the color standards have been preserved for from 4 to 8 weeks or more.

2. The method is particularly suited to the average technical laboratory because of its accuracy, simplicity of technique, and the use of ordinary C. P. "Analyzed" salts instead of the highly purified buffer salts recommended by Clark.[1]

HIGHLAND PARK, MICHIGAN

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

THE DETERMINATION OF THE DISTRIBUTION OF SIZE OF PARTICLES IN DISPERSE SYSTEMS[1]

BY THE SVEDBERG AND HERMAN RINDE

Received January 24, 1923

Some years ago one of us[2] pointed out the eminent importance of determinations of the distribution of size of particles in disperse systems and suggested two experimental methods founded on the resistance law,

$$f = 6\pi\eta r v \qquad (1)$$

where f is the friction developed when a sphere of radius r is moving with the velocity v in a liquid possessing the viscosity η.

The force applied to keep the particle in constant motion was, as suggested in the paper referred to, (1) the force of gravitation, (2) the shocks from the surrounding molecules of the liquid. In other words, the first method applies Stokes' law,

$$r = \sqrt{\frac{9\,h\eta}{2(s_p - s_d)gt}} \qquad (2)$$

where h is the height of fall, s_p and s_d the density of the particles and the dispersion medium, respectively, g the gravitational constant and t the time of fall. The second method applies the Einstein law for the Brownian movements

$$r = \frac{RT}{N} \cdot \frac{1}{3\pi\eta\,\overline{(x_2 - x_1)^2}} \cdot t \qquad (3)$$

where R is the gas constant, T the absolute temperature and $\overline{(x_2 - x_1)^2}$ the average square of the displacement of the particle in the time t.

Some series of measurement illustrating the use of the first method were given. The fall of a statistically sufficient number of particles was measured with the aid of a microscope provided with a dark-field condenser and the frequency curves of the size of particles were drawn. The particles studied in this investigation were all of spherical shape (mercury, gamboge, fats).

A preliminary attempt to utilize the second procedure was made by

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

[2] Svedberg and Estrup, *Kolloid-Z.*, **9**, 259 (1911).

Svedberg and Nordlund, but the rather great number of determinations necessary for sufficiently reliable statistics discouraged us from pursuing the work.

In order to simplify the application of the first method Odén has worked out a very ingenious procedure of recording the amount of sediment accumulating on a plate immersed in the disperse system and suspended on a balance, and has shown how the frequency curve can be found from the data given in the sedimentation curve.[3] For the percentage mass of particles, dS, corresponding to the radius interval, dr, of the frequency or distribution curve we have

$$\frac{\mathrm{d}S}{\mathrm{d}r} = -\frac{2t^2}{r}\cdot\frac{\mathrm{d}^2P}{\mathrm{d}t^2} \tag{4}$$

where t is the time of fall, P the total weight of sediment corresponding to the time t, and where r can be found from Equation 2.

Odén has with great success used this method for the study of the distribution of size of the particles of various coarse-grained sols, clays and precipitates of barium sulfate, etc.[4] Nordlund has measured the distribution curves of mercury emulsions.[5]

Odén's apparatus was originally devised for the study of rather coarse systems such as clays, and required comparatively large quantities of particles, namely, about 1 g. The importance of size-frequency curves in colloid chemistry is constantly increasing and we have, therefore, again taken up the problem of the determination of such curves. On the one hand, we have tried to introduce such improvements in Odén's procedure as to make it meet the requirements of the colloid chemist better than in its original form, and on the other hand, we have tried to find some other variation in Method 1, Equation 2, capable of being extended to particles of such smallness as occur in fine-grained disperse systems, that is, in true colloid solutions.

Weighing of Sediment. Odén's Procedure Applied to Small Quantities

Börjeson using the method of direct weighing of sediment showed that Odén's procedure is capable of being extended to such small quantities as 0.1–0.2 g.[6] For our purpose we desired if possible to be able to use only 0.02 g. and to have the deposition of such small quantities recorded automatically. In such a case Odén's procedure of adding automatically small counterweights was not practicable. We therefore adopted the principle of compensating automatically for the disturbance of equilibrium of the balance by means of a coil acting on a tube of iron suspended on the arm

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[3] Odén, *Proc. Roy. Soc. Edinburgh*, **36**, 219 (1916); *Int. Mitt. Bodenkunde*, **1915**; *Bull. Geol. Inst. Upsala*, **16** (1918).

[4] Odén, *K. Vetenskapsakad. Ark. Kemi*, **7**, No. 26 (1920).

[5] Nordlund, "Quecksilberhydrosole," *Dissertation*, Upsala, **1918**.

[6] Börjeson, "Electrical Synthesis of Colloids," *Dissertation*, Upsala, **1921**, p. 13.

of the balance, and we recorded the current in the coil by means of a self-registering milliammeter. In this way we obtained a diagram showing the variation of compensating current with time; this curve can easily be transformed into a curve giving the variation of weight of sediment with time.

Fig. 1 gives a diagram of the apparatus used.

The particles settle on a plate of silica or very resistant glass Q, attached to a fine glass or silica rod suspended on one arm of the balance and immersed in the sedimentation vessel of resistant glass. It is essential to have those parts made of silica or insoluble glass in order to prevent coagulation. On the other arm of the balance is suspended the iron tube B with its end reaching into the coil E. The latter is connected with the regulation resistance R, the precision self-registering milliammeter P, an accumulator battery of 40 volts and the drum resistance T. At the beginning of an experiment the resistance R is adjusted so as to give equilibrium. When the weight of the plate increases, because of the particles settling on it, the contact k_1, consisting of a platinum point and plate, is closed and by means of a very sensitive system of relays the motor M is set in motion revolving the drum T. By this means the sliding contact D is carried toward the left, a certain portion of the resistance wire is cut out and thus the equilibrium reëstablished. The most difficult point in the construction of apparatus was to make the contact k_1 work satisfactorily. The resistance to direct current of such a contact is dependent on the pressure exerted on it. To overcome this difficulty we applied a high frequency potential of about 20 volts between point and plate. This potential was produced by means of an Audion generator of frequency 600, and the direct current of the contact was cut off from the generator by inserting the two condensers c_1 and c_2. When the contact k_1 is closed the Audion relay AKGK sets the electromagnetic relay r_1 in action

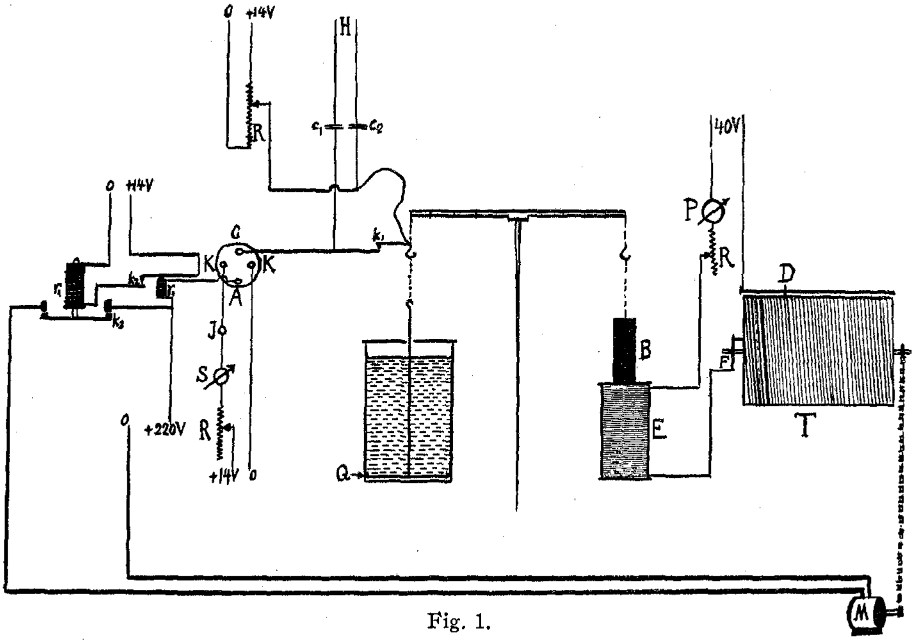

Fig. 1.

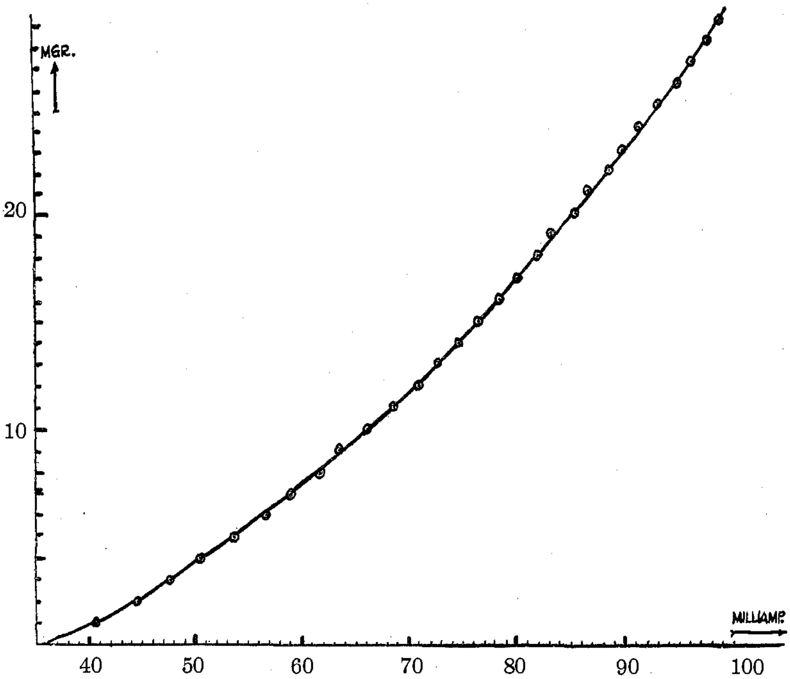

Fig. 2.

and this acts on the second electromagnetic relay r_2, which closes the current to the motor. In this way the compensating arrangement was made to work automatically without affecting the sensitivity of the balance. The whole apparatus was installed

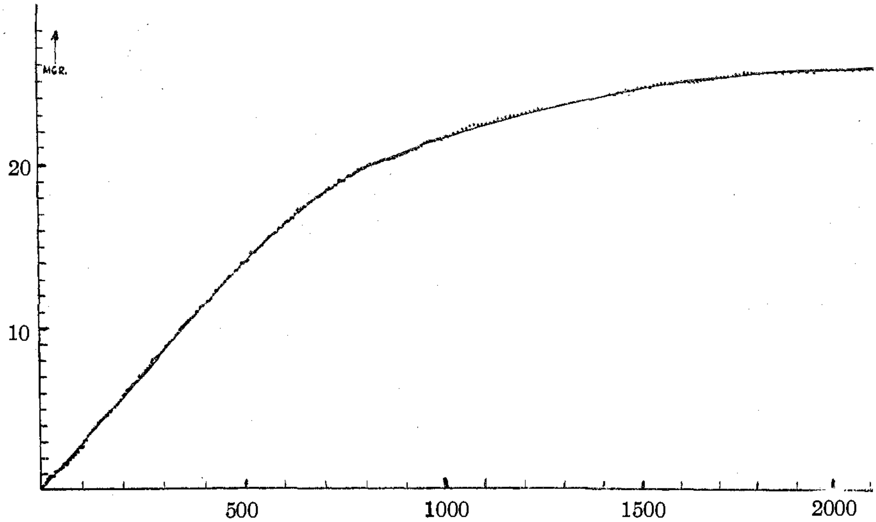

Fig. 3.

in a room of fairly constant temperature and the balance with the sedimentation vessel was covered by a double-walled hood of cardboard during the deposition of the sediment.

The following two experiments may serve as an illustration of the method. The first refers to a mercury hydrosol prepared by electrical pulverization (continous current arc), the second to a gold hydrosol prepared by means of Zsigmondy's well-known nuclear method.

For these two experiments a coil and iron cylinder giving the relation between current and weight indicated in the results plotted in Fig. 2 were used.

In Fig. 3 is the sedimentation or fall curve for mercury sol.

To construct the distribution curve we used the following simple procedure. Tangents were drawn from points of the fall curve corresponding to successive time intervals of 50 minutes. The difference between the cuts by successive tangents on the weight axis then represents the amount of sediments, ds, that corresponds to the radius interval, dr, where dr in its turn corresponds to the time interval in question.[7] That is, we calculate

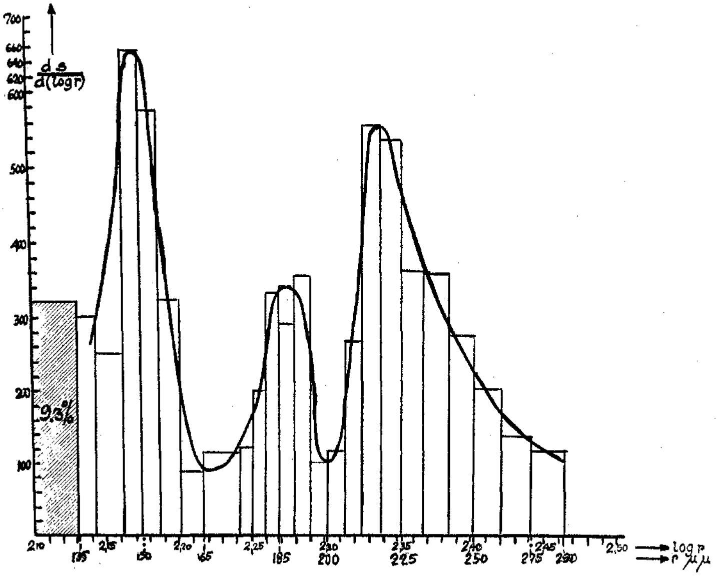

Fig. 4.

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[7] This can easily be shown as follows. The length on the weight axis cut off by the tangent is $w = P - t\frac{dP}{dt}$ where P is the total sediment corresponding to the time t. By derivation we have

$$\frac{dw}{dt} = \frac{dP}{dt} - t\frac{d^2P}{dt^2} - \frac{dP}{dt} \text{ or } \frac{dw}{dt} = -t\frac{d^2P}{dt^2}$$

but according to Stokes' law $r^2 = ct$ or $dt = \frac{2t}{r} \cdot dr$; thus $\frac{dw}{dr} = -\frac{2t^2}{r}\frac{d^2P}{dt^2}$; but according to Equation 4 the expression on the right-hand side is actually equal to ds/dr; thus $\frac{dw}{dr} = \frac{dS}{dr}$.

from Stokes' law of fall the radii r corresponding to the times t and the constant height of fall and with these radii we coördinate the weight differentials, ds, read off on the weight axis by means of the tangents drawn from the points on the fall curve corresponding to the readings on the time axis. The values of $\frac{\mathrm{d}s}{\mathrm{d}(\log r)}$ in percentages of the total are then plotted against $\log r$. Table I gives the data and Fig. 4 the distribution curve for the mercury sol studied.

TABLE I

DATA ON MERCURY HYDROSOL

Temperature during the sedimentation, 15.2°; height of fall, 58 mm.; t = time of fall in minutes; r, radius of particles in $\mu\mu$; ds = weight of sediment corresponding to particles between $\log r$ and $[\log r + \mathrm{d}(\log r)]$; d$S$ = % ds.

Total weight of sediment was 28 mg. 9.3% of the sol consists of particles of radius less then 135 $\mu\mu$.

t	r	$\log r$	d log r	ds	dS	$\frac{\mathrm{d}S}{\mathrm{d}\log r}$
450	291	2.464	...	...	...	...
500	276	2.441	0.025	0.8	2.9	116
550	263	2.420	0.021	0.8	2.9	138
600	252	2.401	0.019	1.1	3.9	202
650	242	2.384	0.017	1.3	4.7	276
700	233	2.367	0.017	1.7	6.1	358
750	225	2.352	0.015	1.5	5.4	360
800	218	2.338	0.014	2.1	7.5	535
850	212	2.326	0.013	2.0	7.2	555
900	206	2.314	0.012	0.9	3.2	266
950	200	2.301	0.012	0.4	1.4	116
1000	195	2.290	0.011	0.3	1.1	100
1050	190	2.279	0.011	1.1	3.9	354
1100	186	2.269	0.010	0.8	2.9	290
1150	182	2.260	0.009	0.8	2.9	321
1200	178	2.251	0.009	0.5	1.8	200
1250	175	2.242	0.009	0.3	1.1	122
1400	165	2.217	0.025	0.8	2.9	116
1500	159	2.201	0.016	0.4	1.4	88
1600	154	2.187	0.013	1.2	4.2	322
1700	150	2.175	0.012	1.9	6.8	575
1800	146	2.163	0.012	2.2	7.9	655
2000	139	2.143	0.020	1.4	5.0	250
2100	135	2.131	0.012	1.0	3.6	300

In Fig. 5 the values for the fall curve of the gold hydrosol are represented and in Fig. 6 the corresponding distribution curve.

We do not intend to discuss here the character of these distribution curves. We should like, however, to call attention to the fact borne out by these measurements that even in sols that have been looked upon as consisting of particles of fairly equal size, as for instance in the case of the gold hydrosols prepared by the nuclear method, there can actually be present groups of particles of sizes varying over a considerable range in radius.

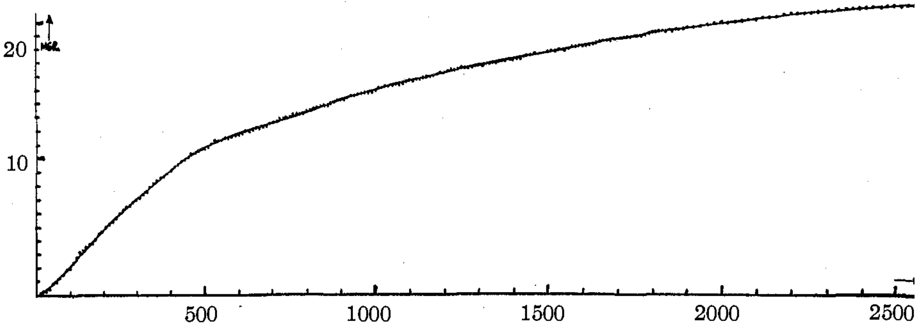

Fig. 5.

Determination of the Variation of Concentration with Height in a Sedimenting Disperse System

If we wish to extend the use of Equation 2 to a fine-grained system we must adopt a procedure which will give us high values of g, that is, we must be able to expose the system to a centrifugal force. Determinations of the weight of the sediment formed with the time become almost impossible in such a case. A more promising procedure is the determination of the variation of the concentration of the disperse phase with height.

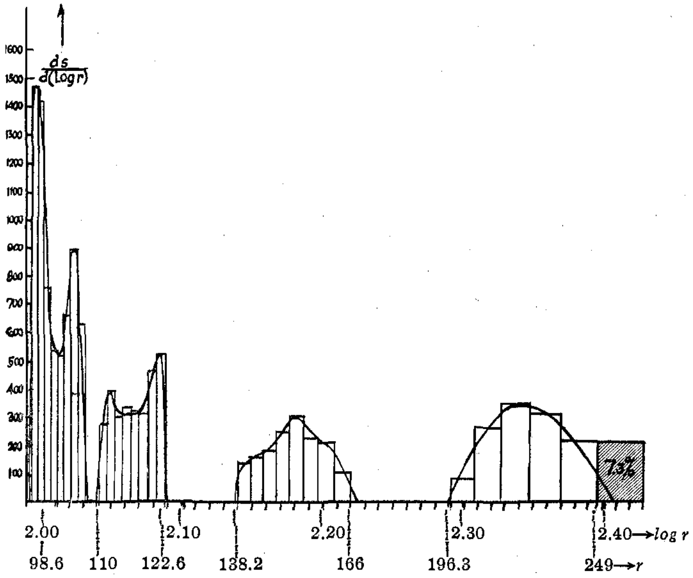

Fig. 6.

Let us consider a thin layer, dx, in a sedimenting column of a disperse system. The change in concentration dc from the section at x to the section at $x + \mathrm{d}x$ arises from the particles with radii between r and $r + \mathrm{d}r$, the values of r being determined by Stokes' law. A determination of the variation of c with x enables us to find $\mathrm{d}c/\mathrm{d}x$ and the distribution function $\mathrm{d}c/\mathrm{d}r$ as a function of r, for we find $\frac{\mathrm{d}c}{\mathrm{d}r} = \frac{\mathrm{d}c/\mathrm{d}x}{\mathrm{d}r/\mathrm{d}x}$, and in case of a constant acceleration throughout the whole height of the column, $\frac{\mathrm{d}r}{\mathrm{d}x} = \frac{A}{2\sqrt{x}}$, where $A = \sqrt{\frac{9\eta}{2(S_p - S_d)t}}$ and

$$\frac{\mathrm{d}c}{\mathrm{d}r} = \frac{2\sqrt{x}}{A} \cdot \frac{\mathrm{d}c}{\mathrm{d}x} \tag{5}$$

If the acceleration varies with x, as is the case in centrifuging experiment we have $\frac{\mathrm{d}r}{\mathrm{d}x} = B\frac{d}{\mathrm{d}x}\sqrt{\frac{x}{x + a}} = \frac{Ba}{2(x + a)\sqrt{x(x + a)}}$ where

$$B = \sqrt{\frac{9\eta}{2(S_p - S_d)\omega^2 t}}, \text{ and } \frac{\mathrm{d}c}{\mathrm{d}r} = \frac{2(x + a)\sqrt{x(x + a)}}{Ba} \cdot \frac{\mathrm{d}c}{dx} \tag{6}$$

Here a is the distance from the axis of rotation to the point $x = 0$, and ω the angular velocity.

The variation of concentration c with height x is best measured by some optical method which permits the determination to be made from outside without disturbing the sedimentation. An ideal method would be to measure the absorption of X-rays of a suitable wave length, a procedure which would give the mass concentration directly and be independent of the various sizes of the particles. In such cases where the relation between size of the particles and absorption of ordinary light is known or in cases where the light absorption does not vary much with the sizes of particles, a determination of the variation of light absorption with height can be used. If k is the absorption constant at the concentration c, and k_1 the absorption constant at unit concentration we have, according to Beer's law which holds good also for colloids, $k = k_1.c$; $\frac{\mathrm{d}c}{\mathrm{d}x} = \frac{1}{k_1} \cdot \frac{\mathrm{d}k}{\mathrm{d}x}$, and

$$\frac{\mathrm{d}c}{\mathrm{d}r} = \frac{2\sqrt{x}}{A} \cdot \frac{1}{k_1} \cdot \frac{\mathrm{d}k}{\mathrm{d}x} \tag{7}$$

in case of constant acceleration, and

$$\frac{\mathrm{d}c}{\mathrm{d}x} = \frac{2(x + a)\sqrt{x(x + a)}}{Ba} \cdot \frac{1}{k_1} \cdot \frac{\mathrm{d}k}{\mathrm{d}x} \tag{8}$$

in case of the acceleration varying with the height.

Before starting measurements on the centrifuge we made some observations in the field of gravity using gold sols of the same kind as the one recorded with the balance method.

A plane parallel cell 10cm. high and 2cm. deep was filled with the gold hydrosol and placed in a room of constant temperature. After a time of appropriate length the light absorption was measured as a function of the height, as follows. A beam of light 1-mm. high and 10mm. wide, from a mercury quartz lamp fed from an accumulator battery of constant potential, was made parallel by means of two quartz lenses and passed through the sector wheel S (Fig. 7) rotating with constant velocity, and then through the photographic shutter B which is opened just before the aperture of the wheel passes the beam of light and is shut after the passage. This device enabled us to duplicate exactly the times of exposure. The light beam then passed through the cell A containing the gold sol and then a system of 3 nicol prisms N_1, N_2, N_3. These prisms are cut with the end surfaces orthogonal to their length and are cemented with glycerol. Finally, the light beam enters the quartz spectrograph Q and its spectrum is photographed on the plate F. The cell with the sol was mounted on a table which by means of a screw and sliding arrangement could be moved vertically. Fig. 8 gives a photographic view of the apparatus.

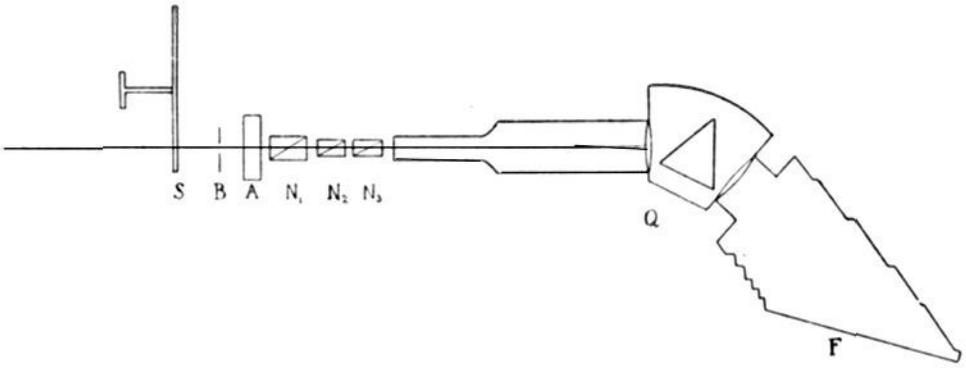

Fig. 7.

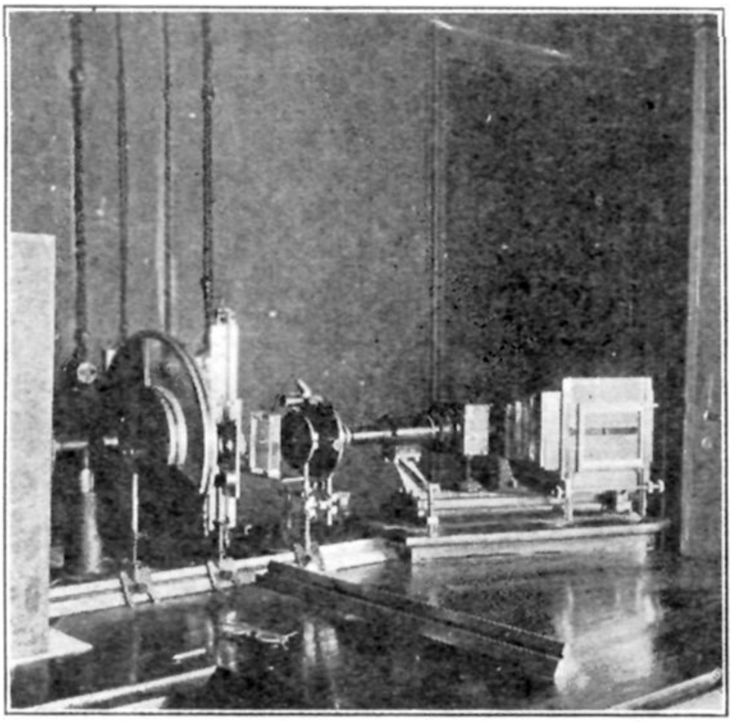

Fig. 8.

A series of equally timed exposures at different heights and with parallel nicols was taken. The sol was then removed and the cell filled with water. A new series of spectra was photographed on the same plate and the intensity of the light varied by turning the nicol N_2 a certain angle φ. If I_0 denotes the intensity of the light striking the photographic plate when the nicols are parallel, then $I = I_0 \cos^4 \varphi$ is the intensity when the nicol N_2 is rotated the angle φ. With parallel nicols and the sol in the cell we have $I' = I_0 e^{-kd}$ where I is the intensity of the light striking the plate after having passed through the sol, k the absorption constant and d the depth of the cell.

On comparing the two sets of spectra and selecting for a certain line the spectrum from one set that shows the same intensity on the plate as a spectrum from the other, we find $I' = I$ and $e^{-kd} = \cos^4\varphi$; that is, $k = -\frac{4}{d}\cdot\log\cos\varphi$.

The absorption constant has not yet been measured accurately for gold sols with radii above $100\mu\mu$. However, it has been found to be approximately constant, decreasing slowly with increasing radius. For the wave length $366\mu\mu$ and a concentration of 10^{-4} N, k can be taken as 0.1.

In Table II the results of such a series of measurements are given, and they are plotted in Fig. 9.

TABLE II

DATA ON GOLD HYDROSOL

Time of sedimentation: 1040 minutes

a	r	$\log r$	$d \log r$	φ	$k = c \times 10^3$	$dc \times 10^3$	$\frac{dc \times 10^3}{d \log r}$
20	93	1.9659	....	..	...	...	..
21	97	1.9877	0.0218	47	0.765	0.765	35.0
23	102	2.0074	0.0197	52	0.970	0.215	10.9
25	106	2.0255	0.0181	53	1.016	0.046	2.55
31	118	2.0722	0.0487	54	1.063	0.047	0.97
34	124	2.0923	0.0201	54	1.063	...	..
37	129	2.1107	0.0184	54	1.063	...	..
40	134	2.1276	0.0171	55	1.112	0.049	2.88
45	142	2.1532	0.0256	55	1.112	...	..
50	150	2.1761	0.0229	55	1.112	...	..
55	157	2.1968	0.0207	56	1.163	0.051	2.46
60	164	2.2156	0.0188	59	1.327	0.164	8.73
67	174	2.2396	0.0240	61	1.448	0.121	5.05
74	183	2.2612	0.0216	63	1.579	0.131	6.06
81	191	2.2808	0.0194	64	1.649	0.070	3.61
89	200	2.3013	0.0205	65	1.723	0.074	3.61
97	211	2.3200	0.0187	65	1.723	...	..

Here a is the distance from the surface of the sol to the point of observation in mm.; r, radius of particles corresponding to this height of fall according to Stokes' law of fall, in $\mu\mu$; φ, the angle of rotation of the nicol N_2 required for reducing the intensity of light to the extent it is reduced by the gold sol at the distance a mm. from the surface of the sol; k, the absorption constant calculated from φ as described above; c, the concentration of the colloid in moles/liter $= k \times 10^{-3}$; and d is the depth of the cell $= 20$ mm.

As has already been pointed out, the sol measured here is similar to the one referred to in Fig. 6; both were prepared by means of the Zsigmondy nuclear method. It is of interest to note that the distribution curves are of the same general type. It is evident that the method of obtaining the distribution curve from the relation between concentration and height in a sedimenting sol is by far more convenient than the method of weighing the sediment and will, therefore, probably be of use in the investigation of such systems. Furthermore, by combining the results obtained with a single sol by the two methods, the constant of light absorption can be calculated for the various sizes of particles in a polydisperse sol. Investigations of this kind are being undertaken and the extension of the method to colloids under the action of centrifugal force is in preparation.[8]

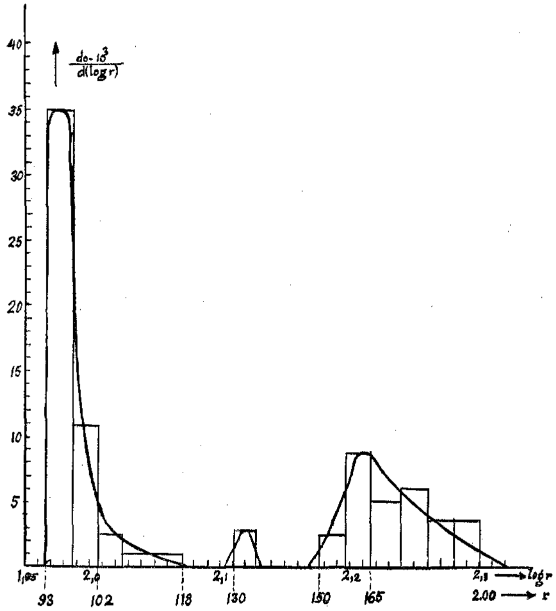

Fig. 9.

Summary

1. The principles for the determination of distribution of size of particles in a disperse system have been discussed.

2. An improved form of Odén's method using a self-recording sedimentation balance, which permits recording quantities as small as 0.02 g. with an accuracy of 0.0001 g. has been described.

3. This method depends on the compensation of the increasing weight of sediment by means of an electric current passing through a coil acting

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[8] The method of measuring light absorption used here has been worked out by Rinde for his as yet unpublished extensive investigations of the light absorption and the Tyndall effect of colloids through the visible and the ultraviolet spectrum. It has the great advantage of avoiding the error introduced when comparing the blackening of the plate for exposures of different lengths of time.

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.

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[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

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[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).