the present work are not to be regarded as very accurate, and so the low specific rate of alcoholysis of pentaglycine ester may be partly due to the change in experimental technique which had to be adopted owing to the sparing solubility of the pentaglycine. The increase in the rate of the reaction involving hexaglycine ester hydrochloride from a value of about 0.05 to more than 0.10 appears to be outside the range of experimental error.

It is of interest to recall that in passing from penta- to hexa-glycine the dissociation constant[5] also undergoes an unexpected change, and it is possible that both this and the increase in the specific rate of the alcoholysis are due to a change in the structure of the polyglycine chain when a sixth unit is added. The increased reaction rate may perhaps be due to the breaking of peptide linkages other than the extreme ones; this is, however, not certain since the results could be explained equally well by an appreciable increase of entropy due to a change of structure accompanying the formation of the activated state for the $G_6 \rightarrow G_5 + G_1$ reaction.

On the basis of the arguments presented above, a general parallelism may be expected between the rates of alcoholysis of the polyglycine esters in acid solution and the hydrolysis of the peptides themselves in the presence of either acid or alkali. Some studies[8,10] have been made of the fission of the glycine peptides in acid and alkaline solution. The results, however, do not appear to be very reliable, and so a reinvestigation of the kinetics of hydrolysis of the simpler polypeptides is being undertaken.

Summary

1. The kinetics of the alcoholysis of di-, tri-, tetra-, penta- and hexa-glycine esters have been studied in the presence of hydrochloric acid as catalyst.

2. The specific rate of the first stage of the reaction, which is believed to involve the splitting off of a single glycine residue in each case, increases fourfold from the di- to the tri-glycine ester, is almost constant for the tri-, tetra- and penta-esters, and increases again with the hexa-glycine ester.

3. The change from di- to tri-glycine ester is analogous to that observed in the dissociation constant of glycine and diglycine and is attributed to electrostatic forces; the increase in the rate of alcoholysis observed with hexaglycine ester may be due to a structural change.

4. The fission of the polyglycine esters is shown to be a true alcoholysis, and not a hydrolytic reaction followed by esterification.

(10) (a) Abderhalden and Suzuki, *Z. physiol. Chem.*, **173**, 250 (1928); (b) Levene, *J. Biol. Chem.*, **82**, 167 (1929).

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, STANFORD UNIVERSITY]

Diffusion of Electrolytes and of the Ions in their Mixtures

BY JEROME R. VINOGRAD AND JAMES W. MCBAIN

Data have been lacking for the diffusion of many of the simplest electrolytes. These are now supplied.

Mixtures also have been studied both experimentally and theoretically with some very striking new results. In contrast to the fairly minor effect of concentration upon single electrolytes are the relatively enormous effects of admixtures with other electrolytes which may bring an ion to a standstill or make the hydrogen travel faster than even a completely freed hydrogen ion would do.

The present studies begin with cases in which all the diffusion is proceeding in the same direction from salt solution into water or more dilute solution. These are especially simple because several of the effects so prominent in the Debye–Hückel theory of conductivity where ions travel in opposite directions are here minimized because they travel together.

We then discuss cases where ions diffuse through buffer salts or against other electrolytes, and show that all are embraced in the principles implicit in the classical equations of Nernst,[1] Arrhenius[2] and Planck.[3] Very much later Debye[4] related diffusion phenomena to Einstein's 1906

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(1) Nernst, *Z. physik. Chem.*, **2**, 613 (1888).

(2) Arrhenius, *ibid.*, **10**, 51 (1892).

(3) Planck, *Wied. Ann.*, **39**, 161 (1890); **40**, 561 (1890); *Sitzber. preuss. Akad. Wiss.*, p. 285 (1927); p. 9 (1929); p. 367 (1930); p. 113 (1931). For references to later authors who have published particular forms of these equations see McBain and Dawson, THIS JOURNAL, **56**, 53 (1934), and also T. Theorell, *Proc. Natl. Acad. Sci.*, **21**, 152 (1935).

(4) Debye, *Physik. Z.*, **18**, 144 (1917).

theory of Brownian motion. Since some of these results have not previously been made explicit, and since they serve to clarify and correct some common conceptions, we have illustrated them with numerical calculations and point out some conclusions that are particularly interesting in biological applications as well as in other fields of physical chemistry.

Experimental

Materials and Analysis.—Kahlbaum "zur Analyze" and Merck "Reagent" chemicals were used throughout. The dilute diffusates, often as dilute as 0.01 *N*, were analyzed in the Zeiss interferometer using a 4-cm. cell. The solutions were always measured against at least two known concentrations to avoid fringe skipping which occurs for inorganic electrolytes within two hundred divisions. Chemical methods were used to analyze the more concentrated solutions: chlorides and thiocyanates were determined by silver nitrate titrations; sulfates by evaporation to dryness, bicarbonates, bitartrates, and tartaric acid solutions were analyzed by acid-base titrations. Tartrate, citrate and iodide solutions were analyzed in the interferometer after suitable dilution. Traces of thymol were added to all solutions suceptible to micro-organisms.

Diffusion Method.—The diffusion measurements were carried out in the two compartment cells described by McBain and Dawson.[5] With these cells, it is possible to change the liquid on each side of the Jena G4 sintered glass disk without disturbing the concentration gradients established in the pores.

Preliminary to each run, the pores were carefully rinsed for at least ten minutes to cleanse them and remove air bubbles. All solutions used were prepared with boiled-out water. The upper and lower compartments are successively rinsed and filled with solution and distilled water, respectively. Diffusion is allowed to proceed for three to five hours depending on the electrolyte until the diffusion gradients are established in the pores of the disk. The compartments are rinsed and filled again with solution and water at the temperature of the experiment, and the cells are returned to the thermostat maintained at 25.00 ± 0.03°, where the diffusion proceeds for twenty to fifty hours. The cells are mounted in brass clamps and are suspended free from vibration in the thermostat. Cylindrical cells having ratios of principal axes that vary by as much as 100% were used in duplicate experiments. The checks obtained demonstrate that measured diffusion coefficients are independent of the geometry of the cells.

The cell constants were redetermined at frequent intervals. Tenth normal solutions of potassium chloride were diffused against pure water. The original solutions and the diffusates were analyzed in the Zeiss portable interferometer. Using the value 1.631 sq. cm./day obtained by Cohen and Bruins,[6] the cell constants could be reproduced within ±0.15%. For example the *KD* values for cell X were 697.3, 695.8 and 698.3. There is a tendency for the cell constants to rise slightly with time. This rise was about 2% in one year.

Since the volumes of the two compartments are not equal, an analogous expression to one derived by McBain and Liu[7] for equal volumes was used to calculate the diffusion coefficients from the analytical data. This expression is

$$KD = \frac{\log c_u^0 - \log [c_u^0 - c_l(1 + F)]}{t}$$

where K is cell constant; D, diffusion coefficient; c_u^0 initial concentration in upper compartment; c_l, final concentration in lower compartment; F, ratios of volumes in upper and lower compartments; t, time elapsed.

In our calculations we have expressed D in sq. cm./day, t in hours, and use ordinary logarithms, and this together with the actual geometry of the cell determines K. It will be noted that the units of concentration cancel out.

Diffusion Results for Simple Electrolytes.—The integral diffusion coefficients of ten salts and of tartaric acid diffusing into water are listed in Table I and Fig. 1. The limiting values at infinite dilution are calculated from Haskell's extension[8] of the Nernst formula (see later)

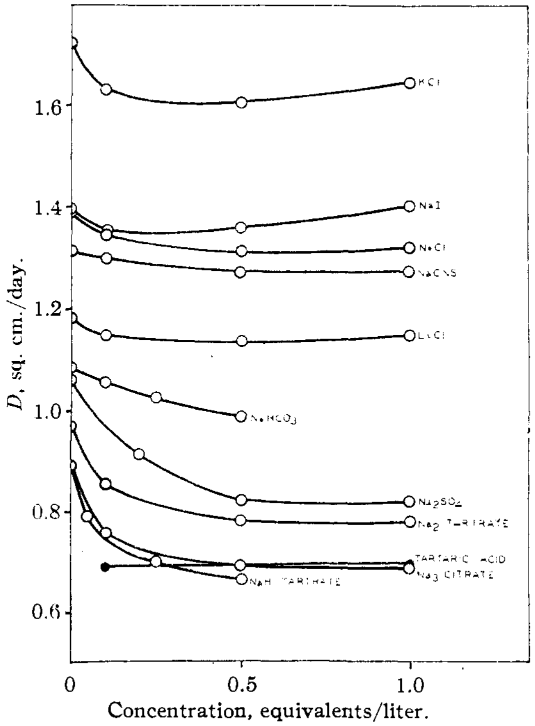

Fig. 1.—Integral diffusion coefficients at 25.0°.

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[5] McBain and Dawson, *Proc. Roy. Soc.* (London), **A148**, 32 (1935).

[6] Cohen and Bruins, *Z. physik. Chem.*, **113**, 159 (1924).

[7] McBain and Liu, THIS JOURNAL, **53**, 59 (1931).

[8] *Phys. Rev.*, **21**, 149 (1908).

using the most recent mobility data given in the Landolt–Börnstein "Tabellen," namely, K^+ 73.5, Na^+ 50.1, Li^+ 38.7, H^+ 349.7, Cl^- 76.3, I^- 76.9, CNS^- 66.5, HCO_3^- 44.5, $^1/_2C_4H_6O_4^{--}$ 64, $C_4H_5O_4^-$ 32, $^1/_2SO_4^{--}$ 79.8, $^1/_3\ C_6H_5O_7^{---}$ 71.5, $^1/_2HPO_4^{--}$ 57, $C_7H_5O_3^-$ 35. Taking RT as 0.02300 at 25°, the values of D at zero concentration are for Na_2HPO_4 0.920, and for sodium salicylate 0.949 sq. cm./day.

TABLE I

DIFFUSION COEFFICIENTS, D IN SQ. CM./DAY AT 25°

Salt	Normality	D		Mean diff. coeff.
KCl	0.0			1.722
	.1	"20°" determined as calibration		1.631
	.5	1.608	1.605	1.607[a]
	1.0	1.643	1.656	1.650[b]
NaCl	0.0			1.391
	.1	1.350	1.342	1.346
	.5	1.306	1.317	1.312
	1.0	1.314	1.328	1.322
LiCl	0.0			1.181
	.1	1.144	1.152	1.148
	.5	1.135	1.138	1.136
	1.0	1.143	1.151	1.147
NaI	0.0			1.396
	.1	1.357	1.340	1.349
	.5	1.368	1.369	1.368
	1.0	1.401	1.406	1.403
NaCNS	0.0			1.316
	.1	1.297		1.297
	.5	1.277	1.265	1.272
	1.0	1.260	1.290	1.275
$NaHCO_3$	0.0			1.084
	.10	1.053	1.059	1.056
	.25	1.016	1.039	1.027
	.50	0.989	0.989	0.989
NaH tartrate	.0			.898
	.05	.791		.791
	.10	.783	.783	.783
	.25	.708	.696	.701
	.50	.664	.671	.667
Tartaric acid	.0			(1.348)
	.1	.691	.690	0.691
	.5	.692	.696	.694
	1.0	.695		.695
Na_2 tartrate	0.0			.969
	.1	.857	.851	.854
	.5	.779	.785	.782
	1.0	.780	.780	.780
Na_2SO_4	0.0			1.062
	.2	.916	.910	0.913
	.5	.821	.826	.823
	1.0	.818	.822	.820
	2.0	.857		.857
Na_3 citrate	0.0			.896
	.1	.762	.757	.760
	.5	.699	.693	.696
	1.0	.693	.687	.690

[a] Same as Dawson's final result, ref. 5. [b] Dawson's final result was 1.653.

The curves for the alkali chlorides form a family each exhibiting a minimum which becomes less pronounced as the molecular weight of the salt decreases.

The two primary factors affecting the diffusion of these strong and weak electrolytes over this wide range of concentration are the mobilities of the ions and the extent of dissociation, the latter modified by activity coefficient and other secondary factors. The measured diffusions for the binary univalent electrolytes may all be arranged in the order predicted by the simple Nernst equation

$$D = 2RT\Big/\left(\frac{1}{u_+} + \frac{1}{u_-}\right)$$

where u_+ is the *equivalent* conductivity of the cation and u_- that of the anion. The numerator refers to the osmotic driving force as dependent upon the number of diffusing ions per mole, the denominator to the electrical and frictional effects.

Haskell's extension[8] of the Nernst equation to polyvalent electrolytes is

$$D = \left(\frac{1}{n_+} + \frac{1}{n_-}\right) RT\Big/\left(\frac{1}{u_+} + \frac{1}{u_-}\right)$$

where n_+ and n_- are the respective valences, because electrical forces still refer to unit charge, whereas the osmotic effects refer to a smaller number of ions per equivalent of salt and the diffusion is correspondingly slowed. This equation is correct as it stands for infinitely dilute solutions of such electrolytes as barium chloride or copper sulfate where the numerical value of the first term by which RT is multiplied is 1.5 and 1, respectively. However, it is illuminating to consider incompletely dissociated electrolytes such as sodium bicarbonate or sodium acid tartrate or sodium acid sulfate, where Haskell's equation is not always immediately applicable. The more general equation[9] is

$$D = iRT\Big/\left(\frac{1}{u_+} + \frac{1}{u_-}\right)$$

where i is the total number of ions or ion complexes or molecules produced by one *equivalent*

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[9] For its application to partly dissociated or associated uni-univalent electrolytes, see M. E. L. McBain, THIS JOURNAL, **55**, 546 (1933).

of salt under the specified conditions. For the type potassium chloride, $i = 2$; for barium chloride 1.5; for copper sulfate 1; for sodium tartrate 1.5; for sodium acid tartrate 1; etc. Sodium tartrate diffuses faster than the bitartrate, because the hydrogen ion in the latter is undissociated. Tartaric acid is still slower and shows little dependence upon concentration because it is little dissociated in the range measured. Of course an acid salt may often be calculated as a uni-univalent salt if the equivalent conductivities are defined accordingly and the acid ion is itself completely undissociated.

It is of interest to note that the diffusion coefficients follow approximately the lyotropic series, falling in the order: thiocyanate or iodide or chloride; bicarbonate; sulfate, tartrate, salicylate, phosphate; bitartrate or citrate. This becomes of importance in considering mixtures, because there the faster constituents are accelerated and the slower ones still further retarded.

The Diffusion of Ions in Mixed Electrolytes Diffusing in the Same Direction.—In the diffusion of any particular ion in a mixture of ions the operative factors are: its concentration gradient (here always expressed in equivalents per liter per cm.), its valency n, its frictional resistance to movement (expressed as the reciprocal of its equivalent conductivity u), and the electrical gradient $\mathrm{d}\psi/\mathrm{d}x$ produced by and a function of all ions present.

These fundamental principles are quantitatively expressed most generally as follows.

For any cation the movement in gram equivalents, N, of ions through a square centimeter is

$$n_+\frac{\mathrm{d}N_+}{\mathrm{d}t} = u_+\frac{c_+}{n_+}\left[-RT\,\frac{\mathrm{d}\log c_+}{\mathrm{d}x} - n_+F\,\frac{\mathrm{d}\psi}{\mathrm{d}x}\right] \qquad (1)$$

and for any anion

$$n_-\frac{\mathrm{d}N_-}{\mathrm{d}t} = u_-\frac{c_-}{n_-}\left[-RT\,\frac{\mathrm{d}\log c_-}{\mathrm{d}x} + n_-F\,\frac{\mathrm{d}\psi}{\mathrm{d}x}\right] \qquad (2)$$

It will be noted that the equations comprise measurable quantities all based upon one gram equivalent of ion, including the faraday, F = 96,500 coulombs. However, by subtracting the equations (2) for all anions from the equations (1) for all cations, we can get an explicit expression for the electrical gradient $\mathrm{d}\psi/\mathrm{d}x$. In making this subtraction all the terms on the left-hand side of equations such as (1) and (2) cancel out because the condition of electroneutrality requires that equal numbers of positive and negative charges pass through any cross section, so that in algebraic summation

$$\Sigma n_+\,\mathrm{d}N_+ - \Sigma n_-\,\mathrm{d}N_- = 0$$

Thus we obtain

$$F\frac{\mathrm{d}\psi}{\mathrm{d}x} = -RT\left[\frac{\Sigma u_+G_+/n_+ - \Sigma u_-G_-/n_-}{\Sigma u_+c_+ + \Sigma u_-c_-}\right] \qquad (3)$$

where G is the concentration gradient for the respective ion, $\mathrm{d}c/\mathrm{d}x$.

By substituting this common value for the electrical gradient in any or all of the equations (1) and (2) we obtain for the movement of each cation the final equation

$$n_+\frac{\mathrm{d}N_+}{\mathrm{d}t} = -RT\,\frac{u_+}{n_+}\left[G_+ - n_+c_+\,\frac{\Sigma u_+G_+/n_+ - \Sigma u_-G_-/n_-}{\Sigma u_+c_+ + \Sigma u_-c_-}\right] \qquad (4)$$

It will be noted that equation (4) gives the actual movement of each and every cation in terms of the very simple measurable quantities, mobilities, valences, concentrations, and concentration gradients of all ions present; similarly for each anion, but with plus substituted for the minus sign after G_+.

This equation (4) for actual movement including actual movement for unit concentration gradient is more important than any attempt to set up a "diffusion coefficient." The latter should refer only to the movement of one constituent uninfluenced by anything else present. Then, according to Fick, $n\mathrm{d}N/\mathrm{d}t = -DG$. However, it has become customary to set up other "diffusion coefficients," D, which describe the response of the ion to unit concentration gradient under the actual circumstances, as for example when hydrochloric acid is diffusing into water and the hydrogen ion is slowed down and the chloride ion speeded up to the same intermediate value. Following this custom, we may write for the practical diffusion coefficient of any cation in any mixture of ions

$$D_+G_+ = RT\,\frac{u_+}{n_+}\left[G_+ - n_+c_+\,\frac{\Sigma u_+G_+/n_+ - \Sigma u_-G_-/n_-}{\Sigma u_+c_+ + \Sigma u_-c_-}\right] \qquad (5)$$

and for any anion

$$D_-G_- = RT\,\frac{u_-}{n_-}\left[G_- + n_-c_-\,\frac{\Sigma u_+G_+/n_+ - \Sigma u_-G_-/n_-}{\Sigma u_+c_+ + \Sigma u_-c_-}\right] \qquad (6)$$

If we assume linear gradients and if the diffusion is into pure water, the numerical value of the concentration may be substituted for that of the gradient of each ion whereupon both values disappear from the equation, except that the concentrations then remain under all summation terms. Although these equations are strictly valid only for very dilute solutions, we have found them to yield interesting and unsuspected information which has been experimentally substantiated in solutions of moderate strength. The effects are a function of relative rather than absolute concentration of ions.

It will be noted that all the equations begin with the term *RTu/n* which is the value of the true diffusion coefficient of that ion when completely released from its partner and not under the influence of a potential gradient. For example, for hydrogen ion $D = 8.05$, and for chloride ion 1.75 (as compared with the mutual compromise, $D = 2.54$ for hydrochloric acid). The same result would be achieved when the first ion is present in very small quantity in a large excess of other ions, but only if there is then no electrical gradient. This latter condition which we have made explicit in the development of the equations has often been overlooked in various applications, as for example in many buffer effects.

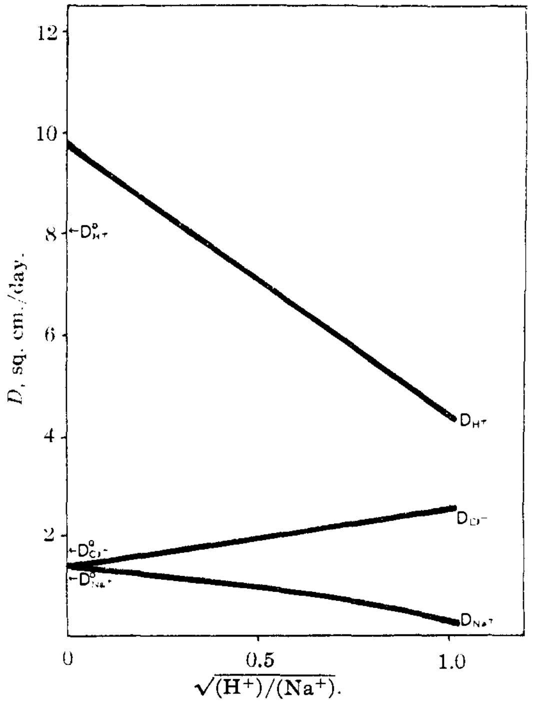

Fig. 2.—Calculated ion diffusion coefficients in mixtures of hydrochloric acid and sodium chloride.

Thus depending upon the electrical gradient produced by the buffers or other ions, the diffusion coefficient of a given ion may be below, or equal to, or above its true value. This is most striking when we take the fastest known ion, H^+, and find it diffusing much faster than its supposed limit $D = 8.05$, merely because there is a large admixture of slow cations, especially those of high valency with faster ions such as chloride ion.

As an illustration, the velocity of diffusion of hydrogen ion in a large excess of potassium chloride must be 1.8% greater than it is in its liberated state, $D = 8.05$. Similarly we predict that in sufficient excess of magnesium chloride solution the diffusion coefficient for hydrogen ion is raised to a value no less than 11.2, that is, 39% greater than its free value.

Before turning to experimental verification, we present in Fig. 2 the calculated "diffusion coefficients" of each of the ions in mixtures of hydrochloric acid and sodium chloride, where the liberated values of *D* are 8.05, 1.75 and 1.15 for hydrogen, chloride and sodium ions, respectively. Instead of these, on the left-hand side of the diagram where the sodium chloride is present in great excess the hydrogen ion value is 9.75, while sodium and chloride move together with a value of 1.39. On the right side of Fig. 2 where the amounts of hydrochloric acid and sodium chloride are equal, the diffusion coefficient of hydrogen is brought down to 4.45, that of chloride brought to 2.5 and, very significantly, that of the sodium ion depressed right down to 0.3. This clearly shows how the electrical gradient accelerates or retards the ions according to its sign, which in turn depends upon whether the salts present in excess have faster or slower cations than their anions. Only at one particular relative concentration where the electrical gradient or diffusion potential is zero does any ion move at its true liberated value, and then all do. For this particular mixture this occurs where the concentration of hydrochloric acid is $^1/_9$ that of sodium chloride.

Summarizing, and referring as an example to Fig. 2, unless the diffusion potential or electrical gradient is zero, no ion can move at its proper rate. If the diffusion potential is positive, all cations will be accelerated and all anions retarded. If it is negative, the converse will be the case. The effect is greatest for any ion that is present in relatively least amount.

In Fig. 3, similar calculations have been made for mixtures of hydrochloric acid with each of the

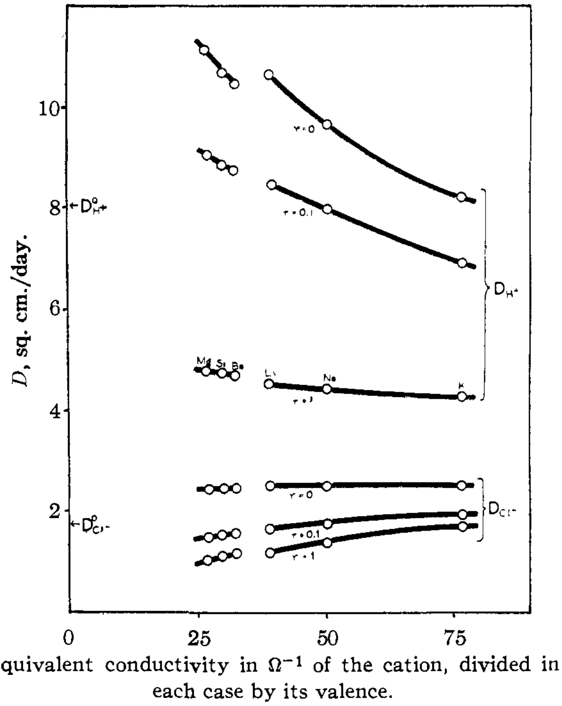

Fig. 3.—Calculated diffusion coefficients of H^+ and Cl^- in mixtures of hydrochloric acid with alkali and alkaline earth chlorides, $r = (H^+)/(\text{cation})$.

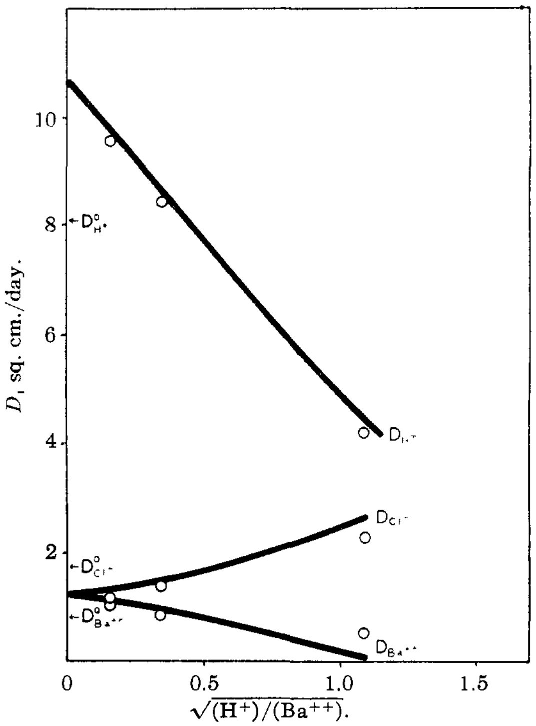

Fig. 4.—Ion diffusion coefficients in mixtures of barium chloride and hydrochloric acid (curves are calculated from theory, points are experimental values).

chlorides of the alkaline earths and the alkali metals. It is seen that the greatest effects are produced by, and upon, the alkaline earth cations on account of their valency; and again in diminishing order by lithium, sodium and potassium as their mobilities approach that of chloride ion.

In Fig. 4 our data for mixtures of hydrochloric acid with barium chloride are given as experimental points for comparison with the calculated curves, and it will be noted that there is quite good agreement with the simple theory. Each measurement was made in quadruplicate for all three ions, and in the very dilute solutions the concentration of hydrogen ion was determined with a Beckman glass electrode. The data are given in detail in Table II. Whereas the barium chloride speeds up the hydrogen ion, conversely hydrochloric acid retards barium ion. When one equivalent of hydrochloric acid is added to one equivalent of barium chloride, the predicted diffusion coefficient for barium ion has sunk from its true uninfluenced value 0.74 to only 0.143.

TABLE II

DIFFUSION COEFFICIENTS OF EACH ION IN 0.426 N SOLUTIONS OF BARIUM CHLORIDE TO WHICH PROGRESSIVE AMOUNTS OF HYDROCHLORIC ACID ARE ADDED

Cell	r^a	Initial H^+	Diffusate concentration Ba^{++}	Cl^-	H^+	Ion diffusion coefficients Ba^{++}	Cl^-	H^+
T	0.0235	0.0100	0.0201	0.0231	0.0030	1.00	1.12	9.37
W	.0235	.0100	.0234	.0267	.0033	1.00	1.12	9.45
T	.0235	.0100	.0199	.0229	.0030	1.00	1.12	9.53
W	.0235	.0100	.0232	.0265	.0033	1.00	1.12	9.58
R	.120	.0513	.0172	.0325	.0153	0.79	1.37	8.42
D_2	.120	.0513	.0134	.0252	.0118	0.86	1.48	8.49
R	.120	.0513	.0170	.0323	.0152	0.99	1.37	8.42
X	1.20	.5107	.0179	.1548	.1369	0.47	2.21	4.16
Z	1.20	.5107	.0151	.1365	.1214	0.48	2.26	4.19
X	1.20	.5107	.0182	.1550	.1368	0.48	2.22	4.16
Z	1.20	.5107	.0153	.1364	.1211	0.49	2.26	4.20

[a] r = ratio $HCl/{}^1/_2\ BaCl_2$.

If the ratio were increased to ten, the diffusion coefficient would decrease and even reverse to -0.16, and similarly in the limit for large ratios to -0.21.

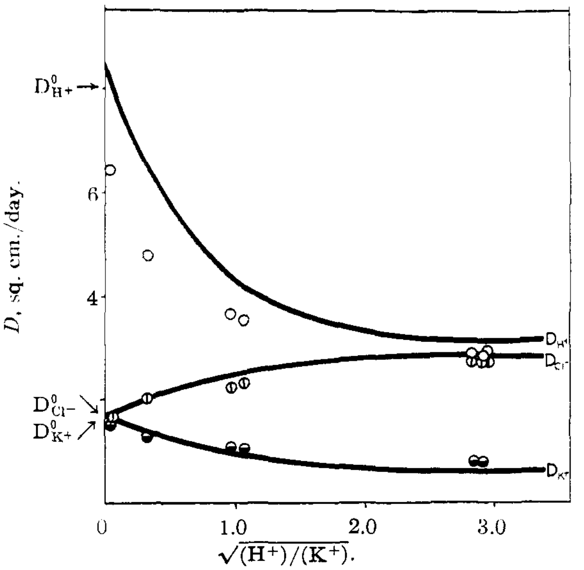

Fig. 5.—Ion diffusion coefficients in mixtures of hydrochloric acid and potassium chloride (curves are calculated from theory, points are experimental values of McBain and Dawson, ref. 5).

Figure 5 gives the curves calculated by the same simplified theory for mixtures of hydrochloric acid with potassium chloride, accompanied by the experimental points of McBain and Dawson.[5] The latter, however, perhaps because they were obtained after longer diffusion periods, show fairly large deviations from the curves calculated assuming initial unchanging gradients. The right-hand side shows clearly how the hydrochloric acid depresses the diffusion of potassium ion to one-third of its normal value, but enhances that of chloride ion.

These findings are of interest in physiology, for example, in intestinal absorption, or in transport of ions through membranes even when other factors are operative. In a saline laxative as has been shown experimentally by Visscher,[10] although interpreted differently, since fast ions are accelerated and slow ions retarded, sodium chloride will move quickly and oppose the movement of such salts as sulfate, tartrate, citrate or phosphate.

Diffusion of Mixed Electrolytes, Some of which are Diffusing in Opposite Directions.—The simple equations already developed adequately account for mixtures of electrolytes diffusing against any other mixture of electrolytes. The only modification in using the formulas is that the gradient for each ion must be taken with the proper sign. Illustrative data for this case have been published by McBain and Dawson.[5] When decinormal hydrochloric acid was diffused against decinormal potassium chloride, the diffusion coefficient for H^+ was 4.40, that for K^+ 2.51, both faster than the 2.54 for hydrochloric acid alone into water and the 1.7 for potassium chloride alone into water. Clearly the fast moving hydrogen ion produces a diffusion potential which moves the potassium ion in the opposite direction and the negative ion in the same direction. Since the hydrogen ion is moving faster than the potassium ion, the chloride ion which was uniformly distributed must now move in the same direction as the hydrogen ion to preserve the electroneutrality of the system. Since there was originally no concentration gradient of chloride ion, it is not really legitimate to calculate it as a "diffusion coefficient" of chloride ion, even when taken with negative sign.[5] This is further discussed in the following section.

Diffusion of Ions through Salts or Buffers which are Originally Present in Uniform Concentration.—It is clear from equation (3) that every diffusing electrolyte produces some diffusion gradient no matter how heavily buffered and that therefore, as in equation (4), every ion moves even when it has originally no concentration gradient. Indeed, this movement must continue until a temporary steady state is reached, after which all ions will gradually revert to uniform distribution.

The initial actual rate of movement in each case is given by equation (4) which does not include any "diffusion coefficients." Similarly the movement of any succeeding stage can be calculated from the same equation by inserting the gradients produced, with proper sign.

It is clear that sufficient relative excess of any salt in uniform concentration throughout no matter how unequal its ionic mobilities may be, will suppress the electrical gradient and permit a relatively low concentration of a diffusing ion to travel in the limit at liberated rate correspond-

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(10) Visscher and Ingraham, *Am. J. Physiol.*, **113**, 134 (1935); Burns and Visscher, *ibid.*, **110**, 490 (1934); Dennis and Visscher, *ibid.*, **131**, 402 (1940); see also F. Verzar and E. J. McDougall, "Absorption from the Intestine," Longmans, Green and Co., New York, N. Y., 1936.

ing to its true diffusion coefficient, $D = RTu/n$. Thus excess of a strong acid or of a strong base could form a perfect buffer, say, for sodium ion diffusing through it, with a rate corresponding to $D = 1.15$.

Since in these cases the movement of the buffer may be comparable to the movement of the particle or ion being studied, an optical method of examination can give erroneous results.

In all of the foregoing discussions such relatively less important influences as activity coefficient and collision effects are omitted; also the influence of ion pairs or ionic complexes[11] is left out of account. The foregoing factors do not affect results of electrolytic migration because in all such measurements the middle portion of the system is free from any concentration gradients. Likewise it does not affect measurements of hydration of ions by movement of a non-electrolytic reference substance, except in so far as collision effects may account for some of the minute movements observed.[12]

Summary

Diffusion data are supplied for sodium chloride, iodide, thiocyanate, bicarbonate, acid tartrate, tartrate, sulfate and citrate, lithium chloride and tartaric acid.

Developing the classical relations of Nernst and Arrhenius, equations are given for calculating diffusion of ions with, through or against other ions. Effects due to mere concentration gradient are explicitly kept separate from the electrical factor produced by diffusion potential. Only when the latter is zero do ions move at their true intrinsic velocity. In other mixtures fast ions are accelerated and slow ions are retarded and may be almost completely stopped. The fastest diffusion is that of hydrogen ion which may thus be pushed up to 40% above its previously supposed limit of $D = 8$ sq. cm./day.

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[CONTRIBUTION FROM THE EVANS MEMORIAL, MASSACHUSETTS MEMORIAL HOSPITALS, AND BOSTON UNIVERSITY SCHOOL OF MEDICINE]

Amperometric Measurement of the Potency of Catalase

BY BURNHAM S. WALKER

The purpose of the present study was to devise a suitable electrical method for the measurement of catalase, which might offer advantages in precision and convenience over titration methods. Such a method has been reported for the measurement of peroxidase,[1] in which the amount of hydroquinone oxidized to quinone is determined by a measurement of electromotive force. Catalase has been shown to have an effect upon the hydrogen peroxide component of the oxygen wave obtained by the polarograph.[2] We were unable, with the equipment available to us, to make a quantitative application of that particular effect. It was found to be a relatively simple matter to follow the course of the decomposition of hydrogen peroxide by catalase, using a dropping mercury electrode.

Experimental

The electrical circuit used was that of Petering and Daniels[3] using as a source of measured applied e. m. f. a Leeds and Northrup type K potentiometer. A box type of galvanometer was used, equipped with a shunt of nichrome wire. The length of the shunt wire was empirically selected to give full-scale deflection with a measured current of 0.2 milliampere. A fixed resistance of 2000 ohms was introduced into the circuit to aid in the suppression of maxima.[4] The dropping mercury electrode was constructed of capillary tubing obtained from broken clinical thermometers. The mercury was of "C. P." grade, and was cleaned for re-use with a current of air. No specially designed electrode vessel was used. The majority of experiments were carried out in an ordinary 50-cc. beaker. The amount of substrate solution was 25 cc., to which was added during the experiment a freshly prepared dilute solution of crystalline catalase in 0.1 cc. volume. The dropping mercury electrode was made the cathode, the anode being either a quiet pool of mercury at the bottom of the beaker, or a saturated calomel electrode connected by a potassium chloride–agar bridge.

In contrast to most measurements at the dropping mercury cathode, all determinations were carried out in solutions kept saturated with oxygen at atmospheric pressure. This was considered necessary since the decomposition of hydrogen peroxide by catalase yields oxygen, and the final measurements must be made under conditions of saturation with oxygen. All solutions were $M/30$ in phosphate buffer (Sørensen) at *p*H 7.

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(2) Brdička and Tropp, *Biochem. Z.*, **289**, 301 (1937).

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