Dawson for his help and constructive criticism in preparing the material for publication.

Summary

1. A method is described for the preparation of the enzyme, laccase, from the wild mushroom, *Russula foetens*. A typical preparation has a Q_{O_2} of 3000.
2. A measurement of units using hydroquinone as the substrate is outlined. Common plant oxidases do not appear to interfere in this procedure.
3. The unusual stability of the laccase preparations over a wide *p*H range is demonstrated.
4. The optimum *p*H for measurement of laccase activity is shown to be in the vicinity of *p*H 6.0.
5. The enzymatic oxidation of hydroquinone, *p*-phenylenediamine, catechol, dimethylcatechol, and potassium ferrocyanide has been described.
6. The behavior of the laccase preparations is contrasted when possible with tyrosinase (*campestris*).
7. The importance of substrate concentration in studying the behavior of the enzyme has been emphasized.

NEW YORK, N. Y. RECEIVED MARCH 4, 1940

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF WASHINGTON]

The Ionization Constant of Monoethanolammonium Hydroxide at 25° from Electrical Conductance Measurements

BY V. SIVERTZ, R. E. REITMEIER AND H. V. TARTAR

In this investigation use has been made of the work of several investigators[1-5] who have shown that accurate conductance measurements of solutions of weak electrolytes are adequate for the determination of limiting equivalent conductance and of thermodynamic ionization constants. The method finally developed is based upon the solution of three simultaneous equations. The first is

$$K = \frac{Cx^2f^2_\pm}{1 - x} = K'f^2_\pm$$

where K and K' are the thermodynamic and stoichiometric ionization constants, respectively, x is the degree of dissociation, C is the concentration of base in equivalents per liter of solution and $f_\pm$ is the mean activity coefficient of the ions. The activity coefficient of the undissociated base is assumed to be unity. The second equation is

$$-\log f_\pm = a\sqrt{Cx}$$

where a is the Debye and Huckel coefficient. Lastly

$$x = \frac{\Lambda}{\Lambda_0}\left[1 + \frac{(\alpha\Lambda_0 + \beta)}{\Lambda_0}\sqrt{Cx}\right]$$

where α and β are the Onsager coefficients.

The authors have determined the electrical conductance at 25° of aqueous solutions of monoethanolammonium hydroxide. Since we were unable to obtain from the literature an accurate value for the equivalent conductance of the hydroxyl ion, the conductances of sodium and potassium hydroxide solutions have also been measured over a limited range of concentrations.

Experimental

Apparatus and Technique.—A Leeds and Northrup conductivity bridge[6,7] was used. The conductance cell, used with nitrogen, was similar in design to a cell described by Shedlovsky[8] and made of Pyrex glass. The electrode chamber met the specifications given by Jones and Bollinger.[9] Because of the magnified effects due to adsorption at very dilute concentrations, platinization was limited to the minimum value to prevent polarization. The electrodes were given a light gray coating, using the procedure suggested by Jones and Bollinger.[9] The cell constant at 25° was of the order of 0.26228 and was determined by comparison with a cell which had been calibrated with 0.01 demal potassium chloride, the specific conductance of which was taken from the recent work of Jones and Prendergast.[10] The cell was cleaned before each run with redistilled alcohol and ether and dried under nitrogen. This procedure produced no undesirable effects either on the character of the platinized electrodes or in the operation of the cell.

The current was supplied to the bridge by a Leeds and Northrup a. c. oscillator, the frequency of which could be varied in steps over a range of 500 to 2000 cycles. Several trials in each series of measurements showed that the conductance was essentially independent of the frequency. A Leeds and Northrup two-stage amplifier and telephone formed the detector circuit.

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(1) MacInnes and Shedlovsky, THIS JOURNAL, **54**, 1429 (1932).

(2) Fuoss and Kraus, *ibid.*, **55**, 476 (1933).

(3) Shedlovsky and Uhlig, *J. Gen. Physiol.*, **17**, 549 (1934).

(4) Fuoss, THIS JOURNAL, **57**, 488 (1935).

(5) Shedlovsky, *J. Franklin Inst.*, **255**, 739 (1938).

(6) Dike, *Rev. Sci. Instruments*, **2**, 379 (1931).

(7) Jones and Josephs, THIS JOURNAL, **50**, 1049 (1928).

(8) Shedlovsky, *ibid.*, **54**, 1411 (1932).

(9) Jones and Bollinger, *ibid.*, **53**, 411 (1931).

(10) Jones and Prendergast, *ibid.*, **59**, 731 (1937).

The temperature of the oil thermostat, controlled to ±0.001° by a Thyratron relay, was established by a thermometer certified by the Bureau of Standards.

All solutions were made up by weight. An analytical balance accurate to 0.1 mg. was used for the more accurate weighings, and a large balance which was read to 1 mg. was used for weighing the water and solutions. The weights were calibrated against a set standardized by the Bureau of Standards.

Materials.—The water used in the preparation and dilution of solutions had a specific conductance of 5.0 (10^{-7}) mho. Since the measurements were made on alkaline solutions, it was necessary to know the carbon dioxide content of the water used. The conductivity water was drawn into a weight pipet, paraffined with material which had been specially purified, and nitrogen, free from carbon dioxide, was passed through for sixty minutes. This procedure gave water with a constant specific conductance which was assumed to be due to the presence of residual carbon dioxide and the specific conductance of pure water. The water correction was made accordingly, using the method suggested by Kolthoff,[11] and by Jeffery and Vogel.[12]

The monoethanolamine was from two sources and, after fractional crystallization and distillation, had a boiling point of 171.1° at 760 mm. pressure and a melting point of 11.52°. The density was found to be 1.0117 at 25° and the refractive index measured with an Abbé refractometer was 1.4539 at 20°. As a check on its purity, the monoethanolamine was analyzed by potentiometric titration against hydrochloric acid and by weight titration using methyl orange indicator. Several titrations gave the purity as 99.97% by the potentiometric titration and 99.96% by the weight titration. The standard hydrochloric acid used was a carefully prepared constant boiling mixture using the data of Foulk and Hollingsworth.[13] As a check on its standardization, the chloride content was determined by precipitation as silver chloride.

Paraffin was purified by shaking the melted material with hot concentrated sodium hydroxide. It was then leached thoroughly with conductivity water until the conductance of the wash water reached that of the original.

The solutions of monoethanolammonium hydroxide were prepared from monoethanolamine that was freshly distilled under nitrogen, care being taken that it did not come in contact with carbon dioxide at any time.

The stock solutions of sodium hydroxide were prepared by two methods. One solution was prepared by permitting pure metallic sodium to react with carbon dioxide-free conductivity water in a paraffined flask. The metallic sodium was cut under benzene, transferred under an atmosphere of nitrogen to the paraffined flask and the reaction permitted to take place under nitrogen. A second stock solution was prepared from sodium amalgam prepared by the electrolysis of a solution of c. p. sodium chloride over pure mercury. The stock solution of potassium hydroxide was prepared in a similar manner from c. p. potassium chloride. All solutions were stored in Pyrex bottles lined with specially purified paraffin and were maintained carbon dioxide-free. These stock solutions were standardized by titrating potentiometrically against hydrochloric acid and by weight titration using methyl red indicator.

The monoethanolammonium chloride solutions were prepared by neutralizing standard hydrochloric acid (to *p*H of 7.5) with monoethanolammonium hydroxide. It was necessary to know the *p*H of these solutions in order to make the proper correction for the trace of excess monoethanolammonium hydroxide, a procedure adopted to control hydrolysis.[8] The measurement was made electrometrically using a Leeds and Northrup *p*H meter that had been checked against standard *p*H buffers over the range used.

Results

Table I gives the data for monoethanolammonium hydroxide. The columns are, respectively, the concentration in equivalents per liter,

Table I

Equivalent Conductance and Ionization Constant of Monoethanolammonium Hydroxide at 25°, Λ_0 = 239.79

Concn. $C \times 10^3$	Λ	x	$f_\pm$	$K' \times 10^5$	$K \times 10^5$
0.22761	74.874	0.31351	0.9902	3.259	3.195
.26771	70.087	.29351	.9897	3.265	3.198
.38478	60.123	.25190	.9886	3.264	3.190
.49003	54.142	.22693	.9878	3.264	3.185
.77751	44.163	.18523	.9861	3.274	3.184
1.0182	39.072	.16395	.9851	3.274	3.177
1.2754	35.104	.14735	.9842	3.248	3.146
2.2535	26.995	.11344	.9815	3.271	3.152
2.6870	24.931	.10481	.9806	3.297	3.171
3.6628	21.550	.09065	.9790	3.310	3.173
3.9557	20.680	.08701	.9786	3.280	3.141
5.3468	17.947	.075564	.9768	3.303	3.152
6.4121	16.462	.069341	.9757	3.313	3.154
6.5739	16.204	.068261	.9756	3.288	3.129
7.5866	15.170	.063929	.9747	3.313	3.147
7.6417	15.122	.063729	.9746	3.315	3.149
9.0240	13.918	.058681	.9735	3.301	3.130
9.2932	13.705	.057787	.9734	3.294	3.121
13.546	11.438	.048282	.9706	3.318	3.126
14.334	11.132	.046999	.9702	3.323	3.127
16.759	10.279	.043419	.9690	3.303	3.102
26.015	8.297	.035100	.9654	3.322	3.096
26.063	8.326	.035223	.9653	3.352	3.123
30.603	7.667	.032455	.9639	3.332	3.096
33.872	7.310	.030956	.9626	3.350	3.106
36.033	7.062	.029911	.9625	3.323	3.079
45.308	6.335	.026858	.9602	3.359	3.097
57.955	5.586	.023707	.9577	3.337	3.060
60.745	5.472	.023229	.9572	3.356	3.074
81.261	4.7250	.020084	.9540	3.345	3.045
89.213	4.4990	.019132	.9530	3.329	3.024
89.486	4.4999	.019135	.9529	3.341	3.034
109.014	4.0642	.017300	.9506	3.321	3.001
122.92	3.8096	.016225	.9493	3.290	2.964
162.97	3.2949	.014055	.9457	3.265	2.921
198.47	2.9453	.012576	.9434	3.179	2.830
199.75	2.9453	.012577	.9432	3.200	2.847
326.83	2.2577	.009668	.9366	3.085	2.706

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[11] Kolthoff, *Rec. Trav. Chim.*, **48**, 664 (1929).

[12] Jeffery and Vogel, *Phil. Mag.*, **15**, 395 (1933).

[13] Foulk and Hollingsworth, This Journal, **46**, 1220 (1923).

the equivalent conductance, the degree of dissociation, the mean activity coefficient of the ions, the stoichiometric ionization constant and the thermodynamic ionization constant. This table is based on a Λ_0 value obtained from the conductance of the strong electrolytes, sodium and potassium hydroxides, by the additive method.

Equivalent conductances of sodium and potassium hydroxides and monoethanolammonium chloride over a limited concentration range are given in Tables II, III and IV, respectively.

TABLE II

EQUIVALENT CONDUCTANCE OF SODIUM HYDROXIDE AT 25°

Concn. $C \times 10^3$	Λ	Concn. $C \times 10^3$	Λ
0.25339	246.34	1.0034	244.94
.26417	246.14	1.7954	243.58
.29973[a]	246.11	2.7419	242.67
.31230	246.11	3.2893	242.27
.57934[a]	245.04	3.8729	241.65
.75295	244.83	5.8921[a]	240.12
.77058	244.92	8.5340	238.82
.80892[a]	245.00	10.211	237.93
.81373[a]	245.22	10.513	237.85

$\Lambda_0 = 247.83$

[a] The values from conductance measurements made on the stock solution prepared from sodium amalgam.

TABLE III

EQUIVALENT CONDUCTANCE OF POTASSIUM HYDROXIDE AT 25°

Concn. $C \times 10^3$	Λ	Concn. $C \times 10^3$	Λ
0.15581	269.60	1.2503	267.60
.17545	269.51	1.4395	267.44
.19920	269.34	2.3630	266.32
.23780	269.68	3.5242	265.41
.32192	269.18	4.4166	264.48
.34780	268.90	5.9956	263.57
.58624	268.67	9.3414	261.68
.79877	268.22	11.165	260.90
1.0525	267.84		

$\Lambda_0 = 270.94$

TABLE IV

EQUIVALENT CONDUCTANCE OF MONOETHANOLAMMONIUM CHLORIDE AT 25°

Concn. $C \times 10^3$	Λ	Concn. $C \times 10^3$	Λ
0.28495	117.55	7.7008	111.34
.77892	116.44	7.7412	112.11
.88910	116.38	12.492	109.97
1.0842	116.03	13.068	109.98
1.4052	115.51	19.821	107.84
2.1825	114.83	22.726	106.73
2.6130	114.55	62.597	101.21
3.0936	114.52	109.010	97.21
3.7512	113.56	112.110	96.90
4.3850	113.38	116.120	96.46
6.7983	112.54		

$\Lambda_0 = 118.58$

The limiting conductance values of potassium and sodium hydroxides and monoethanolammonium chloride were determined by using Shedlovsky's method of extrapolation in which $1/\Lambda$ is plotted against $\sqrt{C}$. This gives $\Lambda_0 = 247.83$ for sodium hydroxide, $\Lambda_0 = 270.94$ for potassium hydroxide and $\Lambda_0 = 118.58$ for monoethanolammonium chloride. These values, combined with limiting conductance for the Na^+, K^+ and Cl^- as determined by MacInnes, Shedlovsky and Longsworth[14] (corrected to the Jones and Prendergast basis[11]), give the Λ_0 value for monoethanolammonium hydroxide which was used in Table I, as well as 197.71 and 197.41 for the limiting conductance of the hydroxyl ion, obtained from sodium hydroxide and potassium hydroxide, respectively.

The mean of the values of K in the concentration range below 0.0015 N gives 3.19×10^{-5}. Other weak electrolytes show a constant value of K below this concentration.[15,16] The values of K in this range show a variation to be expected with a weak base. It is improbable that this variation is due to the solution of glass, since we were able to confirm the findings of Jeffery and Vogel[12] and Wynne-Jones[17] that the change in conductance of the more concentrated solutions (0.005–0.01 N) was inappreciable over the period of time required for a series of measurements. It is more likely that this variation results from fluctuations in the carbon dioxide content of the water used in preparing solutions, *i. e.*, a failure to comply strictly with the assumption made earlier in this paper regarding constancy of carbon dioxide content of the water.

Table I also shows a tendency for the constant, K, to decrease as the concentration of the monoethanolammonium hydroxide increases. This decrease is expected due to the assumption made that the activity of the undissociated base is unity.

Summary

1. Conductances of sodium and potassium hydroxides, of monoethanolammonium chloride and of monoethanolammonium hydroxide have been measured over a limited range of concentrations at 25°.

2. A new value for the limiting conductance of hydroxyl ion is reported.

3. From these measurements the thermody-

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(14) MacInnes, Shedlovsky and Longsworth, THIS JOURNAL, **54**, 2758 (1932).

(15) Shedlovsky, *ibid.*, **54**, 1429 (1932).

(16) Martin and Tartar, *ibid.*, **59**, 2672 (1937).

(17) Wynne-Jones, *J. Chem. Soc.*, 630 (1926).

namic ionization constant of monoethanolammonium hydroxide has been calculated and found to be 3.19×10^{-5} at 25°.

SEATTLE, WASHINGTON RECEIVED MARCH 5, 1940

[CONTRIBUTION FROM THE RESEARCH LABORATORY OF THE GENERAL ELECTRIC COMPANY]

Vapor Pressures of Nickel and of Nickel Oxide

BY H. L. JOHNSTON* AND A. L. MARSHALL

Vapor pressures of copper and of iron, determined by measuring the rates of evaporation from metal rings heated inductively in a vacuum, were recently published from this Laboratory.[1] We have completed a similar investigation with nickel between the temperatures of 1300 and 1600°K., and have also determined vapor pressures of nickel oxide, by measuring rates of nickel and of oxygen loss from nickel rings coated with a thin film of the oxide. From these data, and thermodynamic and quantum-statistical data on both the solid and gaseous forms of nickel and of nickel oxide, we have computed their heats of sublimation and have derived vapor pressure formulas.

Vapor pressures, p, are computed from the measured rates of evaporation, m, by use of the relationship[1,2]

$$\log p + \log \alpha = \log m - {}^1/_2 \log M + {}^1/_2 \log T - 1.647 \qquad (1)$$

where p is in atmospheres; m is in grams per square centimeter per second; M is the molecular weight in the vapor phase; T the absolute temperature; and α, termed the *accommodation coefficient*, is a measure of the efficiency with which molecules that strike the equilibrium surface from the vapor phase are condensed. The accommodation coefficient is unity if condensation results at every collision.

As with copper and with iron, the results with nickel establish this coefficient as unity and support the general validity of the Langmuir method in its application to metals. Vapor pressures, and the accommodation coefficient, could not be determined for the oxide with the same accuracy as for the metal, but the accommodation coefficient appears to be unity within the limits of experimental error.

Nickel

Experimental.—Apparatus and procedure were described previously.[1] Except as noted below the same description applies here.

The nickel used, in all but one of the runs, was electrolytic in origin and had been vacuum melted in an alundum crucible. Spectrographic analysis indicated the absence of any impurity, in significant amount, other than cobalt. The latter was present to the extent of 0.64% in the original specimen and, within limits of a colorimetric method employed for the analysis, this percentage remained unchanged in the evaporated film.[3] The results obtained in a single (subsequent) run[4] with a ring turned from pure carbonyl nickel[5] were undistinguishable from those obtained with the electrolytic nickel.

Dimensions of the nickel ring turned from the vacuum ingot were: outer diameter, 2.23 cm.; inner diameter, 1.13 cm.; thickness, 0.63 cm.; total surface, 12.46 sq. cm. Correction for condensation of atoms which strike the inner surface of the ring reduces the effective surface area to 11.74 sq. cm.

Evaporation losses were determined by weighing the approximate 16 g. ring on a sensitive balance. Weighings were reproducible to within 0.02 milligram.

Temperatures were determined by setting the filament of the optical pyrometer on two holes, 1.2 mm. in diameter, drilled vertically into the face of the specimen to a depth of about four millimeters. Measurements on a specimen drilled with holes of 0.6 mm., 1.2 mm. and 1.8 mm. diameter proved that the emissivity from the 1.2 mm. hole was indistinguishable from that from the 0.6 mm. hole to the highest temperature to which measurements were carried, 1600°K., and was therefore the equivalent of black body radiation. On the other hand, the emissivity from the 1.8 mm. hole was definitely less than black body at 1100° and yielded brightness temperatures more than twenty degrees in error at 1600°.

Temperatures determined as described above were normally reproducible within limits of approximately three degrees (each determination the average of five to ten successive observations). This corresponds to limits of approximately 10% on individual vapor pressure determinations. Temperatures were taken continuously during

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* Now at Ohio State University, Department of Chemistry.

(1) Marshall, Dornte and Norton, THIS JOURNAL, **59**, 1161 (1937).

(2) Langmuir, *Phys. Rev.*, **2**, 329 (1913).

(3) The influence of this amount of cobalt on the measurements of the evaporation of nickel must lie well within the accuracy of the latter. The observation that the composition of the evaporated metal is identical with that of the original specimen within limits of the analytical error indicates that vapor pressures of cobalt and of nickel are nearly equal, which is in agreement with the data of Ruff and Bormann (*Z. anorg. Chem.*, **88**, 386, 397 (1914)) on liquid nickel and cobalt.

(4) Run M in the tables. This run was made by Dr. F. J. Norton and Dr. R. W. Dornte.

(5) The metal for this ring was furnished through the courtesy of the International Nickel Co. Its composition was stated to be: 99.89% nickel, 0.048% iron, 0.035% carbon, 0.014% copper and 0.008% sulfur.