logarithm of specific viscosity against the logarithm of the molar concentration for the individual zinc halide solutions were very nearly linear up to concentrations of 1.5 to 2.0 molar. Above that limit, the curves become quite sharply concave upward. Within the region of linearity, a series of rough empirical relations can be obtained, the slopes being approximately constant for a given solute, and the intercepts being a function of temperature. Study of the variation of the intercepts with temperature permits introduction of temperature into the expression for the specific viscosity. By means of this kind of stepwise treatment, an empirical equation has been developed for each of the three solution systems, having the form

$$\log \eta_{sp} = a \log C + b - \frac{1}{T}(d + eT^2 - T^3)f \qquad (2)$$

where

η_{sp} = specific viscosity = $\eta_{relative} - 1$
C = molar concentration
T = absolute temperature
d = $E_{vis.}/2.303R$ for the solvent alone
$E_{vis.}$ = activation energy for viscous flow of the solvent

The parameters, a, b, e and f, for each salt are presented in Table I.

From Eq. (2), it follows that the specific viscosity may be written as

$$\eta_{sp.} = C^a F(T) \qquad (3)$$

TABLE I

PARAMETERS IN THE EMPIRICAL RELATION FOR SPECIFIC VISCOSITY AS A FUNCTION OF CONCENTRATION AND TEMPERATURE[a]

Salt	a	b	e	f
$ZnCl_2$	1.39	5.46	0.013	~0
$ZnBr_2$	1.40	5.78	.014	~0
ZnI_2	1.26	8.70	.053	−0.000077

[a] $d = 542$.

which is very similar in form to the function which is graphed to obtain the "intrinsic" viscosities of high polymers.[13] While it is known in the polymer field that the non-concentration term $F(T)$ is in fact a function of temperature, no adequate theoretical explanation of the functional relationship seems to be available. It is also known that the same function is dependent upon solute particle size.[14] The variations of the parameters, b, e and f, with solute species in the present study reflect this dependency but, again, a quantitative interpretation must await the development of a more comprehensive theory.

(13) E. O. Kraemer and W. D. Lansing, *J. Phys. Chem.*, **39**, 153 (1935).

(14) M. L. Huggins, *Ind. Eng. Chem.*, **35**, 980 (1943).

LEXINGTON, KY. RECEIVED JANUARY 24, 1951

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF KENTUCKY]

The Conductivities of Solutions of the Zinc Halides in Methanol from −50 to 20°[1]

BY L. R. DAWSON, A. TOCKMAN, H. K. ZIMMERMAN, JR., AND G. R. LEADER

Data are reported for the conductance of solutions of zinc chloride, bromide and iodide in methanol from about 0.1 to 4 or 5 molal over the temperature range between −50° and 20°. It is shown that in the greater part of the temperature and concentration range studied, conductance is not strictly a function of viscosity, but probably depends, in addition, upon the kinds and amounts of zinc halide complexes formed.

For a considerable time, it has been known that the conductance of zinc chloride displays a maximum as a function of concentration in non-aqueous medium.[2] Moreover, Getman and Gibbons[3] have shown that, for two temperatures at least, the conductance of zinc chloride in methanol has a negative temperature coefficient. In addition, Mead and Fuoss[4] have found a maximum in the conductance of aqueous zinc chloride solutions, as a function of concentration, using technical grade materials. Apparently, however, no attempt has been made to carry out a systematic study of the conductances of all three of the zinc halides in the same solvent over a wide range of temperatures and concentrations. As a consequence, the study reported here was undertaken in order to extend the existing data and thereby to obtain a fairly comprehensive picture of the conducting behavior of concentrated solutions of the halides in methanol.

Materials and Apparatus

The materials used in this work were prepared as described in a previous communication.[5] In order that the results might be strictly comparable, the solutions used were exactly the same as those previously employed.

Conductivities were measured by means of a Wheatstone bridge circuit consisting of the following components: a variable resistance consisting of two resistance boxes, a Leeds and Northrup 10,000 ohm coil-type decade resistance box inductance compensated and graduated in 0.1 ohm intervals, and a Shallcross No. 935 megohm decade box with a resistance of 1,100,000 ohms graduated in steps of 10,000 ohms; a Leeds and Northrup Kohlrausch-type slide-wire of 10 ohms resistance, drum-wound in a helix of ten turns; a compensating capacitor consisting of tandem type air condenser; Western Electric Model 509 headphones; and wiring consisting of shielded cable. The source of current was a 1000 cycle Cenco A. F. vacuum tube oscillator with taps of 50, 500 and 5000 ohms impedance; the 500 ohm tap was employed. The detector circuit also made use of a Masco Model MA-25 three-stage audio amplifier set in a Signal Corps FT-250-A mounting to protect the amplifier from (extraneous) vibration.

Results and Discussion

The results of the conductivity measurements are presented in Figs. 1, 2 and 3 for the chloride, bromide and iodide, respectively. Because of the virtual certainty that a significant amount of complex

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(1) Based on research performed under contract No. W36-039-sc-38184 for the U. S. Army Signal Corps.

(2) S. J. Lloyd, *J. Phys. Chem.*, **17**, 264 (1912).

(3) F. H. Getman and V. I. Gibbons, *Am. Chem. J.*, **48**, 124 (1912).

(4) D. J. Mead and R. M. Fuoss, *J. Phys. Chem.*, **49**, 480 (1945).

(5) L. R. Dawson, H. K. Zimmerman, W. E. Sweeney and G. P. Dinga, THIS JOURNAL, **73**, 4326 (1951).

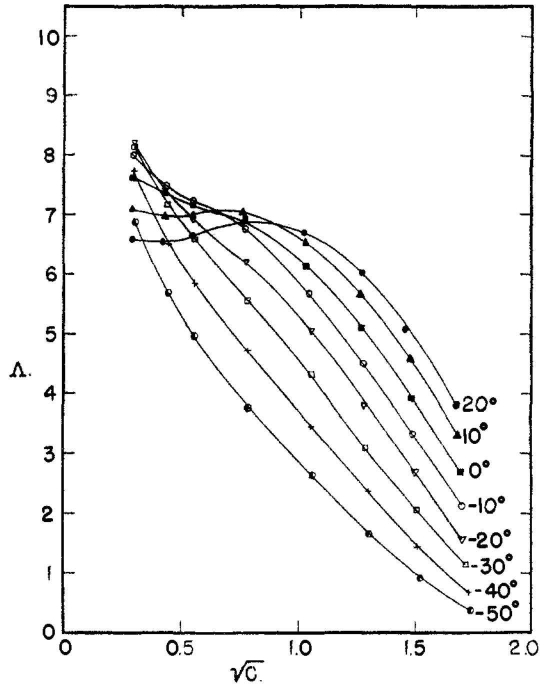

Fig. 1.—Conductance isotherms for concentrated solutions of zinc chloride in methanol as a function of square root of concentration.

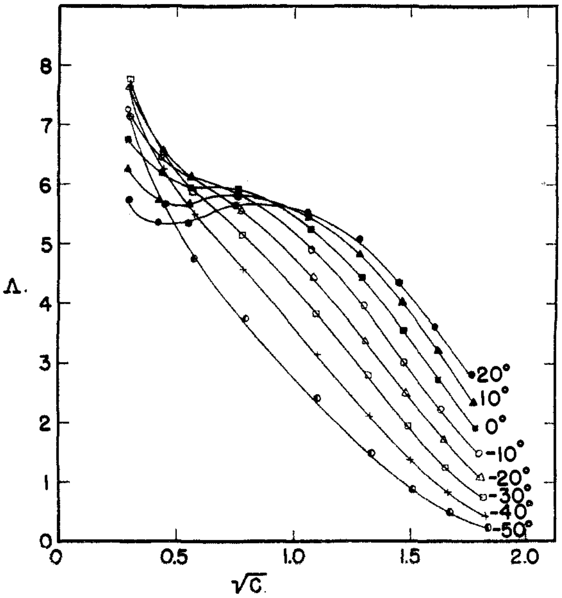

Fig. 2.—Conductance isotherms for concentrated solutions of zinc bromide in methanol as a function of square root of concentration.

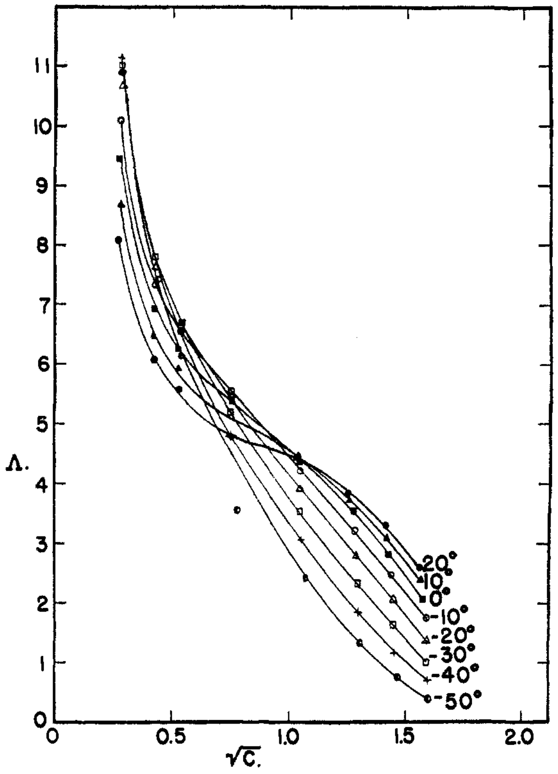

Fig. 3.—Conductance isotherms for concentrated solutions of zinc iodide in methanol as a function of square root of concentration.

formation exists in these solutions,[6] molar conductance has been presented rather than an equivalent conductance which probably is a very complicated function of the complex equilibria present in the solutions.

From the figures, it will be seen that in all cases at the lower concentrations the conductance rises as the temperature is diminished, passes through a maximum, and then decreases as the temperature drops still farther. In the specific case of the zinc chloride solutions, this provides a partial explanation of the negative temperature coefficient observed by Getman and Gibbons.[3] At the higher concentrations, on the other hand, an inversion of this behavior has occurred, and all the systems display a positive temperature coefficient. Between these two extremes there lies a region where the shape of the isotherm depends very markedly upon the temperature, the precise complexion of such shape depending even further upon whether the solute be the chloride, the bromide, or the iodide.

If one considers the bromide as a standard upon which to base comparisons, it appears that in the case of the chloride, the entire family of isotherms is shifted toward the left (*i.e.*, toward lower concentrations); and in the case of the iodide the entire family is shifted upward (*i.e.*, toward higher temperatures). These observations are admittedly quite general, insofar as they neglect the absolute magnitudes of the conductivities and consider only the over-all characteristics of the three families of curves. However, at the present time our knowledge is not sufficient to allow us to attach any quantitative significance to the real magnitudes of the conductances; these values depend too much both on the natures of the complexes present in their effects on the real concentrations of conducting species, and on the absolute mobilities of those conducting species under the existing conditions of temperature and concentration.

The occurrence of the minimum–maximum effect in the 20 and 10° isotherms for the chloride and the bromide is of especial interest. Considering

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[6] *Cf.* L. G. Sillen and B. Liljeqvist, *Svensk Kem. Tid.*, **56**, 85–95 (1944).

again the bromide, which is the best defined of the three cases in this respect, one notes that as the temperature is decreased, this effect is gradually wiped out until in the isotherms at the lowest temperatures there remains no vestige of it. The situation for the chloride is very similar, differing only in the smaller details of the curves. And for the iodide one notes the interesting fact that the effect is not observed at all, even at the highest temperature attained; at 20° only the beginning of the effect is to be seen, corresponding roughly to the state of development found in the −10° isotherm for the bromide.

In considering the causes of conductance maxima occurring in measurements at one temperature, it is usual to attribute them to the changing viscosity of the solution.[7] While there is little doubt that this interpretation is correct for relatively simple systems, such as 1–1 electrolytes, in which complexing does not occur, its accuracy in cases such as those presented here is very doubtful. Figures 4, 5 and 6 present isotherms of log (fluidity) *versus* conductance for the zinc chloride, bromide and iodide solutions, respectively.[5] It is evident from the lower left section of such a loop as is depicted that there exists a region of temperature and concentration in which conductance is a single-valued function of fluidity, regardless of whether that fluidity is altered by either change of temperature or change of concentration. In the upper section of the loop, on the other hand, a change of fluidity by means of a change in temperature has a vastly different effect on conductance than does a change in fluidity by means of a change in concentration. It would seem that this phenomenon can be interpreted only as meaning that conductance in that region is influenced not only by fluidity, but also by some other factor which is affected differently by temperature changes than it is by concentration changes. At least one factor which answers such a description is that of complex formation. Moreover, a consider-

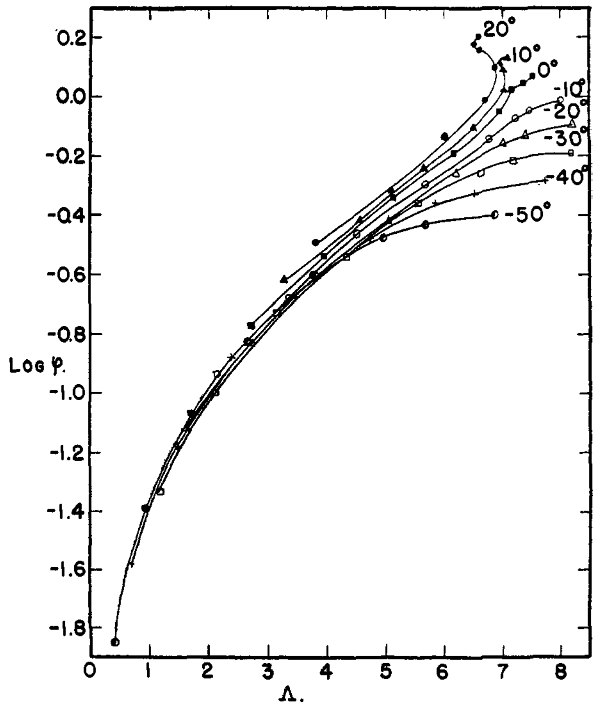

Fig. 4.—Log (fluidity) *vs.* conductance for zinc chloride in methanol.

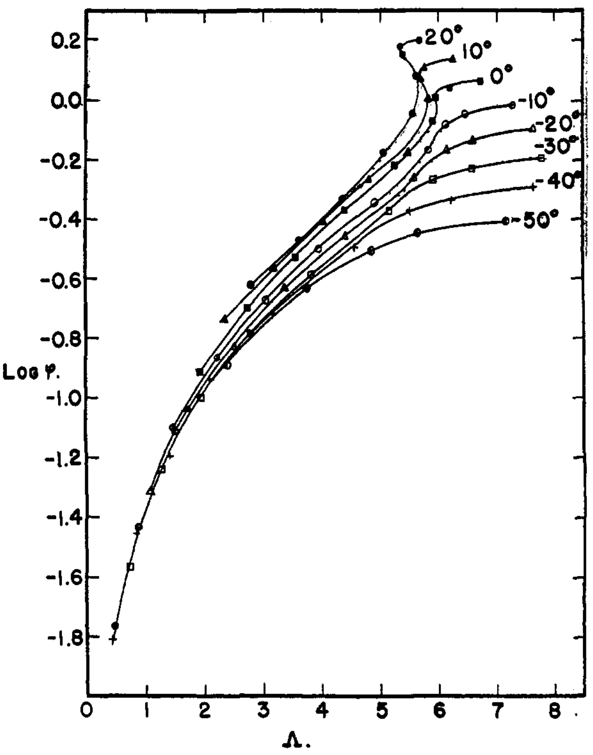

Fig. 5.—log (fluidity) *vs.* conductance for zinc bromide in methanol.

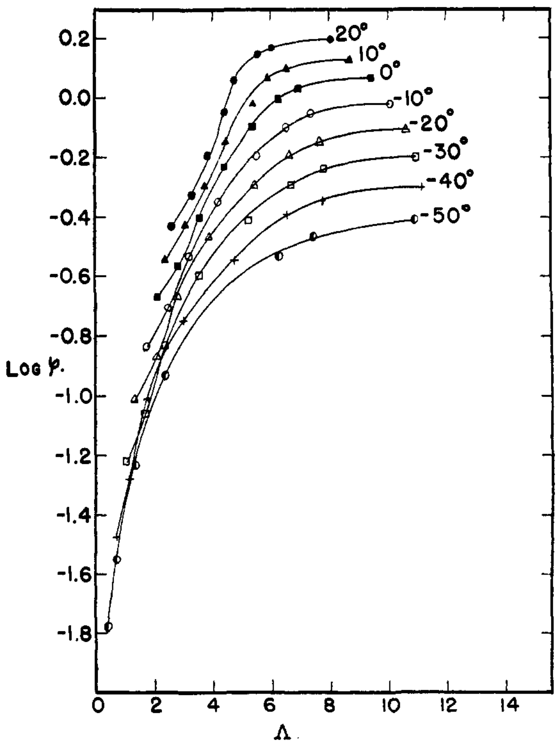

Fig. 6.—log (fluidity) *vs.* conductance for zinc iodide in methanol.

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[7] See, for instance, L. E. Strong and C. A. Kraus, This Journal, 72, 166 (1950).

ation of the relative locations of the points in, for instance, Figs. 1 and 4 shows that those in the region of the fluidity–conductivity plot which are interpreted as falling in a region of changing complex formation correspond rather well with those in the conductivity–concentration plot which display the minimum–maximum effect. By induction, therefore, it appears that the effects noted in the first three figures are probably due more to complex formation than to viscosity alone.

A completely quantitative development of these ideas probably will result in confirmation of the complexing hypothesis. However, the correlation must await the accumulation of further information, both on the exact nature and strength of whatever complexing does occur, and also on further conductance studies in the form of the investigation of systems containing mixed solutes involving a common ion. Such conductance studies are now in progress and will be reported in a subsequent communication.

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[CONTRIBUTION FROM THE LABORATORY FOR THE STUDY OF HEREDITARY AND METABOLIC DISORDERS AND THE DEPARTMENTS OF BIOLOGICAL CHEMISTRY AND MEDICINE, UNIVERSITY OF UTAH COLLEGE OF MEDICINE]

Kinetics of Carboxypeptidase Action. I. Effect of Various Extrinsic Factors on Kinetic Parameters[1]

BY RUFUS LUMRY,[2] EMIL L. SMITH AND RUBY R. GLANTZ

Investigation of the kinetics of crystalline pancreatic carboxypeptidase has shown that there is a marked effect of ionic strength on the activity of this enzyme. Normal rate parameters are obtained with carbobenzoxyglycyltryptophan and carbobenzoxyglycylphenylalanine only at substrate concentrations of 0.02 *M* and below; above this range there is strong substrate inhibition. With the corresponding leucine substrate, inhibition does not occur. Orthophosphate has a small effect as a competitive inhibitor of this enzyme. The effect of temperature on the rate constants for carboxypeptidase has been measured and values for the activation parameters are presented. Evidence is presented for interpreting K_m (the Michaelis constant) as a ratio of the dynamic constants of the consecutive reactions rather than as a thermodynamic equilibrium constant. The effect of *p*H and D_2O are considered in terms of the mechanism of this enzyme and the role of water and its component ions in the hydrolytic process.

I. Introduction

It has generally been found that the kinetic scheme suggested by Henri[3] but developed by Michaelis and Menten[4] and later by Briggs and Haldane[5] holds for the steady state velocities of most enzyme systems. The usual formulation of this scheme is

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_0} P + E$$

where E is enzyme, S is substrate and P the products of the reaction. The velocity is described as

$$\frac{d[P]}{dt} = \frac{k_0 e[S]}{[S] + K_m} \tag{1}$$

where $K_m = (k_{-1} + k_0)/k_1$ and e is the total concentration of enzyme, always small with respect to the substrate concentration.[5]

Evaluation of velocity–substrate relationships under different conditions provides potential data for the elucidation of enzymic mechanisms, especially for enzymes where it is possible to compare different substrates. Although it has been customary to measure K_m, it has not been possible to interpret such data because of a lack of knowledge as to the meaning of K_m which as k_{-1}/k_1 may have thermodynamic significance or as k_0/k_1 may be a ratio of possibly unrelated specific rate constants. In recent years there have been a few integrated kinetic studies, for example, those of Laidler and co-workers.[6] Unequivocal interpretation of K_m has been secured only where it has been possible to make direct estimates of the kinetic constants which are part of K_m, as in the work on catalase and peroxidase.[7] It is a purpose of this paper to describe experiments which lead to a highly probable interpretation of K_m for carboxypeptidase. The technique depends on the retardation of reactions involving water in the presence of deuterium oxide and a critical study of the temperature coefficients of catalysis velocity. With an interpretation of K_m, it is possible to obtain values for the rate constants for the reactions involving formation of the intermediate compound ES and its decomposition to products.

Carboxypeptidase was selected because a large body of data for this enzyme already exists.[8,9] In our work, it was found that many of our data were not in accord with earlier kinetic studies by Neurath and his co-workers.[8] Control experiments demonstrated that the lack of agreement is probably due to various combinations of three factors: (1) Although carboxypeptidase is inhibited by some component of phosphate buffer,[10] this has been interpreted[11] as due to a decrease in the rate of product–enzyme dissociation. However, there is also a weaker effect of phosphate which can be interpreted as competitive inhibition. (2) The

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[1] This investigation was aided by research grants from the National Institutes of Health, United States Public Health Service.

[2] Merck Fellow in the Natural Sciences.

[3] V. Henri, "Lois generales de l'Action des Diastases," A. Hermann & Cie, Paris, 1903.

[4] L. Michaelis and M. L. Menten, *Biochem. Z.*, **49**, 333 (1913).

[5] J. B. S. Haldane, "Enzymes," Longmans Green and Co., London, 1930.

[6] (a) K. J. Laidler and J. P. Hoare, THIS JOURNAL, **71**, 2699 (1949). (b) E. J. Casey and K. J. Laidler, *ibid.*, **72**, 2159 (1950).

[7] B. Chance, *J. Biol. Chem.*, **151**, 553 (1943); *Acta Chem. Scand.*, **1**, 236 (1947).

[8] H. Neurath and G. W. Schwert, *Chem. Revs.*, **46**, 96 (1950).

[9] E. L. Smith, *Advances in Enzymol.*, **12**, 191 (1951).

[10] E. L. Smith and H. T. Hanson, *J. Biol. Chem.*, **179**, 803 (1949).

[11] H. Neurath and G. de Maria, *ibid.*, **186**, 653 (1950).