per g. nitrogen as a "representative" value. Since pepsin has a molecular weight of 35,000, this amounts to 85 kcal. per mole of protein, roughly half of that observed for methemoglobin. Thus the heat of denaturation per gram of protein material is about the same for both compounds, which may be interpreted to mean that alkali has a similar effect on molecular structure in both cases.

We wish to thank the Rockefeller Foundation for its grant in support of this work.

Summary

The heats of reaction of native and denatured pepsin with potassium hydroxide have been measured at 30°. The difference between these heats, which may be called the heat of denaturation, was found to depend on the initial and final *p*H of the experiment. Between *p*H 4.3 and 8.2 the heat of denaturation is +16.06 cal. per g. of nitrogen, or 85 kcal. per mole of pepsin. The heat of denaturation was found to decrease with increasing *p*H more rapidly than the enzymatic activity. It is concluded that the effects of previous treatment with alkali on the heat of denaturation and on the enzymatic activity are to some extent independent.

CAMBRIDGE, MASS. RECEIVED APRIL 28, 1941

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY OF THE UNIVERSITY OF MINNESOTA]

An Application of the Ilkovic Equation to the Analysis of Mixtures of Reducible Substances with the Dropping Mercury Electrode

BY I. M. KOLTHOFF AND E. F. ORLEMANN

The current–voltage curve of a solution of two substances which are reducible at the dropping mercury electrode at different potentials has the general appearance of that given in Fig. 1. When the plotted values of the current are corrected for the residual current the following relations are found.

$$i_{d_1} = k_1c_1 \qquad (1)$$

$$i_{d_2} = k_2c_2 \qquad (2)$$

The subscript (1) refers to the first component reduced and subscript (2) to the second component. i_d is the diffusion current, k is the diffusion current constant and c is the concentration of the substance. The total current i_t can be measured at a certain potential π_t and is equal to the sum of i_{d_1} and i_{d_2} at this same potential π_t. We may therefore write

$$i_t = (i_{d_1})_{\pi_t} + t(i_{d_2})_{\pi_t} \qquad (3)$$

Obviously i_{d_1} cannot be measured directly at the potential π_t but can be measured only at potentials between π_a and π_b as shown in Fig. 1. It is necessary therefore to find the value of i_{d_1} at the potential π_t from the value of i_{d_1} obtained at some more positive potential in order to use equation (3) for the determination of the diffusion current of component two.

Various empirical procedures have been proposed to meet the above problem which in effect either assume that i_{d_1} is independent of the potential or assume an extrapolation of i_{d_1} which is not fundamentally correct.[1] For a given empirical procedure the error involved may be very small or very large dependent upon the ratio of i_{d_1}/i_{d_2} and the potential region involved in the measurements of i_{d_1} and i_{d_2}. In the present paper a general discussion of the problem based on the Ilkovic equation is given and a procedure based on fundamental principles is described.

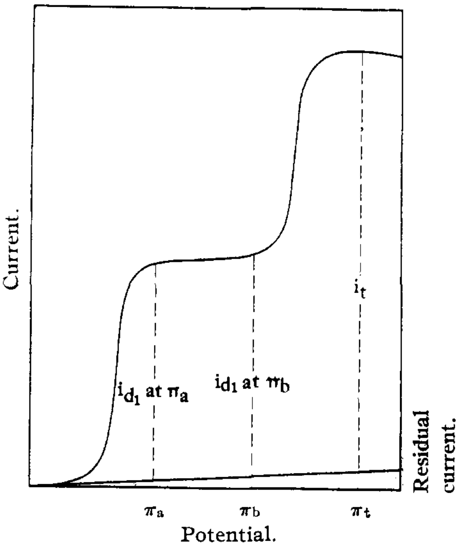

Fig. 1.—C. v. curve of a mixture of two reducible substances.

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[1] I. M. Kolthoff, *Chem. Rev.*, **24**, 1 (1939).

The Ilkovic[2] equation is

$$i_d = 0.627nFD^{1/2}Cm^{2/3}t^{1/6} \qquad (4)$$

In this equation m is the mass of mercury, usually given in mg., flowing through the capillary per second and t is the drop time in seconds. The other factors in equation (4) are independent of the potential and need not be considered in the present application. From a practical point of view m is found to be essentially independent of the potential of the dropping mercury electrode. However, the value of t depends markedly upon the potential and has a maximum value at a potential of about −0.55 v. (*vs.* S. C. E.) at the so-called electrocapillary zero point of mercury.[1] The potentials refer to the saturated calomel electrode (S. C. E.).

Suppose i_{d_1} is measured at the potential π_1 as shown in Fig. 1, where π_1 might have any value from π_a to π_b and the total current i_t is measured at a potential π_t. From equation (4) we find that the value of i_{d_1} at the potential π_t can be expressed as

$$(i_{d_1})_{\pi_t} = (i_{d_1})_{\pi_1}\frac{(m^{2/3}t^{1/6})_{\pi_t}}{(m^{2/3}t^{1/6})_{\pi_1}} \qquad (5)$$

If we substitute this expression into equation (3) we find

$$(i_{d_2}) = i_t - (i_{d_1})_{\pi_1}\frac{(m^{2/3}t^{1/6})_{\pi_t}}{(m^{2/3}t^{1/6})_{\pi_1}} \qquad (6)$$

From a practical point of view the application of equation (6) would be somewhat of a nuisance since it would involve the determination of the values of m and t for each capillary used over the potential range involved. Fortunately expression (6) can be simplified for practical purposes in the following way. It was found in this Laboratory that the relative change of $m^{2/3}t^{1/6}$ with the potential was practically the same for various capillaries. Since this is true a table of relative values of $m^{2/3}t^{1/6}$ can be prepared which is applicable to any ordinary capillary used. For convenience the maximum value of $m^{2/3}t^{1/6}$ at −0.5 v. (electrocapillary zero) is taken as unity and the relative values of $m^{2/3}t^{1/6}$ at all other potentials are then calculated on this basis. If we denote the relative value of $m^{2/3}t^{1/6}$ at a potential π by the symbol $(\text{r. v.})_\pi$ we may write equation (6) in this form

$$i_{d_2} = i_t - (i_{d_1})_{\pi_1}\frac{(\text{r. v.})_{\pi_t}}{(\text{r. v.})_{\pi_1}} \qquad (7)$$

The following procedure for finding the value of i_{d_2} is recommended: after correction for the residual current, measure i_{d_1} at a suitable potential π, and the sum of the diffusion currents (i_t) of both constituents at a potential π_t, then calculate i_{d_2} with the aid of equation (7) using the values of r. v. given in Table I.

It is evident from Table I that in general the correction factor $(\text{r. v.})_{\pi_t}/(\text{r. v.})_{\pi_1}$ is negligibly small when i_t is measured at a potential more positive than −1.0 v. (*vs.* S. C. E.) if the ratio of the concentrations of constituents one and two is of the order of one or smaller than one. However, when the ratio is much greater than one (use of compensation method[3]) the correction has to be applied even in this case. It is also evident that the magnitude of the correction factor increases rapidly with increasing negative value of the potential at which i_t is measured, and with increasing separation of the potentials at which i_t and i_{d_1} are measured.

Experimental

All experiments were carried out in a thermostat at a temperature of 25 ± 0.02°. The manual apparatus was used for the determination of the current voltage curves.[1] The measured values of the current were reproducible to within 1%. Values of m and t were determined in 0.1 M potassium chloride solution using the method of Lingane and Kolthoff.[4] All chemicals used were of reagent quality and tested for impurities.

Table I gives the values of m and t and the relative values

TABLE I

VALUES OF m AND t AND OF RELATIVE VALUES OF $m^{2/3}t^{1/6}$ (r. v.) AT VARIOUS POTENTIALS

π	m, mg. sec.$^{-1}$	t, sec.	r. v. K. and O.[a]	r. v. L. and K.[b]	r. v. W[c]	r. v. adopted
0	1.925	3.26	0.983	0.991	0.980	*0.985*
−0.2					0.99	*.99*
− .3	1.922	3.58	0.997			*.995*
− .5	1.921	3.64	1.00	1.00	1.00	*1.00*
− .8				0.995		*0.995*
−1.0	1.931	3.34	0.989		0.98	*.985*
−1.1				.978		*.98*
−1.3	1.933	2.97	.971			*.97*
−1.5	1.942	2.65	.955	.952	.953	*.95*
−1.6	1.941	2.46	.944			*.94*
−1.7	1.940	2.25	.930			*.93*
−1.8	1.941	2.12	.910			*.92*
−1.9				.908		*.91*
−2.0					.894	*.89*

[a] Kolthoff and Orlemann, pressure 90 cm. of mercury. [b] Lingane and Kolthoff,[4] pressure 21.8 cm.; m = 2.62; t = 3.08 at π = −0.5 v. [c] Watters, pressure 63 cm.; m = 0.908, t = 6.27 at π = −0.5 v.

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[2] D. Ilkovic, *Collection Czechoslov. Chem. Commun.*, **6**, 498 (1934).

[3] D. Ilkovic and G. Semerano, *ibid.*, **4**, 176 (1932); J. J. Lingane and H. Kerlinger, *Ind. Eng. Chem., Anal. Ed.*, **12**, 750 (1940).

[4] J. J. Lingane and I. M. Kolthoff, THIS JOURNAL, **61**, 825 (1939).

TABLE II

EFFECT OF $m^{2/3}t^{1/6}$ UPON DIFFUSION CURRENT OF CUPRIC COPPER

π (*vs.* S. C. E.)	−0.3	−0.5	−1.00	−1.30	−1.50	−1.60	−1.70	−1.80
i_d in microamp.	6.12	6.14	6.10	5.98	5.88	5.84	5.70	5.56
$i_d/m^{2/3}t^{1/6}$	6.13	6.14	6.16	6.16	6.15	6.18	6.13	6.05

(r. v.) of $m^{2/3}t^{1/6}$ at various potentials found with our capillary. In the fifth column values of r. v. obtained by Lingane and Kolthoff[4] with a different capillary are reported, and in the sixth column those recently obtained by J. J. Watters in this Laboratory. The seventh column gives the adopted values of r. v. which can be used quite generally in applying equation (6). When the potential is more negative than 2.0 v. (determination of alkalies) the values of r. v. vary with the characteristics of the capillary used and should be determined separately by the experimenter. In solutions of strongly capillary active ions such as iodide or cyanide there may be deviations from Table I and care should then be exercised in using Table I. In such cases it would be better to measure the drop time t at the potentials π_1 and π_t.

In order to test equation (6) and the reliability of the data of Table I, current–voltage curves were determined of solutions of copper sulfate and of manganese chloride in 0.1 *M* potassium chloride to which 0.008% of gelatin had been added to suppress maxima. The residual current of the medium was determined separately. All values of the current reported below were corrected for the residual current.

Table II gives the decrease of the diffusion current of 0.001 *M* cupric copper in 0.1 *M* potassium chloride (containing gelatin) with increasing negative potential. The data again confirm the validity of the Ilkovic[1] expression (equation 3) regarding the $m^{2/3}t^{1/6}$ effect, as is evident from the constancy of $i_d/m^{2/3}t^{1/6}$. The values of r. v. used in these and the following calculations were those reported under the heading K. and O. in Table I.

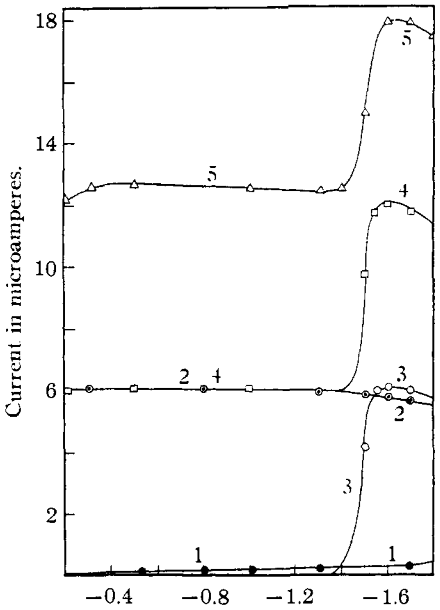
The current–voltage curves obtained with copper and manganese solutions alone and with mixtures of both ions are given in Fig. 2. Curve 1 represents the residual current of the medium; the values of the current in the other curves are corrected for i_r. Curves 4 and 5 represent the c. v. curves of mixtures of copper and manganese; in curve 4 the ratio of the two was 1 to 1 and in curve 5 it was 2 to 1. The diffusion current of copper (i_{d_1}) was taken at a potential of −0.5 v., while the total current (i_t) was taken at −1.7 v. The data are summarized in Table III. From lines 5 and 6 it is readily seen that the amount of manganese found is too small if i_d of manganese is taken equal to the total current at −1.7 v. minus i_d of copper at a potential of −0.5 π. The error increases with increasing ratio of copper to manganese. The last two lines show that satisfactory results are obtained when i_d of manganese is calculated with the aid of equation (6).

TABLE III

DETERMINATION OF i_d OF MANGANESE IN THE PRESENCE OF COPPER

Molar concn. Cu	10^{-3}	..	1.01 × 10^{-3}	2.04 × 10^{-3}
Molar concn. Mn	..	10^{-3}	10^{-3}	10^{-3}
i_d at π = −0.5	6.19	..	6.19	12.76
i_t at π = −1.7	..	6.00	11.85	18.00
i_d of Cu calcd. at π = −1.7	..	..	5.75	11.90
$i_t - (i_{d_1})$, π = −0.5	..	..	5.66	5.24
Error of Mn in %	..	..	−5.6	−12
$i_t - (i_{d_1})$, π = −1.7	..	..	6.10	6.10
Error of Mn in %	..	..	1.7	1.7

Instead of mixtures of copper and manganese, we also have investigated other systems and obtained good results by application of equation (6). It should be emphasized that the correction becomes very large in the polarographic determination of alkali and earth alkali metals in the presence of other constituents which yield waves at more positive potentials.



Potential of dropping electrode in volts (*vs.* S. C. E.).

Fig. 2.—C. v. curves of copper and manganese alone and in mixtures, supporting electrolyte 0.1 *M* potassium chloride + 0.008% gelatin: Curve 1, residual current in supporting electrolyte; Curve 2, 10^{-3} *M* cupric sulfate; Curve 3, 10^{-3} *M* manganous sulfate; Curve 4, 10^{-3} *M* cupric sulfate + 10^{-3} *M* manganous sulfate; Curve 5, 2.04 × 10^{-3} *M* cupric sulfate + 10^{-3} manganous sulfate. All currents have been corrected for the residual current shown in Curve 1.

Acknowledgment is made to the Graduate School of the University of Minnesota for a grant which enabled us to carry out this study.

Summary

When dealing with mixtures of two electroreducible substances the diffusion current of the second constituent should be found by subtracting from the total current (sum of i_{d_1} and i_{d_2}) the value which the first diffusion current i_{d_1} would have at the potential at which the total current is measured. Application of equation (7) using the values of r. v. given in the last column of Table I yields satisfactory results in such mixtures.

MINNEAPOLIS, MINN. RECEIVED APRIL 21, 1941

[CONTRIBUTION FROM THE MASSACHUSETTS INSTITUTE OF TECHNOLOGY]

Reaction of Olefins with Solid Cuprous Halides

BY E. R. GILLILAND, H. L. BLISS AND C. E. KIP

Experimental results on the reaction between the olefins, ethylene, propylene, and isobutylene and solid cuprous halides have been reported by Gilliland, Seebold, FitzHugh and Morgan.[1] They found that the olefins combined mole for mole with solid cuprous halides yielding a compound which could be written empirically as $C_nH_{2n}\cdot CuX$. The equilibrium pressure curves for the various olefin compounds with solid cuprous chloride were almost identical and it was therefore concluded that the reaction was characteristic of the unsaturated structure and not markedly influenced by molecular weight.

The work reported in this paper summarizes the results obtained on the reaction of diolefins and acetylene with solid cuprous halides.

Experimental

Procedure.—The apparatus used and the procedure followed were essentially the same as described for the low pressure studies in the previous work.[1] Pressure readings were considered accurate within 1 mm. and temperature readings within 0.5°. The low equilibrium pressures were the least accurate both because of an extremely slow rate of approach to equilibrium and because they were calculated by taking small differences between large numbers.

It was found that acetylene would not react directly with solid cuprous halides. The addition compounds however, were easily prepared in solution, following the procedure given by Chavastelon.[2] For example, with cuprous chloride, the procedure was as follows: cuprous oxide was dissolved in 38% hydrochloric acid and acetylene bubbled through the solution. Dilution with water gave a white precipitate, which was removed, washed with alcohol and ether and dried in a current of acetylene. A purple color indicated formation of the complex.

Butadiene was obtained from the Standard Oil Development Company and was estimated to be over 98% pure. Isoprene, purity about 74% (the rest being pentanes and pentenes), was obtained from the United Gas Improvement Company. It should be noted that in both cases the reaction with cuprous chloride proved to be a very simple and effective method of purification. Cyclopentadiene of a high degree of purity was prepared by depolymerization of dicyclopentadiene obtained from the United Gas Improvement Company.

Results

The results show that such solid reactions are not limited to the mono-olefins. One mole of butadiene reacts with two moles of cuprous chloride (CuCl) yielding a compound which may be empirically represented as $C_4H_6\cdot 2CuCl$. Isoprene forms a compound with cuprous chloride, but the maximum reaction observed was 0.336 mole of isoprene per mole of cuprous chloride. This ratio would correspond approximately to the formula $C_5H_8\cdot 3CuCl$, although the low isoprene content may be due to the fact that reaction was not complete. From the similarity of the unsaturated structure of isoprene and butadiene and from the heat of reaction data it seems probable that the compound may be $C_5H_8\cdot 2CuCl$. This behavior is similar to that of isobutylene reported in the previous work,[1] where it was found that only 0.62 mole of isobutylene reacted per mole of salt while both ethylene and propylene reacted practically mole for mole. No reaction of cyclopentadiene with cuprous chloride at 0° could be detected, and this may also be due to steric hindrance.

Koblyanskii, Shul'ts, and Piotrovskii[3] have investigated the composition of the complex compounds formed by the reaction between CuCl and butadiene. They report the preparation of the compound $C_4H_6\cdot 2CuCl\cdot 4H_2O$ from aqueous solution as a finely crystalline, yellow precipitate. From an anhydrous medium or by washing the hydrate with alcohol an anhydrous compound $C_4H_6\cdot 2CuCl$ was obtained.

Christgau[4] studied the reaction of butadiene

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[1] Gilliland, Seebold, FitzHugh and Morgan, THIS JOURNAL, **61**, 1960 (1939).

[2] Chavastelon, *Compt. rend.*, **126**, 1810 (1898).

[3] Koblyanskii, Shul'ts and Piotrovskii, *Trudy Gosudarst. Opyt. Zavoda Sintet. Kauchuka*, Litera B, IV; *Synthetic Rubber*, 20–23 (1935).

[4] Christgau, M. I. T. Thesis (1939).