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[CONTRIBUTION FROM THE COLLOID LABORATORY, UNIVERSITY OF WISCONSIN]

THE STRUCTURE OF GELATIN GELS FROM STUDIES OF DIFFUSION[1]

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The behavior and properties of gelatin systems have been the subject of a good deal of study by colloid investigators because they serve as a prototype of gel-like bodies occurring in living organisms. Further studies into the mechanism and necessary conditions for gel formation, the structure and characteristic properties of gels, and the changes in state which gels undergo should throw much light onto related biological problems.

In general, gel formation may be considered to be an unsuccessful or incomplete precipitation of a solid phase from a liquid system, and it is always preceded by the sol form. The rigidity and elasticity of a gel is due to the presence of a solid or semi-solid phase, which in some gels is indicated by ultramicroscopic observations and by the marked Tyndall effect; but gelatin gels can be prepared that show no structure in the ultramicroscope nor any Tyndall effect. This and the general behavior of gelatin gels indicate a very fine grained structure. The high vapor pressure of water above a weak gel and the permeability to ions and molecules show that on a molecular scale the gel does not possess the elasticity and rigidity that it exhibits in bulk.

By assuming gelatin gels to consist of threads or short chains loosely knit together in three dimensions, Kraemer[1b] calculated the average value of the interval between two threads. By such an approximation he found this to be of the order of magnitude of 100 millimicrons in a very dilute gel.

It was the purpose of this investigation to study further the micro-structure of gelatin gels and the order of magnitude of the discontinuities within them. Attempts to study directly the micro-structure of weak

[1] The substance of this article was included in a paper presented at the Midwest Regional Meeting of the American Chemical Society, University of Chicago, May 27 and 28, 1927.

[1a] An extract from a thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy, University of Wisconsin.

[1b] Kraemer, *J. Phys. Chem.*, 19, 1523 (1925).

gelatin gels have been very few. Bachmann's[2] ultramicroscopic observations of gelatin gel formation are perhaps not so significant, because it is possible to prepare gelatin gels which show no structure in the ultramicroscope. Freundlich and Seifriz[3] investigated the micro-rigidity and elasticity of weak gels by observing the behavior of small nickel particles in a magnetic field. It was found that on a micro scale the rigidity and elasticity of gelatin gels are far from uniform.

In order to decrease the scale of observation still further, Kraemer,[4] by means of the cinematograph, studied the Brownian motion of very small mercury particles imbedded in a weak gel. From these observations it appears that the structure of weak gelatin gels is even considerably finer than the indicating mercury particles and their displacements.

None of the above techniques enable one to detect the heterogeneities of these weak gelatin gels, for the magnitude of the structural units lies somewhere between the scale of Brownian motion experiments and the molecular size. The purpose of this investigation was to explore this particular region by the study of the diffusion of molecules into and through gels. It was sought to give a detailed and quantitative interpretation of diffusion data in terms of heterogeneous gel structure and the possible dimensions of the pores.

As has been pointed out by Miss Lloyd,[5] investigations into gel structure should be carried out in systems uncomplicated by ionic reactions present in aqueous solutions. In this research it was attempted to conform with her suggestion as much as possible, by using only electrodialyzed materials free from electrolytes, while still working in aqueous solution. In this way it was hoped to observe the gel properties and not the influence of electrolytes on these properties.

Method for Study of Diffusion in Gels.—In search of more exact values of diffusion constants in gels than can be obtained by the methods described in the literature,[6] a method was developed which has many advantages over those previously used. A mass M of material was uniformly distributed at time zero in a gel of depth a. This gel was covered with an equal depth of water which was kept continually stirred. The material diffused through the gel and into the water, where its concentration was determined as a function of the time by analysis of very small

[2] Bachmann, *Z. anorg. Chem.*, **73**, 125 (1911).

[3] Freundlich and Seifriz, *Z. physik. Chem.*, **104**, 233 (1923).

[4] Kraemer, "Colloid Symposium Monograph," **1924**.

[5] Alexander, "Colloid Chemistry," Chemical Catalog Co., New York, **1926**, Vol. I, p. 781.

[6] (a) Graham, *Ann.*, **121**, 5, 29, 36 (1862); (b) Bechhold and Ziegler, *Z. physik. Chem.*, **56**, 105 (1906); (c) Herzog and Polotsky, *ibid.*, **87**, 449 (1914); (d) Stiles and Adair, *Biochem. J.*, **15**, 621 (1921); (e) Stiles, *Proc. Roy. Soc.* (London), **103A**, 261 (1923); (f) Fricke, *Z. Elektrochem.*, **31**, 430 (1925).

samples by means of the Zeiss Immersion Refractometer. More often the system was reversed, in which case the mass of diffusing material was in the water at the beginning of the experiment and the rate at which it diffused into the gel was measured. Since at infinite time, if no reaction took place between the gel and the material diffusing, half of this material would be in the gel and half in the water, it made no difference which system was used.

In this method it was not necessary to avoid convection currents due to light and vibration, as is the case in systems where a liquid column is supposed to remain undisturbed. Diffusion took place more rapidly than in systems previously described in the literature, because here the maximum possible concentration gradient was always maintained at the interface. In one experiment lasting as little as four days it was possible to obtain 10 or 12 values of the diffusion constant from the same system, which showed any variation or steady shift that had taken place with time, and gave a "best value" which was the mean of 10 or 12. By introducing a third substance into the preliminary soaking bath where a swelling equilibrium was supposed to be attained, an equilibrium with respect to this third material could also be reached, and its effect upon the diffusion process studied. Since no warm solution was ever poured upon the gel after it had set, no melting could take place at the diffusion boundary, and a sharp interface was maintained. No precipitation was taking place, therefore diffusion was not being hindered by clogging in the pores of the gel. These advantages, coupled with the accurate analysis possible by use of the Immersion Refractometer, made this method capable of very exact results. This system was, however, of no use for the study of diffusion in very concentrated or very dilute gels, for the former would swell too much after diffusion had started and the stirring would tear the surface of the weak gels.

Description of Apparatus.—The experimental determinations of the diffusion constants were carried out in ordinary wide-mouthed glass bottles with a capacity of 500 cc. and of known cross sectional area. The aqueous layer above the gel was constantly and uniformly stirred by small glass stirrers, thus keeping the concentration of diffusing material at the top surface of the gel the same as that of the whole layer. A capillary pipet was used to extract two or three drop samples of the solution above the gel. During the determinations the bottles were supported in a thermostat kept at the desired temperature. The Zeiss Immersion Refractometer equipped with auxiliary prisms was used to determine the concentration of diffusing material in the aqueous layer. Only two or three drops of the liquid were necessary and the analysis was correct to within $\pm 0.01\%$ of the material.

Experimental Procedure

The purified gelatin was diluted to approximately the desired concentration, cooled to 5°, cut into small pieces and allowed to soak in water at 5° for a long enough period to reach a swelling equilibrium. Then the water was drained off and saved for future

use while the gelatin was again melted. In some of the work it was found unnecessary to go through this preliminary soaking treatment, as the gels showed no tendency to change volume during diffusion. Measurements were made with a depth gage at the start and the end of the experiments to be certain that the changes in volume of the gel were small enough to be of no influence on the results. In five tests the maximum change was 0.08 cm. and the mean 0.04 cm. while the total depth of gel was about 5 cm. Two hundred cc. of molten gelatin was poured into a calibrated bottle and this was placed in the thermostat at 5°. In order to prevent hardening of the surface, the gel, as soon as it had set, was covered with a little of the water that had been saved. After twenty-four hours, a time which was found sufficient for the system to reach a gelling equilibrium, the water was removed and the gel covered with 200 cc. of a solution of non-electrolyte.

The refractometer readings for water and the non-electrolyte solution were known. In order to determine the amount of non-electrolyte that had diffused into the gel as a function of the time, refractometer readings of the solution were taken from time to time during the experiment. When the original water was stirred in contact with the gel as a blank experiment, the refractive index remained constant for the length of the test (seventy-six hours). In some of the earlier work the diffusing substance was placed in the gel and allowed to diffuse out into pure water. Results obtained from this type of system gave practically the same constants as for the reversed system, as can be seen by study of Fig. 2 and Table III (d). The determinations marked with an asterisk show results for diffusion from the gel, the others into the gel. These are plotted together in Curve d, Fig. 2. When it was desired to study the effect of a third material on the diffusion process, this was introduced into the gelatin and the water before the preliminary soaking treatment and an equilibrium for this material thus obtained before the diffusate was added.

Calculation of the Diffusion Constant.—The equation connecting the coefficient of diffusion with the time concentration data in the liquid layer was derived for this system from an entirely theoretical standpoint by Dr. W. Weaver, mathematical physicist, University of Wisconsin.[7] This equation may be put in the form

$$V = {}^1/_2 - \Sigma \frac{8}{(2n+1)^2\pi^2} e^{-(2n+1)^2\frac{\pi^2}{4}T}$$

where V is the fraction of the material that has diffused into the gel per unit of area, starting with all the material in the liquid layer, and $T = kt/a^2$, where k is the coefficient of diffusion, t is the time in seconds, and a is the depth of the gel layer and also that of the liquid. In order to use this series, it was expanded to twelve terms.

Substituting into this equation values for T and solving for V, it is possible to plot the theoretical curve shown in Fig. 1. In actual use, in order to obtain greater accuracy, this curve was cut up into small segments, each plotted on a separate sheet. To determine the diffusion coefficient, k, for any sample of gel, the value of T corresponding to the experimentally determined value of V was read from this theoretical curve. k can then be calculated from the previously mentioned relation $k = a^2T/t$. In this way a value of the diffusion coefficient was obtained from each

[7] Weaver, *Phys. Rev.*, **31**, 1072 (1928).

determination of the concentration of the aqueous layer and the mean value of 10 or 12 such determinations was used as the "best value."

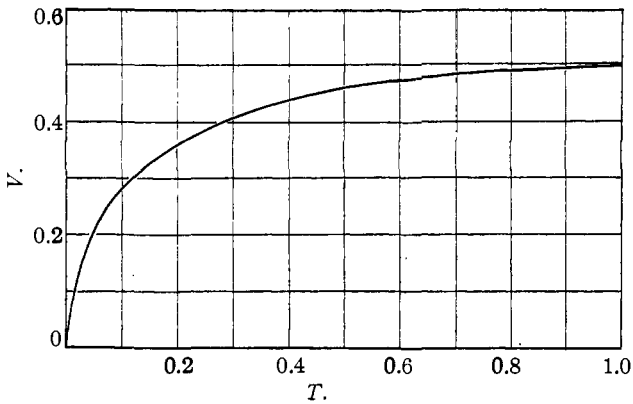

Fig. 1.—Theoretical diffusion curve.

The details of the calculation can best be shown by giving the data and calculations for one of the experiments on the diffusion of sucrose into a gelatin gel, Table I. Diffusion took place from a 5% sucrose solution into

TABLE I

DIFFUSION OF SUCROSE IN A GELATIN GEL

Time in hours	Refractometer shift	V	T	$K \times 10^5$
8.55	0.98	0.0511	0.00228	0.154
21.08	1.49	.0777	.00538	.149
29.77	1.74	.0908	.00752	.148
45.42	2.01	.1048	.01025	.132
53.90	2.24	.1169	.01305	.142
70.50	2.57	.1340	.01785	.148
79.25	2.68	.1397	.01945	.144
93.90	2.97	.1549	.02450	.153
117.20	3.26	.1700	.03050	.152
144.87	3.65	.1904	.03990	.161
Area of bottle = 43.59 sq. cm.			Mean =	0.148

a 6.91% gel at a temperature of 5°. The total shift of the refractometer reading from that of water caused by the sucrose in the solution at zero time was 19.17. The refractometer shifts listed in Column 2 are shifts from the reading of the aqueous solution at zero time and represent the amount of sucrose that had left the liquid and penetrated into the gel. V, the fraction of sucrose that had penetrated into the gel, was obtained by dividing the shift given in the second column by the total shift 19.17. It was not necessary to change refractometer readings to concentrations by means of calibration curves, for over the range of concentrations used

there was a linear relation between these two. Values for T and K were obtained as described above.

Table II shows, for several experiments, the maximum and average deviations of any one value from the mean and the accuracy of the check that can be obtained for duplicate experiments by this method.

TABLE II

DIFFUSION IN GELATIN GELS

Diffusing substance	Gel concn., %	Diffusion constants ($K \times 10^5$) Expts.	Max.	Min.	Mean	Max. dev., %	Av. dev., %	Variation of mean, %
Sucrose	6.9	10	0.161	0.132	0.148	10.8	3.7	
Urea	2.65	6	.807	.693	.744	8.5	3.9	
Urea	2.65	6	.794	.685	.740	8.8	3.5	0.5
Urea	2.9	6	.718	.590	.645	11.3	5.9	
Urea	2.9	6	.681	.617	.644	5.7	3.6	0.2
Urea	4.0	7	.584	.477	.541	11.8	6.1	
Urea	4.0	7	.598	.437	.539	18.9	6.9	0.4

Diffusion of Non-Electrolytes in Gelatin Gels.—The gelatin used in these studies was purified by soaking in $N/128$ acetic acid and water, and finally by electrodialysis. The purified gelatin obtained in this way had an ash content of 0.1% or less. In order to show the effect of concentration of gelatin upon the structure of the gels, determinations of the rates of diffusion of 3 non-electrolytes, urea, glycerin and sucrose, were made in various concentrations of gels. Curve a, Fig. 2, shows the results for diffusion of urea from a gelatin gel (Silver Label Gelatin) where the gel originally contained 6% of urea. A decrease in the rate of diffusion of 20% as the concentration of gel increased from 2.5 to 5% is shown, contrary to the statement by Stiles and Adair[6d] that above 2% there is very little change. It is probable that below 2% the line would bend upward and cut the zero axis at the diffusion constant for water. It was, however, impossible to work with such dilute systems by the method used in this investigation.

Curve b also shows the diffusion of urea from gels (Silver Label Gelatin) originally containing 6% of urea. The slope of the line here is different from that in Curve a, as would be expected, since the gelatin used was from a different supply and had been subjected to different preliminary treatment. It is impossible to prepare two samples of gelatin to give comparable diffusion results unless material from the same source, purified under the same conditions and given exactly the same previous heat treatment is used. In all cases, data used for any one curve or given in any table for comparison were obtained from gelatin from one large stock supply of purified material and from samples treated in exactly similar manner.

Curve c shows the diffusion of urea into gels (gelatin for this and all following work was of calf skin stock supplied by the U. S. Gelatin Com-

pany) from a 3% urea solution. The line would intercept the axis for zero diffusion at a concentration of about 30%, so an attempt was made to see whether diffusion were truly zero at this concentration of gelatin. The

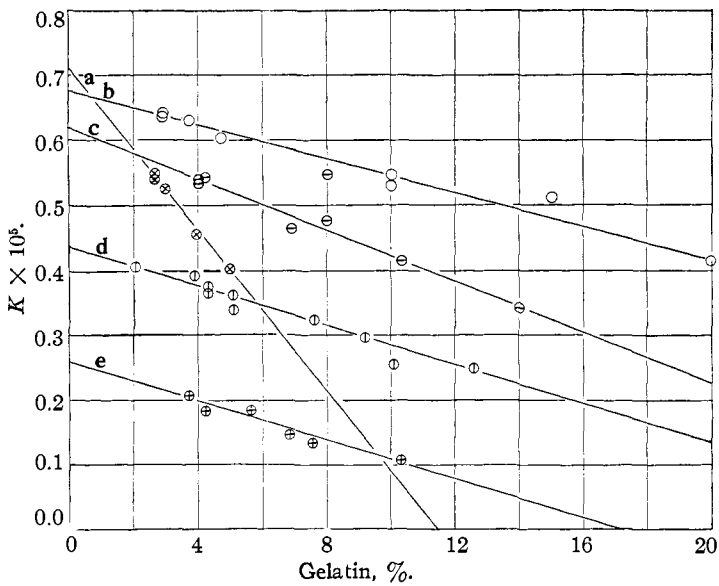

Fig. 2.—Diffusion in gelatin gels.

results are shown in Part c, Table III. The gel used cannot be compared to the others, for it was an unpurified gel. The remarkably high value,

TABLE III

DIFFUSION IN GELATIN GELS *vs.* CONCENTRATION OF GEL

Gelatin concn., %	Total diff., hours	Expts.	Diff. constants ($K \times 10^5$) Max.	Min.	Mean
		(a) 6% Urea from gel			
2.65	137	6	0.807	0.693	0.744
2.65	137	6	.794	.685	.740
3.0	94	4	.758	.709	.725
4.0	118	5	.689	.617	.657
5.0	118	5	.625	.585	.604
		(b) 6% Urea from gel			
2.9	107	6	.718	.590	.645
2.9	107	6	.681	.617	.644
3.7	107	6	.658	.615	.637
4.7	107	6	.664	.560	.608
10.0	112	5	.603	.469	.533
10.0	112	4	.578	.530	.550
15.0	112	5	.554	.436	.513
20.0	112	5	.456	.362	.416

TABLE III (*Concluded*)

Gelatin concn., %	Total diff., hours	Expts.	Diff. constants ($K \times 10^6$) Max.	Min.	Mean
	(c)	Urea into gel from 3% solution			
4.0	107	7	.584	.477	.541
4.0	108	7	.598	.437	.539
4.2	65	5	.618	.506	.547
6.9	120	8	.522	.400	.468
8.0	111	8	.655	.410	.549
8.0	113	9	.581	.407	.479
10.35	146	9	.483	.363	.419
14.0	110	8	.390	.280	.347
29.8	25	3	.387	.228	.320
	(d)	Glycerin into gel from 3% solution			
2.0*	209	15	.462	.354	.406
3.8	71	6	.415	.363	.390
4.2	69	6	.403	.314	.364
4.2	122	11	.421	.287	.373
5.0	97	4	.351	.324	.338
5.0*	255	13	.460	.287	.362
7.5*	255	15	.443	.210	.323
9.1	94	7	.327	.237	.296
10.0*	135	7	.271	.231	.253
12.5*	255	12	.301	.187	.249
	(e)	Sucrose into gel from 5% solution			
3.8	216	8	.264	.144	.209
4.3	144	13	.213	.145	.184
5.7	94	8	.205	.167	.186
6.9	145	10	.161	.132	.148
7.6	81	5	.151	.124	.135
10.35	145	10	.110	.098	.107
	(f)	Sucrose into gel from 3% solution			
28.8	48	6	.037	.025	.032
	(g)	Lactose into gel from 3% solution			
25.0	29	2	.038	.035	.037

however, for the diffusion constant would indicate that diffusion does not cease at this concentration. This would be expected if diffusion takes place through capillaries of many sizes and the diffusion constant is a measure of the average.

Curve d shows the course of the diffusion of glycerin in various concentrations of gels. Curve e shows the diffusion of sucrose into gels, the results being in good agreement with those for the diffusion of urea and glycerin.

Using sucrose and lactose, larger molecules than urea, another attempt was made to see whether diffusion would be zero in gels so concentrated that Curve e would indicate no diffusion. The results are shown in Parts f

and g of Table III and, although diffusion is very slow, there is no question that some of the material is penetrating the gel.

Dimensions of the Gel Framework.—As shown by the curves in Fig. 2, diffusion is considerably slower in a gelatin gel than in pure water. The diffusion coefficient for urea in water at 5° as calculated from Öholm's[8] data is 0.880×10^{-5}, that for glycerin is 0.552×10^{-5} and for sucrose 0.285×10^{-5}. The two phase solid–liquid theory best explains the results obtained in this investigation. If it is assumed that this theory is correct, and that diffusion takes place through the liquid in the pores of the gel, this slowing down of diffusion may be due to three different causes. First, the solid gelatin in the gel mechanically blocks part of the area across which diffusion can take place; second, since the diffusion is taking place through very tiny capillaries, there will be a drag on the molecules due to the proximity of the solid walls; and third, the viscosity of the liquid in the gel is different from that of water at the same temperature. This last factor also includes any specific attraction of the gel material for the diffusing substance.

To take care of these three influences, the following relation was set up:

$$K_{\text{water}} = K_{\text{gel}} (1 + 2.4r/R)(1 + \alpha)(1 + \pi)$$

where r is the radius of the diffusing molecule, R is the average radius of the pores in the gel, α is the correction factor for the viscosity and π is the correction factor for the mechanical blocking. At zero concentration of gelatin the difference between the diffusion constants in pure water and the extrapolated values for the gelatin is due only to the difference in viscosity of the liquids through which diffusion takes place and any specific action. Therefore the correction factor α can be calculated from these values of the diffusion constant. The correction for mechanical blocking was calculated from the formula given by Dumanski[9]

$$\pi = \sqrt[3]{(g/d)^2}$$

where g is the number of grams of gelatin in 1 cc. and d is the density of the gelatin or the inverse of the partial specific volume. The correction factor $(1 + 2.4r/R)$ is the best approximation that can be obtained for the size of the pores. It is from Ladenburg's correction for the fall of bodies in capillary tubes and was originally intended to be applied to Stokes' law.

TABLE IV

SIZE OF THE PORES IN A GELATIN GEL

Substance diffusing		Sucrose	Glycerin	Urea	Naphthol Yellow S
Radius of the pores in millimicrons	5% gel	5.5	5.7	4.7	0.8 (Herzog and
	10% gel	1.4	1.7	1.5	... Polotsky)
	15% gel	0.5	1.0	0.8	...

[8] Öholm, *Medd. Vetenskapsakad. Nobelinst.*, 2, n23; Öholm, *Z. physik. Chem.*, **70**, 399, 401 (1909).

[9] Dumanski, *Kolloid-Z.*, **3**, 210 (1908).

The correction has been used by Westgren and Nordlung and found to hold very well. The radii of the diffusing particles were determined by calculation from Einstein's formula

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

Using Curves c, d and e of Fig. 2 and the values from Öholm given above, calculations were carried out in this manner and the results shown in Table IV were obtained.

The following values for the diffusion of the dye Napthol Yellow S were taken from the work of Herzog and Polotsky.[10]

In water at 7.3 °C., $K \times 10^6 = 0.468$
In 5% gelatin gel at 1.2° = 0.148

Using these values for the calculations outlined above a value of 0.83 mμ was found for the radius of the pores in their gel. This is in excellent agreement with this investigation; a difference of this magnitude would be expected because of the difference in gelatin used and the greater adsorption of diffusate.

Summary

1. A new method for the study of diffusion rates in gels has been described and its advantages pointed out.

2. An equation connecting the time–concentration data with the diffusion constant has been presented and its use described.

3. The data and calculations presented show the method to be capable of a degree of accuracy of about 1%.

4. It has been shown that a very marked decrease in the rate of diffusion in gelatin gels is brought about by increase of concentration of the gel.

5. A relation has been set up connecting the diffusion constants in water and in gel in such a manner as to include the radii of the openings in the gel framework.

6. Calculations have been made of the size of these openings in the gel framework for 5, 10 and 15% gels from the results of diffusion experiments with urea, glycerin and sucrose.

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[10] Freundlich, "Colloid and Capillary Chemistry," **1926**, p. 549.