LXXV. THE PENETRATION OF ELECTROLYTES INTO GELS.

III. THE INFLUENCE OF THE CONCENTRATION OF THE GEL ON THE COEFFICIENT OF DIFFUSION OF SODIUM CHLORIDE.

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INTRODUCTION.

THE influence of the concentration of a gel on the rate of diffusion of salts through it is a question on which the most divergent opinions have been expressed. Whereas de Vries [1884], Hagenbach [1898], Bechhold and Ziegler [1906], and Öholm [1905, 1913] concluded that the presence of gelatin had a retarding effect on the rate of diffusion, the determinations of Morse and Pierce [1903] suggest the contrary, while Henri and Calugareanu [1903] came to the conclusion that if the gelatin had not set to a gel the rate of diffusion of organic dyes was the same as in water, but that if the gelatin had set the rate of diffusion was slower. Voigtländer [1889] concluded that in agar-agar gels any differences observed in the coefficients of diffusion of a number of electrolytes in gels of different concentrations were within the limits of experimental error. Incidental observations recorded in previous papers of this series support the conclusion of the comparatively small influence of the gel on diffusion in agar-agar gels. [Stiles, 1920; Adair, 1920.]

Since recent investigations on the structure of protoplasm all point to the conclusion that the living materials of cells may in some cases exist in the gel condition; it is of physiological as well as of chemical interest to obtain exact information with regard to the influence of the gel on the diffusion of electrolytes and other substances of importance in the life of the organism.

METHOD OF EXPERIMENT.

Most of the determinations already made of coefficients of diffusion, have been made by the chemical analysis of different layers of the medium through

which diffusion is allowed to take place. While the indicator method used for the determinations described in this paper has found considerable employment in the past for observing the penetration of electrolytes, definite measurements of the coefficient of diffusion by its means have only become possible by the application of Fourier's equations to this special case as worked out in the previous contribution in this series of studies [Adair, 1920]. The advantages of the indicator method are described in that paper and need no repetition here.

Measurements were made with both gelatin and agar-agar gels containing silver nitrate as indicator. As experimental salt sodium chloride was employed as in the earlier work previously described. The concentrations of gel, indicator and diffusing salt employed are recorded in the section dealing with the experimental results. It speaks for the validity of the method that the values obtained with the stronger silver nitrate concentration in the gel are practically identical with those obtained with the weaker strength of silver nitrate.

The diffusion was allowed to proceed from a large volume of salt solution into inverted narrow tubes containing the gel; care has to be taken in the performance of the experiments that evaporation does not take place either from the surface of the liquid or from the upper end of the gel. The formulae necessary for the calculation are to be found in the second paper [Adair, 1920].

The experiments were carried out in thermostats maintained at 0° and 20° in the case of gelatin and at 0°, 20° and 40° in the case of agar-agar. The last named temperature is too high for experiments with gelatin. During the time of the experiment the temperature of the bath rarely changed more than 1/10 of a Centigrade degree, and generally not nearly so much.

EXPERIMENTAL RESULTS.

1. *Gelatin.*

Experiments were conducted on two samples of gelatin. In both cases the gelatin on analysis proved to contain chloride, although in the first sample the quantity of this was much greater than in the second sample. As the presence of any substance that will remove the silver nitrate from solution, apart from the experimental diffusing salt, will vitiate the results of the subsequent calculations, it was necessary to remove the chloride by successive washing with distilled water, until this gave no precipitate with silver nitrate after it had remained for some time in contact with the gelatin. This process was carried out in all cases.

The experimental data are given in the tables below. Each determination is the mean of six or more readings, but it was not considered necessary to publish the results for each individual tube; in order to show the degree of variation between different tubes treated in the same way the results for the first set of readings in three experiments (Nos. 3-5) are given in full in Table I.

Table I. Penetration of sodium chloride from *N* solution into gelatin gels containing 0.01 *N* silver nitrate at 0° C.

Concentration of gel	...	...	...	4 %	8 %	16 %
Time of diffusion	...	...	...	22.63 hours	22.525 hours	22.71 hours
Penetration in cm.	...	...	...	2.70	2.50	2.32
				2.71	2.53	2.28
				2.70	2.60	2.28
				2.71	2.53	2.30
				2.70	2.58	2.28
				2.70	2.54	2.28
				2.72	2.59	2.31
				2.70	2.52	2.31
				2.70	2.55	—
				2.72	2.59	—
Mean penetration in cm.	...	...	...	2.706	2.553	2.295
Probable error of mean...	...	...	...	0.002	0.007	0.004
Coefficient of diffusion in c.g.s. units...				0.675×10^{-5}	0.604×10^{-5}	0.483×10^{-5}

The numbers given above will give sufficient idea of the degree of concordance between different tubes in the same experiment. Similar agreement was obtained in all the results recorded in Table II, p. 623.

2. Agar-agar.

In determining coefficients of diffusion in gels of agar-agar the experimental difficulties are greater than in the case of gelatin. One fruitful source of error is evaporation from the upper end of the tube of gel especially in the case of low concentrations and at higher temperatures. In gels of high concentrations bubbles in the gel are often difficult to eliminate and the presence of these may lead to incorrect results. In the third place, any heating of the gel after addition of silver nitrate leads to a darkening of the gel. If this is to be interpreted as due to decomposition of some of the silver nitrate, its incidence will lead to values of the coefficient of diffusion which are higher than the true ones. The results given below are of experiments free from these sources of error. Experiments were conducted at three temperatures—0°, 20° and 40°. The values obtained for the coefficient of diffusion at these three temperatures in gels containing respectively 0.5, 1, 2 and 4 per cent. of agar-agar are summarised in Tables III and IV.

Table III. Coefficient of diffusion of sodium chloride at different temperatures in agar-agar gels of different concentrations. (Shred agar-agar.)

Concentration of agar-agar in %	Coefficient of diffusion in c.g.s. units $\times 10^5$		
	0.0°	20.1°	40.2°
0.5	0.781	1.386	—
1.0	0.765	1.357	2.143
2.0	0.738	1.328	2.084
4.0	0.711	1.290	2.061

Table II. Penetration of sodium chloride from *N* solution into gelatin gels with silver nitrate as indicator. (Concentration of gelatin in percentage by weight, time in hours, penetration in centimetres of precipitate. Diffusion coefficient, *k*, in c.g.s. units $\times 10^5$.)

Experiment	Concentration of gelatin. %	Concentration of AgNO ₃	Temperature °C.	Time	Penetration	<i>k</i>
1	5	0.01 <i>N</i>	0	{ 20.65 43.58	2.575 3.735	0.670 0.668
2	15	"	"	{ 20.28 43.15	2.237 3.258	0.515 0.513
3	4	"	"	{ 22.63 46.57	2.706 3.903	0.675 0.683
4	8	"	"	{ 22.525 46.64	2.553 3.705	0.604 0.614
5	16	"	"	{ 22.71 46.70	2.295 3.312	0.483 0.490
6	2	0.005 <i>N</i>	"	{ 17.775 41.57	2.6525 4.1025	0.697 0.713
7	4	"	"	{ 17.85 41.625	2.630 4.034	0.6835 0.688
8	8	"	"	{ 17.93 41.69	2.533 3.880	0.630 0.636
9	16	"	"	{ 17.99 41.74	2.289 3.487	0.513 0.513
10	2	"	"	17.905	2.703	0.719
11	4	"	"	17.98	2.605	0.664
12	8	"	"	18.075	2.530	0.624
13	16	"	"	18.15	2.342	0.532
14	5	0.01 <i>N</i>	20	{ 20.37 43.43	3.435 5.040	1.209 1.221
15	15	"	"	{ 20.00 43.03	3.043 4.490	0.967 0.978
16	4	"	"	{ 22.18 48.075	3.651 5.385	1.254 1.260
17	8	"	"	{ 22.29 48.15	3.507 5.120	1.152 1.137
18	16	"	"	{ 22.39 48.22	3.164 4.666	0.934 0.943
19	2	0.005 <i>N</i>	"	{ 22.53 46.32	4.049 5.816	1.281 1.285
20	4	"	"	{ 22.61 46.40	4.0025 5.710	1.248 1.237
21	8	"	"	{ 22.675 46.48	3.864 5.481	1.160 1.139
22	16	"	"	{ 22.76 46.55	3.500 4.974	0.950 0.936
23	2	"	20.3	{ 17.31 41.99	3.577 5.607	1.302 1.318
24	4	"	"	{ 17.41 42.04	3.504 5.456	1.243 1.247
25	8	"	"	{ 17.51 41.93	3.334 5.209	1.118 1.140
26	16	"	"	{ 17.62 42.11	3.150 4.899	0.9915 1.003

Table IV. Coefficient of diffusion of sodium chloride at different temperatures in agar-agar gels of different concentrations. (Powdered agar-agar.)

Concentration of agar-agar in %	Coefficient of diffusion in c.g.s. units $\times 10^5$		
	0.0°	20.0°	40.0°
0.5	0.782	1.382	2.205
1.0	0.761	1.371	2.126

The agreement between these two sets of results is very good; there is evidently no difference between shred and powdered agar-agar in regard to the diffusivity of sodium chloride through gels made from these two kinds of agar-agar.

Taken by themselves these results form a perfectly regular series indicating a progressive reduction in the diffusivity with increasing concentration of the gel. But when the values for diffusivity in a gel of concentration 0 are obtained by extrapolation they are found to differ both from those obtained in a similar way by the use of gelatin gels and those obtained by extrapolation from Öholm's determinations for the diffusivity of sodium chloride. Some discussion of the results is obviously necessary.

DISCUSSION OF EXPERIMENTAL RESULTS.

In the preceding section of this paper we have recorded the values obtained by the indicator method for the coefficient of diffusion in gels of gelatin and agar-agar of various concentrations. From these data the following conclusions can be drawn:

1. In gelatin the relation between the coefficient of diffusion (k) and the concentration of gelatin (c) can be expressed by the equation

$$k = a(1 - bc)$$

where a and b are arbitrary constants, and when $c > 2\%$. The values of a and b calculated from the data presented are as follows:

At 0°	for a gel containing 0.01 <i>N</i> silver nitrate,	$a = 0.74$;	$b = 0.0207$.
" 0°	" " 0.005 <i>N</i> " "	$a = 0.734$;	$b = 0.018$.
" 20°	" " 0.01 <i>N</i> " "	$a = 1.35$;	$b = 0.0185$.
" 20°	" " 0.005 <i>N</i> " "	$a = 1.35$;	$b = 0.0185$.
" 20.3°	" " 0.005 <i>N</i> " "	$a = 1.36$;	$b = 0.017$.

2. The results for agar-agar indicate that the coefficient of diffusion does not decrease uniformly with increase in the concentration of the gel when the concentration is less than 2%. Between concentrations 0.5 and 2% the relation between coefficient of diffusion and concentration of the gel is roughly expressed by the equation $dk/dc = -0.04k$. For concentrations of agar from 2% upwards the curve approximates more closely to that for gelatin, but it appears to fall less steeply, that is, b is smaller, but the range of 2 to 4% is too short to admit of accurate determinations of this constant in the case of agar-agar.

3. Despite the greater solidity of agar gels, the calculated coefficient of diffusion generally exceeds that of a gelatin gel of equal strength by about 0.03.

4. The extrapolated values for pure water, using the agar figures, are as follows:

$$0^{\circ}, k = 0.8 \times 10^{-5}; 20.1^{\circ}, k = 1.41 \times 10^{-5}; 40.2^{\circ}, k = 2.22 \times 10^{-5}.$$

It may be noted that these extrapolated figures are all greater than the constant a in the gelatin results, this constant being the value for the coefficient of diffusion in gelatin of concentration zero. The reason for this divergence is found in the steeper slope of the curve in the case of agar when c is less than 2. The constant a in the equation for gelatin gels is only applicable when the concentration is greater than 2 %. The extrapolated value for k at 20° calculated from Öholm's results is 1.31×10^{-5} .

The ideal method of treating these results would be to compare the values here given for the coefficient of diffusion with those to be derived theoretically from a consideration of the various theories of gel structure. The present extent of our knowledge is however scarcely adequate for this and a first essential is to determine whether the indicator method gives accurate and reliable results. We shall therefore first consider the various possible sources of error in the method, and the magnitude of the errors.

Errors of Measurement.

Mathematical investigation shows that the maximum error due to errors in measurement is 1 % of k , and it is improbable that the error in k due to this cause would exceed 0.3 %, a figure which is trifling in comparison with the gap of 7.5 % between the interpolated values obtained by Öholm and by us for the coefficient of diffusion of sodium chloride in water at 20° .

Errors arising from the conditions of experiment.

In our experiments it is assumed that convection and diffusion keep the concentration of the sodium chloride at the surface of the gelatin constantly at normal concentration. It is also assumed that no evaporation is taking place. As with careful work the values of the coefficient of diffusion calculated from measurements made at different times are the same within the limits of experimental error, it would appear that no serious error arises from these assumptions. Error may also arise from imperfect temperature control. The maximum variation in our experiments was about 0.2° , but generally considerably less than this. An error of 0.2° brings about an error of about 0.5 % in the determination of the coefficient of diffusion.

Theoretical errors.

In deducing the formula from which the coefficient of diffusion is calculated, a number of simplifying assumptions were made to which reference should here be made. The most important of these is that silver nitrate diffuses at the same rate as sodium chloride. It was shown in the second paper in this series [Adair, 1920] that no appreciable error is introduced by this assumption; even doubling the rate of diffusion of silver nitrate has little effect on the

calculated value of the coefficient of diffusion of sodium chloride when the indicator is present in relatively low concentration. The errors arising from simplification of the formula are negligible when N sodium chloride is used and the concentration of the indicator in the gel is only $0.01\ N$ or $0.005\ N$.

Adsorption of silver nitrate.

Reactions between the silver nitrate and the gel are sources of complication in the phenomena we are investigating, rather than sources of error, but it will be convenient to consider the possibility and consequences of such reactions in this place. Two possible cases will be considered.

1. The silver nitrate forms a reversible compound with the gelatin or agar-agar.

Mathematical investigation [see Adair, 1920, formula IV] shows that if this suggestion is correct, the calculated coefficients are 2.3% too high.

2. A portion of the silver nitrate forms an irreversible compound with the gelatin.

In this case the calculated coefficients will similarly be too high. The following numbers illustrate the magnitude of the effect produced on the calculated values of the coefficient:

Per cent. of $0.005\ N\ AgNO_3$ adsorbed ...	2	4	6	8	12	20
Per cent. increase in calculated value of k ...	0.49	0.94	1.64	1.89	2.94	4.0

The presented material does not enable us to come to a definite decision on the numerical error in the coefficient of diffusion resulting from adsorption, but some indirect evidence is available.

A glance at the values for the constant b , the rate of decrease in k per unit increase in gelatin concentration, shows that b may be slightly different for gels containing $0.005\ N$ and $0.01\ N$ silver nitrate, though the difference at most is slight, and the recorded difference might be the result of small errors in temperature control or differences in the samples of gelatin used. With such small differences it would appear most improbable that there is any very appreciable removal of silver nitrate from the action of the diffusing sodium chloride.

CONCLUSION.

A survey of the various sources of error in the determination of the coefficient of diffusion of sodium chloride in gels by the indicator method has yielded the following results:

- (1) Errors in measurement: 0.3% of the value of k .
- (2) Errors in temperature control: 0.5% of the value of k .
- (3) Errors due to adsorption: of the order of 1.2% of the value of k .

These fail to account for the difference between Öholm's extrapolated value for normal sodium chloride at 20° , namely $k = 1.31 \times 10^{-5}$, and that given by the indicator method, namely 1.41×10^{-5} , a difference of 7.5% .

Öholm's value for the coefficient of diffusion of 0.05 *N* NaCl is about the same as the value given for a normal solution by the indicator method. Here we may have the explanation of the apparent discrepancy.

Diffusion coefficients stated for a definite concentration are not strictly accurate. By the ordinary method of determining the coefficients chemical analyses are made of solutions of varying concentration, and for a set of experiments such as that of Öholm, all that can be said with justification is that the coefficients refer to some concentration between the initial value and zero. As the free ions diffuse about twice as fast as the undissociated molecules, the coefficient calculated by the indicator method which measures the rate of penetration of the ions, is bound to be greater than the coefficient calculated on the basis of chemical analyses in which the total quantity of salt, dissociated and undissociated, is considered.

The differences between the values found for the coefficient of diffusion of sodium chloride by different methods could be utilised in an attempt to obtain a more complete description of the diffusion of that salt, but all that can be said at present is that the investigator has a choice of two values of the diffusion coefficient; one if he is rather concerned with the total quantity of salt, and the other if he is concerned with phenomena depending on diffusion gradients near the limit of salt penetration.

Perhaps the most significant fact from the physiological point of view, brought out by work on the diffusion of sodium chloride in gelatin and agar-agar gels, is the smallness of the resistance which even a stiff jelly offers to the diffusing salt. The viscosity of a jelly is of course extremely great, yet the rate of diffusion is reduced by less than 2 % for each gram of gelatin added to 100 cc. when the concentration exceeds 2 %. It has been shown by Einstein [1905, 1906], Sutherland [1905] and von Smolukowski [1906] that k is inversely proportional to η the viscosity, and so we must seek for some explanation of this discrepancy. Although a formula showing the effect of concentration of the gel cannot be deduced at present from the various theories of gel structure [see Lloyd, 1920] a tentative suggestion should perhaps be made with regard to the mechanism which retards diffusion, because this question will require consideration in certain types of diffusion experiments.

The first additions of gelatin to distilled water will have a double effect. There will be (1) an increase in the concentration of ionised gelatin with increase in the gelatin concentration until a maximum value is reached, and (2) an increase in the solid phase. Subsequent additions of gelatin will merely increase the latter. Now it has been shown by Pauli and others that solutions with the greatest proportion of ionised protein have the greatest viscosity, therefore it is unlikely that the (k, c) curve will have the same slope throughout.

The Arrhenius [1892] equation connecting viscosity with coefficient of diffusion, namely $K = K_0(1 - \frac{a}{2}x)^2$, certainly does not agree with the results we have obtained. Öholm also found that the constant " a " of that formula

diminished from 3.6 to 2.3 as the concentration increased. Over the range 2 to 16 % concentration of gel the formula

$$K_c = K_{\text{water}}[0.965(1 - 0.0186c)]$$

gives a much better representation of the facts.

It is interesting to compare this result with the value calculated from Einstein's theory [1906] of the viscosity of suspensions of small rigid spheres,

$$\eta' = \eta(1 + 2.5f).$$

If 1.4 is the specific gravity of the spheres, then a 10 % suspension would reduce k by 15 %. The observed reduction is 18.6 %, and considering that the particles of gelatin are probably arranged in some kind of structure, it is remarkable that the two numbers agree as well as they do, especially as the Einstein formula does not agree exactly with results obtained with the capillary viscometer. Bradford's theory of gel structure [1918] is in agreement with the calculation given above, but the problem is so complicated that the agreement may be no more than a coincidence.

SUMMARY.

The coefficient of diffusion of sodium chloride from normal solution into gels of gelatin and agar-agar of a number of different concentrations has been measured by the indicator method previously described.

The values obtained by extrapolation from these results for diffusion in water are higher than those obtained for the same temperatures by extrapolation from the results of Öholm. The probable reason for this apparent discrepancy is stated.

With increasing concentration of gel, the coefficient of diffusion decreases at first rapidly according to an approximate exponential relation, but with concentrations above 2 % the curve connecting coefficient of diffusion and concentration is approximately a straight line, the rate of decrease of the diffusion coefficient with increasing concentration of the gel being less than with concentrations less than 2 %. It is shown how this result can be harmonised with the relation between diffusion and viscosity found by Einstein, Sutherland and von Smolukowski.

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