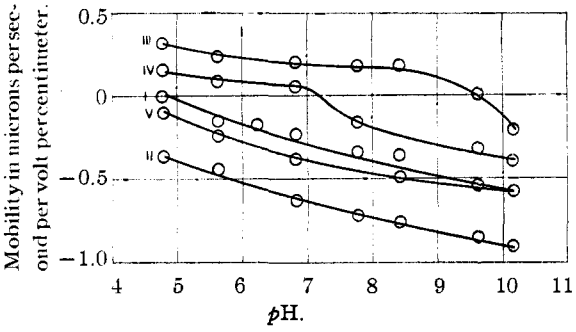



Fig. 2.—Mobility–*p*H curves for five protein components of latex serum.

**Relationship between *p*H and Electrophoretic Mobility.**—Figure 2 shows the mobility–*p*H curves for five of the seven protein components of whole unpreserved latex serum. These curves were plotted from data obtained from the electrophoresis of unconcentrated serum before the discovery of the minor components VI and VII, which were detectable only after concentration. It was found impossible to follow these curves below *p*H 4.79. Some insoluble material was precipitated below *p*H 5.7, but schlieren diagrams continued to exhibit peaks corresponding in an orderly way to five components down to *p*H 4.79. Below this level it is not possible on the basis of data in hand to correlate the diagrams obtained with the behavior of the proteins above *p*H 4.79.

(9) Longsworth, Shedlovsky and MacInnes, *J. Exptl. Med.*, **70**, 399–413 (1939); Moore and Lynn, *J. Biol. Chem.*, **141**, 819 (1941).

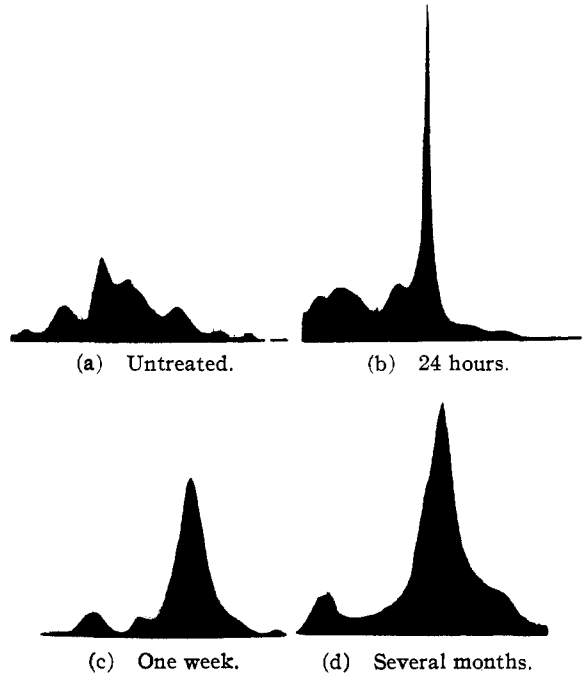
TABLE II

MOBILITY *versus* *p*H DATA ON SUMATRA LATEX SERUM PROTEINS

| *p*H | Component.. I | II | III | IV | V |
|---|---|---|---|---|---|
| 4.79 | 0 | −0.360 | +0.330 | +0.160 | −0.105 |
| 5.66 | − .150 | − .445 | + .245 | + .089 | − .245 |
| 6.85 | − .230 | − .630 | + .200 | + .064 | − .385 |
| 7.81 | − .340 | − .735 | + .170 | − .170 | ..... |
| 8.43 | − .365 | − .755 | + .177 | ..... | − .495 |
| 9.67 | − .530 | − .850 | | − .325 | − .530 |
| 10.18 | − .575 | − .910 | − .212 | − .395 | − .575 |

The wide distribution of isoelectric points on the *p*H scale is a matter of interest. Since two of these isoelectric points occur at *p*H 7.2 and *p*H 9.7, the persistence of positively charged protein components at high *p*H values is demonstrated. This may be accountable for the known stability of fresh unpreserved latex toward the addition of acid, whereas the negative components stabilize with respect to added alkali.

**Effect of Ammonia Treatment of Latex Serum.**—The progressive effects of free ammonia at high *p*H levels on the proteins from unpreserved latex serum are shown by a comparison of the schlieren diagrams (falling boundaries at *p*H 6.85) contained in Fig. 3. The final effect of am-



(a) Untreated. (b) 24 hours.

(c) One week. (d) Several months.

Fig. 3.—Schlieren diagrams showing effects of treatment of latex serum with 1% $NH_3$ for various lengths of time: (a), (b), (c) concentrated 3/1; (d) concentrated 5/1; *p*H = 6.85.

monia treatment is to reduce the number of resolvable components from seven to two.

Ammonia is of interest because of its commercial importance as a latex preservative. In its effect on latex proteins the presence of ammonia constitutes an abnormal condition in the serum and would be expected to produce departures from the characteristic normal pattern for untreated latex serum. It has been found that pathological conditions in human sera sometimes cause an abnormal change in the ratios of the various protein components of the sera. Although the detailed nature of the change in latex protein dissolved in ammonia solution is not known, one important observation may be made. The principal component of the exhaustively ammoniated protein solution has a higher numerical value of negative mobility than the principal component of unpreserved latex protein on the alkaline side of the isoelectric point. This is shown in Table III and probably accounts at least in part for the well-known rise in mechanical stability of latex within a short time after ammonia preservation is applied on the plantations.

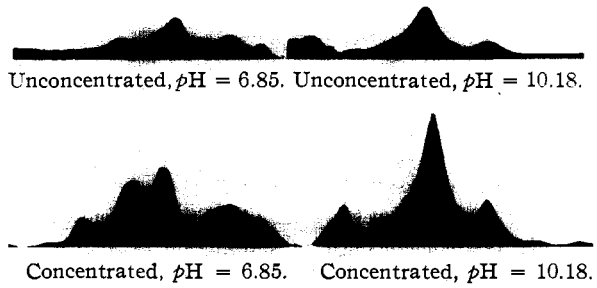
TABLE III

MOBILITY VALUES OF PRINCIPAL PROTEIN COMPONENT OF LATEX SERUM AFTER VARIOUS LENGTHS OF TIME IN 1% $NH_3$ SOLUTION *p*H 6.85

| Time in 1% $NH_3$ | Mobility in microns per sec. per volt per cm. |
|---|---|
| Untreated | −0.210 |
| 24 hours | − .332 |
| 7 days | − .565 |
| Several months | − .465 |

It should be noted that soaps produced by the hydrolysis of resins may also be partially responsible for this increase in stability.

These experiments were all carried out on vacuum dried protein redissolved at a concentration three times that occurring in serum from frozen latex, except as indicated in Fig. 3.



Unconcentrated, *p*H = 6.85. Unconcentrated, *p*H = 10.18.

Concentrated, *p*H = 6.85. Concentrated, *p*H = 10.18.

Fig. 4.—Schlieren diagrams showing effects of vacuum sublimation on latex serum proteins.

**Effects of Vacuum Sublimation.**—Figure 4 shows a comparison of schlieren diagrams obtained with unconcentrated serum and diagrams obtained with concentrated solutions of vacuum dried serum solids.

The diagrams made in experiments with concentrated protein solutions show more detail than those made with dilute solutions, and tend to confirm the latter. The correspondence of the peaks representing the various components is taken to be an indication that no important change in the electrophoretic properties of the proteins was produced by the sublimation process.

**Acknowledgment.**—The writers wish to acknowledge their indebtedness to Mr. H. F. Loomis, of the United States Department of Agriculture, for his courtesy and coöperation in furnishing the fresh unpreserved latex from Florida. The writers are also indebted to Dr. L. G. Longsworth, Dr. Dan Moore and Dr. E. J. Cohn for helpful discussions of experimental methods.

## Summary

1. The serum from unpreserved rubber latex (*Hevea brasiliensis*) contains seven electrophoretically distinct protein components. The proteins from whole serum originating in Sumatra and Florida give very similar results in electrophoresis experiments.

2. The relationship between electrophoretic mobility and *p*H has been determined for five of the seven protein components of unpreserved total latex serum. The results are considerably different from those reported by workers with ammonia preserved latex, and tend to clarify observed differences in the stability behavior of unpreserved and preserved latex.

3. Ammonia preservation treatment rapidly alters the electrophoretic behavior of the native protein components of latex serum and reduces the number of resolvable components from seven to two.

4. The preparation of dry latex protein from rubber free latex serum can be accomplished by the vacuum sublimation of frozen serum. This process does not appear to produce important changes in the electrophoretic properties of the total serum proteins.

5. Minor modifications of the electrodes and of the standard illumination system in the electrophoresis apparatus are described.

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