TABLE II

DIFFUSION OF GLUCOSE AT 25°

Concn.	D in cm.²/day	Calcd. mol. wt.
0.60	0.535	237
.50	.539	232
.40	.543	227
.355	.546	223
.30	.550	218
.25	.552	216
.20	.556	211
.10	.566	200
.00 (extrap.)	.586	180

The experimental values are reported graphically in Figs. 1 and 2, in which it is evident that
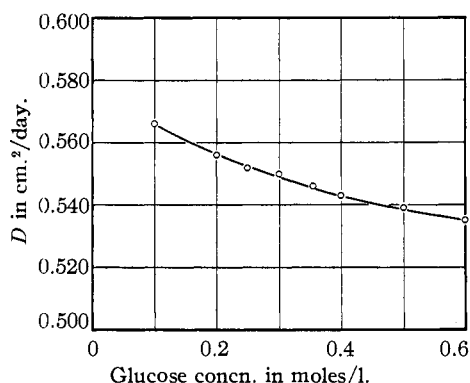

Fig. 1.—Diffusion of glucose at 25°.

when sufficiently accurate diffusion data are obtained, it is possible by plotting diffusion coefficient against square root of the concentration to extrapolate to infinite dilution and thus obtain the value of diffusion coefficient that will yield the correct molecular weight when substituted in the Stokes–Einstein diffusion equation.

Even more striking is the fact that this relationship holds for a substance of molecular weight of 180 whereas most investigators have felt that the Einstein relationship could not be applied to substances of such low molecular weight.

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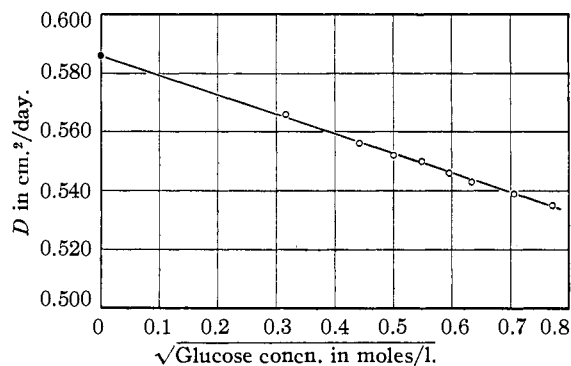

Fig. 2.—Diffusion of glucose at 25°.

Summary

It has been shown that molecular weights of simple non-electrolytes can be calculated from diffusion coefficients determined at infinite dilution, (1) by an examination of the values of diffusion coefficient to be found in the "International Critical Tables," and (2) by experimental study of the diffusion velocity of glucose.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, OREGON STATE COLLEGE]

Diffusion Velocity and Molecular Weight. II. The Effect of pH upon Particle Size in Gelatin Solutions

BY LEO FRIEDMAN AND KARL KLEMM[1]

Change of hydrogen ion concentration of a gelatin solution brings about marked changes in many of the properties of the solution. In seeking explanations of these changes, some investigators have attributed them to increased or decreased hydration with changing pH or to increased or decreased particle size with changing pH. Investigators of diffusion of colloidal materials have observed marked changes in diffusion velocity with changing pH.[2–4]

Experimental

In order to investigate the effect of pH upon particle size in gelatin solutions, diffusion experiments have been carried out using 3% solutions of Eastman Kodak Co. electrodialyzed gelatin, adjusted to various pH's by the

(1) Present address: The Paraffine Companies, Inc., Emeryville, Calif.

(2) H. R. Bruins, Kolloid Z., **54**, 265, 272 (1931); **57**, 152 (1931).

(3) J. Lazle and J. Groh, Biochem. Z., **173**, 248 (1926).

(4) J. W. McBain, C. R. Dawson and H. A. Barker, THIS JOURNAL, **56**, 1021 (1934).
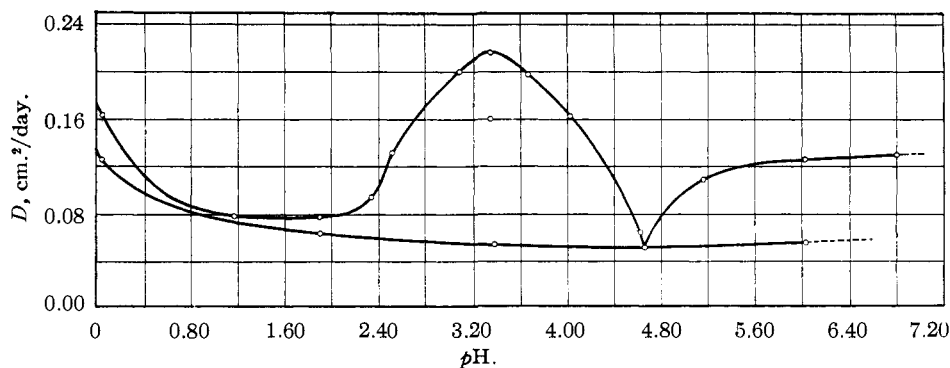
Fig. 1.—Effect of pH on diffusion velocity of gelatin.

addition of hydrochloric acid. A similar series of experiments has been carried out in which there was added to the above solutions a sufficient amount of potassium chloride to eliminate the electrical charge effect upon diffusion velocity.[4]

Diffusion coefficients were determined following the method and technique of McBain and co-workers[4,5] with the exceptions that the cells were suspended by a metal rod from a universal joint, making leveling of the cells easier and more certain, and that the sealing of the systems to prevent evaporation between the cell and the outside beaker was improved. Diffusion cells containing fused-in sintered Jena glass membranes (G_4) were used. The cells were calibrated with 0.1 N potassium chloride solutions using values from Öholm as listed in the "International Critical Tables." All diffusion measurements were made at 35° and extended over twenty-five hours. Gelatin concentrations after diffusion were determined by the colorimetric micro-method of Koch and McMeekin,[6] with the following modification. After the digestion, the material was diluted to 25 ml. instead of 100 ml., which allowed use of samples containing 0.1 to 0.25 mg. of nitrogen. This permitted shorter diffusion times. All nitrogen determinations were made in quadruplicate.

Since the source of the material and the previous history, especially thermal treatment, have such a marked effect upon the properties of gelatin solutions, these factors were controlled carefully and conditions were duplicated as nearly as possible in all determinations.

The results are given in Table I and are shown graphically in Fig. 1.

Discussion

A study of the two graphs shown in Fig. 1 reveals the cause of the abnormally high values of diffusion coefficient obtained by many investigators of lyophilic systems.[2,3] The lower curve reveals the cause of the large decreases in value of diffusion coefficient frequently found by investigators upon the addition of salts to lyophilic solutions.[2,3] The lower curve also reveals the effect of pH upon the aggregation–deaggregation equilib-

(5) McBain and Liu, This Journal, **53**, 59 (1931).

(6) Williams, "Introduction to Biochemistry," D. Van Nostrand Co., New York, N. Y., 1931, p. 456.

Table I

Electrolytes added	Concn. KCl, N	pH soln.	D, cm.^{2}/day	
HCl		0.054	0.163	± 0.005
HCl		1.17	.078	± .003
HCl		1.90	.077	± .003
HCl		2.33	.093	± .001
HCl		2.51	.130	± .002
HCl		3.08	.200	± .003
HCl		3.34	.216	± .007
HCl		3.65	.197	± .008
HCl		4.04	.162	± .013
HCl		4.60	.064	± .003
None		4.66	.051	± .004
NaOH		5.16	.108	± .003
NaOH		6.03	.125	± .004
NaOH		6.80	.129	± .002
HCl + KCl	0.250	0.054	.125	± .001
HCl + KCl	.100	1.90	.063	± .001
HCl + KCl	.020	3.35	.160	± .002
HCl + KCl	.100	3.38	.053	± .003
NaOH + KCl	.100	6.03	.055	± .002

rium of 3% gelatin solution at 35° within the pH range of 0.05 to 6.40. Below pH 2, the values of D indicate a rapid disintegration or deaggregation of the gelatin micellae, whereas if pH is above 2 since D remains nearly constant, there is a much smaller change in the particle size in gelatin solutions with changing pH. The point at pH 3.34, $D = 0.160$, shows that the addition of an insufficient amount of salt to a system of given concentration and pH will decrease the value of D, but will not reduce it to the minimum value for that pH.

An excellent explanation for the shape of the upper curve has been presented by McBain, Dawson, and Barker[4] in discussing their results obtained in the study of the diffusion of egg albumin. The results obtained in this study are an excellent corroboration of their work.

It is quite evident from the above results that any investigations of the diffusion of lyophilic colloidal substances must be carried out at the iso-

electric point or the solutions must contain a sufficient amount of salt, if the results are to be used for the calculation of particle size.

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Summary

The effect of pH upon diffusion velocity of gela-
tin solutions has been measured over the pH range 0.05 to 6.4.

The effect of added potassium chloride at the various pH's upon the diffusion coefficient has been measured.

Results obtained indicate that between pH's 2 and 6.4 only a small change in particle size takes place in 3% gelatin solution at 35°, but below pH 2, there is a rapid decrease in particle size.

The Effect of Non-electrolytes upon the Time of Setting of Gelatin Gels

BY LEO FRIEDMAN AND W. N. SHEARER[1]

In an earlier investigation[2] a study was made of the effect of non-electrolytes upon diffusion velocity in gelatin gels. Interpretations of the results obtained involved the hypothesis that non-electrolytes present in gelatin gels actually influenced gel structure. With the view to further investigate this possible influence the following study of the setting of gelatin gels was undertaken.

Experimental.—Gelatin solutions containing 25 g. of electrodialyzed calfskin gelatin in 475 ml. of water were made up. The gelatin was first placed in 200 ml. of water in a one-liter flask and permitted to stand for twenty-two hours in a thermostat at 25°, after which 275 ml. of water was added and the solution heated to 55° until the gelatin was dissolved completely. This solution was then cooled to 25° in the thermostat.

Varying amounts of the non-electrolytes to be used were dissolved in enough water to make up 10 g. of solution. These non-electrolyte solutions were then placed in wide-mouthed bottles, approximately 6.3 cm. in diameter, and 90 ml. of the gelatin solution was poured into each bottle. The bottles were placed immediately in the thermostat and tightly stoppered.

In order to determine when the gels had set a penetrometer was used. The penetrometer consisted of a glass tube about 45 cm. long and 8 mm. in diameter sealed and rounded on the end which was used to test the gel. At the other end of the glass tube was fastened a larger glass tube 18 cm. long and 12.5 mm. in diameter. Around

(1) Present address: Western Waxed Paper Co., North Portland, Oregon.

(2) Leo Friedman and W. N. Shearer, THIS JOURNAL, **56**, 1323 (1934).

the first tube was a coil consisting of 25 loops of steel wire 0.035 mm. in diameter so fastened that the spring resisted the movement of the smaller glass tube into the larger. As an indication that a gel had set the following criterion was followed.
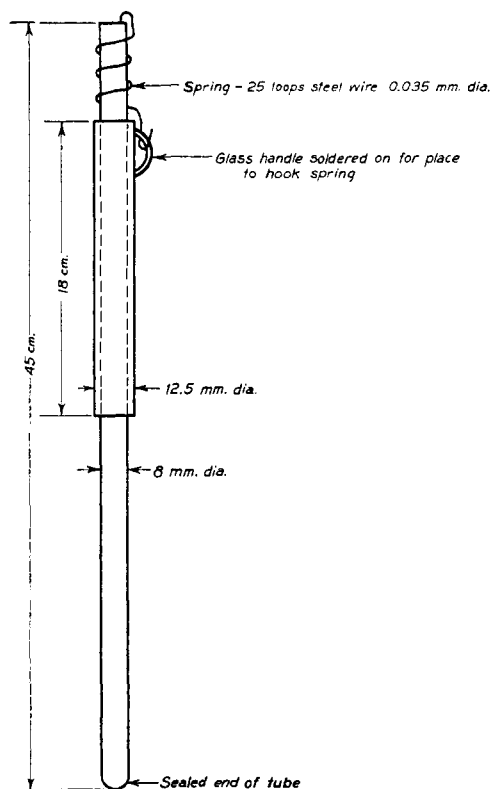

Fig. 1.—Penetrometer.

When a gel had set enough so that the tube would go into the gel far enough to stretch the spring to the 2.6 cm. mark from the starting point without