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Martin R. Broadbooks for the M.S. in Chemistry
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(Major Professor)

The outstanding work of Svedberg with the ultracentrifuge has centered considerable attention on particle sizes and molecular weights of proteins.

By means of the Stokes-Einstein equation the radius of a solute particle may be calculated from the diffusion coefficient. The particle size and molecular weights calculated from diffusion data compare favorably with those determined by other methods.

The present work is an attempt to get diffusion data under conditions that will give a value for the weight of gelatin particles deprived of their hydrated sheath of water.

It was found that if a charge is imparted to gelatin particles by making a gelatin solution slightly acid in the presence of KCl the solution would remain stable in 20 percent ethanol.

With such a solution, diffusion data was obtained under a condition which would dehydrate gelatin particles yet allow a stable sol to exist and at the same time prevent anomalous diffusion due to unequal velocities of ions.

A curve plotting concentration of gelatin against diffusion velocity has been extrapolated to zero concentration. The diffusion value at that point (0.0402 cm^2/day) resulted in a value for a molecular weight of 181,000.

THE DIFFUSION OF GELATIN

by

MARTIN ROBERT BROADBOOKS

A THESIS

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APPROVED:

Professor of Chemistry

In Charge of Major

Head of Department of Chemistry

Chairman of Committee on Graduate Study

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INTRODUCTION

The outstanding work of Svedberg (8,9,10) with the ultracentrifuge has centered considerable attention on the particle sizes and molecular weights of proteins. If accurate values can be obtained for the particle size under a variety of conditions these values can be correlated with other behavior under the same conditions and will be of great help in understanding and predicting colloidal phenomena.

Gelatin was chosen for this present investigation because it possesses more of the characteristics of a lyophilic colloid than any other single substance, and because it is available in comparatively pure form.

By means of the Stokes-Einstein equation the radius of a particle may be calculated from its diffusion coefficient, providing the particle is assumed to be spherical and large. The particle size and molecular weights calculated from diffusion data compare favorably with those determined by other methods (8,3,6,7).

Following the introduction of the method of separating solutions by means of a sintered glass diaphragm by Northrup and Anson (6), and the extensive work done by McBain and Liu (4) in standardizing the technique incident to the use of the method, diffusion data need no longer be the result

of a painstakingly slow and oftimes inaccurate process that it once was. A perusal of the International Critical Tables will reveal a scarcity of data, which is sometimes conflicting and subject to more or less error.

In a Doctorate dissertation presented by Karl Klemm (1), the diffusion of gelatin under various conditions and concentrations was discussed. It was found that at pH values below that of isoelectric gelatin the diffusion velocity showed a marked increase. This was explained as being due to the dragging effect of the Cl^- ion resulting from the formation of Gelatin Chloride in HCl solution. The Cl^- ion diffuses rapidly but as the ions must maintain electrical neutrality the Cl^- ion is held to the $Gelatin^+$ ion which in turn is dragged along at a faster rate. If there were an excess of Cl^- ions on both sides of the diffusion membrane the $Gelatin^+$ ion would not be dependent upon a few Cl^- ions but could pass from one to another and carry on normal diffusion.

This theory checked with experimental facts, for by making the gelatin solution 0.1 N with respect to KCl the diffusion was the same even at pH values 2 and 3 units below or above the isoelectric point.

The present work is an attempt to get diffusion data under conditions that will give a molecular weight of the gelatin particle without a hydrated sheath of water. Hydrophylic colloids depend upon charge and hydration for

stability, more particularily the latter. When gelatin particles are deprived of their water layer they will flocculate if the charge is small.

It was found that if the charge is increased by the addition of HCl to a pH of approximately 3.5, gelatin will not flocculate in alcohol solutions up to 50 percent ethanol by weight. If such a solution is made 0.1 N with respect to KCl, diffusion data can be obtained under conditions which would dehydrate the gelatin particles, yet permit a stable sol to exist, and at the same time give results which are not anomalous due to unequal diffusion velocities of ions.

It has been shown by McBain and co-workers (3) at Stanford University that satisfactory values of molecular weights can be calculated from diffusion data. The effect of pH on the diffusion coefficient of egg albumin was investigated at pH's above and below the isoelectric point. It was found that the diffusion increased rapidly on leaving the isoelectric point but that if the solutions were made 0.02 N with respect to KCl, the diffusion would be lowered to a value closer to diffusion at the isoelectric point.

The molecular weight calculated from the diffusion coefficient at the isoelectric point was 34,000, but if calculated at pH's other than the isoelectric point (which calculations are not justified due to the charge

on the particle), the values ranged all the way down to 500.

Apparently McBain did not try the effect of more KCl to see if the diffusion values could be brought down to equal those of isoelectric albumin. Perhaps the difficulty of analysis by means of the interferometer in the presence of electrolytes accounts for this.

A different method of analysis was used by Klemm (1,2) (who did much the same work with gelatin) so that the diffusion of gelatin in solutions at various pH's could be compared to diffusion in solutions at the same pH's but with varying amounts of KCl present. It was found that in solutions 0.1 N with respect to KCl that the diffusion was the same as it was for gelatin at the isoelectric point down to a pH 3.5 from which value it slowly rose with further lowering of the pH.

A calculation of the molecular weight of gelatin from the diffusion coefficient at zero concentration obtained by extrapolation of a curve from values at 0.5 percent to 3.0 percent gelatin solutions resulted in a value of 68,200.

In comparing molecular weight values obtained from diffusion through a porous membrane with those obtained by other methods (8,3,6,7) it was found that they check quite well for albumin and hemoglobin. The value of 34,000 for albumin obtained by McBain (4) checks with Sørensen's value of 34,000 (8) from osmotic pressure values and with 34,500 ± 1000 by Svedberg (8) from sedimentation equilibrium

in the ultracentrifuge. The value of 68,500± 1000 for hemoglobin by Northrup and Anson (6) checks with 68,000 by Svedberg and Nichols (10).

EXPERIMENTAL PROCEDURE

The experimental method used is based upon the use of a cell introduced by Northrup and Anson (6), the technique for which has been studied by McBain and Liu (4). The cell consists of a resistance glass cylinder with a sintered glass diaphragm sealed into the base and a stopcock sealed into the opposite end. The cell is placed in contact with the outside solution contained in a tall form beaker of slightly larger size so that the diaphragm separates two solutions of different concentrations.

Eastman Kodak electrodialyzed gelatin was used throughout the work. Because of the effect of thermal treatment on gelatin all samples were handled in a uniform manner. The water used in all the work was degassed by heating to 60°C. and shaken under reduced pressure. The gelatin was weighed and placed in tared beakers with about the amount of water needed. Then before anything else was added the gelatin was put into solution by heating for 35 minutes in a water bath kept at 90°C. This is slightly more than sufficient time to dissolve the gelatin for a 3 percent solution. The solutions were cooled and the KCl, HCl and alcohol added. The KCl was added by weight, the alcohol

by volume to give 20 percent by weight, and the HCl added in sufficient amount to give a pH 3.5. The solution was then made up to final weight with water.

The outside solution was made up with the same proportions of KCl and alcohol, and made up to the same pH with HCl. All pH's were adjusted finally by means of a quinhydrone electrode to within 0.02 pH unit. As the solutions had to be stirred they were degassed again by placing under reduced pressure before placing in the cells and beakers.

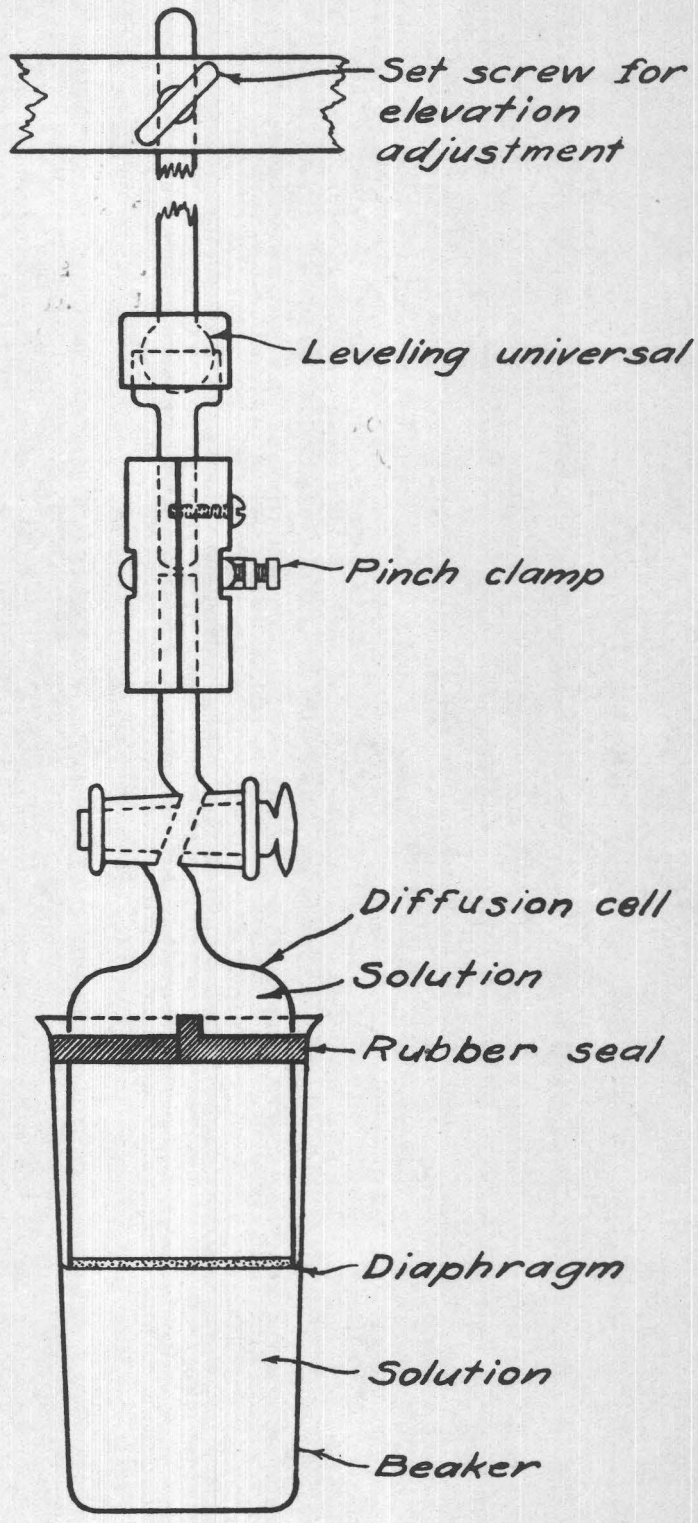
The filled cells were placed in contact with the outside solutions for twelve or more hours in order to set up a diffusion gradient within the diaphragm. The cells were then placed in contact with fresh solutions and the diffusion allowed to continue until enough gelatin had diffused to be analyzed accurately.

The cells were calibrated by means of a molar solution of KCl the diffusion values for which are well known (5).

The cells were cleaned thoroughly and swept clean of air with degassed water. By never sucking any more air through the diaphragms, it was possible to keep them free of air for practically all the runs, so that a minimum of degassed water needed to be drawn through them.

A microkjeldahl method was used (2) with a slight modification. By making up to 25 cc. instead of 100 cc., samples containing as little as 0.07 mg. nitrogen could be analyzed. This modification made it necessary to

diffuse a 0.2 percent solution for only 5 days.



Diffusion Cell

TABLE I

% Gelatin	Run	D cm^2/day	Average D
3.00	IX	0.0289	
3.00	X	.0294	
3.00	X	.0284	
			0.0289
2.00	II	0.0313	
2.00	X	.0285	
2.00	X	.0276	
			0.0291
1.00	III	0.0303	
1.00	V	.0306	
1.00	VIII	.0298	
			0.0302
0.50	V	0.0315	
0.50	VIII	.0311	
			0.0313
0.35	VI	0.0315	
0.35	VII	.0357	
0.35	IX	.0357	
			0.0343
0.20	VII	0.0379	
0.20	VII	.0370	
0.20	IX	.0344	
			0.0365

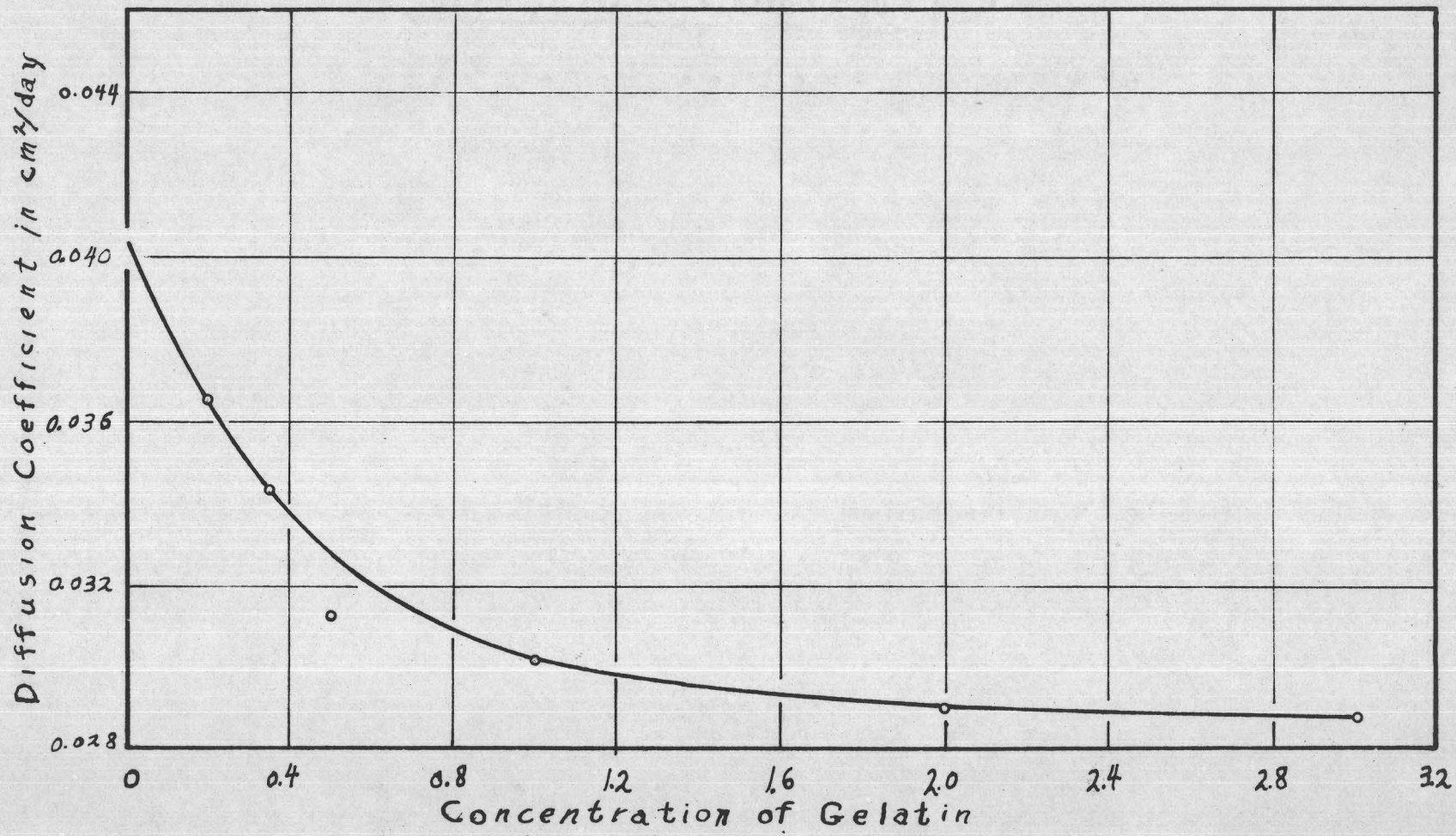

Diffusion Coefficient in cm²/day
0.044
0.040
0.036
0.032
0.028
0
0.4
0.8
1.2
1.6
2.0
2.4
2.8
3.2
Concentration of Gelatin

Fig. I

Table I gives the values of D expressed in cm^2/day for gelatin concentrations ranging from 0.2 percent to 3 percent. The alcohol concentration in all cases is 20 percent by weight, the solutions are 0.1 N with respect to KCl and are adjusted to a pH of 3.5 with HCl. The outside solutions correspond to the inside solutions but lack the gelatin.

Figure I is a curve plotting the values of D against the concentration. The curve is extrapolated to zero concentration for the purpose of calculating a molecular weight value.

By using a modification of Koch and McMeekin's (10) microkjehldahl method it was possible to determine the nitrogen concentration of the gelatin solutions after diffusion had occurred, concentrations were expressed in milligrams nitrogen per cc. instead of concentration of gelatin itself as the values needed in calculating D need only be relative.

A special form of Fick's equation (5,6) is used in calculating diffusion values from the data obtainable by the membrane method.

A form of Fick's equation:

$$ds = DA \frac{dc}{dx} dt$$

Integrated modification:

$$D = \frac{\log C_o - \log (C_o - 2c)}{Kt}$$

Where ds is the amount diffusing per interval of time dt. A is the effective area of the pores. The diffusion gradient dc/dx is equal to $\Delta C/\Delta X$ where ΔX is the thickness of the disk. D is the diffusion coefficient, K is a constant individual to each cell, which is obtained by calibration with molar KCl. C_o is the inside concentration at zero time, c is the outside concentration at time t. C_o is obtained by adding c to the inside concentration at time t for the volume of the outside solution is adjusted to equal that of the inside solution so that a decrease in concentration in one solution is equal to the gain in concentration in the other.

DISCUSSION OF RESULTS

By means of the Stokes-Einstein equation which equates the diffusion coefficient to the radius of a particle, in combination with the equation for the volume of a sphere it is possible to calculate the volume of a particle from diffusion data. By multiplying the volume by the density and then by Avagadro's number a molecular weight value may be obtained.

Stokes-Einstein equation:

$$I \quad D = \frac{RT}{N} \times \frac{1}{6\eta\pi r}, \qquad r = \frac{RT}{N} \frac{1}{6\pi\eta D}$$

Where D equals the diffusion coefficient in $cm^2/sec.$, R is equal to 8.316×10^7 ergs deg^{-1}, T equals degrees

Absolute, N is 6.06 x 10^{23}, n is the absolute viscosity of the dispersion medium in poises and π is 3.1416.

The equation for the volume of a sphere is $4/3\pi r^3$; the weight would be $4/3\pi r^3 d$, and if each sphere were a molecule the molecular weight would be $N.4/3\pi r^3 d$. A substitution of the value for r from I results in:

$$M = N \frac{4}{3} \pi d \left[\frac{RT}{N} \frac{1}{6\eta\pi D} \right]^3$$

$$M = \frac{4}{3} \frac{\pi}{N^2} \left(\frac{R}{6\pi} \right)^3 \left(\frac{T}{\eta} \right)^3 \frac{d}{D^3}$$

As all of the work was done at 35°C., T is 308. The value for n of a 20 percent alcohol solution at 35° is 0.0132 poise.(5) Svedberg (9) gives a value of 0.682 for the partial specific volume of gelatin so d = 1/.682. D is 0.0402 cm^2/day which must be divided by 86,400 to convert it to cm^2/sec.

Using these figures the molecular weight is 181,000. This value is higher than was expected. Klemm (1) obtained a value of 68,200 by extrapolating from 0.5 percent. A possible explanation of the high value (assuming Klemm's extrapolation to be correct) is as follows: In water solutions where gelatin particles are hydrated it would be possible for small particles to be present. (Gelatin is polydispersed (9) so that a range of sizes is present.)

In alcohol solutions some or all of the water is

removed. It is entirely possible that any small particles present would aggregate, although flocculation is prevented by the charge, so that the average molecular weight of the gelatin is higher in alcohol solutions instead of being lower as might be expected.

A great deal more work can be done on this problem; more concentrated alcohol solutions can be used, and gelatin in water alone can be investigated at 0.2 percent and 0.35 percent so that a more accurate extrapolation to zero concentration can be made.

SUMMARY

The diffusion coefficients for various concentrations of gelatin in 20 percent alcohol solutions have been determined. A curve plotting concentration against diffusion rate has been extrapolated to zero concentration and the resulting diffusion coefficient has been used to calculate a molecular weight by means of the Stokes-Einstein equation. A value of 0.0402 cm^2/day at zero concentration resulted in a molecular weight of 181,000.

A possible explanation has been made for the magnitude of the value as compared to results from other work.

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