on an iron cylinder, recorded on a recording milliammeter. The compensation apparatus works automatically by means of a system of relays and a drum resistance rotated by a motor. From the relation between time and current the sedimentation curve can be found and from that curve the distribution curve can be calculated.

4. To illustrate the procedure, the sedimentation and distribution curves of a mercury hydrosol and a gold hydrosol have been given.

5. A method has been described for determining the distribution of size of particles, depending on the variation of concentration with height in a sedimenting system. As an example, a gold hydrosol was studied, the concentration at different heights being measured by means of the light absorption.

6. The theory for the development of an analogous method depending on the use of centrifugal force has been discussed.

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

[CONTRIBUTION FROM THE LABORATORY OF PHYSICAL CHEMISTRY OF THE UNIVERSITY OF UPSALA]

THE CATAPHORESIS OF PROTEINS[1]

BY THE SVEDBERG AND ERIC R. JETTE

Received January 24, 1923

Introduction

In view of the important position which the proteins at present occupy in the field of colloid chemistry, it has seemed that the publication of this preliminary paper on the behavior of the proteins in an electric field would be of value, as it gives a new method for attacking the problem. That proteins migrate in an electric field and that the rate and direction of this migration varies with hydrogen-ion concentration and possibly other factors, is well known. Michaelis[2] and others have used these facts to determine the iso-electric point of various proteins. However, as the methods previously used[3] depended on a chemical analysis of a solution into which the protein migrated, a direct observation of the rate of movement of the protein boundary has never been possible. For the purposes of theoretical consideration, such measurement is highly desirable, since by this means it would be possible to calculate the potential difference between the protein particle and the medium in contact with it. By using

[1] Presented before the Physical Chemistry Symposium, Rochester Section, American Chemical Society, January 19th and 20th, 1923.

[2] Michaelis and others, *Biochem. Z.*, **24,** 79 (1910); **27,** 38 (1910); **28,** 103 (1910); **33,** 182 (1911); **47,** 250 (1912).

[3] Michaelis, *Biochem. Z.*, **16,** 181 (1912).

the method described in this paper, such determinations may be made and the various factors affecting the behavior of the protein in an electric field studied more closely.

The Principle of the Method

In ordinary light a dilute protein sol can be distinguished from a colorless solution only with great difficulty. However, it has been observed that proteins absorb the ultraviolet wave lengths very strongly. It was at first intended to apply this fact directly and photograph a cataphoresis tube containing a protein layer under a layer of a solution which did not absorb these wave lengths, using merely the difference in the absorption to mark the position of the boundary on the plate. In the very beginning of the investigation it was observed that the absorption of the ultraviolet wave lengths was accompanied by a strong fluorescence. This is much more readily photographed, but in order to obtain a fluorescence strong enough to define sharply the boundary surfaces between the 2 liquids, a very powerful source of ultraviolet light was required, which required the experimenter to wear protective glasses that very markedly reduced the intensity of the fluorescence that reached the eye. Direct observation on the boundary surfaces was thus made impossible, and measurement by means of the photographic plate was then adopted. This phenomenon, therefore, enables us to measure the cataphoresis of many proteins by a method which is in many respects similar to that used by Burton[4] and others in the case of colored inorganic sols. The appearance of the boundaries may be seen in Fig. 1, which is a fluorescence photograph of the cataphoresis apparatus removed from the thermostat.[5]

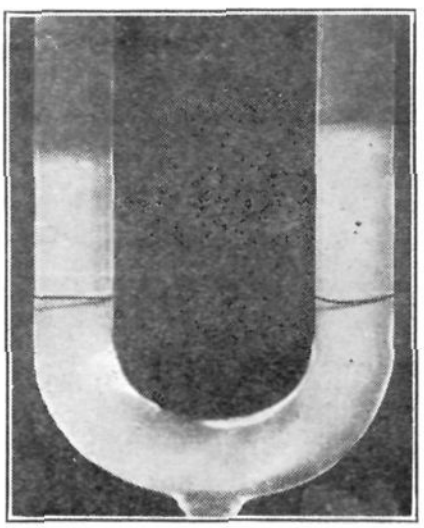

Fig. 1.

The Apparatus

As may be seen from Fig. 2, the cataphoresis tube is of the same type as described by Burton.[4] But since proteins migrate very slowly in an electric field and especially slowly near their iso-electric points, it was necessary to pass the current through the solution for a comparatively long time in the same direction. This required the removal of the electrodes

[4] Burton, *Phil. Mag.*, [6] **11**, 440 (1906).

[5] The intensity of the fluorescence is somewhat reduced when the specimen is inside a water thermostat, due to absorption of the ultraviolet wave lengths by the glass of the thermostat.

as far as possible from the sol and the use of non-polarizing electrodes as described by Michaelis.[3]

In Fig. 2, A is a U-tube with the capillary B entering at the bottom, and the bulb C for allowing the sol to reach the same temperature as the solution in A before being run in. The non-polarizing electrodes DD′ consist of zinc rods immersed in a saturated zinc sulfate solution. The electrode vessels were connected by a liquid bridge K to equalize the levels. Tube E served as the outlet tube for the excess solution from both arms of the U-tube during filling. Tube F was for convenience in breaking the liquid bridge between the intermediate vessels HH′ which would otherwise serve as a short circuit. Tubes GG′ allowed the air to escape from the intermediate vessels while the apparatus was being filled.

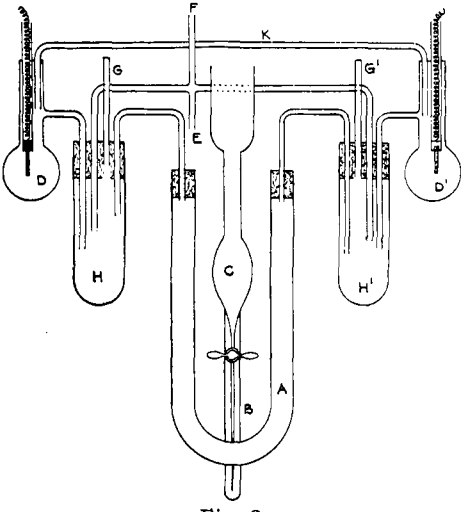

Fig. 2.

The bulbs of the electrode vessels were filled with the saturated zinc sulfate solution and the remainder of the apparatus with a buffer solution of the desired hydrogen-ion concentration, except Bulb C. The solution containing the protein was placed in C and after reaching the proper temperature, it was slowly run into the U-tube under a slight pressure from an outside source. After removing the connecting bridge and breaking the liquid column connecting HH′ the apparatus was ready for photographing.

The arrangement of the apparatus for photographing included a small water thermostat, the box containing the quartz mercury lamp and special glass screens and the camera. The glass screens were Wratten's new ultraviolet filters which passed practically only the wave length $\lambda = 366\mu\mu$ and a small amount of $\lambda = 334\mu\mu$. The quartz mercury lamp was made by Hereaus and gave 3000 candle power with 3.5 amperes and 185 volts. The camera lens was a Zeiss-Tessar (1:4.5) with a focal length of 21 cm. To prevent the ultraviolet light reflected from the glass walls of the thermostat from entering the camera, a cell 2mm. thick, filled with a 5% solution of quinine bisulfate in 5% hydrochloric acid, was placed before the lens.

Results

Fig. 3 shows the position of the protein boundaries before A and after B the current has passed through an egg albumin sol containing 0.3% of dry albumin in an acetic acid-sodium acetate buffer solution with a hydrogen-ion concentration of 3.8×10^{-5}. The time interval between A and B was 429 minutes. Fig. 4 shows the movement of a similar sol. in a hydrogen-ion concentration of 0.3×10^{-5} during a time interval of 40 minutes. The amount of displacement indicates that the iso-electric point is not far from the former value, for this particular sample of egg albumin.

Since this paper is of a preliminary nature, discussion of the previous

literature on the subject and presentation of quantitative data will be reserved for later publication. Work is now in progress with apparatus designed to allow greater ease of manipulation, the formation of sharper

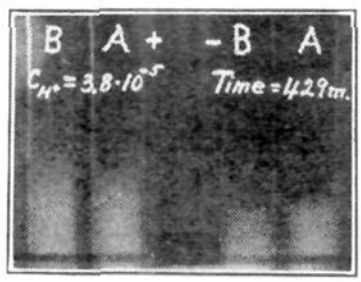

Fig. 3.

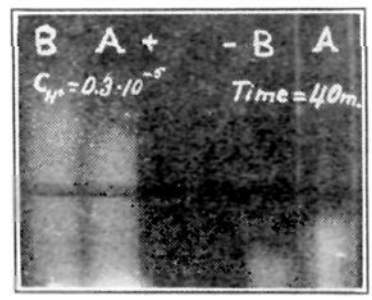

Fig. 4.

boundaries between the two liquids, and to decrease still further the electrode effects.[6]

Summary

1. A method has been described by which the rate of migration of a protein particle under the influence of an electric field may be determined experimentally.

2. The method depends on the fact that under the illumination by ultraviolet light, proteins fluoresce. This fluorescence may readily be photographed and the position of the boundaries of the protein sol measured on the photographic plate.

3. Photographs are included showing qualitatively the effect of changing the hydrogen-ion concentration of a 0.3% egg albumin sol on its rate of migration.

CHEMICAL LABORATORY
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

[6] The junior author has been enabled to carry on this work through the grant of a stipend from the American-Scandinavian Foundation to which he wishes to express his appreciation.