and Zwicky[23] to calculate this effect quantitatively are a step in the right direction, but do not yet account for the general linearity with $c^{1/2}$ or for the individuality of the lines.

It also seems unsafe to try to account for *all* the anomalous behavior of electrolytes on the basis of the free ions, since we showed[19] that the apparent molal volumes and compressibilities of sucrose and urea, from 30 to 70° obey the square root law. The same must be true of the apparent molal expansibilities of these substances, although the present density data do not warrant a numerical calculation.

Summary

We have defined the apparent molal expansibility of a solute, which is a linear function of the square root of the volume concentration for all the seven electrolytes studied.

The limiting value $\Phi^0(E_2)$ is more positive and the negative slope greater at low temperatures and for high valence type electrolytes.

Whenever the apparent molal expansion and volume each follows the square root law, the coefficient of expansibility (thermal expansion) $\alpha = \alpha_1 + Ac + Bc^{3/2}$.

We have derived equations by which the partial molal expansibility of solute and solvent may be calculated from $\Phi(E_2)$.

We have derived from the Debye–Hückel limiting law an expression which predicts the linearity of $\Phi(E_2)$ with $c^{1/2}$. An estimate of the coefficient, based on our present incomplete knowledge of the dielectric properties of water under pressure, indicates that the slope predicted at 20° is *positive* instead of *negative*.

The general theory of the apparent molal expansibility is incomplete but the order of increasing slopes of the $\Phi(E_2)$ lines here reported is shown to parallel that of the $\Phi(V_2)$, $\Phi(K_2)$ and $\Phi(C_{p2})$ lines.

EVANSTON, ILLINOIS RECEIVED NOVEMBER 6, 1933

(23) Evjen and Zwicky, *Phys. Rev.*, **33**, 860 (1929).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, STANFORD UNIVERSITY]

The Diffusion of Colloids and Colloidal Electrolytes; Egg Albumin; Comparison with Ultracentrifuge[1]

BY J. W. MCBAIN, C. R. DAWSON AND H. ALBERT BARKER

Colloids may diffuse quite as fast as ordinary molecules.[2] This is due to the influence of the oppositely charged ions which accompany all charged particles and drag them along just as hydrogen ions accelerate sulfate ions in the diffusion of sulfuric acid. Hence, ferric hydroxide or soaps may diffuse faster than sucrose.[3] These effects are diminished by the presence of other electrolytes or buffers, and they vanish at the isoelectric point. A number of investigators have arrived independently at the same formulation for diffusion of colloidal electrolytes and their admixtures with salts (at infinite dilution).

Under suitable conditions, diffusion through porous membranes of constant properties becomes one of the simplest, quickest and most accurate methods of determining particle size or molecular weight of many materials and substances of biological interest.

Egg albumin has been chosen for demonstrating some of the factors involved. It is one of the most thoroughly studied of colloids, and it forms clear, stable solutions which do not coagulate upon dialysis or the addition of moderate amounts of acid or base.

The experiments comprise a series with electrodialyzed egg albumin, brought to definite values of *P*H with hydrochloric acid or sodium hydroxide, diffusing into a solution of the same *P*H; and other series in which the egg albumin was not electrodialyzed and diffusion took place into water.

Experimental

The method and technique were those previously described,[4] in which diffusion cells ob-

(1) Read at the meeting of the Pacific Intersectional Division at Salt Lake City, June 13, 1933.

(2) The contrary impression has been emphasized by the usual lecture demonstrations in which diffusion takes place into jellies or through colloidal diaphragms, thus introducing the wholly extraneous factor of ultrafiltration and sieve action.

(3) McBain and Liu, THIS JOURNAL, **53**, 59 (1931); M. E. Laing McBain, *ibid.*, **55**, 549 (1933); see also Hartley and Robinson, *Proc. Roy. Soc.* (London), **A134**, 20 (1931); Svedberg, *Kolloid-Z.*, **36**, Ergänzungsbd., p. 63 (1925), equations 13b and 14. We find for a ferric hydroxide sol ["Sol No. 20," McClatchie, *J. Phys. Chem.*, **36**, 2088 (1932)] a diffusion coefficient of 0.613 as compared with 0.46 for sucrose. The chlorine present diffused still faster than the iron. Herzog and Polotsky obtained similar high values for some dyes [*Z. Elektrochem.*, **17**, 680 (1911)].

(4) McBain and Liu, THIS JOURNAL, **53**, 59 (1931); M. E. Laing McBain, *ibid.*, **55**, 549 (1933); Dawson, *ibid.*, **55**, 432 (1933); McBain and Dawson, *ibid.*, **56**, 52 (1934).

tained from the Fish-Schurman Corporation, New York, containing fused-in sintered Jena glass membranes G4, were employed. All diffusion was carried out at 25° and was continued from twenty-one to twenty-three hours. Analysis was made with a Zeiss interferometer.

First Series.—The egg albumin for the first series was prepared by the method of Sørensen,[5] being dialyzed in Cellophane tubes for eighteen days and electrodialyzed for three days. Before electrodialysis a 1% solution exhibited a P_H of 5.25 (glass electrode, ±0.02) and a conductivity of 6.45×10^{-5} mhos (25°), and afterwards P_H 4.81 and conductivity 1.1×10^{-5}. For diffusion the 7.1% stock solution (preserved with toluene in a refrigerator) was diluted with sufficient boiled-out distilled water to give a 1% solution (2.94×10^{-4} N if $M = 34{,}000$).

The experimental data are collected in Table I. The first column names the added electrolyte used to adjust the P_H; the second and third give the P_H; the fourth, the diffusion coefficient of the egg albumin; and the last column, the apparent molecular weight. It is seen at once that the true molecular weight is only obtainable at the isoelectric point or in the presence of buffers; otherwise, the values are low and may be only a few per cent. of the true molecular weight.

TABLE I

DIFFUSION COEFFICIENTS OF 1% ELECTRODIALYZED EGG ALBUMIN IN THE PRESENCE OF HCl OR NaOH OR KCl

Added	P_H Cell	P_H Beaker	$D_{Obs.}$	Apparent molecular weight
HCl	1.62	1.63	0.099	31,900
HCl	1.99	1.98	.104	27,500
HCl	2.45	2.41	.130	14,100
HCl	3.06	3.07	.230	2550
HCl + KCl[a]	3.51	3.51	.0935	38,000[a]
HCl	3.75	3.80	.256	1850
None[b]	4.81	4.66	.0975	33,500[b]
[c]	5.71	5.90	.296	1190
NaOH	7.55	7.53	.410	450
NaOH	11.35	11.38	.187	4740

[a] Buffered by the addition of 0.02 N KCl, therefore approaching the true molecular weight.

[b] Minimum diffusion value, therefore nearly isoelectric, therefore true molecular weight.

[c] Not electrodialyzed.

Second Series, Egg Albumin not Electrodialyzed.—This dialyzed egg albumin exhibited P_H 5.1 and conductivity 6.1×10^{-5} in 1% solution. Not more than four observations were made with any cell before rechecking its cell constant.[6] The data are collected in Table II. It will be noted that the P_H was at first lowered by the addition of hydrochloric acid, and the diffusion coefficient correspondingly decreased, passing through a minimum at P_H 4.53. Once more there clearly appears the effect of potassium chloride, added to the extent of 0.01 N in both beaker and cell, in lowering the diffusion in the direction of the value for isoelectric albumin.

TABLE II

DIFFUSION COEFFICIENTS OF 1% EGG ALBUMIN (NOT ELECTRODIALYZED) IN THE PRESENCE OF HCl OR NaOH OR KCl, DIFFUSING INTO WATER

Added	P_H	$D_{Obs.}$	Apparent molecular weight
HCl	3.26	0.148	9570
HCl	3.52	.173	5980
HCl	3.76	.176	5690
HCl	3.98	.158	7860
HCl	4.04	.144	10,400
HCl	4.14	.129	14,400
HCl	4.25	.125	15,900
HCl	4.35	.119	18,400
HCl	4.41	.105	26,800
HCl[a]	4.53	.0965	34,500[a]
HCl	4.61	.100	31,000
HCl	4.66	.100	31,000
HCl	4.82	.110	23,300
KCl only[b]	5.10	.120	17,900[b]
None	5.12	.184	4980
NaOH	5.15	.191	4450
NaOH	5.32	.202	3770
NaOH + KCl[b]	5.38	.140	11,300[b]
NaOH	5.39	.233	2450
NaOH	5.40	.302	1120
NaOH + KCl[b]	5.79	.156	8160[b]
NaOH	5.85	.327	890
NaOH	6.37	.375	590
NaOH	8.14	.421	410
NaOH	9.07	.328	880

[a] Isoelectric.

[b] Buffered by the addition of 0.01 N KCl to solutions in both cell and beaker.

Third Series, not Electrodialyzed, with Sulfuric Acid.—This series differed from the second only in that sulfuric acid was added (in the amounts indicated) instead of hydrochloric acid. The data are collected in Table III and plotted in Fig. 3.

Formulation

Uncharged colloid, charged colloid with one other ionic species present, and charged colloid

(5) Sørensen and Høyrup, *Compt. rend., trav. lab. Carlsberg*, **12**, 12 (1917).

(6) Dichromate cleaning solution does not alter the cell constant but it does not seem readily or entirely to remove egg albumin from the glass membranes. On the other hand, alkali is dangerous, for membranes that have been exposed even only in the cold to deci-normal alkali tend to become less permeable during subsequent use.

TABLE III

DIFFUSION COEFFICIENTS OF 1% EGG ALBUMIN (NOT ELECTRODIALYZED) IN THE PRESENCE OF H_2SO_4, DIFFUSING INTO WATER

P_H	N H_2SO_4	D	P_H	N H_2SO_4	D
3.56	0.0043	0.0804	4.58	0.00072	0.105
3.78	.0036	.0852	4.60	.00090	.091
3.95	.0028	.0929	4.65	.00054	.100
4.01	.0025	.082	4.66	.00072	.109
4.12	.00216	.106	4.73	.00061	.102
4.22	.0018	.087	4.78	.00039	.143
4.26	.0018	.0892	4.79	.00054	.097[a]
4.33	.00144	.0957	4.81	.00047	.107
4.36	.00144	.0936	4.82	.00036	.112
4.39	.00108	.0864	4.87	.00036	.138
4.50	.00108	.105	4.97	.00018	.155
4.51	.00108	.107			

[a] Isoelectric.

with two or more other ionic species present give very different rates of diffusion. For uncharged colloidal particles or large molecules such as sucrose, or even for virus or phage at the isoelectric point, the Sutherland[7] or Stokes–Einstein equation, $D = RT/N6\pi\eta r$, is valid, provided that the particle is massive and approximately spherical. The same value may be approached, with far less certainty, when a sufficient amount of suitable buffer or other electrolyte is added.

Charged colloidal particles or colloidal ions with only one other ionic species present very rarely occur but then they may be considered as polyvalent electrolytes. Several authors[8] have arrived at a common formulation, differing only in notation, using the fundamental assumptions first made by Nernst[9] and stated more formally by Planck;[10] namely, the total movement of an ion is equal to the sum of the movements due to osmotic and electrostatic forces

$$\frac{\partial c'}{\partial t} = u'RT\frac{\partial^2 c'}{\partial x^2} + u'E\frac{\partial}{\partial x}\left(c'\frac{\partial \psi}{\partial x}\right) \qquad (1)$$

where t is the time, E the charge on an individual ion, R the gas constant, T the absolute temperature, ψ the electrostatic potential, c' the concentration and u' the mobility of the ion.

In practice nearly all colloids belong to the third group, and misleading results would follow from considering them as pure solutions of polyvalent electrolytes. The correct formulation is that for a mixture of a polyvalent electrolyte with one or more other electrolytes. This is of importance, for here the phenomenon of accelerated and retarded diffusion, first emphasized by Arrhenius,[11] becomes prominent. This formulation is discussed and illustrated in the preceding communication[12] (see equation (2) below). So far it has been developed only for conditions of infinite dilution as in the Nernst equation. In real solutions diffusion is somewhat slower, owing to diminished activity, and it may be somewhat hastened or retarded by the collision effect noted in previous communications from this Laboratory.

Almost every colloid serves as an example of such a mixture. It is impossible to prepare a solution of a protein in the form of a pure polyvalent electrolyte. This is true even at the only favorable value of P_H, namely, the neutral point of water, owing to impurities, hydrolysis and the necessary addition to bring the P_H to the value 7. Hence the diffusion observed will always be less than that so expected, a circumstance which renders impracticable Svedberg's[13] suggestion that his equation for a polyvalent electrolyte might be used as a method of determining the valency of charged colloidal particles, since the result is always an underestimate.[14]

These points are illustrated in Fig. 1, in which the number of charges per egg albumin ion of particle weight 34,000 derived by the different methods is plotted for comparison. The full curve is that found by the potentiometric activity determinations of Frisch, Pauli and Valkó[15] whose values agree with those of Hitchcock[16] between P_H 2.6 and the isoelectric point. The dashed curve represents the low values which would result from applying the Svedberg equation for pure polyvalent electrolyte alone. The dotted line is that obtained from the equation for a mixture of polyvalent electrolyte with another electrolyte of common ion (egg albumin chloride with hydrochloric acid), neglecting unknown impurities which, of course, tend to make the calculated results too low

(7) Sutherland, *Australasian Assoc. Adv. Science*, **10**, 117 (1904); *Phil. Mag.*, [6] **9**, 781 (1905).

(8) Haskell, *Phys. Rev.*, (1) **27**, 145 (1908); Svedberg, *Kolloid-Z.*, **36**, Ergänzungsbd., p. 53 (1925); Hartley and Robinson, *Proc. Roy. Soc.* (London), **A134**, 20 (1931); Bruins, *Kolloid-Z.*, **57**, 158 (1931); M. E. Laing McBain, THIS JOURNAL, **55**, 545 (1933); McBain and Dawson, *ibid.*, **56**, 52 (1934); Guggenheim, unpublished.

(9) Nernst, *Z. physik. Chem.*, **2**, 613 (1888).

(10) Planck, *Wied. Ann.*, **39**, 161 (1890); **40**, 561 (1890).

(11) Arrhenius, *Z. physik. Chem.*, **10**, 81 (1892).

(12) McBain and Dawson, THIS JOURNAL, **56**, 52 (1934); also in Hartley and Robinson, *Proc. Roy. Soc.* (London), **A134**, 20 (1931); Bruins, *Kolloid-Z.*, **57**, 158 (1931).

(13) Svedberg, *Kolloid-Z.*, **36**, Ergänzungsbd., p. 63 (1925).

(14) Nichols, "Colloid Symposium Monograph," The Chemical Catalog Co., Inc., New York, 1928, Vol. VI, p. 298, expresses the same view.

(15) Frisch, Pauli and Valkó, *Biochem., Z.*, **164**, 412 (1925).

(16) Hitchcock, *J. Gen. Physiol.*, **5**, 383 (1923).

$$D'G' = 2.29 \times 10^{-2} \times \left[G'u' - n'c'u'\left(\frac{n'G'u' - n'G'V_{Cl}}{(n')^2c'u' + n'c'V_{Cl} + c''(U_H + V_{Cl})}\right)\right] \quad (2)$$

where[17] D' is the expected diffusion of egg albumin in cm.^{2}/day, u' the mobility of the egg albumin molecule as determined at the isoelectric point, G' the average concentration gradient of egg albumin during the experiment (proportional to the difference in concentration on the two sides of the membrane), c' the concentration of egg albumin, n' the number of chlorine ions from one molecule of egg albumin chloride, c'' concentration of HCl, U_H the mobility of hydrogen ion and V_{Cl} that of chlorine ion. (For an ionic species diffusing into water, G' equals c' at the beginning, and here was always at least 96% of c'.) It will be noted that for strongly alkaline or strongly acid solutions the number of equivalents of electrolyte is far greater than the number of equivalents of protein present.

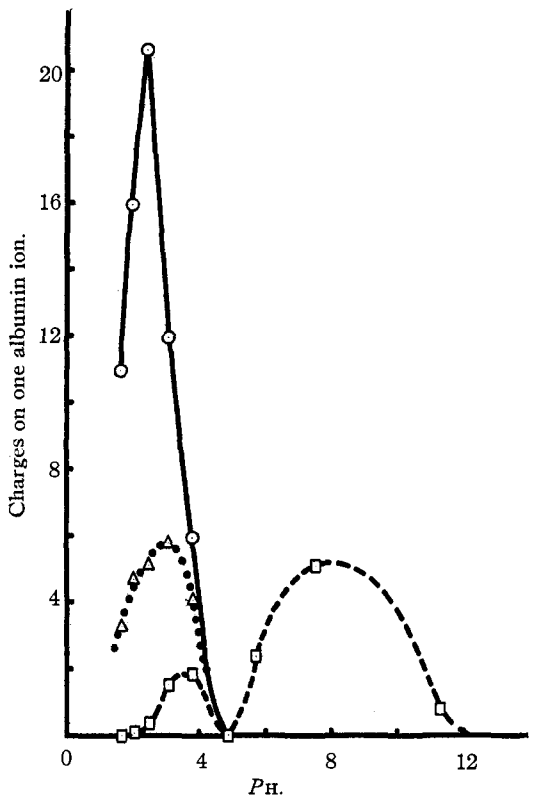

Fig. 1.—The number of charges per ion of egg albumin at various values of P_H: full curve, derived by titration; dashed curve, low values calculated as polyvalent electrolyte, ignoring admixtures present; dotted curve, calculated, taking hydrogen chloride present into account, neglecting residual impurities (equation (2)).

(17) See McBain and Dawson, THIS JOURNAL, **56**, 52 (1934).

Discussion

1. The values here obtained for the diffusion coefficient exhibit a pronounced minimum in the neighborhood of the isoelectric point. This is well shown in Figs. 2 and 3. It is obvious that at the isoelectric point all complications due to ions vanish even when appreciable concentrations of ions are present. Hence, our main conclusion is that for determination of particle size or molecular weight of large molecules the best value is that corresponding to this minimum diffusion rate in the neighborhood of the isoelectric point. The values so obtained should lead to the true molecular weight or particle size for massive particles or large molecules[18] of reasonable approximation to spherical or ellipsoidal shape.

In the present case the minima for D at 25°, when evaluated by the Sutherland–Einstein formula, give values for the molecular weight of egg albumin of 33,500, 34,500 and 34,000 from series 1, 2 and 3, respectively. This is in surprisingly good agreement with expectation[19] and with Svedberg's value of 34,500 ± 1000 as determined by sedimentation *equilibrium* in the ultracentrifuge, as well as with Sørensen's osmotic value of 34,000.[20]

2. The second main point is that now two proteins have been tested with the porous membrane method, hemoglobin by Northrop and Anson, and egg albumin here, in both cases with orthodox results; namely,[21] 69,500 ± 1000 and 34,000, respectively. This is in striking contrast to Svedberg's results for the *diffusion* of the same materials in the ultracentrifuge, where in both cases the diffusion observed was much lower and corresponded to molecular weights[22] of 128,000 for hemoglobin and 166,000 for egg albumin.

(18) Sutherland gives also a formula for very small molecules.

(19) Compare Cohn, Hendry and Prentiss, *J. Biol. Chem.*, **63**, 721 (1925).

(20) Sørensen, Christiansen, Høyrup, Goldschmidt and Palitzsch, *Compt. rend. trav. lab. Carlsberg*, **12**, 356 (1917); Adair, however, would have doubled Sørensen's value [*Proc. Cambridge Phil. Soc., Biological Sciences*, **1**, 75 (1924)]. Sutherland's calculation from Stefan's calculation of Graham's experiment, if the density 1.33 is introduced, leads to the value 36,000.

(21) Northrop and Anson, by not using the exact values of R, η, etc., given in "International Critical Tables," give 68,500 [*J. Gen. Physiol.*, **12**, 549 (1929)].

(22) Nichols, "Colloid Symposium Monograph," Vol. VI, p. 296, inadvertently inverted his ratio and may not have used the value of η for water as is done by ourselves and by Northrop and Anson. Compare McBain and Liu, THIS JOURNAL, **53**, 71 (1931).

It is evident that the *diffusion* values obtained for these substances in the ultracentrifuge are erroneous. In recent years papers from the Upsala laboratory have laid no weight upon diffusion experiments in the ultracentrifuge.

The treatment accorded to these two discrepant results from the ultracentrifuge was different. In the case of hemoglobin it was used to infer that hemoglobin is not a spherical molecule "but may be more or less plate-shaped."[22,23] Svedberg and Nichols[24] wrote that "the diffusion constant and the specific sedimentation velocity are normal." By this was meant that both diffusion constant and specific sedimentation velocity diverged to an equal extent from the behavior to be expected from their molecular weight as deduced from sedimentation equilibrium and from the requirements of the Sutherland–Stokes–Einstein equation. Some might prefer to term the ultracentrifuge value abnormal in view of the fact that it does not agree with the more direct measurements of Northrop and Anson, although acceptance of the value published by the latter would leave the sedimentation velocity unexplained. The observed diffusion, with the ultracentrifuge, is actually 81% of the theoretical, giving a molecular weight twice too large.

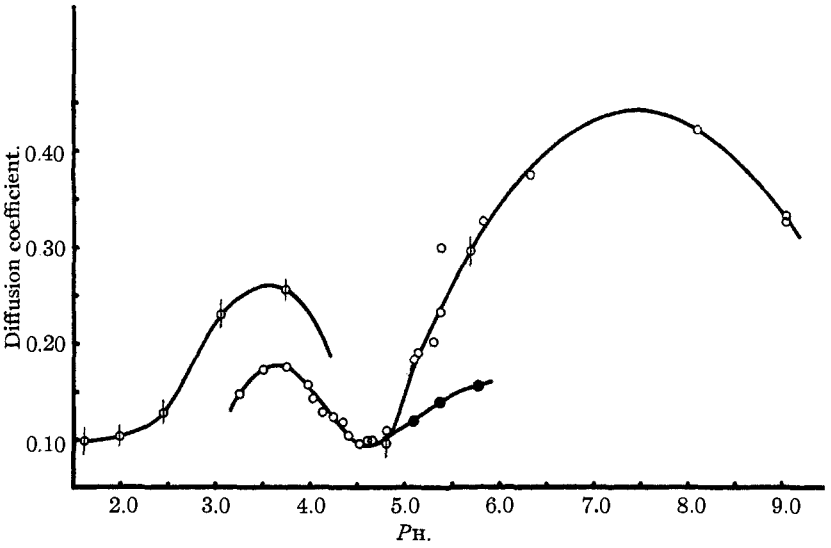

Fig. 2.—Diffusion of egg albumin as affected by addition of HCl, NaOH, or NaOH + KCl: ⊙, first series; ○, second series; ●, second series + KCl.

In the case of egg albumin, on the other hand, the result, which is actually 58% of that predicted, giving a molecular weight now five times too large, was termed "abnormal," and was not used further.

It may be generally known that determinations of sedimentation velocity do not of themselves lead to a value for molecular weight without additional information.[25] It is necessary further to measure, estimate or select a value for the diffusion coefficient. For egg albumin Nichols found that by taking a value predicted for spherical particles of molecular weight 35,000, instead of the diffusion coefficient actually observed, the sedimentation velocity gave the molecular weight approximately 34,500. Had the sedimentation velocity been otherwise, it would have been interpreted as evidence of dissymmetry or lack of sphericity as was done with hemoglobin.[26]

That accurate values for diffusion should ever be obtained in the ultracentrifuge is an astonishing achievement in view of the extraordinary precautions required to get undistorted diffusion columns under optimum conditions.[27] Sedimentation velocity is less precarious than diffusion, and sedimentation equilibrium may even be stabilized by the intense centrifugal field. For some time one of us (J. W. M.) thought that diffusion and, to a much lesser extent, sedimentation velocity, might be distorted through the difference in centrifugal field at top and bottom

(23) Radii are calculated in Table XXXIIIB of Svedberg's "Colloid Chemistry," pp. 164, 165. Compare Kunitz, Anson and Northrop, *J. Gen. Physiol.*, **17**, 369 (1934).

(24) Svedberg and Nichols, THIS JOURNAL, **49**, 2934 (1927).

(25) Compare Svedberg, *Kolloid-Z.*, **36**, Ergänzungsbd., p. 53 (1925); Svedberg and Fåhraeus, THIS JOURNAL, **48**, 430 (1926); Svedberg and Nichols, *ibid.*, **49**, 2920 (1927). However, even where this was considered to be especially emphasized, Svedberg wrote "Die praktische Konsequenz ist nun, dass die Sedimentationsgleichgewichtsmethode oft für die Bestimmung des Molekulargewichts zuverlässiger ist als die Sedimentationsgeschwindigkeitsmethode" [*Kolloid-Z.*, **51**, 12 (1930)].

(26) Nichols, THIS JOURNAL, **52**, 5177, 5180 (1930). Svedberg, by another method, obtained for a more concentrated, 4.5% solution, the value 0.041 at 0°, corresponding to 0.090 at 25° ["Colloid Chemistry," 2d edition, The Chemical Catalog Co., Inc., New York, 1928, p. 142]. Since this paper was submitted, Tiselius and Gross have obtained a diffusion coefficient equivalent to 0.075 at 25°, which is much greater than the ultracentrifuge value, 0.056, but much less than Svedberg's 0.090 or Gróh's 0.092 or than our value, 0.097, which is that for uncharged spheres of weight 35,000. Tiselius and Gross's value is 83% of that which they expected from sedimentation velocity, as compared with the 58% observed in the ultracentrifuge [*Kolloid-Z.*, **66**, 18 (1934)].

(27) Compare also Johnston and Howell, *Phys. Rev.*, **35**, 274 (1930); McDowell and Usher, *Proc. Roy. Soc.* (London), **A138**, 133 (1932).

of the cell, causing a more rapid impoverishment of the outer layers and thus occasioning systematic upset or convection below the meniscus of falling particles. However, it has been pointed out (private communication) by members of the du Pont staff at Wilmington that a slight extension of the analysis presented by Svedberg and Rinde[28] of the combined effects of axial distance and sector shape of cell leads only to a uniform impoverishment of the solution below the meniscus. Nevertheless, the latter is blurred (or perhaps sometimes even sharpened) by diffusion, vibration or any residual convection of the liquid below. Throughout a uniform solution the intense centrifugal field does nothing to avoid convection, and the solution does not tend to be immobilized thereby as in sedimentation equilibrium or even as in measurements of sedimentation velocity where there is a spectrum of particles of different weight. However, even if a sedimentation velocity measurement were sensibly affected, it would not detract from the invaluable function of sedimentation velocity in giving numerical characterization to all classes of particles and molecules in so far as accessible to experiment.

These considerations are of general interest now that the ultracentrifuge developed from the spinning top of Henriot and Huguenard[29] promises to become available in most laboratories.

3. Away from the isoelectric point diffusion values are greatly affected by the amount and nature of ions present not only in the cell but also in the solution into which they are diffusing. Here the phenomena of accelerated as well as of retarded diffusion discussed in the preceding communication become prominent, especially perhaps when the diffusion is taking place into water. Inspection of equation (2) shows that if a sufficient concentration of suitable buffer is added to both cell and beaker, the second term of the right-hand member of the equation tends toward zero as a limit and the equation reduces to $D' = RTu' = RT/6N\pi\eta r$ which is identical with the Sutherland or Stokes-Einstein equation.

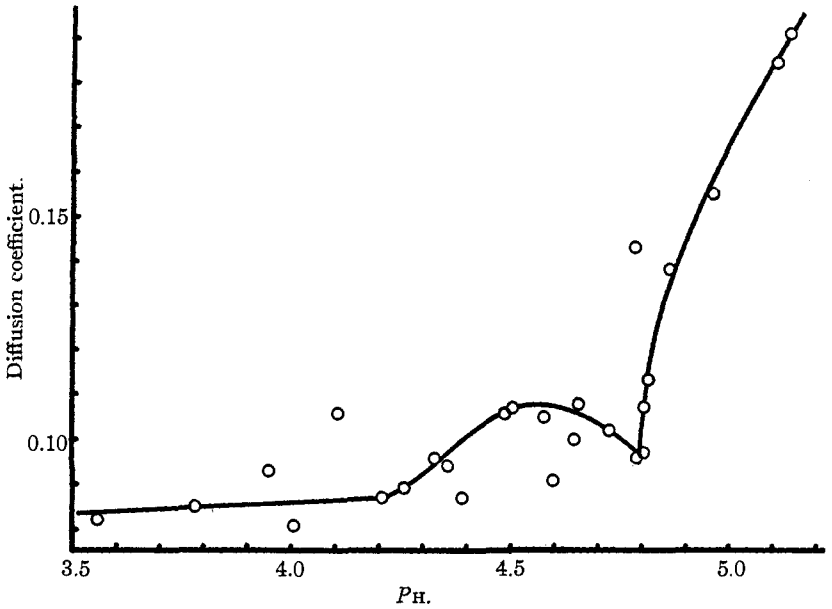

Fig. 3.—Diffusion of egg albumin as affected by addition of H_2SO_4 or NaOH.

From a practical standpoint it is usually better to obtain molecular weights from diffusion studies in the neighborhood of isoelectric points rather than in the presence of buffers for the following reasons. (a) It is very difficult to obtain and maintain exactly the same effective concentrations of added electrolyte on both sides of the membrane, thus still leaving electrical and collision effects of diffusion. (b) A high concentration of electrolyte may change the size of or degree of aggregation or dissociation of the colloid. (c) As a rule analysis, such as by interferometry, becomes less accurate.

In the case of egg albumin Figs. 2 and 3 show that it is possible by suitable additions of hydrochloric acid or potassium chloride to obtain diffusion values similar to that at the isoelectric point, but this is not the case if diffusion is into pure water. For example, with excess of sulfuric acid the albumin is retarded.

For intermediate values of PH or added electrolyte it is seen from the tables and more clearly from Figs. 2 and 3 that values of diffusion widely different from that corresponding to the true

(28) Svedberg and Rinde, This Journal, **46**, 2677 (1924); Svedberg and Nichols, *ibid.*, **49**, 2921 (1927); Svedberg, *Kolloid-Z.*, **36**, Ergänzungsbd., 59 (1925); *Z. physik. Chem.*, **127**, 54 (1927); see also Lamm, *Arkiv. Mat. Astron. Fysik*, **21B**, No. 2 (1929); Faxén, *ibid.*, **21B**, No. 3 (1929).

(29) Henriot and Huguenard, *Compt. rend.*, **180**, 1389 (1925); *J. phys. radium*, **8**, 443 (1927).

molecular weight are obtained. As was discussed in connection with Fig. 1, they can be accurately calculated only if an exact knowledge of all the admixtures and impurities present is available.

Summary

Diffusion through porous membranes of constant properties is one of the simplest, quickest and most accurate methods of determining particle size or molecular weight of molecules larger than sucrose that are approximately spherical.[30] Only at the isoelectric point or, with less certainty, in the presence of buffers is the true molecular weight obtained. For egg albumin 34,000 was found. At other values of P_H or with insufficient or unsuitable buffering widely divergent values result from the phenomena of the mutual acceleration and retardation of ions and charged particles. Thus charged colloids may be found to diffuse faster than ordinary molecules.

(30) Compare the calculation as to the probability of a spherical shape for palmitic acid in aqueous solution by Langmuir ["Colloid Chemistry, Theoretical and Applied," edited by J. Alexander, The Chemical Catalog Co., Inc., New York, 1926, Vol. 1, p. 538]. Protein molecules are not inherently spherical, but as far therefrom as possible in aqueous or mercury surfaces, spreading out to enormous sheets only a few Å. in thickness [for collected references see N. K. Adam, "The Physics and Chemistry of Surfaces," The Clarendon Press, Oxford, 1930, pp. 79–82]; nevertheless, according to sedimentation velocity and our diffusion measurements, egg albumin is spherical in solution at the isoelectric point.

STANFORD UNIV., CALIF. RECEIVED NOVEMBER 20, 1933

[CONTRIBUTION FROM THE PHYSICAL INSTITUTE OF THE UNIVERSITY OF LEIPZIG]

Influence of Dipole Fields between Solute Molecules. I. On Osmotic Properties

BY RAYMOND M. FUOSS[1]

I. Introduction

If we consider electrolytic solutions in solvents of various dielectric constant, we first find, as the dielectric constant decreases, an increasing tendency for the formation[2] of ion pairs.[3] As the dielectric constant of the solvent is further decreased (or as concentration is increased), we next find it necessary to take into account configurations in which three[4] specific ions are involved. The next step is the consideration of the interaction of four ions.[5] This case becomes important at even moderate concentrations in solvents of low dielectric constant, where the minimum in equivalent conductance appears at concentrations well below 0.001 N. (In such solvents, the minimum indicates the concentration at which the triple ion interaction is equally as important as the ion–ion interaction.)

It is the purpose of this article to calculate in first approximation the osmotic properties of electrolytes in solvents of low dielectric constant. In such solvents, the constants K and k_3 describing the equilibria between ions, ion pairs and ion triples are of the order of 10^{-18} and 10^{-4}, respectively (Ref. 5, Table VII). At a total concentration of 10^{-4} mole per liter, for example, the fraction γ of total solute existing as free ions is 10^{-7} and the fraction γ_3 existing as triple ions is 10^{-6}, if we use the above round values for the constants, so that $(1-\gamma-3\gamma_3)$, the fraction of solute existing as ions pairs, is practically unity. As concentration increases, γ decreases and γ_3 increases, but the relative change in $(1-\gamma-3\gamma_3)$ is negligible. For simplicity, therefore, we shall neglect at present all effects due to unpaired charges, and consider the properties of a solution of ion pairs. Except for the fact that an ion pair can dissociate into free ions under suitable conditions, an ion pair resembles a dipole molecule with a fairly large moment. We shall make the further simplification that the ion pairs are assumed to be rigid dipoles; the energy of dissociation of quaternary ammonium salts in benzene, for example, is of the order of 20,000 calories, which is about 30 times RT. We have thus re-

(1) International Research Fellow.

(2) "Formation of ion pairs" is a convenient and short way of expressing the fact that, when the potential energy ϵ^2/aD of two oppositely charged ions at contact becomes large compared to the thermal energy kT, we find more and more configurations of ions in which two such ions spend a relatively large fraction of their existence very near to each other. The phrase does not imply the formation of chemically neutral molecules. There are cases where, after Coulomb forces have brought two ions into contact, the electrons in the two ions redistribute themselves in accordance with quantum restrictions, to form a homopolar bond. Then in these cases the formation of the final neutral molecules cannot be described simply by means of Coulomb's law.

(3) Fuoss and Kraus, THIS JOURNAL, **55**, 476, 1019 (1933); Fuoss, *Physik. Z.*, **35**, 59 (1934).

(4) Fuoss and Kraus, THIS JOURNAL, **55**, 2387 (1933). A more rigorous treatment, which will eliminate the arbitrary limit in the integration, is proposed by Fuoss, Ref. 3.

(5) Fuoss and Kraus, THIS JOURNAL, **55**, 3614 (1933).