ment of high stability in the resulting chelate.

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WORCESTER, MASS.

[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT, UNIVERSITY OF TORONTO]

The Determination of the Adjusted Indicator Concentration behind a Moving Boundary in Dilute Solution; the System Potassium Chloride–Sodium Chloride in Water at 25°

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A method is described for determining the adjusted indicator concentration behind a moving boundary in dilute solutions. To test the procedure, the system KCl–NaCl was investigated. It is shown that the Kohlrausch ratio can be determined with a precision comparable with that obtainable in moving boundary determinations of transference numbers, and that the ratio, measured over a range of concentration, can be extrapolated unambiguously to zero concentration. From the limiting ratio and the limiting equivalent conductances of leading and indicator salts, limiting ionic conductances may be obtained at once. Thus limiting ionic conductances can be determined in any solvent in which precise conductance measurements are possible, without carrying out any transference measurements in the ordinary sense of the term. Possible applications of the method are discussed.

In 1934, Hartley, Drew and Collie[1] reported a method for measuring the transference number of a slow ion by determining the adjusted indicator concentration of that ion behind a boundary for which the transference number of the leading fast ion was known. The basis for the procedure was the familiar Kohlrausch[2] condition for a stable two salt boundary of the type MA/M_iA

$$C_i/C = t_i/t = r \qquad (1)$$

where C and C_i are the concentrations of the leading and indicator ions M and M_i, respectively, t and t_i are the corresponding transference numbers, and r is the Kohlrausch ratio. As originally developed, the method was not capable of high precision, but it did permit approximate determinations of the transference numbers of certain paraffin-chain salts.[3] Years later, and using modern techniques, analogous measurements were carried out by Longsworth[4] to test the Dole theory[5] of polysalt boundaries, but on the whole the possibility of obtaining reasonably precise transference data in this way has received little attention. In a recent paper[6] dealing with adjustment of indicator concentration during moving boundary measurements, it was shown that with the type of cell there employed, it was possible to determine the indicator concentration behind the boundary within a part in 500 or so; this suggested that if the precision of such measurements could be raised to a level comparable with that obtainable in the usual moving boundary determination, and that if the Kohlrausch ratio, measured over a range of concentrations, could be extrapolated to infinite dilution, the resulting limiting ratio could be combined with the corresponding limiting equivalent conductances to yield limiting ionic conductances. Since limiting ionic conductances provide information as to ion–dipole interaction unobtainable from the equivalent conductance data, and since the procedure outlined above would eliminate the need for transference measurements in the ordinary sense of the term, it seemed desirable to investigate this possibility.

The system chosen for investigation was KCl/NaCl in aqueous solution at 25°. The reasons for this choice were first, that for both salts the transference numbers were well established,[7–9] and second, that owing to the relatively rapid change of the transference number for NaCl with concentration as compared with that for KCl, this system provided a good test of any extrapolation procedure.

Experimental

A series of tests with the cells previously employed (see Fig. 2 of ref. 6) and with various modifications of them, showed that the desired precision in the measurement of the adjusted indicator concentration (0.02%) could not be attained, but partly by accident it was found that a much simpler technique could, with care, provide data of the accuracy desired. Essentially the cell is a conventional falling boundary cell of the type used for many years in this Laboratory with a type VIII shearing mechanism (see Fig. 1 of ref. 6). It differs only in that the channel is somewhat longer (approximately 30 cm.) than in earlier cells, and that there is an additional shearing stopcock at the base of the channel. As in earlier cells, the channel is 2.5 mm. internal diameter and cadmium and silver–silver chloride electrodes serve as anode and cathode. To fill, KCl solution is forced upward into the channel until it rises just above the top of the upper shearing stopcock, which is then closed. The reservoir above the stopcock is then repeatedly flushed with the NaCl solution; after filling, the cell is brought to temperature equilibrium in a bath regulated to ±0.01°, the junction is formed at the top of the channel, and the current is started. It should be noted that only an approximate knowledge of the current and the time of electrolysis is necessary. Fifteen or twenty minutes after the time calculated for the boundary to pass the lower stopcock, the current is stopped and both cocks closed, thus isolating a column of indicator solution. For a system for which the transference number of the leading ion was not known, a preliminary series of measurements would obviously be required to determine the length of electrolysis necessary for the concentration of the solution in the channel to become independent of further passage of current.

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[3] G. S. Hartley, B. Collie and C. S. Samis, *Trans. Faraday Soc.*, **32**, 795 (1936).

[4] L. G. Longsworth, THIS JOURNAL, **67**, 1109 (1945).

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[6] A. R. Gordon and R. L. Kay, *J. Chem. Phys.*, **21**, 131 (1953).

[7] L. G. Longsworth, THIS JOURNAL, **54**, 2741 (1932).

[8] R. W. Allgood, D. J. LeRoy and A. R. Gordon, *J. Chem. Phys.*, **8**, 418 (1940).

[9] R. W. Allgood and A. R. Gordon, *ibid.*, **10**, 124 (1942).

The solution above the upper stopcock is then removed under suction with a fine pipet, the stopcock is opened, and the solution in the channel down to a level 2 cm. below the stopcock is removed under suction with a capillary pipet. A second capillary pipet, with a tip 30 cm. long and with a reservoir of 1-cc. capacity, is next inserted well into the channel; the contents of the channel are then removed under suction. It should be noted that the sample of indicator solution removed is only that part between levels 2 cm. below the initial position of the meniscus in the channel and 2 cm. above the lower stopcock. The pipet is then withdrawn, the outside of the tip dried with Kleenex and, after extruding a few drops, the solution is introduced into a small conductance cell of approximately 0.8-cc. capacity. This cell has bright platinum electrodes 8 mm. in diameter, completely filling the ends of the cell and sealed in the glass; the electrode spacing is 9 mm. Obviously in work with a volatile solvent, before employing the procedure described above, both sampling pipet and conductance cell would be flushed with a saturated atmosphere of the solvent to minimize evaporation losses.

The conductance cell is then placed in a bath at 25.000 ± 0.002°, and the apparent resistance determined on an a.c. bridge with Wagner ground at 1000 c.p.s. Since the indicator solution might have its resistance altered by the handling procedure, blank experiments were carried out at each concentration. The transference cell was completely filled with a NaCl solution of the appropriate concentration, current was passed for a time corresponding to the number of faradays required to move the boundary from end to end of the channel, a sample was withdrawn as described, and its resistance determined; this was compared with the resistance of the same solution when the sample was transferred directly from the solution flask into the conductance cell. At the lowest NaCl concentration studied, 0.0016 *N*, the resulting blank correction amounted to 20 ohms on a measured resistance of approximately 6000 ohms; it was however independent of current, was remarkably reproducible for a given concentration, and accordingly was applied before interpreting apparent resistance in terms of concentration.

This increase in conductance could be due to contamination either from the sampling pipet or from the transference cell itself. To distinguish the two effects for reasons discussed below, samples were transferred from the solution flask to the conductance cell by pipet, and (to take the measurements at 0.0016 *N* NaCl as an example) it was found that one-half the decrease in resistance was due to the pipet; the remainder must then be due to contamination in the transference cell, *i.e.*, the effective solvent conductance in the actual measurements was 3.3×10^{-7} greater than the measured conductance of the solvent used in making up the solution. Moreover, it was found that this increase was approximately independent of NaCl concentration, so that in computing the solvent correction, the specific conductance of the solvent was taken as $1.1_0 \times 10^{-6}$ (see below).

The cell was calibrated for each run by measuring the resistance of sodium chloride solutions whose known concentrations were 1 or 2% greater and less than that of the indicator solution. For the narrow concentration ranges involved, calculation showed that linear interpolation of the reciprocal of the resistance against concentration fixed the concentration of the unknown solution within experimental precision—0.01% in the apparent resistance or better. In this way, since all measurements were effected at the same frequency and at the same input, systematic errors in the interpretation of the conductance measurements in terms of concentration were eliminated as completely as possible.

One word of caution is perhaps desirable in connection with such miniature conductance cells. (1) In our experience, a cell should stand for at least three months after manufacture if consistent, reproducible results are to be attained; this is probably associated with the release of slight strains in the glass, which are particularly serious in such a small cell in view of the method of mounting the electrodes. (2) The cell should be protected as completely as possible against changes in temperature; a cell left inadvertently in bright sunlight for an hour, was useless for several days as a result. (3) One cell, after giving excellent service for several months, suddenly gave highly erratic results; after standing for several months, it again performed satisfactorily. For this reason, it is advisable to have a number of cells of different vintages in reserve, in order to avoid loss of time and temper.

The solutions were prepared as previously described.[8,9] The conductivity water, which was equilibrated with atmospheric CO_2, had a specific conductance of 7.5 to 8.0 × 10^{-7}.

Results

As an example of the results obtained, the data from a series of runs with 0.002 *N* KCl are summarized in Table I. In all, the concentration of the leading solution differed only by a fraction of a per cent. from this value, and for this reason, the quantity tabulated is C_i/C, the ratio of the measured indicator concentration to that of the leading solution, since for the concentration range involved this is independent of C. Table Ia shows the results of preliminary measurements such as would be required with a system for which no transference data were available. In the first series a deliberately bad choice of C_i°, the initial indicator concentration, was made—roughly one-half the Kohlrausch value; even so, and in spite of the fact that the measured C_i/C are definitely current dependent, an adjusted concentration in the neighborhood of 0.0016 *N* is indicated. In the second series, with $C_i^\circ = 0.0014$ *N*, the current dependence is less marked, and the ratios are larger, while in the measurements of Table Ib, with C_i°/C between 0.79 and 0.80, the observed C_i/C are independent of current—a criterion which must be satisfied if the true adjusted indicator concentration is being determined.

TABLE Ia

PRELIMINARY VALUES OF C_i/C FOR 0.002 *N* KCl

$C_i^\circ/C = 0.40$		$C_i^\circ/C = 0.70$	
I, μamp.	C_i/C	*I*, μamp.	C_i/C
60	0.8021	60	0.8052
90	.7974	80	.8042
120	.7925	110	.8032
		120	.8029

TABLE Ib

C_i/C for 0.002 *N* KCl, $C_i^\circ/C = 0.79–0.80$

I, μamp.	50	70	85	100	115	140
C_i/C	0.8063	0.8057	0.8060	0.8053	0.8066	0.8057
		.8066	.8063	.8055	.8062	.8059
			.8059		.8061	
			.8059			

It is perhaps significant in both series of Table Ia that C_i/C is linear in the current, and extrapolation suggests that the correct value would be obtained at a current of approximately 35 μamp. for $C_i^\circ/C = 0.4$, and of 50 μamp. for $C_i^\circ/C = 0.7$; hence it is possible that at still lower currents correct current-independent values of C_i/C would result. The inordinate length of time required for such measurements makes them impracticable. Gordon and Kay[6] showed that as long as there was a layer of indicator solution of the correct Kohlrausch concentration in contact with the boundary, correct values of the transference number of the leading ion were obtained from the observed boundary movement, even if the whole column of indicator solution above the boundary were not at this concentration; thus an erroneous measured value of C_i/C does not necessarily imply abnormal boundary movement. We believe the most probable explanation for the results of Table Ia is that convective stirring, due to Joule heating, tends to dilute the column of solution in the channel with the weaker solution in the reservoir above, the effect being more pronounced the higher the current. For higher KCl concentrations, the requirement that C_i° be near the Kohlrausch value if true, current-independent values of C_i/C are to be obtained, is not so stringent; for example, with 0.005 N KCl, current-independent values of C_i/C for a current variation of 100% are obtained with C_i° 7% below the Kohlrausch concentration.

Table II summarizes the results for four KCl concentrations. The second line gives the number of independent measurements, the third the current range, and the fourth the mean observed value of C_i/C; the number in brackets indicates, in units of the fourth decimal place, the mean absolute deviation of the individual results from the average as printed.

TABLE II

THE KOHLRAUSCH RATIO AS A FUNCTION OF KCl CONCENTRATION

C, equiv./l.	0.002	0.005	0.010	0.020
No. meas.	14	12	10	6
I, milliamp.	0.05–0.14	0.22–0.42	0.48–0.74	0.90–1.00
C_i/C	0.8060 (3)	0.8026 (3)	0.8003 (2)	0.7976 (3)
$t_i/t = r$	0.8038	0.8017	0.7999	0.7974
t	.4904	.4903	.4902	.4901
t_i, eq. 1	.3942	.3931	.3921	.3908
t_i, m.b.	.3943	.3932	.3922	.3907
r'	.8076	.8076	.8083	.8092

Discussion

To obtain the Kohlrausch ratio $r = t_i/t$ from the observed C_i/C, a "solvent" correction must be applied. If the specific conductance of the leading and indicator salts be κ and κ_i, and that of the solvent be κ_s, the current, corrected for solvent conductance in the indicator solution, will differ by a factor $(1 - x)$ from the corresponding current in the leading solution, where x is defined by

$$1 - x = 1 - \kappa_s/\kappa_i + \kappa_s/\kappa \qquad (2)$$

For example, for $C = 0.002\ N$, $C_i = 0.001612\ N$, κ and κ_i are 291.52×10^{-6} and 198.32×10^{-6}, respectively,[10] and for $\kappa_s = 1.10 \times 10^{-6}$, x will be 0.0018.

Consider a boundary moving in a channel, bounded by fixed reference planes enclosing the boundary. For the passage of 1 net faraday of charge (the charge transporting the K^+ and Cl^- ions in the leading solution) the boundary will move through a volume V_f, and CV_f will be equal to t, the transference number of K^+ in the KCl solution. In this interval (from conservation) 1 equivalent of Cl^- will cross the boundary into the NaCl solution, and $(1 - x)(1 - t_i)$ equivalents Cl^- will pass upward through the upper reference plane. Hence, if the boundary is moving in a steady state, $1 - (1 - x)(1 - t_i)$ equivalents Cl^- must be contained in the volume V_f of indicator solution formed behind the boundary, and, for electrical neutrality

$$C_iV_f = 1 - (1 - x)(1 - t_i) \qquad (3)$$

After dividing by $CV_f = t$, and rearranging, there results[11]

$$r = t_i/t = (C_i/C - x/t)/(1 - x) \qquad (4)$$

For 0.002 N KCl, t_i/t is thus 0.0022 less than the observed C_i/C.

The resulting t_i/t are given in the fifth line of Table II; the sixth line gives the transference numbers for K^+ in the leading solution,[7,8] and the seventh line the transference numbers for Na^+, computed from these and the values of r. The table also gives for comparison the values of t_i obtained from moving boundary measurements with NaCl solutions,[7,9] and it is at once evident that the agreement is excellent. Thus, in any solvent for which the transference numbers for one salt are available, the transference numbers for any other salt possessing a common ion can be determined readily to a precision comparable with that obtaining in the original transference measurements. While this technique was developed primarily for use in nonaqueous solvents, it nevertheless could prove useful in aqueous solution with electrolytes not easily dealt with by the moving boundary technique; the iodates, cadmium salts and the soaps are obvious examples.

A more important and more general problem arises, however, in cases where no transference data *per se* are available. As a first step, r is plotted against $C^{1/2}$; the plot is definitely curved, but a "best" straight line, drawn through the three lowest points gives a provisional limiting value of the Kohlrausch ratio, r_0, of 0.8067. If Λ^0 and Λ_i^0 are the limiting equivalent conductances of KCl and NaCl, 149.85 and 126.45, respectively,[10] it is easy to show that the limiting transference number for K^+ is given by

$$t^\circ = (\Lambda^\circ - \Lambda^\circ)/(\Lambda^0 - r_0\Lambda_i^\circ) \qquad (5)$$

The resulting provisional values of t^0 and t_i° are 0.4892 and 0.3946.

As a second approximation, the limiting law for transference numbers is employed[7]

$$t = t^\circ - \alpha C^{1/2} \qquad (6)$$

where $\alpha = (1 - 2t^0)\sigma/\Lambda^0$ and $\sigma = 30.09$ for aqueous solutions at 25°. In the corresponding expression for t_i, t^0, Λ^0 and C are replaced by t_i°, Λ_i° and $C_i = rC$. If one neglects second-order terms, it is at once evident that the quantity

$$r\{1 - (\alpha/t^\circ - \alpha_i r^{1/2}/t_i^\circ)C^{1/2}\} \equiv r_0' \qquad (7)$$

should approach r_0 with decreasing C. This quantity, in which α and α_i are computed with the provisional values of t^0 and t_i° given above, is entered in the last line of Table II, and is plotted in Fig. 1. Linear extrapolation gives $r_0 = 0.8075$, which may be compared with the value 0.8077, computed from the limiting transference numbers[7–9] for KCl and NaCl, *viz.*, 0.4905 and 0.3962. The resulting t^0 and t_i°, computed by eq. 5, are 0.4902 and 0.3958, and calculation shows that a further extrapolation, using these values, is unnecessary. The corresponding limiting conductances are 73.46, 50.05

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[10] For self consistency, the equivalent and ionic conductances used are those of Benson and Gordon (*J. Chem. Phys.*, **11**, 473 (1945)). See also Shedlovsky, Brown and MacInnes (*Trans. Electrochem. Soc.*, **66**, 165 (1934)) and H. S. Harned and B. B. Owen "The Physical Chemistry of Electrolytic Solutions," 2nd Edition, Reinhold Publ. Corp., New York, N. Y., 1950, p. 590.

[11] In the case of a solvent where no transference data are available, a preliminary estimate of t can be made by the approximation procedure discussed below.

and 76.39 for K^+, Na^+ and Cl^-, respectively; the values obtained from the extrapolated transference data[10] are 73.50, 50.10 and 76.35.

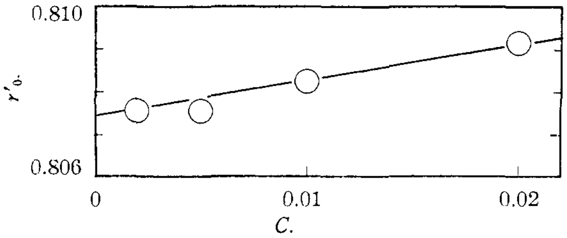

Fig. 1.—The quantity r'_0 as a function of KCl concentration; the radii of the circles correspond to the apparent precision of the measurements.

The results obtained here for the limiting transference numbers and ionic conductances illustrate one restriction on this method. Although the extrapolated r_0 differs from that computed from the limiting transference data by no more than the numerical precision of the latter (a part in 4000) the ionic conductances deviate by a part in 1800 for the faster ions, and by a part in 1000 for the slowest. Inspection of eq. 5 shows that this is due to the relatively small difference between Λ^0 and Λ_i^0, with a resulting limiting Kohlrausch ratio not differing greatly from unity. Thus to attain as high a numerical precision as possible in the ionic quantities, leading and indicator ions should have as large a difference in ionic conductance as possible, and this should be borne in mind in selecting the electrolytes for study in any new solvent.

It should also be noted that while the extrapolation procedure outlined above is probably adequate for strong electrolytes, the extrapolation, if ion pair formation is appreciable, would probably require the use of ionic rather than stoichiometric concentrations in eq. 6 and in the analog of eq. 7. We believe, however, that these results show that limiting ionic conductances can be determined with reasonable precision for any solvent in which precise conductance measurements are possible.

In conclusion, we wish to express our thanks to Mr. R. H. Chappell for constructing the conductance cells, and to the National Research Council of Canada for a grant in aid of this research and for the award to D.R.M. of a studentship and a fellowship.

TORONTO, ONTARIO, CANADA

[CONTRIBUTION FROM THE DEPARTMEMT OF CHEMISTRY, THE UNIVERSITY OF TEXAS]

The Inhibition of Urease by Various Metal Ions

BY WILLIAM H. R. SHAW

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Data on the relative toxicity of metal ions toward the enzyme urease have been collected from the literature. It has been found possible to arrange the common metal ions in a toxicity sequence. Correlation of toxicity with various properties of the metal ions is discussed and illustrated on the basis of a model mechanism.

Introduction

The enzyme urease is highly sensitive to trace quantities of metal ions. Different metals exhibit quite different behavior in their ability to act as enzyme inhibitors. In the case of urease, for example, the silver ion[1,2] is an extremely efficient inhibitor, while the manganous ion is relatively very weak.[3] A search of the literature has revealed that enough data are now available to order the common metal ions in a tentative sequence of relative inhibitory efficiency. It is the purpose of this investigation: to summarize the available data in such a sequence; to define a quantitative functional measure of inhibitory efficiency; and to correlate this quantity, if possible, with some fundamental property of the metals.

Mathematical Development

It was demonstrated in a previous communication[4] that the inhibition index, for a first-order Michaelis–Menten system, was given by the equation

$$\phi = \frac{V_u - V_i}{V_i} = \frac{K_1 K_m i + K_2 S i}{S + K_m} \qquad (1)$$

S is the substrate concentration, V_u is the uninhibited rate of urea hydrolysis by urease and V_i is the inhibited rate, K_m is Michaelis constant, K_1 the equilibrium constant for the combination of the free enzyme with the inhibitor, and K_2 an analogous constant involving the enzyme-substrate. The concentration of inhibitor not bound to the enzyme[5] is designated by "i." At an experimentally fixed substrate concentration, it has been common practice to determine the concentration of inhibitor (I) necessary to produce some arbitrary inhibition (ϕ_A). Thus for all the types of inhibition listed in Table I, equation 1 may be rearranged to read

$$pI = \text{const.} + \log K_i \qquad (2)$$

where K_i may be K_1, K_2 or K, and p refers to the negative logarithm of the quantity involved. For an enzyme obeying the inhibited Michaelis–Menten mechanism with the above restrictions, a comparison of pI's for various inhibitors corresponds to a comparison of functions that are, at a given temperature, linear functions of the free energy of inhibition

$$-\Delta F_i^0 = RT \ln K_i \qquad (3)$$

If $K_i = K$, the total free energy of inhibition will be twice that given by equation 3, since two inhibition reactions are involved. The larger the value of pI the more efficient the inhibitor, since there is a greater loss in the free energy of

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(1) J. B. Sumner and K. Myrbäck, *Z. physiol. Chem.*, **189**, 218 (1930).

(2) J. F. Ambrose, G. B. Kistiakowsky and A. G. Kridl, THIS JOURNAL, **73**, 1232 (1951).

(3) A. L. Dounce, National Nuclear Energy Series, Div. VI, Vol. 1, McGraw-Hill Book Co., Inc., New York, N. Y., 1949, pp. 839–848.

(4) G. B. Kistiakowsky and W. H. R. Shaw, THIS JOURNAL, **75**, 66 (1953).

(5) In what follows it is assumed that the amount of inhibitor bound to the enzyme is small compared to the total inhibitor concentration. For inhibitor concentrations that greatly exceed the total enzyme concentration, this assumption is entirely justified. For very strong inhibitors, such as silver ion, it should be considered as an approximation.