AN ABSTRACT OF THE THESIS OF

Karl Klemm (Name) for the Ph.D. (Degree) in Chemistry (Major)

Date Thesis presented June 1934

Title THE DIFFUSION COEFFICIENTS AND MOLECULAR WEIGHT OF GELATIN

Abstract Approved: [redacted] (Major Professor)

An attempt has been made to determine the feasibility of the use of the type of diffusion in the steady state embodied in the Northrop and Anson type of cell for the measurement of the diffusion velocity of lyophilic colloids. The accuracy to be expected in such measurements has also been determined. An attempt has also been made to study the validity of the apparent molecular weights calculated from diffusion data and the possibility of calculating the true molecular weights of colloidally dispersed lyophilic substances from diffusion measurements.

In order to solve these problems, the changes of several properties of gelatin solutions with change of solution medium have been measured and the changes noted and explained. The changes studied and measured were:

1. Variation of the diffusion velocity (expressed in terms of D) with source.
2. Variation of the diffusion velocity with concentration.

3. Variation of the diffusion velocity with different concentrations of added KCl.

4. Variations of D with different concentrations of added alcohol.

5. Variations of D with different concentrations of added HCl.

6. Variations of D with different concentrations of added HCl and KCl.

7. Variations of D with different concentrations of added NaOH.

8. Variations of D with different concentrations of added NaOH and KCl.

9. Change of the relative viscosity of gelatin solutions with:

   (a) Change of the concentration of gelatin.

   (b) Change of the concentration of added KCl to a constant concentration of gelatin.

   (c) Change of the concentration of added alcohol.

10. Variation of the pH of gelatin solutions with:

   (a) Change of the concentration of gelatin.

   (b) Change of the concentration of added acid or base.

From the above measurements were ~~calculated the~~ the following calculations were made:—

1. Change of the "apparent molecular weight"(M) of the gelatin particles with the change of source of the gelatin.

2. Change of M with change of concentration of the gelatin.

3. Change of M with change of concentration of the added HCl and KCl.

4. Change of M with change of concentration of the added NaOH and KCl.

5. Change of M with change of concentration of the added alcohol.

6. Apparent change of the "apparent molecular weight" of the gelatin micellae which results from the calculation of the M for charged particles by means of the Stokes-Einstein equation.

7. Apparent molecular weight of the gelatin molecule at zero concentration.

8. Effect of the removal of the water mantle from the micellae upon the value of M.

From the measurements and calculations made from them the following results have been obtained:-

1. The changes of D with concentration of the gelatin, concentration of added HCl, HCl and KCl, NaOH, NaOH and KCl, and of added C_2H_5OH have been explained.

2. The possibility of error incurred by the use of the Stokes-Einstein equation for the calculation of M from diffusion data for ions has been shown.

3. The necessity of employing values of D corresponding to zero concentration of diffusate for the calculation of the apparent molecular weight of the diffusate when molecularly dispersed has been demmnstrated.

4. The possibility of the determination of the true molecular weights of lyophilic substances by determining their diffusion velocities after the removal of the water mantle has been discussed.

5. The probable incorrectness of all the calculated values of M for lyophilic colloids has been shown.

6. The possibility of measuring the thickness of the water mantle and the determination of its effect upon the physical properties of the diffusate have been discussed.

7. The probable sources of error possible by this method have been enumerated.

8. The necessity of emen more precise methods for the measurement of concentrations has been mentioned.

THE DIFFUSION COEFFICIENTS AND MOLECULAR WEIGHT OF GELATIN

by

KARL KLEMM

A THESIS

submitted to the

OREGON STATE AGRICULTURAL COLLEGE

in partial fulfillment of
the requirements for the
degree of

DOCTOR OF PHILOSOPHY

June 1934

APPROVED:

[redacted]

Professor of Chemistry In Charge of Major

[redacted]

Head of Department Chemistry

[redacted]

Chairman of School Graduate Committee

[redacted]

Chairman of College Graduate Council

TABLE OF CONTENTS

TABLES

FIGURES

THE DIFFUSION COEFFICIENTS AND MOLECULAR WEIGHT OF GELATIN

I. Introduction

1. The Importance of Diffusion Measurements on Colloids.

The prediction of the behavior and the explanation of the properties of a given substance depend upon a knowledge of the arrangement of the constituent atoms in the molecule. The kinds of atoms present and their existence in the form of the various possible radicles may be determined analytically. The structural units present in complex molecules, which consist of these elements and such radicles as may be formed from them, may be determined by a study of the thermal decomposition and hydrolysis products obtained by the proper treatment of such materials. The final proof of the structure of large complex molecules depends upon a knowledge of their molecular weights.

Substances composed of large molecules, such as agar-agar, gelatin, proteins, and other biologically important materials, generally exist in the dissolved state as colloidal solutions. The limited solubility in water of substances, such as agar-agar, which exist in solution in the colloidal state makes the determination of their molecular weights from boiling point increase, freezing point lowering, vapor pressure lowering, or osmotic

pressure data either very uncertain or even impossible. Some colloidally dispersed materials either coagulate or are thermally decomposed at higher temperatures. Still others exhibit a penchant for aggregation or deaggregation, as the case may be, with change of solvent, concentration, or pH of the solution. Since colloidally dispersed materials exist in such low concentrations, from the molecular standpoint, enormous errors are introduced into boiling point, freezing point, vapor pressure, and osmotic pressure data by the presence in the systems of minute traces of salts or other impurities. In general, lyophilic colloids, especially proteins, are also very susceptible to bacterial action.

One apparently simple and inexpensive method for the measurement of particle radius of colloids, the calculation of molecular weight, and the change of these properties with change of temperature, time, solvent, pH of the solvent, and the method of preparation of the solute is the determination of diffusion velocity data. However, an examination of the literature reveals a bewildering array of material difficult, if not impossible, to correlate. Various investigations of a given substance under the same or comparable conditions of temperature, concentration, solvent, etc., yield widely different values for the diffusion velocity. Other data reveal diametrically

opposite changes in the diffusion velocity of a given substance with change of temperature or concentration. Anomolous results have been obtained with some materials, others, of known molecular weight, have shown diffusion velocities much too high or too low for molecules of their mass. There is, also, seldom any agreement between the molecular weights calculated from diffusion data and those calculated from osmotic pressure, cryoscopic, viscosimetric, x-ray, and ultracentrifugal data.

It is apparent that diffusion data will be of little use for the determination of molecular weights unless it can be more accurately determined and the apparently contradictory and anomolous results can be explained and subsequently eliminated.

2. Statement of Problem and Reason for Studying Gelatin.

This investigation was undertaken in an effort to determine the causes of the above mentioned contradictory and anomolous values obtained for diffusion velocities and to demonstrate the validity of molecular weights calculated from diffusion coefficients.

Gelatin was selected as the material to be studied because of its availability in the very pure form and because it possesses, probably, more of the general

properties of lyophilic colloids than any other single substance. A further factor influencing the selection of gelatin lay in the fact that its diffusion coefficients and molecular weights had already been measured by ultracentrifugal means (92) and thus would serve as a standard with which the results obtained could be compared.

It was also hoped that time would permit the extension of this study to other materials such as typical proteins, but this was found to be an impossibility.

II. Methods of Studying Diffusion in Liquid Systems.

1. Methods Employed

The diffusion of the particles formed as the result of the solution, or dispersion, of any material in a liquid medium is a fundamental phenomenon of liquid systems. It was mentioned first by Berthollet in 1803, but the actual study of this property was not begun until 1850. At that time Thomas Graham (36) set up an apparatus consisting of two bottles of equal volume. A solution was placed in one bottle, pure solvent in the other. The ground tops of the bottles were then placed together, the solution and the pure solvent being separated by a thin layer of sponge which, Graham found, did not change the rate of diffusion appreciably. By placing the denser solution in the lower bottle, Graham was able to study the diffusion of carbon dioxide and of nitrous oxide into water and into each other. Graham also devised a second type of cell. This consisted of a glass bottle, into which was placed the denser solution, and a large cylinder. The bottle was placed in the cylinder which was then filled with solvent.

The results obtained by either method were only qualitative and Graham did not formulate any theory of diffusion nor derive any of the relationships since de-

veloped. He did note that: (1) Solutions of equal density may have widely different rates of diffusion. (2) Most salts diffuse at rates which are proportional to their respective concentrations. (3) The rate of diffusion of a given substance increases with increased temperature. (4) The amount of material diffusing out of the inner bottle decreases with increased time of diffusion, but is constant for the second, third, and fourth days. (5) Acids and bases diffuse more rapidly than the corresponding salts. (6) Egg albumin diffuses very slowly, but the rate is increased slightly by the addition of acetic acid. (7) The viscosity of the solution that is due to the presence of the egg albumin does not retard the rate of diffusion of salts. (8) In a mixture of salts the velocity of the more rapidly diffusing salt is greater than when it is diffusing alone. (9) Alum decomposes during diffusion. (10) Mixtures of salts and double salts may be partially separated by diffusion.

The first mathematical treatment of the phenomenon of diffusion in liquid systems was made in 1855 by Fick (30) who, perceiving the analogy between diffusion in solution and the conduction of heat, applied a modified form of Fourier's equation and obtained:

$$\frac{\partial u}{\partial t} = K \frac{\partial^2 u}{\partial x^2}$$

for diffusion after any time interval, and

$$\frac{\partial^2 u}{\partial x^2} = 0$$

for diffusion in the steady state where u is the amount of diffusate per unit volume, x the distance moved, and t the time during which diffusion has occurred. Fick tested the validity of his relationships by measuring the velocity of diffusion in the steady state. His apparatus consisted of a cell similar to Graham's first type with the end removed from the upper bottle. By means of a glass ball suspended in the liquid of the upper bottle from one arm of a balance beam, Fick measured the diffusion velocity by determining the change in density of the pure solvent as the solute diffused upward into it.

Beilstein (6) devised an apparatus consisting of a cylinder which was bent into a semicircle at one end and closed at the other by means of a glass stopper. The solution of the material to be studied was drawn into the cylinder which, when completely filled, was stoppered. In effect the solution was held in the cylinder by the vacuum above it. The filled cylinder was then suspended in a large vessel of solvent in such a way that the surface of the solution at the open end was horizontal. By this means Beilstein hoped to attain a diffusion layer with the solutions on either side kept uniform by the mixing re-

sulting from the differences in densities in the respective solutions. Assuming that the hoped-for conditions would be attained, Beilstein developed equations for the calculation of the diffusion coefficient independently of the time. Constant values were obtained for KNO_3, but trouble was experienced in the diffusion of acid sulfates.

Semmler (81), by the substitution of a cylinder for the bottle employed in Graham's second type of apparatus (36), embodied in the Graham cell conditions which permitted the application of Fick's equation to the data obtained. Calculations were made by means of Fick's equation in the form:

$$ds = - KQ \frac{du}{dx} dt$$

and by means of an integral of the equation:

$$\frac{\partial u}{\partial t} = K \frac{\partial^2 u}{\partial x^2}$$

which took the form:

$$u = e^{-m^2 k t}(A \cos mx + B \cos mx).$$

Semmler also described a prism-shaped vessel for the measurement of diffusion velocities. In Semmler's apparatus, the solution was to be placed at the bottom of the prism and covered with a layer of pure solvent. Concentration changes at different levels were to be determined optically by viewing the cord of a pendulum through the prism. Since the amount of the apparent dislocation of

the line depended upon the concentration of the diffusate, the concentration at any point could easily be measured.

Graham's third method for studying diffusion (37) consisted of a straight glass cylinder of known volume. The cylinder was filled with pure solvent and then a known volume of solution was introduced into the bottom of the cylinder by means of a pipette. After a suitable interval of time, the liquid was removed in sixteen layers and each layer was analyzed. At the same time, Graham, employing an apparatus similar to his first cell, measured the velocity of diffusion of different salts through parchment paper and in gelatin.

The Graham method was utilized by Marignac (61) in his study of the diffusion of mixtures of electrolytes. Calculations, made by means of Marignac's version of the Beilstein equation,

$$\frac{K}{K_1} = \frac{\log\left(\frac{A}{A-p}\right)}{\log\left(\frac{A_1}{A_1-p_1}\right)},$$

did not give a constant value for the K/K_1 ratio. Marignac realized that the experimental conditions required by Beilstein's equation were not being realized so he arbitrarily introduced 2p for p in the equation and found the K/K_1 ratio to then be nearly constant with time. Marignac also confirmed Graham's statement that salts when mixed

with other materials diffuse at different rates than when they diffuse alone.

Seylor (82) introduced the use of the polariscope for following changes of concentration with time in his measurements of the diffusion velocities of sugar. Stefan (86) utilized Seylor's data to check the validity of Fick's equation. Comparison of these values with those obtained by Johannisjanz (49) for the diffusion of salts showed the latter to be incorrect. Investigation of Johannisjanz's work revealed that his data had been obtained by the optical observation of materials in a glass prism according to the method outlined by Semmler (81). The errors in Johannisjanz's calculations were the result of his not making allowance for the fact that a liquid whose refraction coefficient decreases from bottom to top itself acts like a prism with its refracting edge upward. Stefan also calculated diffusion coefficients from Graham's data by means of an integrated form of Fick's equation which corresponded to the experimental conditions under which Graham's data was obtained:

$$u = u'\frac{h}{h'} + \frac{2u'}{\pi}\sum_{n=1}^{n=\infty}\frac{1}{n}\sin\left(\frac{n\pi h}{H}\right)\cos\left(\frac{n\pi h}{H}\right)e^{-\frac{n^2\pi^2 Kt}{H^2}}.$$

J. J. Coleman (20, 21, 22) originated a method for the study of the free diffusion of such materials as would affect indicators. In this method, the electrolytes to be studied were placed at the bottom of narrow tubes, covered with solvent, and the diffusion of the respective electrolytes was followed by means of indicators originally present in the solvent. Similarly, Weber (104) obtained diffusion coefficients by measuring the changes in the potential of a concentration cell as diffusion proceeded.

An early attempt to set up two solutions with a constant concentration on either side of the diffusion boundary by causing water to flow slowly over a solution contained in a narrow cell (56) gave very unsatisfactory results. With a more accurate control of the rate of flow of the solvent over the solution, combined with a more sensitive method of analysis of the diffusate solution, the apparatus has since yielded a precision method for the determination of diffusion coefficients (11).

Scheffer simplified the study of diffusion by employing the modified Graham apparatus--a perpendicular cylinder placed inside of a large container by dividing the diffusate into four layers for analysis and calculating the diffusion coefficients from Stefan's tables.

By analogy with the diffusion of gases from areas of high pressure to areas of low pressure, Nernst (65) attributed diffusion to osmotic pressure. He then showed that the diffusion coefficient, K , of Fick's equation:

$$S = -K\,Q\frac{dc}{dx}\,dT$$

may be expressed as

$$K = \frac{p_o}{K}\ \text{cm.}^2/\text{sec.}$$

where K is the pressure required to give one gram equivalent of material a velocity of one centimeter per second and p_o is the osmotic pressure. Measurements of K showed it to be of the same order as the force J which Kohlraush found was necessary to move one gram equivalent of material in an electric field. From Ostwald's postulate of electroneutrality (75)—that an equal number of positive and negative charges must be present in any finite volume of an electrolytic solution—Nernst derived the relationship:

$$K = \frac{U \cdot V}{U+V} \cdot 0.04768 \cdot 10^7\ \text{cm}^2/\text{day at } 18^\circ C.$$

which, in the form:

$$D = RT \cdot \frac{2UV}{U+V}$$

has been the basic equation for the calculation of diffusion coefficients of dilute solutions of electrolytes from conductivity data ever since. The equation is valid, as Nernst himself stated, only at extreme dilution where the

osmotic pressure is directly proportional to the concentration. Nernst also developed an equation for the temperature coefficient of diffusion, a relationship for the diffusion at low concentrations, of salts with a common ion (from a study of Marignac's data), and a relationship for the diffusion potential for the case of two solutions of the same salt at different concentrations, namely:

$$\psi = 0.0235 \frac{(U - V)}{(U + V)} \log \frac{p_1}{p_2} \text{ volts.}$$

Planck (78, 79), assuming, as Nernst did, that the total movement of a given ion is equal to the sum of the movement due to osmotic pressure and the movement due to electrostatic pressure, derived an equation for the diffusion of electrolytes which takes the form:

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

where E is the charge on the individual ions, ψ the electrostatic potential, c the concentration of the ion, and U the mobility of the ion. The equation has been assumed by later investigators to be valid for all ions present in a given solution, but a theoretical demonstration of the validity of this assumption has never been developed.

Using the Scheffer method for studying the rate of diffusion and Stefan's tables for the calculation of the diffusion coefficients from the data obtained, Arrhenius (2) investigated the effect of non-electrolytes upon the

diffusion velocities of electrolytes. The results verified the findings of Lenz (54), namely, that the diffusion coefficient of strong electrolytes varies, upon the addition of alcohol, in a manner parallel to the variation of conductivity under similar treatment. His investigations also showed that for the addition of various electrolytes to cane sugar, there is a close agreement between the diffusion velocity of the sugar molecules and the viscosities of the solutions, but that the change in viscosity is greater than the change in the diffusion coefficient. Arrhenius also derived an equation for calculating the effect of a salt, containing a common ion and at constant concentration throughout the diffusion cell, upon the diffusion coefficient of the diffusing salt.

Behn investigated the diffusion of $AgNO_3$ against mixtures of $AgNO_3$ and HNO_3 of the same total concentration (4). He found that in order to determine the absolute values of the diffusion coefficients for the respective salts in a mixture, the thickness of the diffusion layer must be known.

An electrical method for the determination of diffusion velocities was developed by Meyer (64) and employed by him for the measurement of the diffusion velocities of Zn, Cd, and Pb in mercury. The method was improved by Haskel (43) who determined the conductivity of his solu-

tions at various levels and so made direct determinations of the specific diffusion rates of both the dissociated and the undissociated portions of partly ionized substances.

The diffusion, in the steady state, of a number of electrolytic materials was studied by Griffiths (39, 40, 41) by means of a series of diffusion tubes similar to those employed by Coleman (21). The results obtained are fragmentary and not of too great accuracy.

An enormous amount of experimental data was accumulated by Thovert (95, 96, 97, 98, 99, 100, 101) who studied diffusion in a series of glass prisms and employed optical methods for the analysis of his solutions. Thovert not only measured the diffusion velocities of the more common acids, bases, and salts over a wide range of concentrations, as did Öholm, but also secured the experimental data and compiled tables of the diffusion coefficients for a number of organic compounds diffusing into water, methanol, and benzene, respectively, as solvents. The results showed that the product of the diffusion coefficient and the viscosity of the solution, $D\eta$, is a constant for a number of widely different solutions. Thovert next investigated Pickering's conclusion (76) that

$$Dm^{1/2} = k,$$

which results from considering the osmotic pressure of a

solution as being equivalent to the gas pressure exerted by a similar number of molecules in the gaseous state confined within an equal volume, and found that his experimental results did not justify this conclusion. The results of Euler's studies (29) indicated that the $Dm^{1/2} = k$ relationship did hold. In 1902, Thovert showed that the $Dm^{1/2}$ product was relatively constant for nineteen organic compounds lying between methanol and raffinose. A study of Einstein's (27) and Sutherland's (88) work convinced him that the Stokes-Einstein equation did not apply to molecules of the magnitude of those he had investigated. Thovert also extended the work of Arrhenius (2) — the effect of one material at constant concentration upon the movement of a second dissolved material — by applying this type of study to solutions of non-electrolytes. The further extension of this investigation into the region of concentrated solutions revealed a lack of agreement between experimentally determined and calculated results. Thovert realized that the diffusion of one non-electrolyte caused the second non-electrolyte, originally existing at uniform concentration throughout the solution, to move, but reached no conclusion concerning the cause of this phenomenon other than that it was evident that the action was different from electrolytic action or dissociation.

L. W. Öholm (67), employing an improved apparatus of the Graham type and working underground in order to avoid light, vibration, and temperature fluctuations, secured the first really accurate experimental diffusion data.

His apparatus (68), designed for the study of free diffusion upward, consisted of a graduated pipette, equipped with a stop-cock and ending in a capillary tube, which was inserted into the diffusion cell so that the capillary tube just cleared the surface of a layer of mercury which covered the bottom of the cell and formed a plane, level surface. Three volumes of solvent were run into the cell by means of the pipette, then one volume of a solution of the diffusate was introduced into the cell so slowly that it formed a layer below the solvent. After a suitable interval of time, the liquid was removed from the cell, by means of the pipette, in four layers and the respective samples were analyzed. Then, the concentration of the diffusate in the respective layers and the time of diffusion being known, the diffusion coefficient of the diffusate was calculated from Stefan's (86) or from Kawalki's tables.

From his results, Öholm prepared tables of the diffusion coefficients of all the more common acids, bases, and salts for several concentrations and for at least two temperatures. In general, his values show that the diffu-

sion coefficient of every electrolyte, which does not form molecular complexes, passes through a minimum value, the minimum occurring in more concentrated solutions for some electrolytes than for others. Öholm's results, in accordance with those of Arrhenius (2), show that at low concentrations, the experimentally determined values of diffusion velocities approach those calculated from the Nernst equation and that the ratio of the osmotic pressure of a given substance divided by the product of the viscosity of its solution and its diffusion coefficient, $\pi/\eta D$, is a constant for that material at all concentrations.

The employment of diffusion coefficients for the calculation of the molecular weights of substances for which this property reaches a high value was first reported by Sutherland (88). He employed the formula

$$D = \frac{RT}{N} \cdot \frac{1}{6\pi\eta r}$$

which he had obtained from the Nernst equation by substituting Stoke's formula for the resistance to motion of a spherical particle of large diameter, as compared to the diameter of the molecules of the dispersion medium, for the equivalent term in the Nernst equation. Einstein (27) derived a diffusion equation of exactly the same form as that developed by Sutherland. His reasoning was the same as that followed by Sutherland except that he considered

only the case of suspended particles. He specifically stated that the equation applied only to non-dissociated particles (28) and employed it for the determination of Avogadro's constant.

During the interval between the years of 1907 and 1919 a relatively large number of variations of the older methods of studying diffusion were developed: Herzog (45) employed the Öholm apparatus and method (68) for his measurements of the diffusion velocities of a number of proteins, the concentrations of the various layers being determined by gravimetric means. An extension of the method employed by Fick (30) was used by Clack (16) who determined the change in density of a solution with time by placing the solution in a spherical vessel and suspending this vessel in the solvent from the arm of an analytical balance. Öholm (69), studying the diffusion of sugars, determined the concentration of the diffusate in the different layers by means of its optical activity. Microscopic determinations of diffusion coefficients were begun by Westgren (105) who centrifuged the particles of both selenium and of gold hydrosols to one end of a very thin diffusion cell and then observed the movement of single particles. A method for the photographic determination of the diffusion coefficients of substances which exist in solution only in very low concentrations

was developed by Eicke (26). Dummer, in his study of the diffusion of organic substances in organic media (24), made use of a Schuhmeister cell, which was the forerunner of the Zuber type, and measured the concentration changes refractometrically.

A unique method for the measurement of the amount of diffusate present at various depths was that devised by Littlewood (55). His method of procedure consisted of placing the diffusate solution in a closed vessel whose top and one side were of glass. On the glass side of the cell was fastened a vertical scale. The cell was immersed in a water-filled vessel which contained a movable mirror which could be rotated and whose position could be determined on a graduated scale. A telescope was then mounted upon a stand which also carried a horizontal wire illuminated by means of a sodium flame. The mirror in the outer vessel was so adjusted that the image of the wire, after having twice passed through the liquid, was seen on the cross-hairs of the telescope. The corresponding division on the vertical scale was also observed. From these measurements the concentration of the diffusate at different depths was calculated.

In order to secure a more accurate separation of the different layers of the diffusate solution which were taken for analysis, Cohen and Bruins (18) devised a new cell of

the Öholm type. The apparatus consisted of six cylindrical plates fastened together by means of a pin which passed through their centers and about which they were free to rotate. The four inner discs had a hole drilled through each in such fashion that these holes could be "lined up" (by rotating the discs) to form a cylindrical opening. The cylinder formed by the opening in the next to the bottom plate was filled with a solution of the diffusate and cut off from the others. The remaining three cylinders were again "lined up", filled with the solvent; and finally "set" above the solution of the diffusate. When diffusion had progressed for a sufficient length of time, the respective sections of solution were isolated by rotating the discs, and the solution contained in each section was removed and analyzed.

The years from 1926 onward saw another period of activity in the development of different types of cells and better methods for the analysis of the diffusate solutions: Wilke and Strathmeyer (106) measured diffusion velocities by following the changes in density with time of the system at different levels. This was accomplished by means of previously calibrated glass floats, one square millimeter in cross-sectional area and two centimeters in length. The measurement of concentrations by means of an

interferometer was introduced into the study of diffusion in 1927 by Bekesy (5). An entirely different method for the study of the continuous diffusion of materials in solution was developed by Komers (51). In Komers' apparatus, the diffusate passed around a horizontal cylindrical vessel in which the solvent was maintained at a constant level and was caused to travel in a direction counter to that of the direction of motion of the diffusate.

A revival of the study of diffusion in the steady state was begun by Northrop and Anson (17) when they presented a new cell for the study of the passage of a diffusate through a thin porous membrane of sintered glass which prevented any mixing of the solutions it separated and confined the concentration gradient between the solutions to a sharply defined and constant distance. The method was later standardized and successfully applied to the measurement of the diffusion coefficients of electrolytes, non-electrolytes and colloidal electrolytes (59), to mixtures of electrolytes and colloidal particles (57), and,more recently, to a study of the problem of accelerated and retarded diffusion in aqueous solutions (58). The diffusion of electrolytes through collodion membranes was investigated by Butkevitch (15), but use of such membranes was limited and it was found necessary to calibrate a given membrane each time it was used by means of a diffusate of known diffusion velocity.

An ultracentrifugal method for the determination of diffusion velocities either from sedimentation equilibrium data or from sedimentation velocity data was developed by Svedberg (89) and employed for the determination of the diffusion coefficient, molecular weight, and the pH stability range of a large number of proteins (90, 91, 92).

In 1931, Bruins developed an improved apparatus of the type devised by Long (56) for the measurement of the diffusion coefficients of materials having very low diffusion velocities (11). The apparatus consisted of a narrow cell built into a small interferometer through which the light traveled in a perpendicular direction. The diffusate solution was placed in the cell and pure solvent was passed at constant velocity over the top of the diffusion cell. In this way a constant and maximum concentration gradient was maintained.

Non-spherical particles when under mechanical stress, tend to orient themselves in such fashion as to reduce that stress. The orientation of such particles is accompanied by the phenomenon of birefringence. In 1932 Boeder (9) developed a method for calculating diffusion velocities from measurements of the mechanical birefringence induced by the flow of colloids which gave experimental values that agreed well with calculated values.

A microscopic method in which the concentrations at different levels were measured by determining the angles of total reflection was invented by Zuber (107). Values of the diffusion coefficient could be determined within a very few minutes, but the accuracy was only of the order of about 6% for materials showing diffusion velocities in the neighborhood of that of sodium chloride.

An examination of the foregoing examples will show that the methods for studying the velocity of diffusion of materials in liquid systems may be divided into three general classes:

(a) Diffusion in the steady state: When this method is employed, measurements are made only after a steady rate of diffusion has been established between crystals of the diffusate at one end of a diffusion tube and pure solvent at the other.

(b) Dynamic diffusion: This method depends upon measurements taken upon systems in which the equilibrium considered in the "steady state" method has not been established.

(c) Membrane diffusion: The membrane method is a special case of diffusion in the steady state, except that here the diffusion gradient is confined to within a membrane placed between two solutions whose concentrations

do not change appreciably between the beginning and the end of the determinations.

A second manner of classification that might be used is to divide them into:

(a) Free diffusion: Here the diffusate solution is placed beneath, or above (depending upon the respective densities of the solution and the solvent), the pure solvent in such manner that mixing does not occur and diffusion is allowed to take place unhindered. The main advantage of this method, namely, that the apparatus does not require standardization, is more than offset by the following facts: (1) Such a system must be kept free from all light, vibration, and temperature changes, which would produce convection currents. (2) It is impossible to place the pure solvent above the solution without producing a certain amount of mixing. (3) Since the less dense liquid must be placed above the denser, diffusion must generally take place upward, which is a slow process which requires a long period of time before measurable results can be obtained.

(b) Membrane diffusion: In this method, the diffusate solution and the pure solvent are separated by a membrane. After a diffusion gradient has been set up within the membrane, a fresh solution of the solvent is placed in contact with the membrane and the velocity of diffusion of any given substance can be measured. An apparatus con-

taining a membrane for the separation of solution and solvent must be standardized against a substance of known diffusion velocity. But, unlike the methods for free diffusion, there can be no errors due to convection currents resulting from light, temperature changes, or vibration; the diffusion gradient is confined to a certain definite, measurable distance; there can be no errors introduced as a result of the mixing of the diffusate solution and the pure solvent either when diffusion is begun or when the final samples are taken for analysis; and a constant, maximum diffusion gradient may be maintained throughout the duration of the experiment.

2. The Results Obtained.

The results obtained by the various investigators have been of such fragmentary nature and have been determined at so many different temperatures that correlation of the data and the development of general, theoretical relationships between such things as the velocity of diffusion and the viscosity of the solvent, the relative sizes of the diffusate and solvent molecules, the hydration of the diffusate particles and the concentration of the diffusate, have been impossible. When to this is added the fact that the diffusion coefficients obtained by different investigators for a given material and under com-

parable conditions not only do not agree in magnitude, but also indicate an opposite change in diffusion velocity for the same change of concentration or temperature, the necessity for more accurate and more complete data is at once evident.

In general, it may be said that the diffusion coefficient, D, for electrolytes changes with change in concentration (17, 87) and usually passes through a minimum (34). The diffusion coefficient for non-electrolytes is generally lower than that of electrolytes of comparable molecular weight (44) and the change with change of concentration is also less than for electrolytes.

The change of diffusion velocity of electrolytes with change of concentration is proportional, not to the total concentration, but more nearly to the relative number of undissociated molecules (15). A more extended investigation of this phase of diffusion by Wilke and Strathmeyer (106) indicates that the diffusion coefficient periodically increases and decreases with change of concentration. The work of Herzog and Polotzky (46) apparently shows that the diffusion velocity of a given material is determined, not by its molecular weight, but rather by the number of atoms and their configuration in the molecule. Sŭllmann's work on urea (87) shows a minimal value of D at zero concentration, a maximum value at a 2% concentration, and

second minimal value, slightly larger than the first, at a 9% concentration.

In the case of concentrated solutions, the diffusion velocity is not proportional to the concentration gradient, but to an unknown function which is roughly proportional to a power series (103). A decrease of the diffusion coefficient with increasing molecular weight of the diffusate is shown by the work of Heiduschka and Ripper (44). Contrary to the generally prevailing belief, the results of the investigations of Wilke and Strathmeyer (106) show that the influence of vibration upon the value of D, determined by measurements of free diffusion, is of minor importance.

Studies of aqueous sodium oleate solutions (57) show the diffusion velocity to be proportional to the osmotic pressure of the system. This relationship is verified by the work of Jander and Winkel (48) on the diffusion of hydrated amphoteric oxides in aqueous solutions. These latter investigators also found that the hydration of the ions, the temperature, and the viscosity of the solvent have a marked effect upon the velocity of diffusion of a given substance.

As a result of his study of the Stokes-Einstein equation, Öholm (70) concluded that the diffusion velocity

of a given material should be inversely proportional to the viscosity of the solvent. Experimental investigation of this theory shows that the product $D\eta$ is not constant and assumes a considerably lower value for ethyl or amyl alcohol than for water when glycerol is employed as the diffusate. These variations can be explained only partially on the basis of a change in the association of the diffusate and the solvent and the hydration of the diffusate.

The investigations of Öholm (71) upon such electrolytes as HCl, LiCl, KOH, etc., show that the temperature coefficient of the diffusion coefficient D, varies from 0.02 to 0.03 per degree, the average being 0.025. For non-electrolytes such as arabinose, nicotine, raffinose, etc., whose coefficients of diffusion average about 0.3, the temperature coefficient varies from 0.015 to 0.044 per degree. In another series of investigations by Öholm (72), the temperature coefficient of the D of organic materials is given as approximately 2% per degree.

Temperature coefficient relationships for D have been developed by various authors by assuming that the change of the diffusion coefficient with change of temperature is a linear function. The best results are obtained by the use of the relationship developed by Herzog (47) from the Stokes-Einstein equation:

$$\frac{D_2}{D_1} = \frac{T_2}{T_1} \cdot \frac{\eta_1}{\eta_2} = 1 + \alpha (T_2 - T_1)$$

or by the employment of the relationship derived by Freundlich and Kruger (33):

$$D_t = \frac{D_{20^\circ} \cdot \eta_{20^\circ}}{\eta_t \; 293} (273 + t)$$

In one of his later articles Öholm (73) gives the temperature coefficient of D as 3.5% per degree, which, he states, decreases with the decreasing diffusibility of the dissolved materials.

The value of the greater part of the diffusion data extant at the present time and the uncertainty involved in the careless employment of any of it are made very apparent by the preceding summary.

3. Theoretical Bases and Relationships Developed

(a) Fick's Law. The first theoretical and mathematical treatment of the process of diffusion was made by Fick (30) who saw, from the results obtained by Graham (36) and Berthollet, that the rate of diffusion depends upon the nature of the diffusing substance, the temperature of the system, and the concentration gradient of the diffusate. As a result of his observations, Fick reasoned that the quantity of a substance found in a given unit volume of a

diffusion system at a given time is a function of the position of that unit volume. That is,

$$u = f(x,t)$$

Seeing also the analogy between the process of diffusion and the conduction of heat, Fick applied Fourier's equation for the conduction of heat from a point source to the special case of conduction along a cylindrical system of constant cross-sectional area and derived the relationship:

$$\frac{\partial u}{\partial t} = K \frac{\partial^2 u}{\partial x^2} \quad \text{or} \quad ds = DA \frac{\partial c}{\partial x} dt$$

for the general case where ds is the quantity of non-electrolyte material which passes in the time dt through a diffusion cylinder of cross-section A under a concentration gradient dc/dx (a concentration of c in the cross-section at a point x and a concentration of c dc at the point x dx) and D is a constant for a given diffusate. For diffusion in the steady state the equation reduces to

$$\frac{\partial^2 u}{\partial x^2} = 0$$

(b) The Nernst Law. In the case of the diffusion of electrolytes the phenomenon is complicated by the fact that independent diffusion of the ions of different sign cannot occur since the resulting separation would set up

electrostatic forces which would enhance the diffusion velocity of the more massive ions and decrease that of the ions of lesser mass. The result of the action of these opposing forces of diffusion and electrostatic attraction results in the diffusion of both ions through the solvent at an equal rate. With this concept and the consideration that the force determining diffusion in solution is essentially the same as that which is designated as the osmotic pressure of solutions, Nernst (65) developed the following expression for the diffusion of electrolytes:

$$ds = -\left(\frac{2UV}{U+V}\right) RTA\frac{dc}{dx}\,dt \quad ,$$

or

$$D = \left(\frac{2UV}{U+V}\right) .$$

(c) Einstein's Equation. Einstein (27) treated the case of the diffusion of large particles. He assumed that the particles were spherical, uncharged, and very large in comparison with the molecules of the dispersion medium; that the movement of the several constituents of a mixture was independent; and that all the particles possessed the same mean kinetic energy as a gas molecule at the same temperature. On these assumptions, Einstein developed from the equation for the mean displacement of a particle as the result of its Brownian motion the relationship ex-

pressed by:

$$\overline{\triangle}^2 = \frac{2RT}{N}BT \quad , \quad \ldots . \quad (1)$$

where N is Avogadro's constant, R is the gas constant, T the temperature expressed in degrees absolute, B the mobility coefficient (the velocity of motion of a given particle moving through the solution under the influence of unit force), and $\overline{\triangle}^2$ is the mean displacement of a particle. By combining this expression with that for the relationship between molecular motion and diffusion:

$$D = \frac{\overline{\triangle}^2}{2t} \quad , \quad \ldots . \quad (2)$$

the following expression for the diffusion coefficient was obtained:

$$D = \frac{RT}{N} \cdot B \quad , \quad \ldots \quad (3)$$

The value of B, for uncharged, spherical particles which are large in comparison with the solvent molecules is, according to Stoke's law, given by:

$$B = \frac{1}{6\pi\eta r} \quad , \quad \ldots . \quad (4)$$

where η is the viscosity of the solvent, r the radius of the particle. Combination of equations (3) and (4) gave:

$$D = \frac{RT}{N} \cdot \frac{1}{6\pi\eta r} \quad , \quad \ldots . \quad (5)$$

the so-called Stokes-Einstein equation.

(d) Svedberg's Equation. In his determinations of molecular weights by means of ultra centrifugal data, (90) Svedberg, also determines the diffusion velocities of the materials studied by means of the relation developed for the conditions to which a molecule is subjected when studied by this method:

$$ds = - RT \frac{dc}{dx} \cdot \frac{1}{Fdt}$$

where F is the frictional force exerted on a mole of solute material.

Fick's equation applies only to those solutions in which there is not more than one electrolyte or non-electrolyte present and in which there is no change in the state of aggregation either of the diffusate or the solvent. The diffusate molecules must also move independently of one another except that the ions of an electrolyte must move so as to preserve electro-neutrality, which simply means that Fick's law holds strictly - so that the value of D is constant - only at infinite dilution.

The Nernst equation is valid only under the same conditions. Planck and others have formulated equations for the movement of one electrolyte in the presence of another. Öholm and Thovert tried to correlate the change of osmotic pressure and of viscosity, as concentration is increased, with changes of the diffusion coefficient. The modern

theories of electrolytes have been applied to the problem of diffusion with but slight success as yet. Pissarievski and Karp (77) have formulated the general relationship:

$$D\eta\sqrt{M} = \alpha K,$$

where α is the degree of dissociation, η the viscosity of the solvent, and K is a constant.

A general diffusion law and an equation for the diffusion coefficient have been formulated directly from mechanical principles by Brugs (9) and applied to existing data with but slight success.

An investigation of the validity of the Stokes-Einstein equation by Cohen and Bruins (19) for diffusion in molecular solutions in which tetrabromethane in tetra chlorethane was employed, showed a deviation from the law which was three times as great as could be accounted for by experimental error. The temperature coefficient of diffusion velocity was also shown to be less than that required by the Stokes-Einstein equation. Agreement with the Stokes-Einstein equation could not be expected in this case since it was derived for the diffusion of particles large in comparison with the solvent molecules, a classification to which the diffusate employed by Cohen and Bruins does not belong.

Similar investigations have been made by Dummer (24) with a common diffusate dispersed in different solvents.

Calculation of the molecular radius of a given diffusate from measurements of the diffusion coefficient determined in solvents having equal, larger, and smaller molecules than those of the diffusate shows that the radii so calculated vary with the molecular weight of the solvent, the apparent radius being smaller the greater the molecular weight of the solvent. The results of Dummer's investigations confirm Einstein's statement (28) that his equation applies only to the diffusion of particles very large in comparison with the molecules of the solvent.

(e) Arnold's Relation. The latest attempt to place the phenomenon of diffusion upon a firm theoretical basis is that of Arnold (1). The failure of earlier investigators to correlate their experimentally determined diffusion velocities with the other properties of the substances involved was due, according to Arnold, to the lack of a rational theory of diffusion in liquid systems. The formulation, in turn, of a theory of diffusion in liquids was impossible because there existed no kinetic theory of liquids corresponding to that of gases. Attempts at correlation have been made on two bases: the kinetic and the hydrodynamic which are represented by the Exner rule and the Stokes-Einstein equation respectively. Arnold has applied the classical kinetic theory expression for gaseous diffusion to liquid systems, after duly correcting for

the complications introduced by the close packing of the molecules in liquid systems, and has formulated a new diffusion relation by means of a derivation closely paralleling that of Stefan for gaseous diffusion. The final form of Arnold's equation takes the form:

$$D = \frac{B\sqrt{\frac{1}{M_1}+\frac{1}{M_2}}}{A_1 A_2 Z_2^{1/2} S^2} \quad , \ldots \quad (1)$$

where B is a proportionality constant, S the sum of the cube roots of the molecular volumes of solute and solvent respectively, M the molecular weight, and A_1 and A_2 are abnormality factors for solute and solvent, respectively, which must be inserted when either or both substances are associated. A is defined as $D_{caled.}/D_{obsd.}$ Z, expressed in centipoises, is defined by the expression:

$$F = A_1 A_2 \cdot V_2^2 Z_2^{1/2} ,$$

where F is the internal force acting between the molecules, V_2 the molecular diameter of the solute molecule, and Z_2 the proportionality factor.

Equation (1), above, is still not based entirely upon theoretical considerations since, in the derivation, the author has assumed that (1) all the collisions between molecules are binary, (2) the collision rate is unaffected by the volume occupied by the molecules, and (3) that

molecular attractions do not come into play. Such assumptions are permissible in the kinetic theory of gases, but none of them is valid for the treatment of diffusion in liquid systems because of the greater molecular density in the liquid state. Since no molecular analysis of the problem of the collision rate in liquid systems has ever been made, Arnold has accounted for the failure of the three assumptions employed in his derivation by the semi-empirical factor Z_2, the value of which is taken from the expression for F after F has been evaluated by a study of the existing experimental data. The successful analysis, then, of the problem of the collision rate of molecules in liquid systems still remains to be solved before the kinetic theory of diffusion in liquid systems can be placed upon a firm theoretical foundation.

4. Membrane Methods for the Study of Diffusion.

The ideal method for the determination of the diffusion velocity, and from this, the molecular weight of a substance dispersed in a liquid system, or the determination of these properties for each of a series of molecular species present in the solution at the same time, is that involving the use of the ultracentrifuge (93). This method also permits the determination of the assymmetry factor for a given particle. The sedimentation method has the further

advantage that it gives a double check upon the values of the molecular weights obtained.

"The molecular weight analysis by means of sedimentation measurements in strong centrifugal fields requires a complicated and expensive machinery and a trained staff of mechanicians for handling it." (93) This statement automatically eliminates the sedimentation method, but not the problem. The next most accurate method available for the study of diffusion and the calculation of the molecular weight of the diffusate from diffusion data is that of diffusion in the steady state. Even more precise values are obtained by means of a modification of the method of diffusion in the steady state, namely, membrane diffusion.

The first use of a membrane for the separation of solutions was made by Graham (36) who employed a thin sheet of sponge. Fick (30) in testing experimentally the correctness of his diffusion law, tried using animal membranes but found them unsatisfactory. The use of parchment paper by Graham (38) yielded only qualitative results.

Membrane diffusion, as stated above, is a form of diffusion in the steady state since the system consists of two solutions whose respective concentrations do not change appreciably during diffusion. It has the advantage over free diffusion in that the concentration gradient is confined within the membrane. In such a method it is important

that the pores be of such magnitude that neither diffusate nor solvent molecules can be carried through the pores mechanically. That is, the pores must be so small that they prevent convection currents and mechanical mixing within them. That these conditions be met is important since convection currents and mechanical mixing are the chief sources of error in all cases where diffusion in a body of free solution is studied.

If the above conditions can be attained the advantages of the membrane method are that: (1) The thickness of the concentration gradient is a known, non-variable value. (2) Samples can be obtained for analysis without the risk of mechanical mixing. (3) The method may be employed for the study of any diffusate whose concentration may be determined accurately. (4) The time during which diffusion occurs can be measured exactly. (5) Fick's diffusion relationship leads to a simple mathematical equation for this type of diffusion. The only limitation of such a method is that the values obtained are comparative and the diaphragm must be standardized since the size of the pores cannot be measured accurately.

A cell fulfilling the above requirements has been designed by Northrop and Anson (66). The cell further approaches the ideal in that it possesses a membrane of

sintered glass, which not only is unaltered between successive determinations (providing the solutions studied are not so alkaline as to cause solution of the glass which forms the membrane), but also is indifferent to any diffusate. The cell design and the method for using the cell have been improved and standardized by McBain and Liu (59). Further investigations and testings of the method have been carried out (57, 58) which indicate the general applicability of this method.

III. Diffusion of Colloids

After Graham's qualitative study of egg albumin (36) no measurements of the diffusion velocity of colloidally dispersed materials or of substances of large molecular weight were attempted until the beginning of the twentieth century. The first recorded study of colloids (74) was not concerned with their diffusion velocity, but only with the effect of their presence upon the diffusion velocities of the electrolytes in the systems investigated.

In 1907, Herzog and Kasarnowski (45) used an Ōholm type of apparatus to measure the diffusion velocities of invertin and pepsin in 0.5NNaF saturated with toluene and secured results indicating that the presence of foreign materials does not alter the diffusion coefficient appreciably. Herzog (45) later employed the same apparatus and, following the experimental procedure of Graham (36), Stefan (86), and Kawalki, determined the diffusion coefficients for such proteins as ovalbumin, ovomucoid, and clupein and for such enzymes as pepsin, rennet, invertin, and emulsin. Herzog obtained relatively good agreement between duplicate determinations, but worked at such a variety of concentrations and temperatures that no relationships between the values obtained and the other properties of the systems can be detected. Molecular

weights were calculated by the Thovert method - from the relation:

$$D\sqrt{M} = C$$

since at that time the Stokes-Einstein equation was relatively unknown.

Thovert (102) later applied the $D\sqrt{M} = C$ relationship to a number of materials dissolved in methanol. His results show that the equation does not hold in general for all substances in a given solvent or for a given substance in different solvents. Later investigation revealed a variation of the value of the constant even among members of a homologous series. An even more rigid examination was made by Öholm (71), who found that C varies from 6.56 to 7.60. Determinations of the temperature coefficient of the diffusion coefficient for cane sugar, arabinose, nicotine, and raffinose gave values ranging from 0.015 to 0.044, the average being 0.030 per degree. For electrolytes such as HCl, LiCl, and KOH the values ranged from 0.02 to 0.03, the average being 0.025. Studies made upon solutions of dyestuffs by Herzog and Polotzky (46) led them to the conclusion that the diffusion coefficient is determined, not by the molecular weight, but rather by the number of constituent atoms and their arrangement within the molecule.

Still faithful to the $D\sqrt{M} = C$ relation, Öholm (73)

studied the diffusion of nicotine and several sugars and found the $D\sqrt{M} = C$ relation to hold for the materials studied with the value of C equal to 7.0 ± 2%. A temperature coefficient for D of 3.5% per degree was also found. Measurements of the velocity of diffusion of a series of organic substances in ethanol by Öholm (72) applied to the $D\sqrt{M} = C$ equation gave values ranging from 6.1 for resorcinol to 15.9 for bromoform. The use of the empirical equation:

$$D\eta\sqrt{M} = C$$

gave much better agreement. In this work the temperature coefficient was found to be about the same as for non-electrolytes in water, namely, about 2%.

Dummer (24) succeeded in showing that the Stokes-Einstein equation is valid for the diffusion of colloidal particles but not for systems in which the diffusate and solvent molecules are anywhere near equal in size. Similar results were obtained by Cohen and Bruins (19).

The investigations of Laszlo and Groh (53) show that an increase of the hydrogen ion concentration on either side of the optimum for the precipitation of ovalbumin causes an equal diminution in the diffusion rate of approximately 12%. The presence of NaCl in the system increases the diffusion velocity of the ovalbumin. In distilled water (in the absence of buffer solutions) the diffusion

rate is again increased and becomes still greater if NaCl is then added.

Fischer (31), experimenting with the diffusion of hemoglobin into blood serum, found that, in general, electrolytes decrease the diffusion velocity. For the diffusion of hemoglobin into pure water, salt solutions retard diffusion up to a very definite concentration of each salt. At lower concentrations these same salts accelerate diffusion.

Robinson and Hartley (42) extended the Nernst equation to cover the case of multivalent ions of colloidal size and showed that the diffusion coefficient cannot be employed for the calculation of particle size. However, with a high concentration of added electrolyte, a limiting case is obtained in which the real coefficient and the radius of the colloidal ion are given by the Stokes-Einstein equation.

H. R. Bruins (12) devised a new interferometric method for the precise determination of the diffusion coefficients of substances of such molecular magnitude that these values were only about 1% of those for the average material. Bruins (13) then proceeded to try out his methods on various samples of starch and gum arabic. The values obtained for the diffusion coefficients were surprisingly high. Likewise, the particle radii, calculated from the

Stokes-Einstein equation, were so low as to be entirely incompatible with the viscosities and the very slow diffusivity, through membranes, of the solutions investigated. A new series of investigations revealed that the addition of minute amounts of salts reduced the diffusion velocities enormously. For example, the diffusion coefficient of gum arabic in water was found to be 0.225×10^{-5} $cm.^2/sec.$ When the same concentration of gum arabic was placed in a 0.001 molar potassium chloride solution and allowed to diffuse against an equal concentration of the salt, the value of the D dropped to $0.058 \times 10^{-5} cm.^2/sec.$ The same phenomenon was shown by the different samples of starch. In a discussion of his results (14), Bruins used his data to relate the change in diffusion velocity of hydrophilic colloids, upon the addition of salts, to the parallel change in the viscosity of the system upon the addition of the salt, causing the decrease of the diffusion velocity of the colloid.

And, finally, to add one more contradictory note to the conflicting evidence already presented, measurements obtained by McBain (57) apparently indicate that the velocity of diffusion is proportional to the osmotic pressure (the basic postulate employed by Fick in his derivation of the diffusion law) and not to the activity of the colloidal particles.

A careful consideration of the material mentioned above will show that the situation with regard to the measurement of the diffusion velocity of colloidally dispersed materials is in a rather bad shape. The rejection of all data which is in any way open to question and the correlation of the remainder would do much to remove the considerable distrust with which such data have come to be regarded. More accurate methods for the determination of the diffusion coefficient and a more intelligent application of the coefficient, once obtained, would do considerable in establishing the validity and usefulness of this property for the solution of many of the problems of organic and physical chemistry.

IV. Molecular Weights of Proteins

The subject of the molecular weights of colloidally dispersed materials offers an array of evidence as contradictory as that found for the velocity of diffusion for such substances. The more common methods for the calculation of the molecular weights of colloidal materials are listed below. It will be noticed that the number of values obtained for the one particular material considered, namely, gelatin, is greater than the number of methods by means of which it has been calculated.

1. From Osmotic Pressure Measurements.

From measurements of the osmotic pressure developed by 0.5% gelatin solution, Frankel (32) calculated the molecular weight of gelatin to be 53,800 at 6.6°C and 19,800 at 30°C. The same solution, after it had been kept at 37°C for 500 hours gave, from osmotic pressure data, a molecular weight of 24,000. The same solution, if cooled to 22°C before the osmotic pressure measurements were made gave a molecular weight of 16,500.

Eggert and Reitstötter (25) obtained a molecular weight of 30,000 for commercial gelatin and 40,000 for the same material after analysis which corresponds to a molecule of 120 atoms and 6,000 molecules per micelle.

In his investigations, Biltz (7) calculated the molecular weight of gelatin to be 57,000 in a 0.2% solution and 50,000 for a 0.553% solution. Smith (84) obtained a value of 96,000 for a 0.5% solution of electrolyte-free gelatin.

From his measurements of the pressure set up by a 3% gelatin solution in sodium solicylate at 35°C, Schryver (80) calculated a molecular weight of 16,000. For a 0.9% solution under the same conditions, the molecular weight was calculated to be 40,000.

2. From X-Ray Data.

From his study of the thermal decomposition of electro-osmotically purified gelatin, Gerngross (35) decided that the molecular weight of gelatin in solutions of concentrations of less than 0.5% of gelatin in aqueous solution varied between 50,000 and 90,000 if the gelatin were isoclectric. Protracted boiling was found to reduce the molecular weight to a mean value of 4,500.

The x-ray diffraction data of Krishnamurti (52) for highly purified gelatin at concentrations up to one to one gave a value of 3,000 for M.

3. From Viscosity Measurements.

Staudinger (85) defended the values of the molecular weights of proteins when calculated from viscosity data

and claimed that values so calculated agree with those determined by other methods, but gave no values for gelatin.

4. From Determinations on Chemical Combination:

The investigations of Johlin (50) revealed two isoelectric points for gelatin, one at pH 4.68, the other at pH 5.26. An estimation of the molecular weight of gelatin based upon titration data obtained at hydrogen ion concentrations lying between the two isoelectric points gave a value of 50,000.

5 and 6. From Ultracentrifugal and Diffusion Data.

The values of M, calculated directly from sedimentation equilibrium data (92) for a 0.4% gelatin solution at 20°C range from 10,000 to 70,000. Values calculated indirectly from sedimentation velocity data in which case the diffusion coefficient is calculated first and then the molecular weight from D, by means of the Stokes-Einstein equation, ranged from 9,070 to 73,000.

7. From Cryoscopic Measurements.

Cryoscopic determinations for proteins dissolved in anhydrous phenol containing $CaCl_2$ were made by Cohen and Conant (20). Their data shows a molecular weight of 150,000 for gelatin as against a value of 151,125 calculated

from the decomposition products of gelatin.

8. From Dielectric Dispersion and Wave-Length Studies.

By means of an equation derived from the Debye relationship (62), the molecular weights of solutions of colloidally dispersed materials may be calculated from measurements of the dielectric dispersion of the system and the wave length of the dispersed ray. The molecular weights so calculated correspond to the smallest particles of the dissolved material even if it is present in the presence of more complex micellae which possess a very high degree of association. The value of the molecular weight of gelatin, so calculated, is 11,300.

9. From Decomposition Products.

In his calculations, Atkins (3), after a careful study of the most recent data on the amino acid content, assumed that there were two histidine residues per molecule and obtained a value of 34,500, a considerably different value than that obtained by Cohen and Conant (20) calculated on the basis of the cystine content.

10. From Anisotropic Properties.

The calculations of Sheppard and McNally (83), based upon a consideration of the anisotropic properties of

gelatin gels resulted in values ranging from 10,000 to 30,000 for gelatin at temperatures above 38°C.

Values for the molecular weight of gelatin are summarized in the following table:

TABLE I.

Method	Investigator	M
Calcns. from following data:		
Cystine content	Cohen and Conant	151,125
Cryoscopic	Cohen and Conant	150,000
Osmotic pressure	Smith	96,000
X-ray	Gerngross	90,000-50,000-4,500
Diffusion velocity	Svedberg	73,000-9,070
Sedimentation equilibrium	Svedberg	70,000-10,000
Osmotic pressure	Blitz	57,000-50,000
Osmotic pressure	Frankel	53,800-16,500
Chemical combination	Johlin	50,000
Osmotic pressure	Eggert and Reitstötter	40,000-30,000
Osmotic pressure	Schryver	40,000-16,000
Histidine content	Atkins	34,500
Anisotropic	Sheppard and McNally	30,000-10,000
Dielectric dispersion	Marinesco	11,300
X-ray	Krishnamurti	3,000

The values shown in Table I exemplify the possibility of error in the different methods and the necessity of a critical study not only of the method, but of the data obtained, before the measurements secured by any particular method are employed for such calculations. Just how much error has been introduced in any particular calculation

cannot be determined. That such variations are possible, by neglecting, for example, the Donnan membrane effect (which results from the presence of electrolytes in the system) in osmotic pressure measurements to obtain an apparent molecular weight of 100,000 for some substance which actually exists in solution as particles giving a molecular weight of 50,000. Errors of the same order, but in the opposite direction, may be obtained (as will be shown later) by the use of diffusion measurements made upon lyophilic colloidal solutions containing electrolytes. Even in systems permitting free diffusion (89) such, for example, as those set up in sedimentation studies, the addition of an excess of some salt to overcome the Donnan effect[93, 8] may still lead to incorrect values as the result of the partial de-aggregation of the micellae, or the partial decomposition of the molecules when the systems studied are too far from the isoelectric point of the material investigated. The diffusion coefficients obtained and the molecular weights calculated from such data can never be anything but mean values which do not give complete information concerning such systems.

V. Experimental Procedure.

Since the source of the material, the concentration, the temperature, and the previous history, especially with regard to its thermal treatment (20), have such a marked effect upon the properties of gelatin solutions, these factors were very carefully controlled and conditions were duplicated as nearly as possible in all the determinations.

Four different samples of gelatin were employed in the investigation:

1. United States flake gelatin which had been dialyzed against N/128·HCl, then against water, and finally electrodialyzed under an impressed electromotive force of 500 volts until its conductivity approached that of pure distilled water. This sample will be designated hereafter as sample I.

2. Electrodialyzed flake gelatin from the Eastman Kodak Company. The ash content of this material, sample II, was 0.02% on the dry basis. The pH of a 1% solution was 4.74.

3. Eastman Kodak gelatin, sample III, which had the same pH and ash content as sample II, but which varied otherwise.

4. Sample IV had an ash content, on the dry basis, of 0.025% and a pH of 4.80 in a 1% solution. This sample was also an Eastman Kodak Company product.

The moisture content of the respective samples was determined by heating a weighed amount of gelatin to constant weight at 105°C. From this data, gelatin solutions of the desired concentration could be prepared.

Solutions of the desired concentration were prepared by weighing into tared beakers the requisite amount of gelatin, adding water and what other materials were desired, and heating in a water bath at 90°C for 32 minutes, which was the time required at this temperature, to dissolve the gelatin for a 3% solution. At the end of 32 minutes, the solutions were cooled to between 30 and 35°C, placed on a balance, and made up to exactly the desired concentrations by weight by the addition of water. All the systems investigated received this same standard treatment in order that any possible changes which might occur between the properties of one system and those of another as a result of difference in thermal treatment would be eliminated.

The cells employed were calibrated by measuring the rate of diffusion of one molar KCl, whose diffusion coefficient at 35°C is known. The cells were then boiled in cleaning solution, rinsed in distilled water, and finally the air was "swept out" of the pores of the diaphragms by drawing through each cell a liter of distilled water previously degassed by heating to 60°C and

shaking vigorously under a pressure of approximately 10 mm. of mercury. Incidentally, the water used for the preparation of the gelatin solutions and the outside solutions was subjected to this same treatment. The solutions to be studied were drawn into the respective cells and the cells then placed over solutions corresponding to the dispersion medium in each given cell. The procedure employed by Liu (59) and successively by M. E. McBain (57) and Dawson (58) was followed with the exception that the method of suspension of the cells and the scaling of the systems was improved upon.

In the investigations, the rubber tubing suspension employed by Liu (59) was replaced by a metal rod culminating in a universal joint and a clamp (Plate I) which simplified the initial adjustment of the cells and eliminated the necessity of re-leveling the diaphragms when the solutions were changed. The rubber dam employed between the cell and the beaker to prevent the evaporation of the outside solution was found to be entirely inadequate and was replaced by strip rubber which could be set so that an absolutely vapor tight system was obtained (Plate I) and any error due to a change in concentration of the outer solution was entirely prevented. The temperature of the bath in which the systems were placed was maintained at a temperature of 35 ± 0.01°C. All measurements

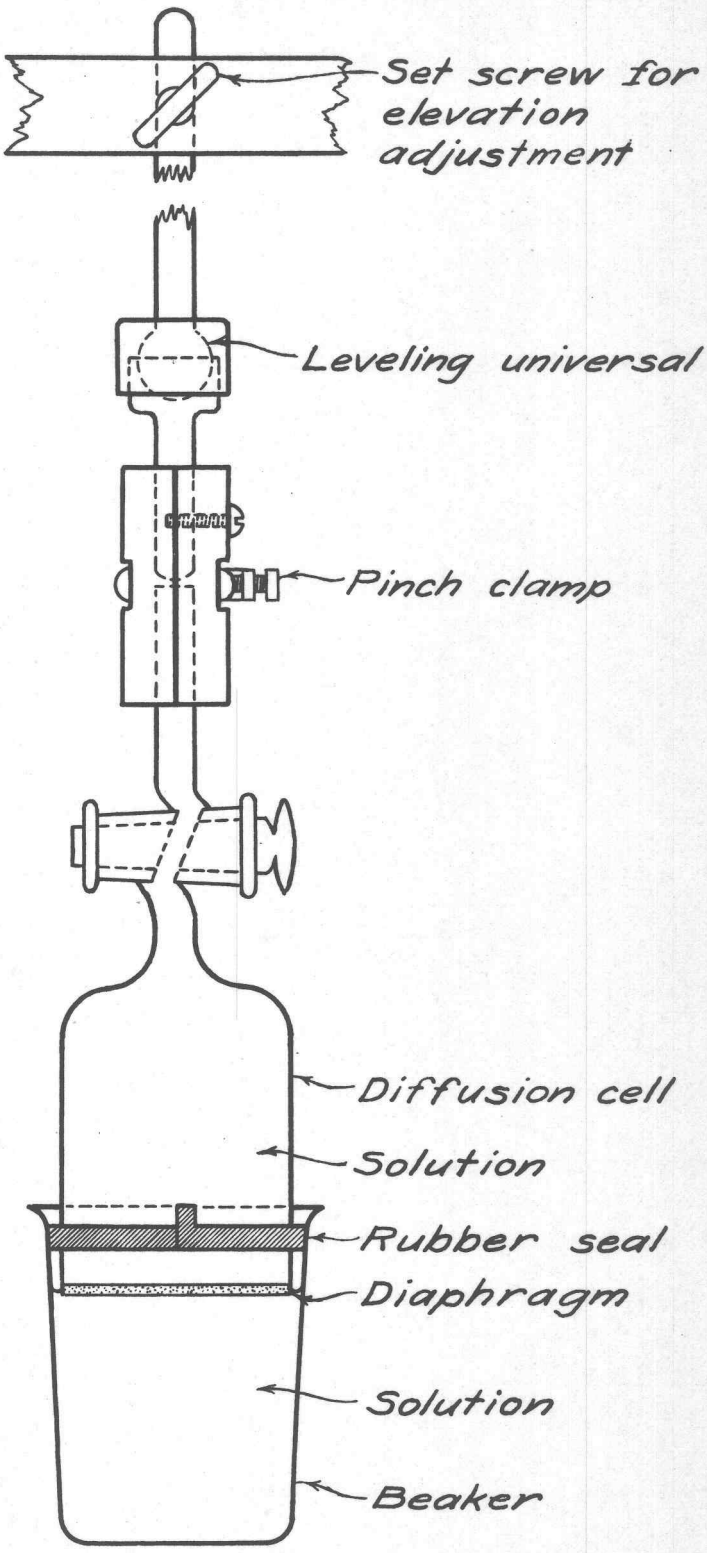

Set screw for elevation adjustment
Leveling universal
Pinch clamp
Diffusion cell
Solution
Rubber seal
Diaphragm
Solution
Beaker

PLATE I

were made at this temperature in order that the results might be directly comparable and to insure that gel formation should not occur in the systems.

Periods of from four to sixty hours were allowed for the setting up of the concentration gradient within the diaphragm. Since no difference could be detected between the diffusion coefficients obtained when four hours were allowed for the establishment of the diffusion gradient and those in which longer time had elapsed, between four and eight hours were generally allowed for the establishment of the gradient.

After the diffusion gradient had been set up, diffusion was allowed to continue until the concentration of the gelatin in the outside solution was sufficiently great that accurate analysis was possible. It was found necessary to resort to the microkjeldahl method of analysis since the change of refractice index change was too small to be measured accurately with the instruments available for that purpose. Even with the micromethod of analysis diffusion had to be continued for periods of approximately 100 hours before a measurable amount of gelatin could be found in the outer solution. In order to cut down the diffusion time, the micromethod was so modified that samples containing only from 0.075 to 0.25 of a mg. of nitrogen could be analyzed. This was accomplished

by making up solutions to only 25 cc instead of to the customary 100. This modification permitted the reduction of the diffusion time from 100 to from 15 to 25 hours.

The relative viscosities at 35°C of part of the solutions employed were determined by means of Ostwald type viscosimeters with large capillaries. The pH values of the solutions were also measured at 35° by means of a quinhydrone electrode set up in a constant temperature air bath. With the temperature of the complete system so controlled, 0.2°, measurements of the pH of a given system could be reproduced to within 0.01 of a pH unit.

Gelatin solutions of any desired pH were prepared by the addition of a calculated volume of standard HCl, if the desired pH lay on the acid side of the pH of isoelectric gelatin, or by the addition of a calculated volume of standard NaOH if the desired pH lay on the basic side. The amount of acid which would combine with the gelatin in a given volume of solution at a given concentration was calculated from tables, prepared in the laboratory, which show the variation of the amount of acid that will combine with one gram of gelatin with change in pH of the solution at a given temperature. The concentration of acid corresponding to the desired pH for the solution was read from Figure I which shows the change of pH of a

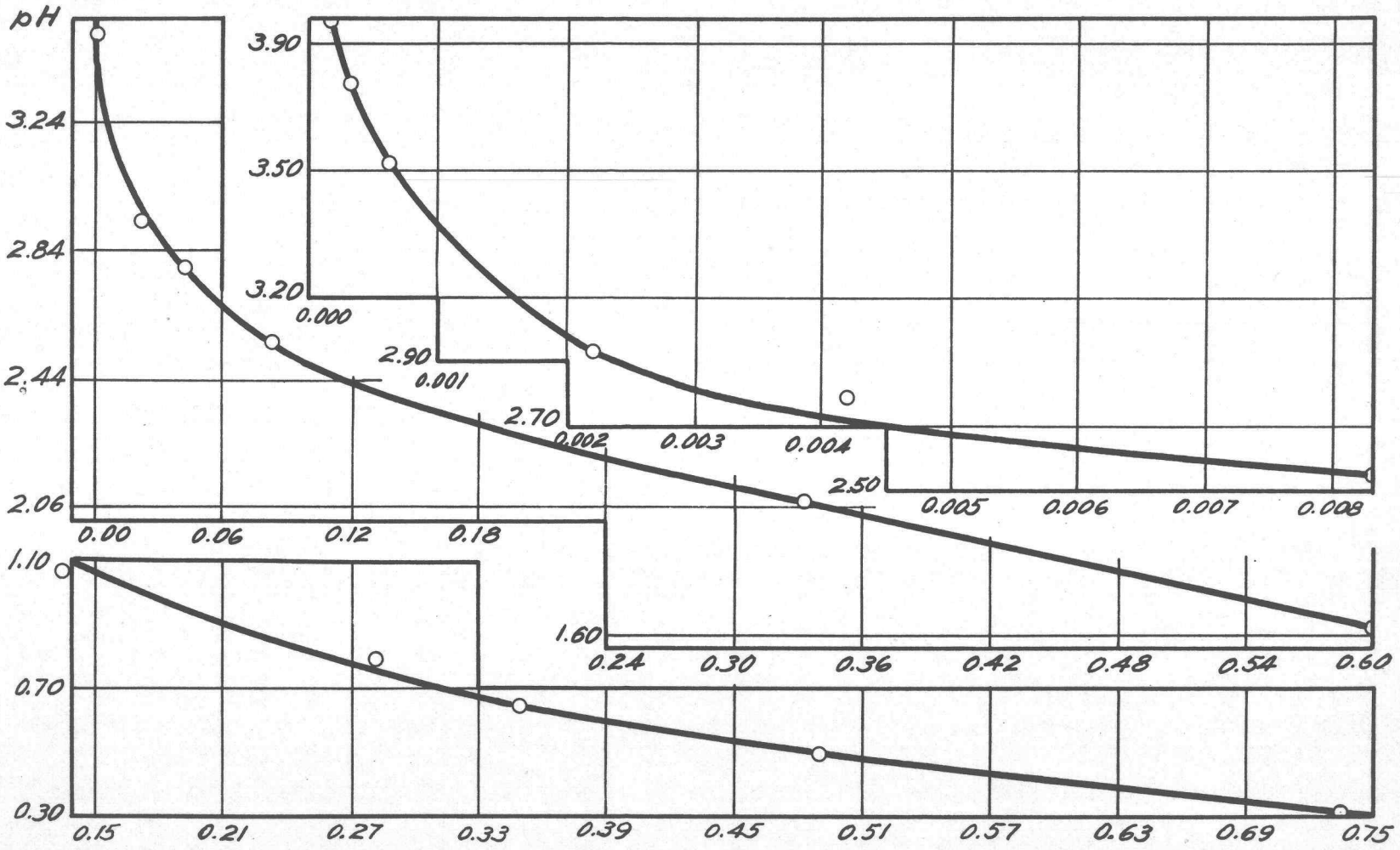

pH
3.24
2.84
2.44
2.06
0.00
0.06
0.12
0.18
3.90
3.50
3.20
0.000
2.90
0.001
2.70
0.002
0.003
0.004
2.50
0.005
0.006
0.007
0.008
1.10
0.70
0.30
1.60
0.24
0.30
0.36
0.42
0.48
0.54
0.60
0.15
0.21
0.27
0.33
0.39
0.45
0.51
0.57
0.63
0.69
0.75
Normality of HCl solution

FIGURE I

HCl solution with change in concentration, the concentrations, as a matter of convenience, being expressed as normalities. From a knowledge of the amount of acid required for combination with one gram of gelatin at the desired pH of the solution, the normality of the standard HCl solution, the concentration of the gelatin in the solution, the volume of the gelatin solution and the normality of the HCl in a solution of the required pH, the volume of the standard acid was readily calculated. For the preparation of an aqueous solution of any desired pH (outside solutions), the required concentration was read from Figure I and the amount of standard acid required for any volume of solution was calculated. As a check upon the accuracy of the calculations, the pH of each solution prepared, both gelatin and aqueous, was measured in duplicate by means of the quinhydrone electrode.

In the preparation of Figure I, a potassium-acid-thallate buffer of pH 3.925 at 35° was prepared. A solution of this buffer gave a reading, with quinhydrone, of -0.2111 volts. By means of the relationship

$$pH = \frac{E_o - E_{cal.} - 0.6918}{0.061103} \quad . \quad . \quad . \quad . \quad (1)$$

where E_o is the electromotive force developed by the cell, expressed in volts, $E_{cal.}$ the electromotive force of the

calomel half-cell, in volts, 0.6918 is the voltage due to the quinhydrone at 35°, and 0.061103 is the RT/nF factor of the Nernst equation. Substitution of the data above in equation (1) gave a value of -0.2409 volts for $E_{cal.}$ The buffer was then replaced by 20cc of standard acid. The electromotive force of the cell was measured and distilled water was then added in varying measured amounts, the electromotive force of the cell being measured after each addition. The results, from which Figure I was plotted are shown in Table II.

TABLE II.

Standard	cc Water Added	Total Volume	N·HCl	E_o(volts)	pH
20cc0.7331N·HCl	0	20	0.7331	-0.4318	0.31
	10	30	.4887	.4204	0.50
	12	42	.3491	.4112	0.65
	10	52	.2819	.4019	0.80
	58	100	.1333	.3858	1.07
20cc0.1333N·HCl	20	40	0.06667	.3496	1.66
	40	80	.3333	.3255	2.05
	80	160	.1667	.3032	2.43
20cc0.01667N·HCl	20	40	0.00833	.2950	2.55
	40	80	417	.2806	2.79
	49.71	89.71	218	.2716	2.93
	80	160	122	.2674	3.03
20cc0.00122N·HCl	20	40	0.00061	.2301	3.52
	40	80	31	.2189	3.77
	80	160	0.00015	-0.2092	3.96

For the preparation of solutions whose pH values lay on the alkaline side of the isoelectric point of pure gelatin, a curve, Figure II, for the change of the pH of a 3% gelatin solution upon the addition of a standard NaOH solution was plotted from the data given in Table III. For the preparation of outside solutions of any desired pH within the ranges investigated, Figure III was plotted from the data in Table IV. The pH values of the solutions prepared from data taken from these curves were checked by means of the quinhydrode electrode.

TABLE III.

Concn. Gelatin	cc 0.1668N·NaOH/100 gms.	E_o	pH
3% (by wt.)	0.	-0.1659	4.66
	0.30	.1556	4.87
	0.90	.1502	4.95
	2.30	.1368	5.16
	3.20	.1283	5.31
	4.80	.1004	5.77
	6.40	-0.0381	6.79

Calculations for the pH's in Tables III and IV are based upon a value of 0.2389 for the calomel half cell.

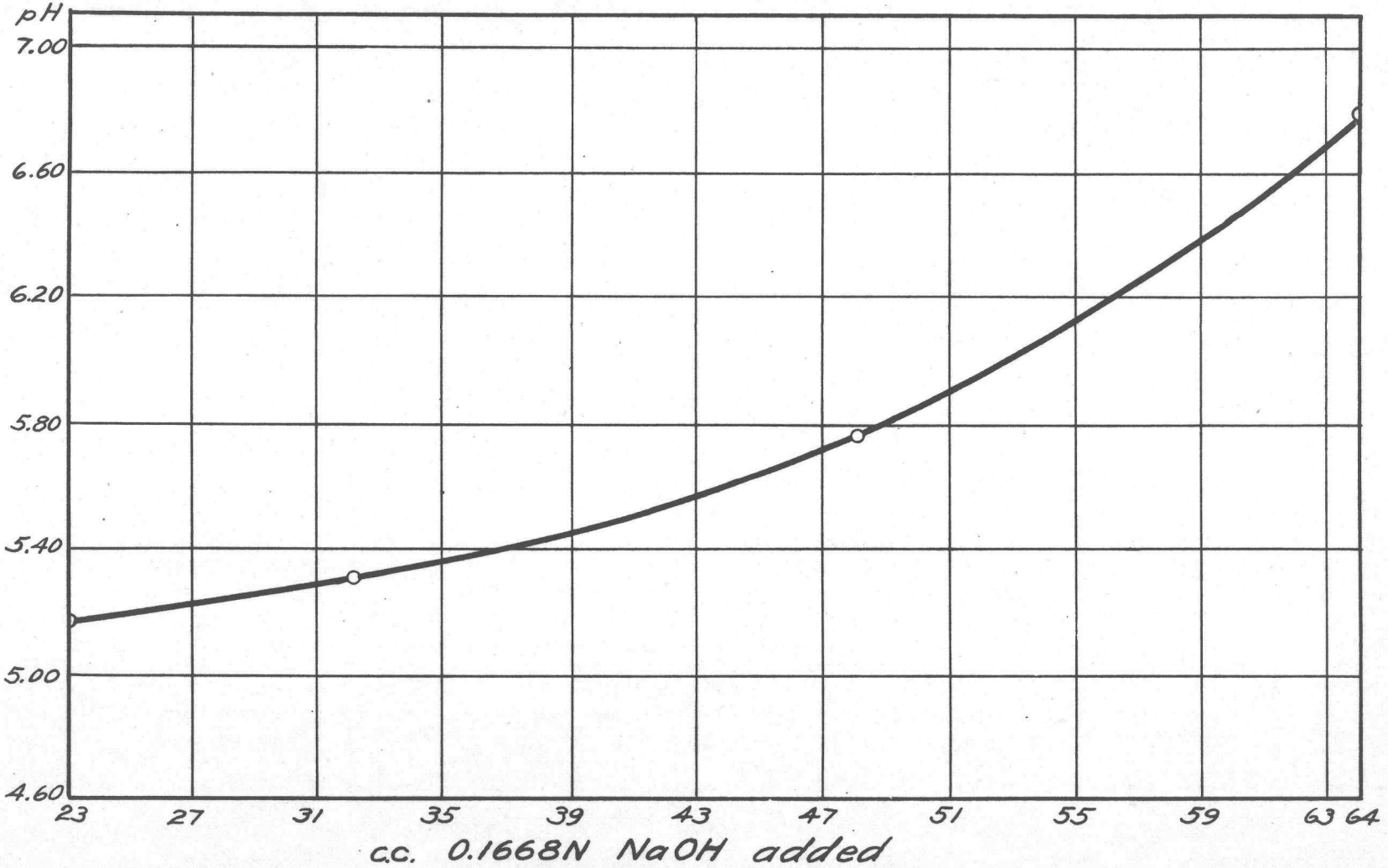

pH
7.00
6.60
6.20
5.80
5.40
5.00
4.60
23
27
31
35
39
43
47
51
55
59
63
64
c.c. 0.1668N NaOH added

FIGURE II

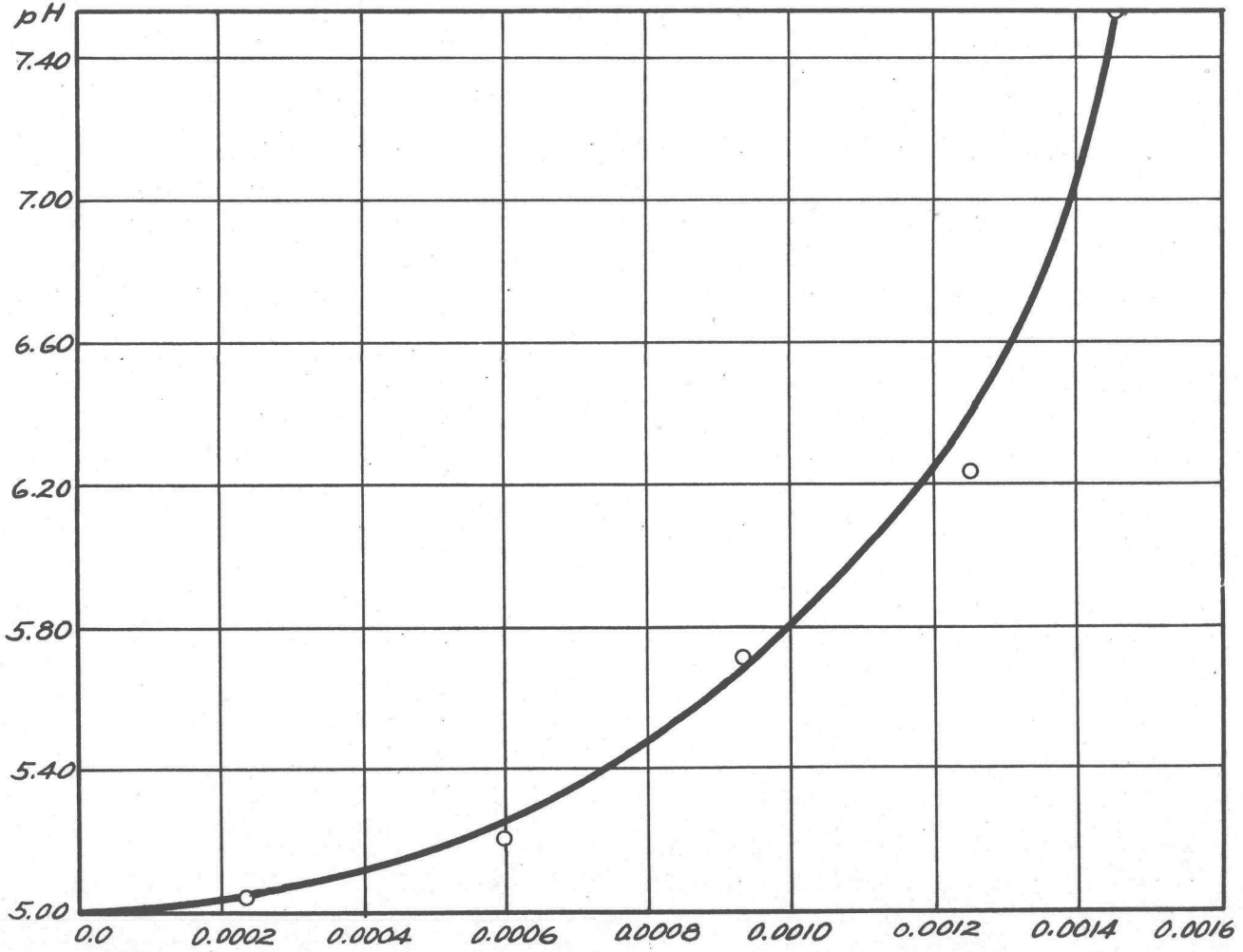

pH
7.40
7.00
6.60
6.20
5.80
5.40
5.00
0.0
0.0002
0.0004
0.0006
0.0008
0.0010
0.0012
0.0014
0.0016
Normality of NaOH

FIGURE III

TABLE IV.

Concn. Gelatin	cc 0.1668N·NaOH/100 ml.	N·NaOH	E_o	pH
3%	0.07	0.00023	-0.1447	5.04
	.18	60	.1349	5.20
	.28	93	.1034	5.72
	.38	0.00130	.0712	6.25
	0.48	0.00160	-0.0013	7.43

In the analysis of the solutions for the gelatin present concentrations were expressed in milligrams of nitrogen per milliliter. Four two to fifteen ml. samples were taken from each outside solution by means of Exax rechecked pipettes for analysis. Four 0.05 ml. samples were taken from each inside solution by taking 5 ml. of each inside solution, diluting to half a liter in a "blue line" rechecked flask, and then taking four 5 ml. samples of the resulting solution for analysis. The accuracy of the sampling and of the color comparisons are shown by the following example taken from cell I in run 9. The usual 0.05 ml. samples were taken from the inside solution, 2.5 ml. samples from the outside. With the standard solution, which contained 0.3 of a milligram of nitrogen per 100 ml. of solution, set at 30, the colorimetric readings were as given in Table V.

TABLE V.

Inside Soln. I	Inside Soln.III	Outside Soln.I	Outside Soln.II
15.4	15.5	35.4	35.0
7	9	34.6	.2
6	8	6	34.5
6	5	5	35.0
8	6	1	34.0
4	8	9	1
15.58	15.68	34.68	34.80

The product of the mg. of nitrogen in the standard and the "setting" of the standard solution in the colorimeter divided by the product of the ml. of the sample of the unknown taken for analysis and the colorimeter "setting" of the solution of the unknown gives the milligrams of nitrogen per 100 ml. of unknown.

The diffusion coefficients were calculated by means of the formula derived by McBain and Liu (59) which is given by :

$$D = \frac{\log C_o - \log (C_o - C)}{Kt_E}$$

where D is the diffusion coefficient (calculated in this investigation in $cm.^2/day$), C_o the concentration of the solution within the cell at t_E equal zero, C the concentration of the solution outside the cell after the elapse of the time t_E, and K is the cell constant.

Blanks were run in order to determine whether or not corrections for the nitrogen present in the C.P. sulfuric

acid used were necessary. Analysis showed no increase in the nitrogen content of the samples with time, either for those "boiled down" or for the untreated samples. Comparison of blanks prepared from the C.P. H_2SO_4 used for the analyses and those prepared from nitrogen-free H_2SO_4 showed, with a standard containing no H_2SO_4 set at 50, an average value of 0.8 for the blank prepared with the nitrogen-free acid and an average value of 2.00 for that prepared from the C.P.H_2SO_4. The difference is 1.20. In the analyses C.P. H_2SO_4 was employed for the preparation of both the standard solutions and the unknowns. The correction factors for the standard set at

50	40	30	25	20 and 15	are
1.20	0.95	0.70	0.60	0.50	0.35	for the

standard. Corresponding corrections naturally, apply also to the comparator readings for the unknowns. Calculations of the diffusion coefficient, D, both from "corrected" and "uncorrected" colorimetric readings revealed that the change in the value of D ranged from zero to only 0.0004 in the most extreme cases. Since the correction involved amounts to from zero to a maximum value of less than one percent, corrections for the nitrogen present in the acid employed have not been made in calculations of D.

The original intention was to measure the concentration of the gelatin in the various solutions by means of a

dipping refractometer in order to secure a check on the values obtained by microkjeldahl analysis. The values of D calculated from refractometer data not only were found to vary considerably among themselves, but were consistently higher than those calculated from analysis of the total nitrogen content. Comparison of the values obtained is shown in Table VI.

TABLE VI.

Run No.	Cell No.	D, (from Refractometric Data)	D, (from Microkjeldahl Data)
2	IV	0.248	0.223
2	III	.302	.209
2	II	.209	.094
2	I	.240	.092
3	IV	.083	.067
3	III	0.110	0.062

Samples containing known amounts of nitrogen were prepared and analyzed both by means of microkjeldahls and by the change of refractive index. From this data the change of refractive index was plotted against the change of concentration of the gelatin. The relationship proved to be a straight line function. The microkjeldahl data was accorded the same treatment, the milligrams of nitrogen in a unit volume being plotted against the gelatin concentration. This relationship was also found to be a straight line function. By means of the curves so ob-

tained, the data, similar to that shown in Table VI, for one of the runs in which the values of D, as calculated from refractive index data were considerably larger than the values obtained by use of microkjeldahl analysis, was used for the calculation of the original concentration of the gelatin present, C_o. The total concentrations for the solutions used in run 3_{II} are shown in Table VII.

TABLE VII.

Soln. No.	Concn. from Micro. Data	Concn. Refractive Index Data
I	0.512% (by wt.)	0.517% (by wt.)
II	1.013	1.020
III	2.068	2.080
IV	3.039	2.999

Since the values for the respective original concentrations from the data secured by one method show good agreement with those obtained by the other, the differences between the values of the diffusion coefficients calculated from the two different sets of values cannot be ascribed to inaccurate analysis. Nor can the too large values for the outside concentrations obtained by refractive index change be ascribed to the presence of faster moving foreign materials which would not only diffuse more rapidly than the gelatin particles, but would be detected

by the refractive index method and not by the micro-kjeldahl analysis. The impossibility of any error from such cause from the fact that the maximum ash content of the sample studied was 0.020% on the dry basis, and in a 3% solution of gelatin, even if the ash were to consist of an electrolyte, the change in refractive index of the solution that would result from the presence of the foreign material would not be detectible.

As no explanation, and therefore no correction, of the error was apparent, analysis by means of refractive index change was not attempted in subsequent analyses and measurements by means of the total nitrogen content were relied upon. Analyses by this method were run in quadruplicate, color comparisons being made upon different samples from a given solution until two samples gave comparable colorimetric readings.

VI. Diffusion of Gelatin.

All the gelatin solutions employed in this investigation were accorded, as nearly as possible, the same treatment, which eliminated any possibility of difference in the properties of the solutions prepared from the different samples of gelatin except that due to the source of the gelatin and its previous thermal history.

1. Effect of Source upon D.

The effect of the source and of the previous treatment upon the diffusion coefficient are shown in Table VIII, the values, which are averages of two or more determinations, having been obtained at 35 ± 0.01°C.

TABLE VIII.

Material	Symbol	Concn.	D(in cm^2/day)
Electrodialyzed	I	3%	0.049
Eastman Kodak	II	3%	0.042
Eastman Kodak	III	3%	0.039
Eastman Kodak	IV	3%	0.051

It is evident from the above table that the study of the change of any particular property of gelatin must be made not only upon samples from the same source, but also upon samples from the same source which have been sub-

jected to the same treatment in their preparation.

2. Effect of Concentration, Viscosity, and pH upon D.

The possibility of reproducing a system which possesses certain physical constants is illustrated by the following table. The measurements given were made upon solutions prepared from Sample II. The values for the pH's and the viscosities were determined from samples taken from the solutions prepared for the measurement of the diffusion velocities. The relative viscosities were measured 8 hours and 108 hours respectively after the preparation of the various solutions in order that the amount of aging might be determined.

TABLE IX.

Concn.	pH	η_s/η_w (After 8 hrs)	η_s/η_w (After 108 hrs)	D	D av.
3%	4.59	4.819	3.803	0.040 .043 .042 .043	0.042 ± 0.002
2%	4.70	2.485	2.073	0.046 .045 .050 .042	0.046 ± 0.004
1%	4.77	1.719	1.545	0.051 .050 .051 .050	0.051 ± 0.001
0.5%	4.91	1.307		0.064 0.057 0.067	0.063 ± 0.006

The pH of 4, 5, 6, 7, 8, 9, and 10% gelatin solutions at 35° were also measured to within 0.01 of a pH unit. This data and that contained in Table IX are shown in Figures IV, V, and VIII. Figure VIII, which illustrates the change of D with concentration, reveals a very slight, straight line increase in D with decreasing concentration between 3 and 1%. With decreasing concentration, from 1 to 0.5%, the increase of D with decrease of concentration is marked, the rate of curvature between the concentrations of 1 and 0.5% indicating that at concentrations lower than 0.5% the change is even more marked. This substantiates the statement made by Krishnamurti (92) that: "At low concentrations (about 0.5%) and above 30° gelatin sols may be regarded as molecular dispersions." The same idea has been expressed by Marinesco (62): "Gelatin molecules are very highly polarized-even more so than water. At concentrations below 0.6% by weight they exist in solution as single molecules. Above 0.6% concentration the gelatin molecules unite into aggregates which have a zero electric moment. The low value of the slope of the curve (Figure VIII) between 2 and 3% concentrations is very similar to that of typical normal non-polar substances of low concentration, the straightness of the curve between these concentrations being a strong indication of

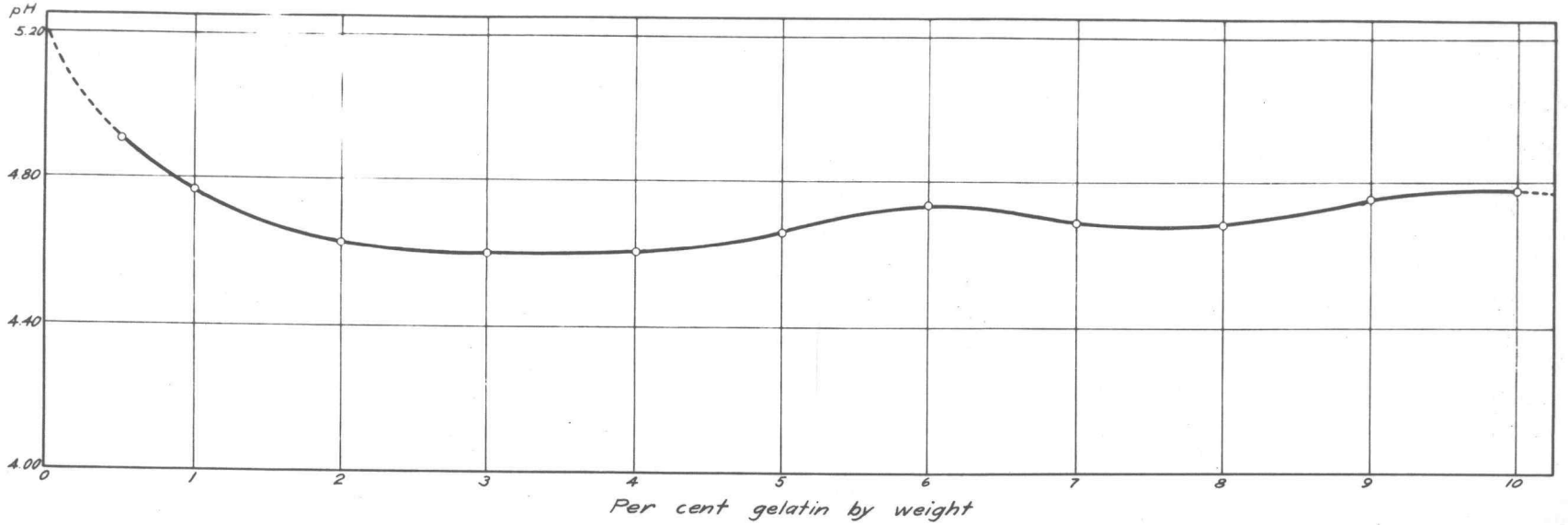


FIGURE IV

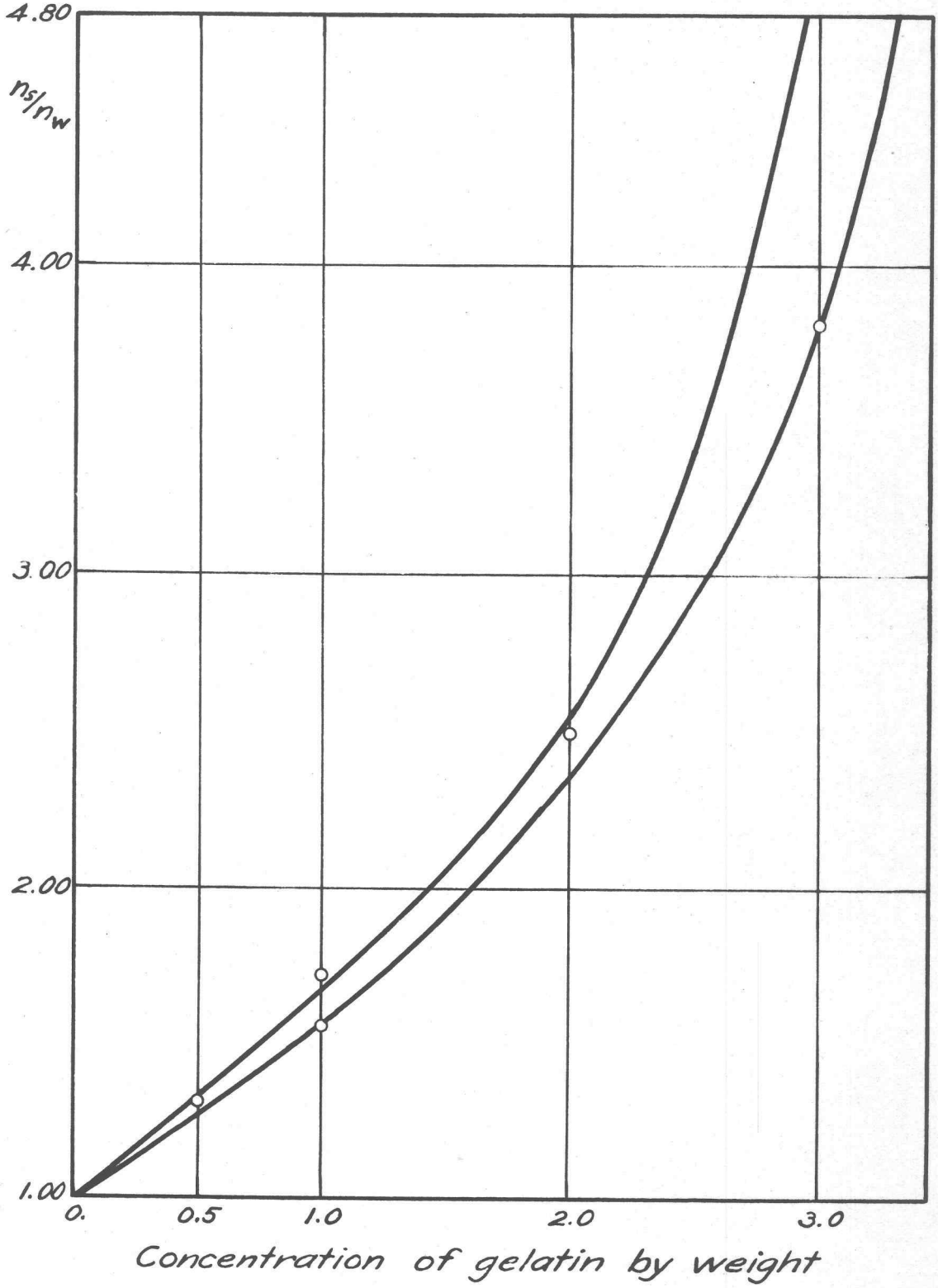

4.80
n_s/n_w
4.00
3.00
2.00
1.00
0.
0.5
1.0
2.0
3.0
Concentration of gelatin by weight

FIGURE V

the lack of polydispersion at concentrations above 0.5% by weight. The curve indicates that when the concentration of the gelatin is increased sufficiently, the aggregation-de-aggregation equilibrium is forced in the direction of a system composed almost entirely of particles of the maximum aggregation value. Further increase of the concentration should force the equilibrium still farther and thus produce a system composed so largely of particles of maximum size that the physical properties exhibited by the system, including the diffusion coefficient, should be almost exactly those which would be exhibited if the system were composed entirely of particles of the maximum magnitude. This consideration, and the fact that the too long periods required for the measurements of the diffusion coefficients of more dilute solutions resulted in the contamination of the solutions by bacteria, led to the employment of 3% solutions for the succeeding investigations.

An inspection of Figure IV which shows the pH's for concentrations between the ranges of 0.5 and 10% shows a change of pH corresponding, roughly, to the change of D. However, the shape of the pH curve for concentrations between 1 and 2%, if the change of D with change of concentration is attributed to the change in pH of the gelatin with concentration, does not correspond to the curve

showing the change of D with concentration within this range. Under these conditions, the change of D with change of concentration of the gelatin cannot be ascribed to the pH of the system which is due to the gelatin in solution. Comparison of Figure VIII with Figure V immediately establishes the independence of the rate of diffusion of gelatin from the viscosity of the system that is due to the presence of the gelatin. This conclusion corresponds to the presence of the viscosity factor, in the Stokes-Einstein equation (27) where is for the viscosity of the dispersion medium. It is opposed to the findings of Bruins (14) who related the change in the diffusion velocity of lyophilic colloids, upon the addition of electrolytes, to the change of the viscosity of the dispersion medium which resulted from such addition.

It is, then, to be inferred that the increase of the diffusion velocity of the particles, in solutions of pure isoelectric gelatin, with decreasing concentration is due to the decreased size of the particles with decreased concentration. The change of size of the micellae is due to the increased de-aggregation of the micellae with decrease in concentration.

3. Effect of Electrolytes and Ethyl Alcohol upon D.

The values given in Tables X and XI were obtained from a study of sample III, and are shown graphically by Figures VI, IX, VII, and X.

TABLE X.

Concn. Gelatin	KCl Added	NKCl	D(cm^2/day)
3%	0	0.000	0.039
	1 milliequivs/liter	0.001	.038
	5 milliequivs/liter	5	.037
	4 milliequivs/liter	0.040	.038
3% in 5% C_2H_5OH	0	0.000	0.039
3% in 10% C_2H_5OH	0	0.000	0.038

TABLE XI.

Concn.	Milliequivs.KCl	η_s/η_w After 8 hrs.	η_s/η_w After 108 hrs.
3%	1	4.371	3.760
	3	.381	.523
	5	.587	.956
	12	.102	.675
	20	.556	.843
	28	.183	.563
	34	.203	.449
	40	.946	4.093
	45	4.262	3.603
	% C_2H_5OH Added		
3%	1	4.006	3.542
	2	3.991	4.003
	5	3.713	3.332
	10	3.610	3.287
	15	3.608	3.371

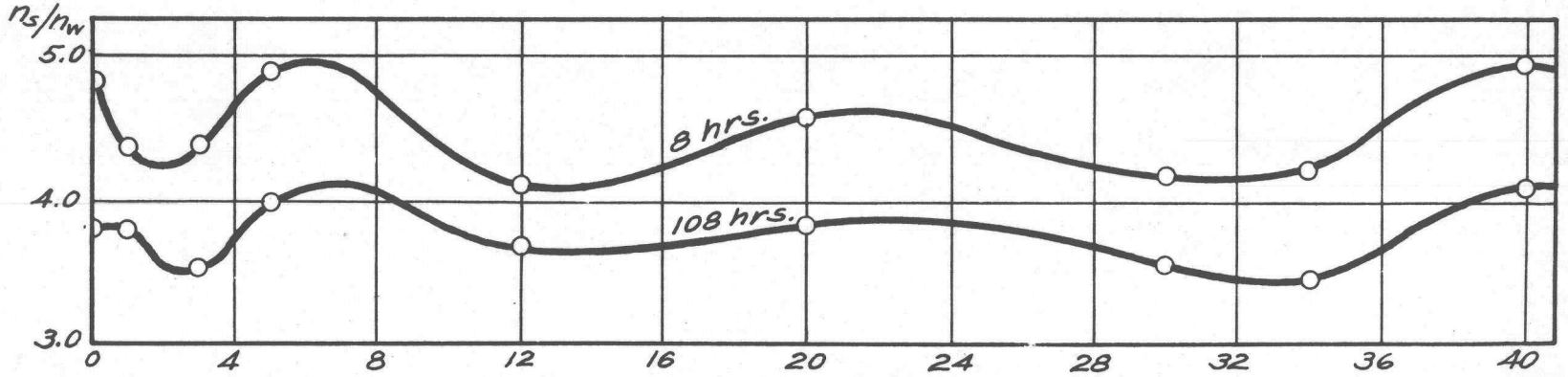


FIGURE VI

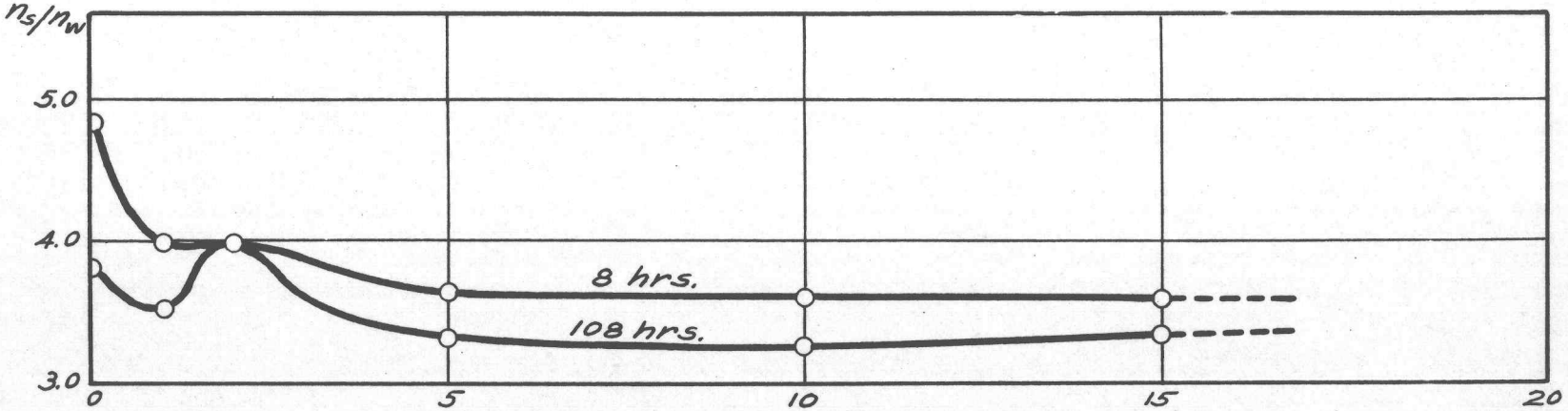
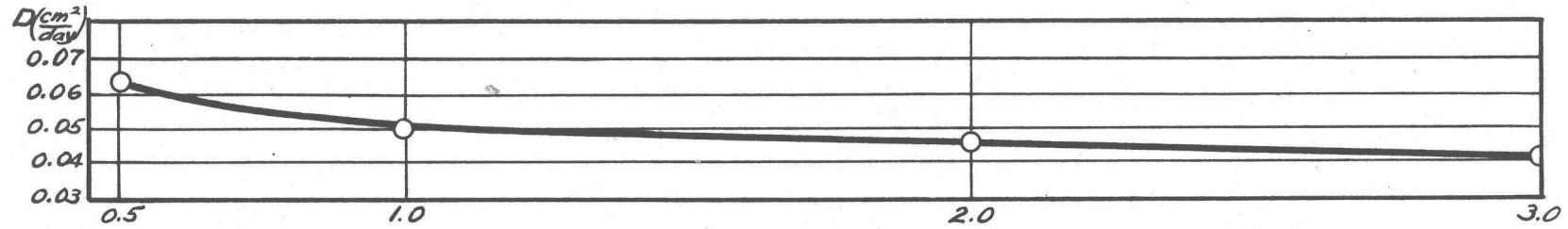
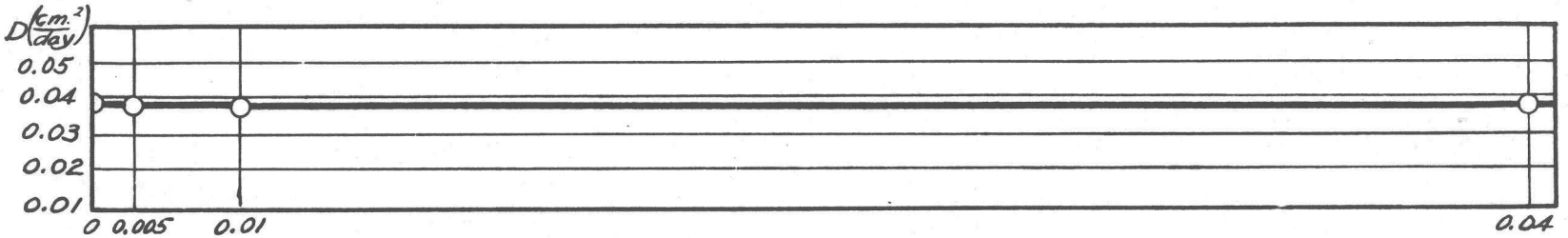


FIGURE VII



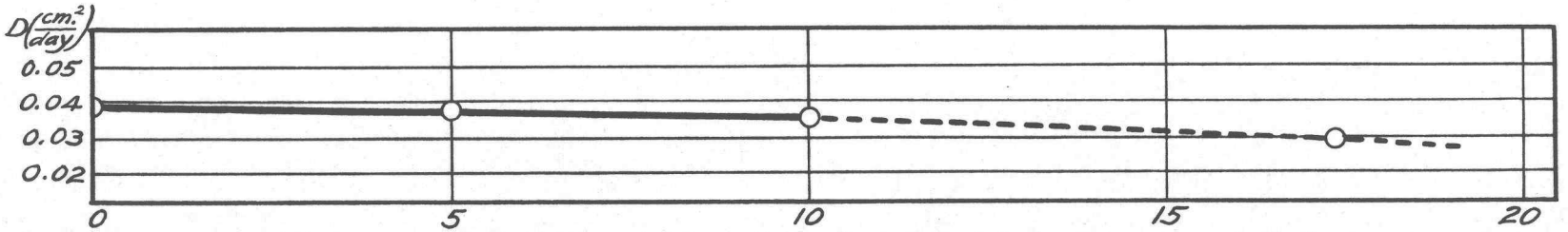
Concentration of gelatin by weight

FIGURE VIII



Concentration of KCl in moles per liter

FIGURE IX



Concentration of ethyl alcohol by weight

FIGURE X

TABLE XII.

% C_2H_5OH in Soln.	η_s/η_w
1	1.026
2	1.095
5	1.154
10	1.341
15	1.572
17.34	1.635

That practically no change is produced in the diffusion velocity of gelatin by the addition of KCl in varying amounts is shown by Figure IX. This finding agrees with that of Herzog and Kasarnowski (45) whose data, for the diffusion of colloidal materials suspended in water containing 0.5% NaF and saturated toluene, show that the presence of foreign materials does not alter the diffusion velocity appreciably. This may be taken, in the case of the addition of KCl to gelatin, to mean that there is no reaction between gelatin and KCl and no adsorption of either of the ions. However, the above statement does not necessarily hold for other substances nor does it hold for gelatin upon the addition of acid on base, as will be shown later.

Contrary results were obtained by Freundlich and Krüger (33) in their investigations of aqueous solutions of quinhydrone and hydroquinone. They found diffusion according to Fick's law for aqueous solutions. Abnormally

high rates of diffusion resulted upon the addition of $NaNO_3$, K_2SO_4, or $NaClO_4$. The increase can be explained, in this instance, on the basis of the formation of highly ionized complexes. In his study of the diffusion of hemoglobin, Fischer (31) discovered that the diffusion velocity of the hemoglobin decreased upon the addition of NaI, $NaOOC \cdot CH_2(HOOC \cdot COH)(HOOC \cdot CH_2)$, or Na-, K,- or Ca-$(C_2H_3O_2)_2$. The decrease, in this case, is explained on the basis of the formation of complex salts which are very slightly ionized. When NaCl, KCl, or $CaCl_2$ were added, the diffusion velocity was found to increase. Here the complex salts formed are highly ionized. The investigations of Laszlo and Groh (53) indicate that in an acid solution, the addition of NaCl to a solution of ovalbumin increases the rate of diffusion, the same effect later being observed in aqueous solutions of ovalbumin.

Comparison of Figures IX and VI again demonstrates the independence of D from the viscosity of the solution due to the presence of the diffusate and shows that the change in the relative viscosity of the dispersion medium, due to the presence of the KCl, has very little influence, if any, upon the value of D within the range investigated. Due to the lack of any appreciable change in the value of D upon the addition of KCl to the gelatin solutions, the

changes in the pH of the solutions upon the addition of KCl, which are relatively small, were not measured.

Figure X illustrates the result of an attempt to measure the magnitude of the effect upon D produced by the removal of the water layer from the surface of the gelatin particles. Since a 17.34% concentration of C_2H_5OH in a 3% gelatin solution at 35° produced practically complete precipitation of the sample (III) diffusion of a 3% gelatin solution dispersed in a medium consisting of 5% C_2H_5OH and 95% water and of 10% C_2H_5OH and 90% water respectively, were studied. Figure VII shows the change of viscosity of a 3% gelatin solution with change in the amount of C_2H_5OH present. The general result of the addition of alcohol is to lower the viscosity of the solution as a whole. The data in Table XII, however, (which has not been graphed) shows a noticeable increase in the viscosity of the dispersion medium upon the addition of alcohol. It is to be seen from Figure X that the addition of alcohol to the system produced a slight decrease in the diffusion velocity. The increase in velocity which resulted from the partial removal of the water mantle from the gelatin particles has been more than offset in this instance by the opposing effect produced by the increased relative viscosity of the dispersion medium.

Continuation of this study from zero percent of C_2H_5OH to percentages approaching precipitation values for the gelatin would yield data from which the amount of water contained in the mantle could be calculated. This was found to be impossible since the gelatin diffusing into even a 15% alcohol solution, as a result of the low concentration of the diffused gelatin, resulted in the precipitation of the gelatin in the outer solution.

4. The Effect of the Addition of Acid, Base, Acid plus KCl, and Base plus KCl.

In order to inquire into the reason for such findings as those of Laszlo and Groh (53) whose results indicated that an increase of hydrogen ion concentration on either side of the optimum for precipitation causes an equal diminution in the diffusion rate of ovalbumin of about 12%, the diffusion velocity of gelatin in the presence of varying amounts of acid, or of base, was measured. Also, in continuation of the search for the reason for the relatively enormous decreases in diffusion velocities of starch and gum arabic upon the addition of minute amounts of electrolytes discovered by Bruins (13), varying amounts of KCl were added to the solutions whose diffusion velocities at different pH's were measured.

The data obtained for 3% gelatin solutions and the results calculated from them are summarized in Table XIII and shown graphically by Figure XI.

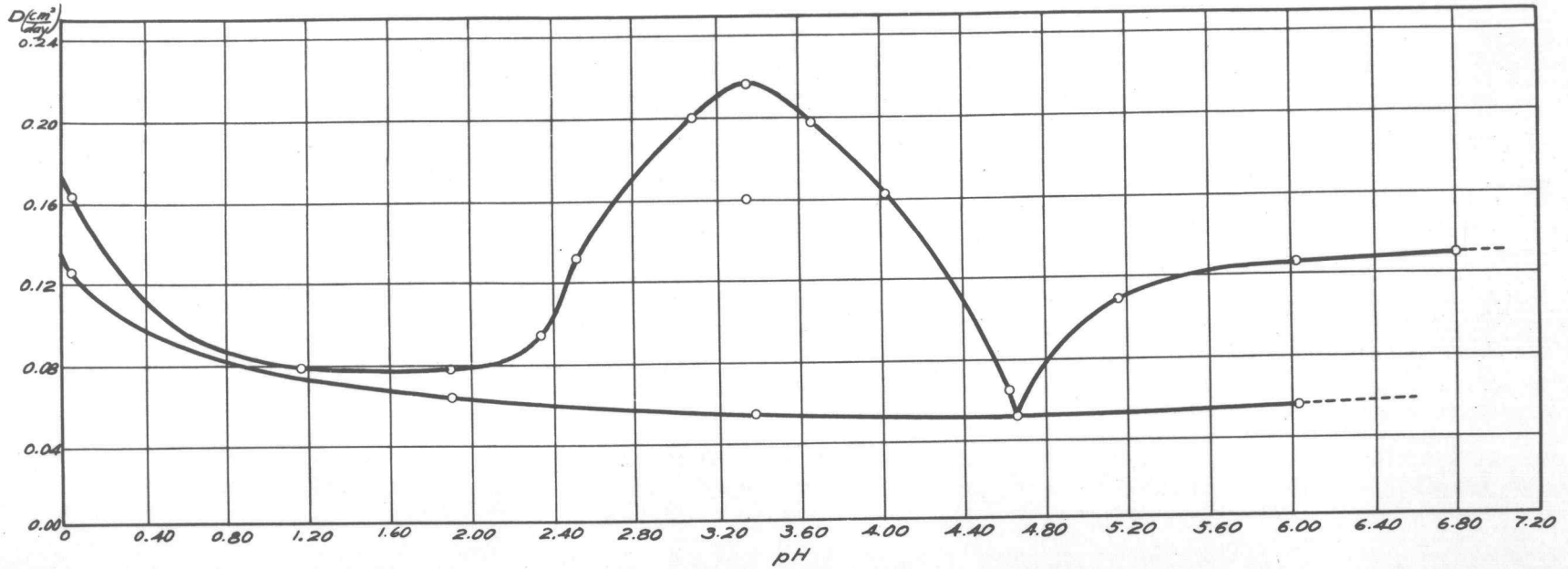

D (cm²/day)
0.24
0.20
0.16
0.12
0.08
0.04
0.00
0
0.40
0.80
1.20
1.60
2.00
2.40
2.80
3.20
3.60
4.00
4.40
4.80
5.20
5.60
6.00
6.40
6.80
7.20
pH

FIGURE XI

TABLE XIII.

Run	Cell	Electrolytes Added	Concn. KCl	pH $Soln_i$	pH $Soln_o$	D cm^2/day	D (av.)
1	III			4.66	5.00	0.055)	0.051 ± 0.004
1	IV			4.66	5.00	0.047)	
2	I	HCl		2.33	2.34	0.092)	0.093 ± 0.001
	II	HCl		2.33	2.34	0.094)	
	III	HCl		3.34	3.34	0.209)	0.216 ± 0.007
	IV	HCl		3.34	3.34	0.223)	
3	I	HCl		1.17	1.17	0.081)	0.078 ± 0.003
	II	HCl		1.17	1.17	0.075)	
	III	HCl		4.60	4.63	0.062)	0.064 ± 0.003
	IV	HCl		4.60	4.63	0.067)	
4	I	HCl + KCl	0.100N	3.38	3.38	0.052)	0.053 ± 0.003
	II	HCl + KCl	0.100N	3.38	3.38	0.055)	
	III	HCl + KCl	0.020N	3.35	3.34	0.161)	0.160 ± 0.002
	IV	HCl + KCl	0.020N	3.35	3.34	0.158)	
5	I	HCl		2.51	2.51	0.128)	0.130 ± 0.002
	II	HCl		2.51	2.51	0.132)	
	III	HCl		4.04	4.04	0.174)	0.162 ± 0.013
	IV	HCl		4.04	4.04	0.149)	
6	I	HCl + KCl	0.250N	0.054	0.054	0.126)	0.125 ± 0.001
	II	HCl + KCl	0.250N	0.054	0.054	0.124)	
	III	HCl		0.054	0.054	0.167)	0.163 ± 0.005
	IV	HCl		0.054	0.054	0.158)	
7	I	HCl		3.08	3.08	0.197)	0.200 ± 0.003
	II	HCl		3.08	3.08	0.203)	
	III	HCl		3.65	3.66	0.189)	0.197 ± 0.008
	IV	HCl		3.65	3.66	0.205)	

(cont'd)

TABLE XIII (Cont'd)

Run	Cell	Electrolytes Added	Concn. KCl	pH $Soln_i$	pH $Soln_o$	D cm^2/day	D (av.)
8	I	HCl+ KCl	0.100N	1.90	1.90	0.063	0.063 ± 0.001
	II	HCl+ KCl	0.100N	1.90	1.90	0.064	
	III	HCl		1.90	1.90	0.074	0.077 ± 0.003
	IV	HCl		1.90	1.90	0.079	
9	I	NaOH		6.80	6.80	0.127	0.129 ± 0.002
	II	NaOH		6.80	6.80	0.130	
	III	NaOH		5.16	5.16	0.108	
	IV	NaOH					
10	I	NaOH+KCl	0.100N	6.03	6.03	0.057	0.055 ± 0.002
	II	NaOH+KCl	0.100N	6.03	6.03	0.054	
	III	NaOH		6.03	6.03	0.121	0.125 ± 0.004
	IV	NaOH		6.03	6.03	0.128	

A study of the upper curve in Figure XI reveals at once the cause of the abnormally high values of D obtained by Bruins (12) for gum arabic and starch and the lack of consistant values for similar measurements made by different investigators upon various lyophilic systems. The lower curve reveals the cause of the relatively high decrease in the value of D upon the addition of salts which was observed by Bruins (12). The lower curve also reveals the effect of the pH upon the aggregation-de-aggregation equilibrium of 3% gelatin solutions at 35° between the pH

range of 6.40 to 0.053. Below pH 2.00, the values of D indicate a rapid disintegration of the gelatin micellae. The point at pH 3.34, D equal to 0.160, shows that the addition of an insufficient amount of salt to a system of a given concentration and pH will cause a decrease in the value of D but will not reduce it to the minimal value for that pH. The lower curve further indicates that although the addition of a sufficient amount of salt will reduce the diffusion coefficient to its normal value at any given pH, only the value obtained for the pure substance at its own pH will be the true D for that material since the increase in the diffusion velocity of the gelatin particles which is due to the ionization of the gelatin itself has been shown, by the immediate increase of D for gelatin on either side of the isoelectric point (Figure XI) to be immeasurably small.

The first increase in the apparent value of D upon the addition of HCl to the gelatin solution results from the formation of gelatin hydrochloride as a result of reaction between the HCl and the superficial amino groups on the gelatin micellae and the ionization of the gelatin hydrochloride to form chloride ion and gelatin ion.

The effect produced is parallel to that which occurs when HCl itself diffuses in dilute solution. In the diffusion of ions, the ions of opposite sign must move

so that electroneutrality is preserved at all times. The result is the movement of the ions of opposite sign through the solution at equal velocities. Consequently, the hydrogen ion never moves with its maximum velocity, the chloride ion always moves at an abnormally great velocity (58). In the case of the gelatin hydrochloride, the velocity of diffusion of the chloride ion is materially reduced, that of the gelatin particle is increased. In effect, the chloride ion is dragging the massive gelatin particle through the solution.

The reaction between gelatin and HCl is subject to the law of mass action. That is, as the solution is made more acid, more of the HCl will combine with the gelatin. The result is the change of value of D from 0.051 cm^2/day for 3% gelatin at 35° and pH 4.66 to 0.216 cm^2/day for the gelatin ion at pH 2.34. At this point practically all the available amino groups may be considered to have reacted with HCl. Further addition of acid now results only in an increase in the acid in the system, since little, or no, reaction is occurring between the HCl and the amino groups of the gelatin. The added HCl, then, distributes itself in the system as hydrogen and chloride ions. As a consequence of the presence of a relatively great number of the faster moving chloride ions, more chloride ions now enter the

electrical sphere of influence of the gelatin ion and no particular chloride ion, or ions, may be considered as attached to the gelatin ion by electrostatic attraction. The result is a lessening of the electrostatic drag produced upon the gelatin ion by chloride ions and a consequent decrease in the velocity of the gelatin ion. The maximum effect of the added acid is reached, for gelatin at a pH of 1.60, but the value of D has now risen, as a consequence of the shift of the aggregation-de-aggregation equilibrium of the micellae resulting from the addition of acid, to a value of 0.064, as shown by the lower curve.

The further increase in the value of D as the pH of the system is decreased is due to the increased breakdown of the gelatin micellae as is also shown by the lower curve. The increase in the value of D for systems on the alkaline side of isoelectric gelatin is explained on the same basis as the increase on the acid side except that here the increase is the result of a reaction between the base and gelatin to form water and highly ionized sodium gelatinate.

The necessity for the complete study of any lyophilic system before any use is made of the measurements secured is very evident from the two curves shown in Figure XI.

The advantages secured by analysis of the gelatin concentrations by means of the microhjeldel method are readily seen by a comparison of the values shown in Figure XI

and those depicted on pages 1025 and 1026 of the Journal of the American Chemical Society (60) which contains the latest results of an investigation of the diffusion velocity of egg albumin under conditions similar to those studied in this work. In this study (60), concentrations were measured by means of an interferometer and the variation of the values obtained for D can be ascribed to the inaccuracies of analysis by this method which result from the presence of electrolytes in the solutions. Such inaccuracy has been noted in the work described here when concentrations of gelatin in the presence of HCl, KCl, or both were measured by means of a refractometer. That such conditions hold has been admitted by the authors of the paper describing the study of egg albumin (60) who state that: "From a practical standpoint it is usually better to obtain molecular weights from duffusion studies in the neighborhood of isoclectric 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 having electrical and colloidal 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."

As may be seen from a comparison of the values obtained by the two methods, the lower sensitivity and accuracy of the microkjeldahl method is more than offset by the errors introduced into the more sensitive optical method by the presence of the added electrolytes.

VII. Calculation of Apparent Molecular Weights

In view of the fact that the particles in gelatin solutions, as well as those in solutions of any other lyophilic materials, are stabilized by either, or both, adsorbed ions and a layer of adsorbed molecules of the dispersion medium, values of the molecular weights of such substances, calculated from data obtained by measurements made upon aqueous solutions, will be only"apparent molecular weights."

The "apparent molecular weight" of gelatin from different sources and under different conditions of pH, dispersion medium, and concentration have been calculated from experimental data by means of the Stokes-Einstein equation (27):

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

and the formulae:

$$D(cm^2/day)/86,400 = D\ (cm^2/sec),$$

$$v = 4\pi r^3/3, \text{ and}$$

$$d = 1/\text{partial specific volume}.$$

The specific volume of dry gelatin has been found by Taffel (94) to be 0.7445 at 32° with a mean temperature coefficient of expansion of 0.000271 per degree. However, since there is a measurable contraction when gelatin is

added to water, the partial specific volume of gelatin was employed in the calculations of M. The value of the partial specific volume of a number of lyophilic substances has been measured by Svedberg (91) who employed the relationship:

$$\text{Partial specific volume, } V = \frac{w-(l-h)}{\rho h},$$

where w is the weight of the solvent in the pycnometer, l the weight of the solution, ρ the density of the solvent, and h the weight of the preotein material. Svedberg (92) obtained a value of 0.682 for gelatin at 30°. This value was employed in calculating the apparent molecular weights since it was found to be independent of temperature change.

The values calculated are summarized in Table XIV. The variation of the apparent radius, molecular volume, and molecular weight of the gelatin particles to be expected from samples from different sources is shown in the first four lines of Table XIV. The maximum difference in the values of M is 63.4%. The importance of accurate measurements of D and the necessity of taking into account all factors influencing the diffusion coefficient are amply demonstrated by the values shown in lines 15, 18, and 14 where a decrease of 0.01 in the value of D results in a 6.2% increase in M and an increase of 0.01 results in a

TABLE XIV.

Sample	Concn.	Subs. Added	Concn. KCl	ph	D	$r \cdot 10^7$ cm.	V(cc)	M(g.m.w.)
I	3%			4.70	0.049	5.363	392,000	574,000
II				4.59	42	6.402	666,000	977,000
III				4.59	39	6.895	832,000	1,220,000
IV				4.66	51	5.272	372,000	546,000
II	3			4.59	0.039	6.402	666,000	977,000
	2			4.70	46	5.859	511,000	749,000
	1			4.77	51	5.272	372,000	546,000
	0.5			4.91	63	4.269	326,000	289,000
	0.0			5.00	0.102	2.636	46,500	68,200
IV	3	HCl+KCl	0.250N	0.054	0.125	2.169	25,900	38,000
		HCl+KCl	0.100N	1.90	.063	4.268	197,000	289,000
		HCl		3.34	.216	1.245	4,900	7,200
		HCl+KCl	0.020N	3.34	.160	1.335	6,000	8,900
		HCl+KCl	0.100N	3.38	.053	5.073	332,000	486,000
				4.66	.051	5.272	372,000	546,000
		NaOH		6.03	.125	2.169	25,900	38,000
		NaOH+KCl	0.100N	6.03	.055	4.889	297,000	435,000
					0.052	5.171	351,000	514,000
III	3	C_2H_5OH	0.0gms.	4.59	0.039	6.895	832,000	1,220,000
			5		39	5.975	541,000	794,000
			10		38	5.277	373,000	547,000
			17.34		0.033	4.939	306,000	448.000

decrease of 5.4% in this particular value of M. The values shown in lines 19 and 20 of Table XIV, reveal the effect of the viscosity of the solution medium upon the calculated values where an increase of 1.341 in the relative viscosity of the dispersion medium lowers the value of the apparent molecular weight by 63.4%. The change in the value of M with change of concentration of the gelatin in solution is shown in lines 5 to 9 inclusive. The molecu-

lar weight of 68,200 for gelatin at zero concentration was calculated from a value of D for gelatin at zero concentration which was obtained by extropolation of the curve in Figure VIII. It is interesting to note that the value 68,200 divided by four gives a value of 17,050, which is very close to the accepted value of 17,000 for the weight of the structural unit of proteins in general. It may also be noted that the division of 17,050 by 16 gives a value of 1066 which is very close to the accepted value of 1070 for the combining weight of gelatin with HCl. However, the inadvisability of too hasty conclusions based upon the above relationships may be seen from the fact that the value of M for a 0.5% solution (line 8) is roughly 4 times that for a zero % concentration (line 9); the value for a 1% concentration (line 7) is 8 times; for a 2% concentration, 11 times; and for a 3% concentration, 15 times. In spite of the fact that these values are practically whole number multiples of the basic weight at zero concentration, the monomolecularity of dispersion in a solution of any given concentration has not been established and values calculated for concentrations between those given would yield molecular weights which are not whole number multiples of 68,200. Further evidence against placing too much weight upon values of M calculated from diffusion or other data obtained from measure-

ments made upon solutions will be introduced in the discussion of values obtained for systems in which alcohol-water solutions functioned as dispersion media.

The values of M shown in lines 10 to 18 illustrate clearly the sources of the differences of the apparent molecular weights calculated for a number of different proteins. The value of 546,000 is the true apparent molecular weight of the gelatin particles in a 3% solution of sample IV at its own pH of 4.66. Line 12 shows the error introduced into the value of diffusion coefficient of the same solution when enough HCl is added to bring the pH of the solution to 3.34. The electrostatic drag impressed upon the gelatin particle as a result of the action of the more rapidly moving chloride ion has increased the diffusion coefficient of the particle from 0.053 to 0.216. This results in a decrease of the apparent molecular weight of the particle, calculated from the diffusion coefficient, from 486,000 to 7,200, an apparent decrease of 98%, whereas, the addition of enough KCl to overcome the drag introduced by the chloride ion, shows that the weight of the particle is still 486,000. The values on lines 13 and 14 show the possibility of error when salts are introduced to overcome the drag of the chloride ion. The introduction of enough KCl to render the solution 0.02N decreases the value of D sufficiently

to raise the value of M to 8,900 which, if the investigation were stopped here might be taken as the true apparent molecular weight at this concentration and pH. However, the addition of sufficient KCl to make its concentration 0.100 N, decreases the value of D to 0.053 and gives a value of 486,000. The values on lines 11, 14, 15, and 17 show the true effect of the changes of pH upon the values of M. These values, like those of D from which they have been calculated (lower line, Figure XI) show no minima in the change of M with pH just as the true values of D show no maxima with change of pH. Values calculated from data corresponding to that shown in the upper curve would show minimal values of M for maximal values of D. That these values would be incorrect is shown by the lower curves of Figure XI which were obtained by measurements made upon similar systems in which all electrostatic drag upon the gelatin particles had been eliminated. It is further to be noticed, especially from Figure XI, that no pronounced disintegration of the gelatin particles occurs between pH's of 3.00 and 6.03. This indicates a situation different than that mentioned in the statement made by Svedberg (92) in which he states that a 0.4% solution of gelatin at 19.5° is not aggregated at pH's of 4.00 and below and at pH's 7.5 and above. It does agree with his claim that there is marked aggregation of the particles between pH's of 4.60

and 6.00, except that the values shown in Figure XI indicate that the aggregation stability range is very little affected between the pH's of 3.00 and 6.40 instead of between 4.60 and 6.00. This difference, may be due to the difference in the concentrations at which the measurements were made.

A very significant phenomenon is disclosed by the data shown on lines 19, 20, 21, and 22, in Table XIV, namely, the removal of the water mantle from the gelatin micellae, its effect upon the apparent molecular weights, and the possibility of the complete removal of the mantle and the determination of the true molecular weight of the particle. This would necessitate the measurement of the diffusion velocity of the gelatin particles at different concentrations of gelatin ranging from 3 to 0.5% in which the dispersion medium was composed of alcohol-water solutions. The concentration of the alcohol would have to approach the precipitation value for gelatin, namely, 17.34%. It would be further necessary to stabilize the micellae by the addition of an electrolyte, being careful to see that no abnormal effects, such as that produced by the addition of acid or base, were introduced.

Some idea of the effect of the removal of the water mantle may be gained by the data secured in this investigation. It is seen that changing the dispersion medium from

pure water to 5% C_2H_5OH by weight decreases the apparent molecular weight by 35%. Changing the medium to 10% C_2H_5OH and 90% H_2O lowers the value of M from 1,220,000 to 547,000, a decrease of 53%. Extropolation of the curve of Figure X to 17.34% C_2H_5OH (the precipitation value for a 3% solution of isoelectric gelatin) gives a value of 0.0333 for D. Due to the inaccuracy of such extropolation the value obtained for D is highly approximate, but the results obtained from calculations based upon this value justify its use. The value of M for the particles of gelatin in a 3% solution dispersed in a 17.34% C_2H_5OH solution is 448,000, a 63% decrease of the value for gelatin in pure water. The value of r drops from $6.895 \cdot 10^{-7}$ cm. to $4.939 \cdot 10^{-7}$cm. The difference between these two values gives the thickness of the water mantle as $1.956 \cdot 10^{-7}$ cm. If it is assumed that the thickness of the water mantle is the same for the particles in a zero concentration of gelatin as it is in a 3% solution, then the calculated radius of the particle at zero concentration minus $1.956 \cdot 10^{-7}$cm. should give the true radius of the particle at zero concentration. The calculated value of r, where M is 68,200 is $2.636 \cdot 10^{-7}$cm. This, minus $1.956 \cdot 10^{-7}$cm, gives $0.68 \cdot 10^{-7}$cm. as the "true" radius. The "true" molecular weight, calculated from the "true" radius is 1171. The assumption and the extrapolation in-

volved in the calculation render its value negligible; however, it demonstrates very clearly the enormous differences between the apparent molecular weight calculated from measurements made upon lyophilic substances dispersed in aqueous media and the true molecular weights of these materials. Such true values, once obtained would undoubtedly eliminate the discripancies between the values of M calculated from decomposition products and those obtained from diffusion, osmotic pressure, and other data. It would also materially simplify the study of the molecular structure of lyophilic substances.

The above calculation also shows very clearly, the danger of attaching too much importance to the fact that the value obtained for the apparent molecular weight of the gelatin particle at zero concentration, 68,200, is equal to four of the basic protein units, 17,000, and that the 17,100 obtained, if divided by 16, gives 1066, which is approximately the value given for the combining weight of gelatin.

The curves in Figure XI also show that the maximal values obtained in viscosimetric and other studies of gelatin are very conducive of misinterpretation.

VIII. Conclusions.

The results obtained in this investigation prove that a very high degree of precision is possible in the measurement of the diffusion velocity by means of the membrane method embodied in the Northrop and Anson (66) type of cell. The excellent agreement between the measurements made upon comparable systems shows not only the accuracy of the method, even for such slowly moving particles as those existing in colloidal solution, but also the possibility of the exact duplication of such systems.

The so-called "abnormally high diffusion velocities" found in this, and other, investigations are as much the true velocities of the respective materials as are the accepted values for HCl, KCl, or other electrolytes at infinite, or any other, dilution. The diffusion velocity of, for example, HCl at infinite dilution is not the diffusion velocity of the HCl molecule at infinite dilution, but a mean value for the diffusion velocity of the swiftly moving hydrogen ion and the more slowly moving chloride ion. This mean value results from the necessity of the preservation of electroneutrality at all points within the solution. As the concentration of the HCl is increased, the possibility of collision is increased and the velocity of both ions is decreased accordingly. The

degree of dissociation is lowered, which results in a decrease in the number of hydrogen and chloride ions per mole of HCl added to the system and the appearance of HCl molecules as such. The HCl molecules as a result of their greater mass and the fact that there is no electrostatic attraction between the molecules such as exists between other ions, diffuse through the dispersion medium more slowly than do the pairs of their oppositely charged ions. The general result is a decrease in the diffusion velocity of HCl with increased concentration. For non-electrolytes, the corresponding decrease, which results from increased collisions alone, is less.

The situation with regard to the "abnormally high diffusion velocities" exhibited by lyophilic colloids is analogous to that of HCl. This investigation has shown that the particles in solutions of pure isoelectric gelatin exhibit a change in diffusion velocity with change in concentration (Figure VIII) analogous to the corresponding change for non-polar materials. Exact coincidence of the curve for gelatin with the general form of the curve for non-electrolytes is not to be expected since the gelatin particles, as shown by the change of the pH of solutions of isoelectric gelatin with change in concentration, show a slight ionization. This ionization, however, is so slight and the mass of the gelatin particle so great that

the resulting deviations from the behavior of true non-electrolytes are immeasurably small.

With the addition of an acid such as HCl or a base such as NaOH, the system is radically altered. The practically unchanged gelatin particles become highly ionized gelatin hydrochloride or sodium gelatinate. The gelatin ions, as is shown by the decomposition products and the molecular weights of the gelatin particles, are multivalent. The result of electrostatic pull of the number of chloride or sodium ions, as the case may be, upon the gelatin ion is the increase of its diffusion velocity from 0.051 cm^2/day for the practically uncharged particle, to 0.216 cm^2/day for the polyvalent ion. The lowering of the diffusion coefficient produced by the addition of more HCl results from the same factors which produce the decrease in the diffusion velocity of HCl itself with increasing concentration coupled with the fact that with increased HCl concentration the number of chloride ions within the sphere of influence of the gelatin particle is increased and the electrostatic drag upon the gelatin particle is decreased accordingly. The lowering of the diffusion velocity of the gelatin particle upon the addition of KCl results from the same change.

The sudden and continued increase of D of the gelatin particles upon the successive addition of small amounts of

acid or base results from the fact that the reaction of gelatin with acid or base is subject to the law of Guldberg and Waage. As more electrolyte is added more of the more swiftly moving gelatin ion is formed. The diffusion velocity measured, which is the mean of the velocities of the uncharged particles and the gelatin ions, increases continuously until all the gelatin particles have become gelatin ions. This condition is indicated by the maximal value at pH 3.34. The second increase of D, which begins at about pH 1.20 is the result of the beginning of de-aggregation of the gelatin particles.

The very low values of the "apparent molecular weights" calculated from diffusion data by McBain (60) to show the necessity of using values for D of isoelectric solutions of lyophilic colloids in order to calculate the correct molecular weights, illustrate the errors introduced by the application of the Stokes-Einstein equation to ions, the error, of course, increasing with the mass and the valence of the more massive ion. The data indicate the possibility of the development of a relationship analogous to the Nernst equation by means of which the apparent molecular weight of the gelatin salt could be calculated.

In addition to the "apparent molecular weights", the pH stability range, and the "apparent molecular weight"

of the molecularly dispersed particles (at zero concentration), diffusion measurements are a potential source of the actual molecular weights of lyophilic substances, if conditions within the systems measured are properly regulated. The differences between the "apparent molecular weights" and the true molecular weights, from the physicochemical standpoint, are relatively enormous, but can be measured to within a few percent (Table XIV) and from such measurements, the thickness of the water mantle and its effect upon the physical properties of the particles can be determined.

IX. Summary

The changes of several properties of gelatin solutions with change of solution medium have been measured and the changes noted and explained. The changes studied and measured were:

1. Variation of the diffusion velocity (expressed in terms of D) with source.
2. Variation of the diffusion velocity with concentration.
3. Variation of the diffusion velocity with different concentrations of added KCl.
4. Variations of D with different concentrations of added alcohol.
5. Variations of D with different concentrations of added HCl.
6. Variations of D with different concentrations of added HCl and KCl.
7. Variations of D with different concentrations of added NaOH.
8. Variations of D with different concentrations of added NaOH and KCl.
9. Change of the relative viscosity of gelatin solutions with:
   (a) Change of the concentration of gelatin.
   (b) Change of the concentration of added KCl to a constant concentration of gelatin.
   (c) Change of the concentration of added alcohol.

10. Variation of the pH of gelatin solutions with:

    (a) Change of the concentration of gelatin.

    (b) Change of the concentration of added acid or base.

From the above measurements were calculated the:

1. Change of the "apparent molecular weight" (M') of the gelatin particles with the change of source of the gelatin.

2. Change of M with change of concentration of the gelatin.

3. Change of M with change of concentration of the added HCl and KCl.

4. Change of M with change of concentration of the added NaOH and KCl.

5. Change of M with change of concentration of the added alcohol.

6. Apparent change of the "apparent molecular weight" of the gelatin micellae which results from the calculation of the M for charged particles by means of the Stokes-Einstein equation.

7. Apparent molecular weight of the gelatin molecule at zero concentration.

8. Effect of the removal of the water mantle from the micellae upon the value of M.

From the measurements and calculations made from them:

1. The changes of D with concentration of the gelatin, concentration of added HCl, HCl and KCl, NaOH, NaOH and KCl, and of added C_2H_5OH have been explained.

2. The possibility of error incurred by the use of the Stokes-Einstein equation for the calculation of M from diffusion data for ions has been shown.

3. The necessity of employing values of D corresponding to zero concentration of diffusate for the calculation of the apparent molecular weight of the diffusate when molecularly dispersed has been demonstrated.

4. The possibility of the determination of the true molecular weights of lyophilic substances by determining their diffusion velocities after the removal of the water mantle has been discussed.

5. The probable incorrectness of all the calculated values of M for lyophilic colloids has been shown.

6. The possibility of measuring the thickness of the water mantle and the determination of its effect upon the physical properties of the diffusate have been discussed.

7. The probable sources of error possible by this method have been enumerated.

8. The necessity of even more precise methods for the measurement of concentrations has been mentioned.

X. BIBLIOGRAPHY

(1) Arnold, J. H. Studies in Diffusion, III. A Kenetic Theory of Diffusion in Liquid Systems. J. Am. Chem. Soc., 52: 3937-3755, 1930.

(2) Arrhenius, S. An Investigation of the Diffusion of Substances Dissolved in Water. Z. physik. Chem., 10: 51-95, 1892.

(3) Atkin, W. R. The Molecular Weight of Gelatin. J. Intern. Soc. Leather Trades Chem., 17:575-582, 1933.

(4) Behn, U. Concerning the Diffusion Velocity of Electrolytes in Dilute Water Solution, Especially with Regard to the Dependence of Diffusion upon the Concentration Gradient. Wied. Ann., 62: 54-67, 1897.

(5) Bekesey, G. van. The Measurement of Diffusion Coefficients in Liquids. Physik Z, 28: 812-814, 1927.

(6) Beilstein. A New Method for the Study of Diffusion. Liebig Ann., 99: 56-73, 1856.

(7) Biltz, W. Concerning the Osmotic Pressure of Colloids, VI - On the Osmotic Pressure of Gelatin. Z physik. Chem., 91: 705-721, 1916.

(8) Boeder, P. A Method for the Determination of the Diffusion Coefficients of Colloids which Show Mechanical Birefringence. J. Rheology, 3: 494-500, 1932.

(9) Bolam, T. R. The Donnan Equilibria. G. Bell and Sons, Limited, London, 1932.

(10) Brugs, B. The Theory of Diffusion. Z. physik. Chem., A 162: 31-43, 1932

(11) Bruins, H. R. A New Method for the Determination of the Diffusion Velocities of Substances of High Molecular Weight. Rec. trav. Chim. 50: 121-128, 1931.

(12) Bruins, H. R. The Diffusion of Colloidal Particles I. Abnormally High Diffusion Velocities in Hydrophilic Sols. Koll. Z., 54: 265-272, 1931.

(13) -------- The Diffusion of Colloidal Particles II. A New Ion Effect in Hydrophilic Sols. Koll. Z. 54: 272-278, 1931.

(14) --------- The Diffusion of Colloidal Particles III. The Investigation, Significance and Interpretation of Abnormally High Diffusion Velocities and the New Ion Effects in Hydrophilic Sols. Koll Z. 57: 152-166, 1931.

(15) Butkevich, Vl. S. and V. V. Concerning the Influence of Ions upon the Diffusion Process. Biochem. Z., 204: 303-321, 1929.

(16) Clack, B. W. On the Coefficient of Diffusion. Phil. Mag., 16:[6], 863-879, 1908.

(17) ---------- Diffusion in Dilate Solutions. Proc. Roy. Soc. (Lon.), 27: 56-68, 1916.

(18) Cohen, E. and Bruins, H. R. A Precision Procedure for the Determination of the Diffusion Coefficient in Any Solvent. Z. physik. Chem. 103: 349-403, 1923.

(19) ---------- The Validity of the Stokes-Einstein Law for Diffusing Molecules. Z. physik. Chem., 103: 404-450, 1923

(20) Cohen, E. J. and Conant, J. B. Molecular Weights of Proteins in Phenol. Proc. Nat. Acad. Sci., 12: 433-438, 1926.

(21) Coleman, J. J. On Liquid Diffusion. Phil. Mag. (Lon.) 23:1-10, 1887.

(22) ---------- Studies of Liquid Diffusion. Proc. Roy. Soc. (Edinburgh), 14: 374-381, 1888.

(23) ---------- Further Studies on Liquid Diffusion. Proc. Roy. Soc.(Edinburg), 15: 248-256, 1889.

(24) Dummer, E. On the Diffusion of Organic Substances in Organic Solution Media and the Proof of the Stokes-Einstein Equation. Z. anorg. allgem. Chem., 109: 31-51, 1919.

(25) Eggert, J. and Reitstötter, J. The Molecular Weight and the Gel Condition of Gelatin. Z. physik. Chem., 123: 363-382, 1926.

(26) Eicke, J. The Photographic Determination of the Diffusion Coefficient by Means of Refracted Light Rays. Beiblätter An. Physik, 40: 535-536, 1916.

(27) Einstein, A. An Elementary Theory of Brownian Motion. Z. Electro-chem., 14: 235-289, 1906.

(28) ---------- The Limits of Application of the Diffusion Coefficient - Molecular Weight Relationship for the Determination of Avogadro's Constant. Ann. der Physik, 19: 289-291, 1906.

(29) Euler, H. Concerning the Diffusion Velocity of Non-Dissociated and Dissociated Molecules. Wied. Ann., 63: 273-277, 1897.

(30) Fick, A. The Theory of Diffusion in Liquids Pogg. Ann., 94: 59-71, 1855.

(31) Fischer, F. P. The Diffusion of Colloidal Particles, the Diffusion of Hemoglobin in Colloidally and Molecularly Dispersed Systems. Koll. Z., 57: 166-173, 1930.

(32) Frankel, M. The Influence of Temperature and the Previous History of a Gelatin Solution upon its Osmotic Pressure and the Weight of its Micellae. Biochem. Z., 240: 149-155, 1926.

(33) Freundlich, H. and Krüger, R. The Diffusion of Organic Substances in Aqueous Electrolyte Solutions. Z. Electrochem., 36: 305-309, 1930.

(34) Gerlach, B. Experimental Investigations of the Diffusion of Liquids. Ann. für Physik, 5: 437-459, 1931.

(35) Gerngross, O., Triangi, O., and Koeppe, P. The Thermal Disintegration of Gelatin. A Röntgenographic Study of its Disintegration. Ber. 63 B: 1603-1614, 1930.

(36) Graham, T. An Apparatus for the Study of Diffusion. Phil. Trans., 1: 805-15, 1850.

(37) --------- An Improved Method for the Study of Diffusion. Phil. Trans., 151: 183-192, 1861.

(38) ---------- Further Studies of Diffusion. Phil. Trans., 183: 90-104, 1861.

(39) Griffiths, S. The Measurement of the Diffusion Velocities for Systems in the Steady State. Phil, Mag., 47: 530-542, 1899.

(40) --------- Steady State by Means of Small Tubes Proc. Phy. Soc. (Lon.), 28: 21-29, 1916.

(41) ---------- Further Studies of Systems in the Steady State. Proc. Phy. Soc. (Lon.) 28: 255-268, 1916.

(42) Hartley, G. S. and Robinson, C. The Diffusion of Colloidal electrolytes and Other Charged Particles. Proc. Roy. Soc. (Lon.), A 134: 20-35, 1931

(43) Haskel, R. The Effect of Concentration and Ionization upon the Rate of Solution of Salts in Aqueous Solution. Phy. Revs., 27: 145-182, 1908.

(44) Heiduschka, A. and Ripper, J. The Use of Diffusion for the Identification and Separation of Fatty Acids. Z. Electrochem., 29: 552-555, 1923.

(45) Herzog, R. O. and Kasarnowski, H. Different Colloids. I. Z. Electrochem., 13: 533-541, 1907.

(46) ---------- and Polotsky, A. Solutions of Dyestuffs, III. The Diffusion of Dyestuffs. Z. Electrochem. 17: 697-684, 1912.

(47) Herzog, R. O. A Contribution to the Knowledge of Solutions. Z. Electrochem., 16: 1003-1004, 1910.

(48) Jander, G. and Winkel, A. The Application of Diffusion Coefficients to the Determination of the Molecular Weights of Ions. - these Relations for Amphoteric Hydrated Oxides in Water Solutions. Z. physik. Chem., A 149: 97-122, 1930.

(49) Johannisjanz. Diffusion Velocities of Sugar Solutions. Pogg. Ann., 11: 24-34, 1868.

(50) Johlin, J. M. The Isoelectric Point of Gelatin and its Relation to the Minimal Physical Properties of Gelatin. J. Biol. Chem., 86: 231-243, 1930.

(51) Komers, K. A New Diffusion Method. Fr., 642: 278-284, June 1927.

(52) Krishnamurti, P. The Nature of Dextrin, Gelatin, and Sodium Oleate Solutions as Revealed by X-Ray Diffraction. Indian J. Physics, 3: 307-329, 1929.

(53) Lazlo, J. and Groh, J. The Rate of Diffusion of Ovalbumin in Different Aqueous Media. Biochem. Z., 173: 248-257, 1926.

(54) Lenz, A. The Diffusion of Strong Electrolytes upon the Addition of Alcohol. Memoirs de l'Acad. de St. Petersburg, 7: 37-50, 1882.

(55) Littlewood, T. H. Diffusion of Solutions. Proc. Roy. Soc. (Lon.), 34: 71-76, 1922.

(56) Long, J. H. Concerning the Diffusion of Salts in Aqueous Solutions. Wied. Ann., 9: 613-641, 1880.

(57) McBain, M. E. Laing. Diffusion of Colloidal Electrolytes; Sodium Oleate. J. Am. Chem. Soc., 55: 545-551, 1933.

(58) McBain, J. W. and Dawson, C. R. Accelerated and Retarded Diffusion in Aqueous Solutions. J. Am. Chem. Soc., 56:52-56, 1934.

(59) McBain, J. W. and Lin, T. S. Diffusion of Electrolytes, Non-Electrolytes, and Colloidal Electrolytes. J. Am. Chem. Soc., 53: 59-74, 1931.

(60) McBain, J. W., Dawson, C. R., and Barker, H. A. The Diffusion of Colloids and Colloidal Electrolytes; Egg Albumin; Comparison with the Ultracentrifuge. J. Am. Chem. Soc., 56: 1021-1027, 1934.

(61) Marginac. Diffusion Velocities of Mixtures of Electrolytes. Ann. de Chimie et de Physique, 2: 50-63, 1874.

(62) Marinesco, N. The Specific Inductive Capacity and the Molecular Weights of Colloids. Compt. rend. 192: 625-628, 1931.

(63) ---------- The Structure of Gelatin Solutions. Compt. rend., 188: 1163-1165, 1928.

(64) Meyer, G. Diffusion Constants. Wied. Ann., 61:225-234, 1897.

(65) Nernst. W. Concerning the Kinetics of Bodies in Solution. Z. physik, Chem., 2: 613-637, 1888.

(66) Northrop, J. H. and Anson, M. L. A Method for the Determination of Diffusion Coefficients and the Calculation of the Radius and the Molecular Weight of the Hemoglobin Molecule. J. Gen. Physiol., 12: 543-554, 1929.

(67) Öholm, L. W. The Hydrodiffusion of Electrolytes I. Z. physik. Chem., 50: 309-327, 1904.

(68) ---------- The Hydrodiffusion of Electrolytes II. Z. physik, Chem., 50: 327-354, 1904.

(69) ---------- Free Diffusion of Non-Electrolytes. Z. physik. Chem., 70: 378-397, 1909.

(70) ---------- The Dependence of Diffusion upon the Viscosity of the Solvent. Medd. K. Vetenskopakad. Nobel Inst., 2, No. 26: 1-21, 1912.

(71) ---------- Free Diffusion of Non-Electrolytes; the Hydrodiffusion of an Optically Active Substance. Z physik. Chem., 70: 397-404, 1910.

(72) ---------- An Investigation of Some Organic Substances in Ethyl Alcohol. Medd. K. Vetenskopad. Nobel Inst., 2, No. 24: 1-31, 1913.

(73) ---------- The Free Diffusion of Non-Electrolytes II. The Hydrodiffusion of Some Organic Substances. Medd. K. Vetenskopad. Nobel Inst., 2, No. 23: 1-52, 1912.

(74) Öholm, L. W. The Influence of Non-Electrolytes upon the Diffusion of Electrolytes and upon Electrical Conductivity. Oeversigt. Findska Vetenskaps. soc. Förhandling ar 55, Afd. A, No. 5: 99-108, 1902.

(75) Ostwald, W. Concerning the Dissociation Theory of Electrolytes. Z. physik. Chem., 2: 270-283, 1888.

(76) Pickering, S. U. Experiments upon the Diffusion of Substances in Solution. Phil. Mag., 35: 127-134, 1893.

(77) Pissarievski, L. and Karp, B. The Relation between the Diffusion Coefficient, the Inner Friction, and the Electrical Conductivity. Z. physik. Chem., 63: 257-268, 1908.

(78) Planck, M. Concerning the Transfer of Electricity and Heat by Electrolysis. Wied. Ann., 39: 161-186, 1890.

(79) ---------- Concerning the Potential Difference between Two Dilute Solutions of Binary Electrolytes. Wied. Ann., 40: 561-576, 1890.

(80) Schryver, S. B. Investigations on Gelatin. Dept. Sci. Ind. Research; Second Report of the Adhesives Committee, 7-16, 1926.

(81) Semmler, Th. A Graham Type of Apparatus Permitting the Use of Fick's Equation. Pogg. Ann., 100: 217-223, 1856.

(82) Seylor, H. The Diffusion of Certain Organic Substances. Medicinish Chemische Untersuchungen, Berlin, 1-47, 1866.

(83) Sheppard, S. E. and McNally, J. G. The Structure of Gelatin Sols and Gels. II. The Anisotropy of Gelatin Gels. Colloidal Symposium Annual, 17-39, 1930.

(84) Smith, C. R. Osmosis and the Swelling of Gelatin. J. Am. Chem. Soc., 43: 1350-1366, 1921

(85) Staudinger, H. The Validity of the Viscosity-Molecular Weight Relationships: Ber., H 67: 92-101, 1933.

(86) Stefan, M. J. The Diffusion Velocity of Sucrose. Sitzungsber, d. k. Akad. d. Wissensch., Wien, 78-88, 1878.

(87) Süllmann, H. Diffusion Coefficients and the Particle Sizes of Colorless Substances. Protoplasma Z., 13: 546-566, 1931.

(88) Sutherland. The Use of Diffusion Coefficients for the Determination of the Molecular Weights of Large Molecules. Aus. Assocn. for Adv. of Sci., 10: 117-127, 1910.

(89) Svedberg, T. and Rinde, H. The Ultra-Centrifuge, A New Instrument for the Determination of Size and Distribution of Size of Particle in Amicroscopic Colloids. J. A. Chem. Soc., 46: 2677- 2693, 1924.

(90) -------- and Fåhraneus, R. A New Method for the Determination of the Molecular W Weights of Proteins. J. Am. Chem. Soc., 48: 430-438, 1926.

(91) Svedberg, T. and Chirnoaga, E. The Molecular Weight of Hemocyanin. J. Am. Chem. Soc., 50: 1401-1411, 1928.

(92) Svedberg, T. and Krishnamurti, P. An Ultrcentrifugal Study of Gelatin Solutions. J. Am. Chem. Soc., 52: 2897-2906, 1930.

(93) Svedberg, T. Molecular Weight Analysis in Centrifugal Fields. Sc., 79: 327-332, 1934.

(94) Taffel, A. The Thermal Expansion of Gelatin Gils. J. Chem. Soc., 121: 1971-1984, 1922.

(95) Thovert, G. Diffusion in Solutions. Ann. de Chemie et de Physique, 26:366-377, 1902.

(96) Thovert, G. Diffusion Velocities of Electrolytic Substances. Ann. de Physique, 2: 369-427, 1915.

(97) --------- Diffusion in Solutions I. Compt. rend. (Paris), 133: 1197-1204, 1901.

(98) --------- Diffusion in Solutions II. Compt. rend. (Paris), 134: 594-601, 1902.

(99) --------- Diffusion in Solutions III. Compt. rend. (Paris), 134: 826-834, 1902.

(100) --------- Diffusion in Solutions IV. Compt. rend. (Paris), 134: 579-594, 1902.

(101) --------- Diffusion in Solutions V. Compt. rend. (Paris), 138: 48-56, 1904.

(102) --------- Diffusion and the Kinetic Theory of Solutions. Compt. rend., 150: 270-272, 1909.

(103) Ullmann, E. Experimental Contributions to the Knowledge of Diffusion in Solutions. Z. Physik, 41: 301-317, 1927.

(104) Weber, H. F. An investigation of the Principles of Hydrodiffusion. Wied. Ann., 7: 469-487, 1879.

(105) Westgren, A. Concerning Diffusion, the Diffusion Velocity and Sedimentation Velocity in Selenium and Gold. Sols. Z. physik. Chem., 89: 63-90, 1914.

(106) Wilke, E. and Strathmeyer, W. An Experimental Contribution to the Theory of Diffusion. Z. Physik, 40: 309-321, 1926

(107) Zuber, R. An Investigation of Diffusion in Liquids. Z. Physik, 79: 280-290, 1933.