A NEW METHOD FOR DETERMINATION OF THE MOBILITY OF PROTEINS

BY THE SVEDBERG AND ARNE TISELIUS

The mobility of egg albumin was determined by Svedberg and Scott[1] with the moving-boundary method, using the fluorescence of the albumin in ultraviolet light to make it visible. The mobilities determined were of about the size to be expected, and the iso-electric point was found to coincide with that found by other methods.

Some work upon the fluorescence of egg albumin performed in this Laboratory by O. Quensel[2] showed, however, that at least some of the fluorescence probably is not due to the albumin itself. The yellow-green fluorescence of the more acid solutions can be separated from the albumin. Possibly some substance partially adsorbed on the protein causes the fluorescence. It seemed, therefore, necessary to find another method of observing the moving boundary in order to test the results obtained by Svedberg and Scott.

All proteins have a very strong absorption in the ultraviolet of wave lengths lower than $300\mu\mu$. Light absorption, in general, is not so sensitive to minute changes in a system, and a considerable absorption is more likely to be caused by a considerable amount of a substance than is fluorescence. Photographing the moving boundary in ultraviolet light should, therefore, give a more reliable method. Moreover, Svedberg and J. B. Nichols in this Laboratory have made determinations of the molecular weight of egg albumin with the ultracentrifuge, using the ultraviolet absorption to determine the changes in concentration of protein produced by the centrifugal force. The value found by this method is in very good agreement with the value 34,000 generally accepted[3] and may therefore be regarded as proof of the applicability of the light absorption method, too.

In order to show the possibilities of the method we have made some measurements of the mobility of egg albumin in acetic acid–sodium acetate buffer solutions of different acidities. On account of the rather crude cataphoresis apparatus and camera used in the present investigation, the results are not of the degree of accuracy obtainable by this method and must be considered as quite preliminary. An apparatus allowing more accurate determinations is under construction.

---

[1] THIS JOURNAL, **46**, 2700 (1924). See also Svedberg and Jette, *ibid.*, **45**, 954 (1923).

[2] Not yet published.

[3] See, for instance, Cohn, *Physiol. Rev.*, **5**, 3, p. 360 (1925). Sørensen, *Compt. rend. trav. Lab. Carlsberg*, **12**, 262 (1915–17).

The Apparatus.—As source of light a quartz mercury-vapor lamp of the vertical type (L, Fig. 1) furnished by Heraeus for use on 220 volts d. c. was used. It was mounted in a double-walled iron box, cooled by water circulating between the walls. The light first passed a water filter (W) to absorb heat radiated from the lamp, then a chlorine filter (C), that absorbs the part 300–380$\mu\mu$ of the ultraviolet,[4] then a mat screen (M) and the cataphoresis tube (T), a bromine filter (B) that absorbs the region 380–530$\mu\mu$ and the camera, (K).
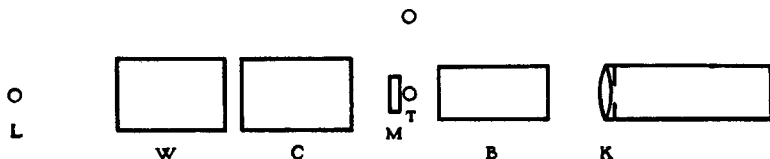

Fig. 1.—L, lamp; W, water cell; C, chlorine cell; M, mat screen; T, cataphoresis tube; B, bromine cell; K, camera.

The windows of the filters, the screen, the tube and the lens of the camera had to be made of quartz, because glass is opaque to the short-waved ultraviolet light used. The filters permit only light of wave lengths lower than 300$\mu\mu$ and higher than 530$\mu\mu$ to reach the camera. The sensitivity of an ordinary (not orthochromatic) photographic plate is however, much greater in the ultraviolet, and therefore the visible light that comes through does not interfere.

The camera lens (K) had 205 mm. focal distance for the ultraviolet used. Just behind the lens was placed an 8mm. stop. The distance from cataphoresis tube to lens was the same as that from lens to plate, namely, 2 × 205 mm. Therefore, the tube and its image were of equal size. Afga Extra Rapid plates, 4$^1/_2$ × 6 cm., were used in the first experiments with 90 seconds' exposure. Later, Imperial Eclipse plates were used, making it possible to

Fig. 2.—Cataphoresis apparatus; ff′, funnels for the zinc sulfate solution; tt′, quartz tubes.

reduce the time of exposure to 15–20 seconds. In neither case could any blackening be obtained on the plate when a glass plate was used in front of the lens, allowing only the visible light to pass. With these short
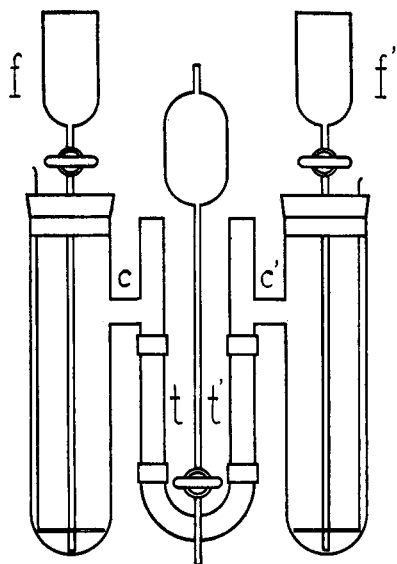
[4] Peskow, *J. Phys. Chem.*, **21**, 382 (1917).

times of exposure any decomposition of the albumin in the ultraviolet would seem improbable.[5]

The cataphoresis apparatus (Fig. 2) was of the ordinary U-tube type with reversible electrodes (zinc rods in a saturated solution of zinc sulfate). Some improvements over the apparatus used by Svedberg and Scott were made. First, the zinc sulfate solution was allowed to form a layer below the buffer solution before starting an experiment by causing it to flow slowly through the capillary tubes from the funnels ff' until the electrodes were completely covered with sulfate. Second, the connections cc' were made as short as possible. In this way the voltage necessary to produce a potential gradient of 1 volt per cm. in the U-tube could be reduced to 47.3 volts from 276.1 volts required in the Svedberg-Scott apparatus.

As mentioned above, it was necessary to use quartz for those parts of the apparatus where the boundaries migrate. Therefore it was made in parts, as shown in the figure: (1) the two electrode vessels with their side tubes; (2) the lower part of the U-tube with reservoir for the albumin, capillary and stopcock; (3) two specially selected quartz tubes tt', as clear, uniform and free from bubbles as possible, that were connected with the other parts by rubber tubing; (1) and (2) were made of Jena "Normalglas." The whole apparatus was mounted on a wooden stand. Experiments were made in a room with sufficiently constant temperature.

The potential required was furnished by a storage battery of 50 volts. The voltage was measured with a precision voltmeter and was in general quite constant during the experiment. The current was only about 0.7 milliampere.

The resistance of the whole apparatus, when filled with 0.02 N potassium chloride solution and the same quantity of saturated zinc sulfate solution above the electrodes as was always used in the experiments, was $w = 33,210$ ohms at 16.0°. The cross section was determined by filling the quartz tube with water to different heights and weighing. The mean cross section in the part used was $q = 0.622$ sq. cm. From these data the potential necessary to produce a potential gradient of 1 volt per cm. in this part is $q \times w \times \kappa = 47.3$ volts, if κ be taken[6] as 0.002294 for 0.02 N potassium chloride at $t = 16.0°$. Since the resistance of the apparatus is inversely proportional to the conductivity of the solution in it, the product $w \times \kappa$ is always the same. Therefore, independent of conductivity, the potential 47.3 volts always produces unit potential gradient in the tube.

Materials.—The egg albumin used was prepared as in Svedberg and Scott's work, but the ordinary dialysis in collodion bags at 0° was interrupted after six days and continued in an electrodialyzer with parchment

[5] Mond, *Pflüger's Arch.*, **196**, 540 (1922).

[6] Landolt-Börnstein-Scheele-Roth, "Tabellen," **1923**, p. 1098.

membranes and graphite electrodes according to Pauli.[7] After two days the conductivity was 1.6×10^{-5} mhos. The slightly yellow solution was filtered and kept in a Jena glass bottle in ice. The concentration of this stock solution was found to be 3.7% by drying a sample of 1 cc. at 105° to constant weight.

The curve in Fig. 3 shows the absorption coefficients at different wave lengths for a 0.1% solution of the egg albumin used. Variation of P_H (4.0–4.8–5.6) had no perceptible effect on the absorption. The measurements were made with a Judd Lewis sector photometer.

The buffer mixtures were prepared from stock solutions of 0.2 N sodium acetate (Kahlbaum "Zur Analyse") and 1 N acetic acid. Toluene was added to the acetate as a preservative. The sodium acetate concentration was always 0.02 N both in buffer and buffer-albumin mixtures. The latter were always 1% with respect to albumin.

Fig. 3.—Absorption coefficient $(1/d \times \log I_0/I)$ at different wave lengths for a 0.1% solution of the egg albumin used.

Sufficient acetic acid was added to give the same hydrogen-ion concentration in each. Under these conditions the conductivity on both sides of the boundary is also very nearly the same. This is a necessary condition for using the moving-boundary method in this form.

P_H **Measurements.**—The acidity was determined by the potentiometer method, the hydrogen electrode being measured against a 0.1 N calomel cell. The measurements were made at 16° and the Sørensen value (P_H) calculated from the values E by the formula $P_H = (E - 0.3382)/(0.0001984 \times 289)$ according to Sørensen.[8] The values of E obtained were always in close agreement with those calculated from tables. Measurements were made with solutions from $P_H = 3.40$ to $P_H = 5.75$.

A Typical Experiment.—When the solutions had been prepared and the Sørensen values measured, the apparatus was filled with buffer solution and the reservoirs were filled with the albumin-buffer mixture and zinc sulfate solution. The filled apparatus was allowed to stand overnight to attain uniform temperature. The experiment was started by opening the stopcocks for the sulfate; after about 20 minutes the stopcock for the albumin could be opened. In order to obtain a very slow rate of
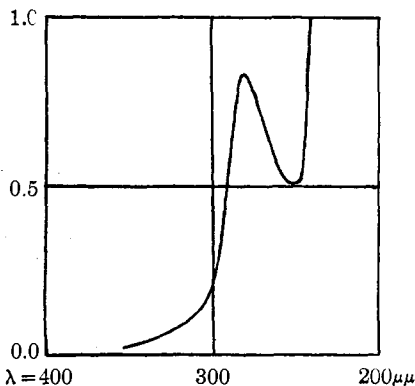
[7] Pauli, *Biochem. Z.*, **152**, 355 (1924).

[8] Sørensen, *Compt. rend. trav. Lab. Carlsberg*, **15**, 6 (1924).

flow in the beginning, which is very important for getting sharp boundaries, a very fine glass capillary was connected with the reservoir by a narrow rubber tubing. By using a set of capillaries of increasing diameter it is possible gradually to increase the rate of flow without disturbing the apparatus itself. When a sufficient volume had passed into the U-tube, the stopcock was closed. Now a photograph of the position of the boundary in the tube was taken, and the voltage was applied. It was impossible to photograph both boundaries with the arrangements used. Therefore, the boundary in most experiments, after having migrated a suitable distance in one direction was allowed to go back again by changing the direction of the current.
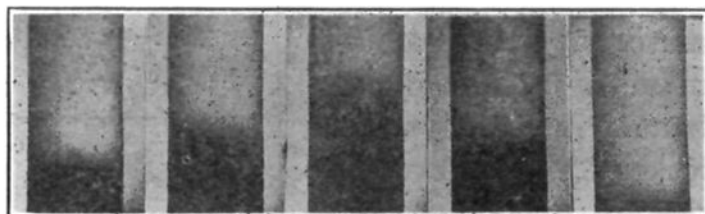

Fig. 4.—Migration of the boundary in an experiment.

All plates were developed over the same length of time. The measurements of movement were made on the plates, not on prints. Fig. 4 is a typical series of photographs for $P_H = 3.39$, 48.9 volts, giving the position of the boundary after 0 minutes, 60 minutes, 120 minutes (change of direction of current), 55 minutes, 145 minutes. The corresponding distances as measured on the plates are 5.5, 11.0, 5.0, 11–12 mm. The last value is, however, very doubtful on account of the blurring of the boundary, and was not used. Generally the experiments were continued until the boundary became too diffuse. As values of v we get, after multiplying the measured distances by 0.983 (the ratio of the size of the tube to that of its image on the plate): $14.5 \cdot 10^{-5}$, $14.5 \cdot 10^{-5}$, $14.4 \cdot 10^{-5}$ ($12.6 \cdot 10^{-5}$) cm.^{2} sec.$^{-1}$ volt^{-1}. In general, the temperature was $13.5°$; in only the last experiments was it somewhat higher (in the case mentioned $15.5°$). To get comparable results all values were reduced to $13.5°$ by multiplying by $\eta_t/\eta_{13.5}$, η_t being the viscosity of water at $t°$.

TABLE I

P_H	Mobility, $v \times 10^5$	Temp., °C.	$v \times 10^5$ at 13.5°
3.40	+14.5	15.5	+13.6
3.96	7.3	13.5	7.3
4.27	2.5	13.5	2.5
4.50	+ 1.3	13.5	+ 1.3
4.81	− 0.7	14.0	− 0.7
4.99	3.4	16.0	3.2
5.25	4.7	13.8	4.6
5.36	5.8	12.6	6.1
5.75	− 7.9	13.5	− 7.9

The values of v have been plotted against P_H values in Fig. 5, Curve I. Evidently the iso-electric point occurs at 4.7. Curve II shows Svedberg and Scott's values, reduced to 13.5°. The very marked difference in form and position of the curves can probably not be explained by the small differences in experimental conditions only, but may be largely due to the properties of the fluorescence mentioned above. The errors of experiment seem to be less in the absorption experiments. In fact, it is much easier to get good pictures with very short times of exposure in the latter case. Fluorescence photography needs an exposure time of about 10 minutes, absorption photography only 15 seconds. Taking more than two pictures in the same fluorescence cataphoresis experiment, therefore, is very difficult, and the degree of accuracy accordingly lower.
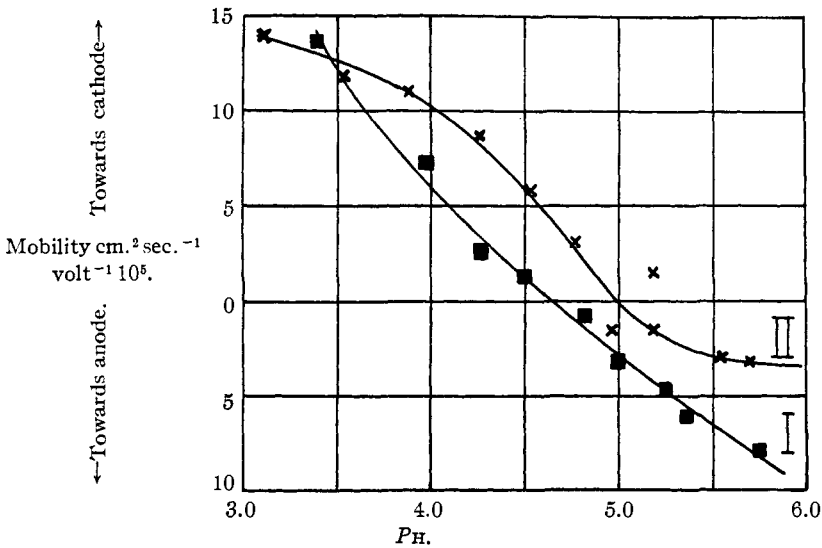

Fig. 5.—Values of the mobility obtained by the absorption (I) and by the fluorescence (II) method.

The expenses connected with these experiments have been defrayed by grants from the Foundation "Therese och Johan Anderssons Minne" and from the Nobel Fund of Chemistry.

Summary

It is proposed to study the mobility of proteins by the moving-boundary method, making the protein visible by photographing the cataphoresis tube in ultraviolet light of wave lengths below $300\mu\mu$, obtained from a mercury lamp after passing through chlorine and bromine filters. The method is used in a preliminary study of the mobility of electrodialyzed egg albumin in a buffer mixture of acetic acid and sodium acetate of differ-

ent acidities. The mobility varied between 13.6×10^{-5} cm.^{2} sec.$^{-1}$ volt^{-1} towards the cathode at $P_H = 3.40$, and 7.9×10^{-5} cm.^{2} sec.$^{-1}$ volt^{-1} towards the anode at $P_H = 5.75$, all at $t = 13.5°$. The values obtained show some departure from those found by Svedberg and Scott using fluorescence to make the protein visible. It is shown that the absorption method is to be considered as more reliable.

Further work by this method with an improved apparatus is in progress.

UPSALA, SWEDEN

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

OXIDATION POTENTIALS IN LIQUID AMMONIA INVOLVING QUATERNARY AMMONIUM RADICALS AND ALKALI METALS

BY GEORGE SHANNON FORBES AND CURTIS ELLIOTT NORTON

This research was undertaken primarily to compare the oxidation potentials of systems of the type $NR_4 - \ominus \rightleftharpoons NR_4^+$, where the R's are various alkyl groups. It appeared that such a potential series would be of value in the systematic study of alkyl groups and of quinquivalent nitrogen alike. Later we included the alkali metals, the better to correlate them with the quaternary radicals.

An examination of the literature indicated the difficulties of such an undertaking. Crotogino[1] electrolyzed water solutions of mono-, di- and trimethyl- and triethylammonium oxalates on a mercury cathode, and compared the positions of the breaks on the polarization curves. McCoy and Moore[2] prepared mono- and tetramethylammonium amalgams by electrolysis of solutions in absolute alcohol; the e.m.f. of their products measured against an aqueous calomel electrode varied with time. Podrouzek[3] electrolyzed water solutions of ammonium, tetramethyl and tetraethyl salts upon dropping mercury cathodes, obtaining results not very similar to those of Crotogino. Examination of the above-mentioned papers, and of papers on ammonium amalgams, revealed many objections to the use of mercury and to working temperatures above its freezing point. On the other hand, the literature concerning the preparation of quaternary ammonium radicals in liquid ammonia offered a better prospect of arranging the former in a potential series.

Palmaer[4] noted blue streaks near a cathode in a solution of tetramethylammonium chloride in liquid ammonia. Eleven years later Kraus[5] observed the same effect with tetramethyl and also with tetrapropyl salts.

[1] Crotogino, Z. Elektrochem., 7, 648 (1901).

[2] McCoy and Moore, THIS JOURNAL, 33, 273 (1911).

[3] Podrouzek, Rec. trav. chim., 44, 591 (1925).

[4] Palmaer, Z. Elektrochem., 8, 729 (1902).

[5] Kraus, THIS JOURNAL, 35, 1732 (1913).