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Summary

The vapor-pressure curve of hydrogen peroxide has been determined with a considerable degree of accuracy and the relation between pressure and temperature may be represented by the equation,

$$\log P = - \frac{0.05223 \times 48530}{T} + 8.843$$

The following physical constants have been calculated for hydrogen peroxide; boiling point, 152.1°; latent heat of evaporation, 341.5 cal.; Trouton's constant, 27.3; and critical temperature, 459°.

MONTREAL, CANADA

[CONTRIBUTION FROM THE LABORATORY OF COLLOID CHEMISTRY OF THE UNIVERSITY OF WISCONSIN]

MEASUREMENTS OF THE MOBILITY OF EGG ALBUMIN AT DIFFERENT ACIDITIES¹

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In an earlier article,² Svedberg and Jette showed that the well-known U-tube method can be applied to the study of the cataphoresis of protein if one determines the position of the protein layer by photographing its fluorescence when illuminated with ultraviolet light.

In this preliminary paper, no quantitative measurements were reported. The apparatus had not been calibrated to determine the effective potential gradient and the pictures were not distinct enough to make possible accurate measurements of boundary positions. A detailed report by Mr. Jette of the faults and difficulties of the former apparatus helped greatly in constructing a new apparatus with which fairly accurate quantitative measurements were possible. The method has now been used to measure the mobility of egg albumin at different acidities with two different buffer mixtures.

The Apparatus

A brief description of the apparatus is given on pp. 185-187 of Svedberg's "Colloid Chemistry," with drawings which show the cataphoresis tube and the general arrangement for illumination and photography.

Pyrex glass was used in making the cataphoresis tube because of its slight fluorescence. It was made in one piece including the connecting siphon tubes which served

¹ A portion of a thesis submitted by Norman D. Scott in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Wisconsin.

² Svedberg and Jette, *THIS JOURNAL*, **45**, 954 (1923).

as salt bridges. The only stopcock was in the capillary and served to let the protein solution run in at the bottom of the U-tube after the whole system had been brought to uniform temperature. In the actual apparatus, the connecting tubes and electrodes were at the back rather than the sides, as shown in Fig. 1, so that they did not interfere with the illumination. The electrodes were zinc rods immersed in a saturated solution of zinc sulfate.

The applied potential was furnished by 270 dry cells in series. At the start it measured 410 volts and after the last experiment was still 405. The current never exceeded a few milliamperes and no fluctuations of potential were observed in any experiment.

The determination of the effective potential gradient in the region of the boundaries involved finding the cross section of this part of the tube by weighing with water to different heights and the resistance of the whole apparatus when filled with a solution of known conductivity. The cross section was 1.175 sq. cm. and the resistance of the tube when filled with 5% potassium chloride solution at 23° was 3400 ohms. Taking the values given by Landolt-Börnstein³ for the conductivity of 5% potassium chloride solution, one finds that each centimeter of the tube would have a resistance of 12.32 ohms and the total resistance is equivalent to that of a U-tube 276.1 cm. long if of uniform diameter like that in the region of the boundaries. The potential gradient was, therefore, very nearly 1.5 volts per cm.

An air thermostat was used because in the earlier work it had been found almost impossible to prevent the water itself from becoming fluorescent. The thermostat was a double-walled box of Beaver Board, with felt packing between the walls. A rapid stream of air was blown into the box, after passing through coils kept at suitable temperatures. The fact that room temperature often rose above 30° together with the heat from the lamps during the photographic exposures made it necessary to have most of the incoming air several degrees below 25° in order to maintain this temperature, which was kept to within 0.2°.

The quartz mercury-vapor lamps were mounted in iron boxes which could be rolled alongside of the thermostat after they attained maximum brightness. The lamps were the horizontal type furnished by the Cooper-Hewitt Company for use on 220 volts d. c. It was found helpful to place silvered watch glasses behind the lamps to act as concave mirrors. Large beakers full of cupric sulfate solution placed in front of the lamps served the double purpose of filtering out heat waves and helping to focus the ultraviolet light toward the U-tube. The side windows of the thermostat were of special glass intended to pass a large amount of the light of the longer wave lengths of the ultraviolet but practically none of the visible.

Wratten's new ultraviolet filters were used for most of the experiments and were

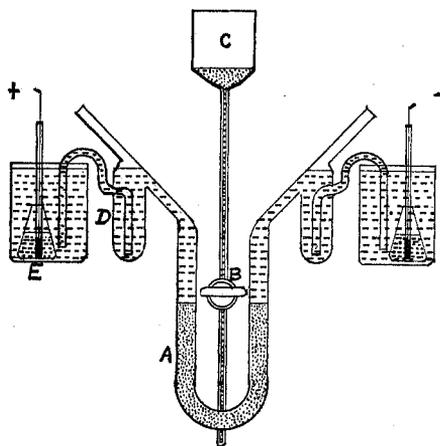


Fig. 1.—Cataphoresis tube. A. Albumin layer. B. Stopcock. C. Reservoir to hold albumin until temperature is uniform. D. Connecting tube filled with buffer solution. E. Zinc electrode in saturated solution of $ZnSO_4$.

³ Landolt-Börnstein, "Tabellen," 1912, p. 1092.

satisfactory. In the last experiments Uviol glass furnished by the Corning Glass Works was used. These filters were practically as selective as Wratten's but were thicker and required longer exposures.

The thermostat was lined with tin foil to minimize extraneous fluorescence. A piece of ruby glass mounted behind the U-tube made an excellent black background. At the front of the thermostat was a window covered with a thin cell filled with a 5% solution of quinine bisulfate in 5% hydrochloric acid. This stopped most of the reflected ultraviolet light, so that practically all of the light that reached the camera was that of the fluorescence of the protein.

A special camera with a Zeiss Tessar 1:4.5 lens was used and Seed's "Gilt Edge 30" photographic plates, 8×10 cm. ($3\frac{1}{4} \times 4\frac{1}{4}$ inches). The exposure averaged ten minutes but varied with the brightness of the fluorescence. The plates were developed to bring out contrast. The measurements of boundary positions were made on the plates rather than on prints. The ratio of the diameter of the tube to that of its image on the plates was 1.109:1.

Preparation of Materials

Eggs were secured less than 24 hours old, the whites separated and diluted with twice their volume of water to cause the globulin to separate. After filtration the solution was transferred to collodion dialysis bags and dialyzed for six weeks. The water was kept saturated with toluene as a disinfectant. The solution was kept in an ice box and for each experiment it was diluted to 0.3% concentration.

The solutions of salts and acids used in the buffer mixtures were made up from the best reagents available. The strength of the acids was determined by titration with barium hydroxide solution and the concentration of the salt solutions determined by analysis.

Inasmuch as the protein and buffer solutions were tested with a hydrogen electrode and brought to within a millivolt of the same reading, these measured values were taken for determining the Sørensen (P_H) values instead of the values given in any published tables.

A Typical Experiment

A typical experiment recorded in detail will make clear both the method of performing the experiment and the measurements and calculations based upon it. The photographs taken in this experiment are shown in Fig. 2 and are somewhat more distinct than any published previously.



Fig. 2.—The boundary movements with solutions of P_H 2.93. Final position superposed at right of original position.

Both the buffer solution and the protein solution were made up with enough disodium hydrogen phosphate to be 0.02 N with respect to sodium. The protein solution contained 0.3% egg albumin. Citric acid was added to each solution until the voltage when tested with a hydrogen electrode was 0.456 volt for each solution.

After the cataphoresis tube had been thoroughly cleaned and steamed out, it was filled up to the stopcock with the buffer solution and the tube and reservoir above the stopcock were filled with protein solution. The tube was then placed in the air thermostat and maintained for an hour at 25° before

taking the first picture showing the original position of the protein layer. When the exposure was finished, 408 volts was applied to the electrodes for an hour. The current was turned off and a final picture taken of the protein position after movement. In all the experiments the positive pole was connected to the right-hand side of the U tube.

Measurement of the plates shows that the level of the boundary in the limbs before the current passed was 9 mm. above the lower reference lines. Afterward on the left it stood 2 mm. below the line and on the right 19 mm. above. It appeared to sink 11 mm. on the one side and to rise 10 mm. on the other with an average movement on the plate of 10.5 mm. toward the cathode.

The mobility is usually expressed as the rate of movement in centimeters per second for unit potential gradient. In this case one has 1.05 cm./3600 sec. divided by 408 volts/276.1 cm. Finally, this quotient

TABLE I

DATA

Series 1. Experiments with buffer mixtures of acetic acid and sodium acetate. Concentration of sodium, 0.02 *N*; of egg albumin, 0.3%

<i>pH</i>	Duration of experiment Minutes	Movement on plate Mm.	Mobility in cm./sec. per volts/cm. $\times 10^5$	Potential difference in volt between particles and solution
6.51	135	6	5.51	-0.0069
3.54	90	11	15.61	.0189
3.12	70	10.5	18.61	.0234
3.12	80	12	18.61	.0234
4.26	100	9.25	11.47	.0141
4.52	120	7.5	7.75	.0097
5.19	60	1	2.07	+ and - .0026
6.58	60	3.25	6.72	- .0084
4.77	60	2	4.13	.0051
3.89	60	7	14.47	.0180
3.89	60	7.25	14.99	.0187
4.97	60	1	2.07	- .0026
5.55	55	1.75	3.96	- .0049
5.70	60	2	4.15	- .0052

Series 2. Experiments with buffer mixtures of disodium hydrogen phosphate and citric acid. Concentration of sodium, 0.02 *N*; of egg albumin, 0.3%

3.47	60	8	16.60	0.0207
2.93	60	10.5	21.79	.0272
5.57	60	1.75	3.66	- .0046
3.93	60	5.75	12.03	.0150
4.44	60	2.75	5.77	.0072
4.93	60	2.25	4.72	- .0059
7.09	50	4.75	11.92	- .0149
4.79	60	1	2.09	- .0026
5.08	60	4	8.37	- .0104
6.26	60	6.75	13.95	- .0174
2.53	60	9	18.83	.0235

must be multiplied by 1.109, the ratio of the size of the tube to its image on the plate. The mobility is thus found to be 21.79×10^{-5} .

One can calculate the difference of potential between the protein particles and the solution by means of the formula used by Burton for other colloids: $V \cdot 1/d = \frac{4 \pi \eta v}{K \cdot X}$, when V is the potential difference between the particle and solution, $1/d$ practically unity, η the viscosity, v the velocity of movement in centimeters per second, K the dielectric constant of the medium and X the potential gradient, both V and X being in electrostatic units. Substitution of the data of this experiment gives 0.0272 volt as the potential difference between the particles and the solution the particles being positive with respect to the solution. In general the figures for mobility must be multiplied by 124.7 to give the potential difference in volts. In Table I a minus sign before the potential difference means that the particles moved toward the anode.

Discussion

Although the electrical behavior of proteins has been studied a great deal, particularly with reference to the sign of the charge on the protein, there have been few, if any, direct quantitative measurements of the mobilities of natural proteins.

Hardy⁴ made some measurements by the U-tube method of the mobility of serum globulin suspensions in acid and basic solutions. Pauli⁵ has attempted to determine the mobility of serum albumin by a combination of conductivity and potentiometric measurements. Loeb⁶ has quite recently published some measurements of the mobility of collodion particles coated with gelatin, which were observed in the field of an ultramicroscope. The fact that the uncoated particles had even greater mobility than the coated ones leaves a doubt as to the significance of the measured mobilities. The measurements which we have recorded are, therefore, practically the first direct quantitative measurements of the mobility of a natural protein.

Since there is considerable difference of opinion as to whether protein solutions are best thought of as true solutions or as colloidal dispersions, it is interesting to note how the observed variations in mobility fit with the two conceptions.

The fact that the boundaries shift as boundaries is not conclusive evidence for either conception, for the moving-boundary method has been used to measure the mobility of both ions and colloid particles. In the case of ions, however, the uniform mobility is probably due to an identity

⁴ Hardy, *J. Physiol.*, **33**, 251 (1905).

⁵ Pauli, "Colloid Chemistry of the Proteins," P. Blakiston's Son and Co., Philadelphia, 1922, p. 127.

⁶ Loeb, *J. Gen. Physiol.*, **5**, 109, 395, 505 (1923).

in the charge, size, hydration, etc., of the various ions. In the case of colloid particles, the size may vary largely and yet the particles move with the same speed because the larger ones have enough extra charge to make up for the extra resistance due to their larger size.

Although more information is greatly needed concerning the size and distribution of sizes of particles in protein solutions, it seems probable that the particles vary considerably in size and that their behavior is enough like that of other colloids to warrant the use of Burton's formula for calculating the potential difference between the particles and the solution.

In Fig. 3 the mobilities have been plotted against the corresponding P_H values for the two buffer mixtures. The curves show that the mobility

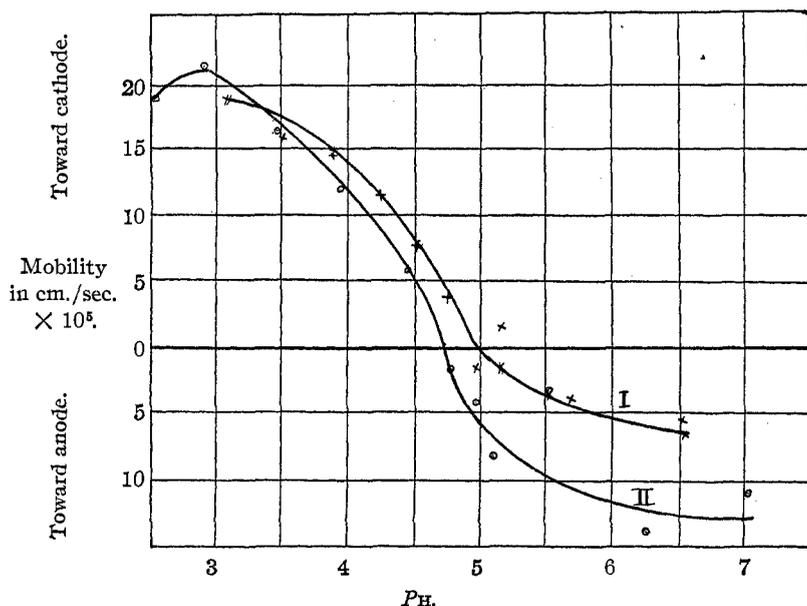


Fig. 3.

increases steadily as one goes in either direction from the iso-electric region. This gradual increase in valence or charge is again more easily thought of as similar to that of colloid particles. When the mobilities of two similar ions which are different in valence are compared, such as those of phosphoric acid $H_2PO_4^-$ and HPO_4^{2-} the mobility of the divalent ion is nearly twice that of the monovalent ion. Our results do not seem to justify considering the protein particles as definitely monovalent, divalent, etc. In Fig. 3 where the movement toward the anode is considered as "negative cataphoresis" even the iso-electric point does not show a very pronounced break.

With regard to the shape of the curves, it is noticed that there seems to be a tendency for the P_H -Mobility curves to approach a straight line over a considerable range on the acid side of the iso-electric point. This part of the curves is the most reproducible. The fact that the P_H scale is logarithmic suggested an analogy to Freundlich's adsorption law. If one attempts to apply a formula of the type, $\text{Mobility} = a.C_H^b$, it will be found that the results do not fit the formula, since the curve is only half logarithmic. However, such a formula would be based on an assumption that the mobility is proportional to the charge on the particles, which is not true even for the simplest similar ions where doubling the valence does not quite double the mobility but increases it, on the average, by more nearly 1.8 times. The charge on the protein may be due to a process very similar to adsorption.

The iso-electric point of egg albumin has been carefully determined by many investigators using different methods, so that our results add little to the knowledge of its location. The method used has the advantage that all the determinations can be plotted so as to help locate the iso-electric point. Curve I shows a region of slight mobility between P_H 4.7 and P_H 5; while Curve II shows a more definite minimum at about P_H 4.7.

A number of investigators have reported that in the iso-electric region there sometimes appears to be simultaneous movement toward both electrodes. Some doubt has been left as to whether the movement was really due to the electrical field or was merely diffusion. The photographic method gives visible evidence in several runs of an upward movement toward both electrodes, which is much greater than that observed when the tube was left for the same time with no potential applied. This point of the coexistence of oppositely charged particles is one of considerable interest and is being studied further.

The general similarity of the two curves indicates that the hydrogen-ion and hydroxyl-ion concentrations are the main factors in determining the mobilities of proteins, but the differences in the shapes of the curves indicate that other ions are not without effect.

In the second series where it was possible to increase the acidity to a P_H of 2.53, there seems to be quite a falling off in the mobility. A similar turnover has often been noted in the properties of proteins in strongly acid solutions. It is interesting to note that the fluorescence itself undergoes a visible change with changes in acidity and appears at a maximum in the region of maximum mobility. In such solutions it would be described as yellow-green, while in the less acid regions it is more blue or violet. The fluorescence itself will be worth further study.

Because of the rapid decomposition of egg albumin in alkaline solutions, no attempts were made to work at a P_H over 7, and the part of the curve

on the neutral and basic side of the iso-electric point is therefore too short and indefinite to enable one to determine whether there is a similar maximum on this side of the iso-electric point, also.

Summary

The method of studying the cataphoresis of proteins by the U-tube method, determining the position of the protein layer by photographing its fluorescence when illuminated with ultraviolet light has been developed and used to measure the mobility of egg albumin at different acidities. Buffer mixtures of sodium acetate with acetic acid and of disodium hydrogen phosphate with citric acid were used over a range of P_H from 7 down to about 3 and 2.5, respectively.

The two buffer mixtures give similar results in general indicating a predominant influence of the hydrogen-ion concentration on the mobility. The sodium acetate and acetic acid mixture gives a broader region of minimum mobility, in which there is evidence of movement toward both electrodes.

The maximum mobility observed was toward the cathode 21.79×10^{-5} cm. per second at a P_H of 2.93.

Further studies by similar methods are in progress at the University of Wisconsin and at Upsala.

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[CONTRIBUTION FROM THE FAYERWEATHER CHEMICAL LABORATORY OF AMHERST COLLEGE]

MOHR'S METHOD FOR THE DETERMINATION OF SILVER AND HALOGENS IN OTHER THAN NEUTRAL SOLUTIONS

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The well-known method of Mohr for the volumetric determination of silver and halides, using potassium chromate as indicator, has the serious limitation that it can be used only in neutral solution. The Volhard method, using ferric alum as indicator and ammonium thiocyanate to precipitate the silver, while applicable to acid solutions, has the disadvantage that when chloride is to be determined it is necessary to filter the silver chloride from an excess of silver nitrate and then titrate the excess of silver nitrate with thiocyanate. It is possible, of course, to use Mohr's method with a solution which is originally acid or alkaline by neutralizing exactly, using methyl red as indicator and titrating to the appearance of a yellow color, but the method is rather tedious.

Apparently it has not been realized that strict neutrality is not necessary for the successful use of Mohr's method. This method depends