[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CALIFORNIA, BERKELEY]

Chemistry of Ru(VI), -(VII) and -(VIII). Reactions, Oxidation Potentials and Spectra

BY ROBERT E. CONNICK AND C. ROBERT HURLEY[1]

RECEIVED APRIL 10, 1952

Aqueous solutions of perruthenate ion, RuO_4^-, were prepared and the absorption spectrum measured. The oxidation state was established by oxidation–reduction reactions involving ruthenate and perruthenate ions. The E^0 value of the couple $RuO_4^{=}$-RuO_4^- is -0.59 volt. The equilibrium between $RuO_2 \cdot xH_2O$, $RuO_4^{=}$ and RuO_4^- was measured and the standard basic potential for the couple $RuO_2 \cdot xH_2O$-RuO_4^- was calculated to be -0.35 volt. The spectra of RuO_4 and $RuO_4^{=}$ were determined.

During the last thirty years practically no new information has been obtained on the aqueous solution chemistry of ruthenium in the +6, +7 and +8 oxidation states. The one exception is the work of Silverman and Levy[2] which was done concurrently with the research reported here and which will be referred to frequently. The older literature is well summarized in Gmelin.[3]

Ruthenium in these oxidation states in aqueous solution exists only as oxygenated ions or molecules, as contrasted with the lower oxidation states which form a variety of complex ions. The +8 state is well characterized as the volatile tetroxide, RuO_4, which has a melting point of 25° and a solubility of 0.13 mole per liter in water at 25°. The +6 state is unstable in acid with respect to disproportionation to the +4 and +8 states. In alkaline solution it exists as the ion $RuO_4^{=}$.

The +7 state is known in the solid compounds $NaRuO_4 \cdot H_2O$ and $KRuO_4$. It has not been well characterized in aqueous solution but is reported to be formed by the reducing action of sodium and potassium hydroxide solutions on RuO_4,[4] and by the oxidation of $RuO_4^{=}$ by chlorine.[5] In the present work the +7 state in solution was identified and its properties studied.

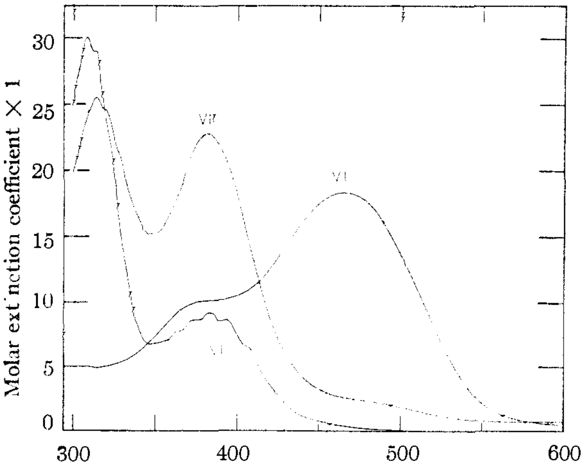

Fig. 1.—Molar extinction coefficients of Ru (VIII), -(VII) and -(VI) as a function of wave length.

+7 Oxidation State—Perruthenate Ion.—When an aqueous solution of ruthenium tetroxide is made alkaline, the absorption spectrum changes at a rate dependent on the concentration of hydroxide ion, the purity of the chemicals, the cleanliness of the containers and the presence or absence of added catalysts. The new species formed has the spectrum labeled VII in Fig. 1. (Those of RuO_4 and $RuO_4^{=}$ are labeled VIII and VI, respectively.) This substance could be ruthenium in the +7 oxidation state or an uncharacterized form of one of the other oxidation states, such as the basic form of ruthenium tetroxide, RuO_5H^-,[2] similar to OsO_5H^-.[6]

It was shown that the new species could be reduced by hydrogen peroxide to $RuO_4^{=}$ by a reaction involving less than a two electron change. Apparently catalytic decomposition of the peroxide occurred as a side reaction. The new species could also be produced by oxidation of $RuO_4^{=}$ by hypochlorite ion with less than a two electron change. The exact stoichiometry could not be measured because the oxidation by ClO^- was slow and the perruthenate ion was simultaneously, but at a lower rate, reduced by water. This combination of reactions leads to the oxidation of water (or hydroxide ion) by hypochlorite ion, catalyzed by ruthenium

$$H_2O + 2RuO_4^{=} + ClO^- = 2RuO_4^- + Cl^- + 2OH^- \quad (1)$$

$$2RuO_4^- + 2OH^- = 2RuO_4^{=} + {}^1/_2O_2 + H_2O \quad (2)$$

The results indicate that the new species is the +7 oxidation state of ruthenium.

In addition it was found that excess hypochlorite ion oxidized, at least partially, RuO_4^- to RuO_4 in alkaline solution. Further proof of the identity of RuO_4^- was obtained from e.m.f. measurements (see next section).

Experimental.—To three separate portions of a 3.1×10^{-4} M perruthenate solution in 1 M sodium hydroxide were added varying amounts of hydrogen peroxide and the solutions were analyzed spectrophotometrically. In all cases the reaction was complete by the time the analysis was performed, which was approximately ten minutes. When the moles of hydrogen peroxide per mole perruthenate ion were 0.35, 0.53 and 1.04, the percentages of the perruthenate reduced to ruthenate were 46, 65 and 73, respectively. For a one electron reduction step these latter figures should have been 70, 100 and 100%, while for a two electron reduction they would be 35, 53 and 100%. Any catalytic decomposition of the hydrogen peroxide would produce a high apparent electron change and therefore the one electron change is presumed to be correct. That catalytic decomposition of the peroxide occurs is shown by the third experiment where reduction to the +6 state was incomplete even though there was sufficient peroxide for a two electron change.

---

(1) From the thesis of C. Robert Hurley, submitted to the University of California, August, 1950.

(2) M. D. Silverman and H. A. Levy, Oak Ridge National Laboratory Report—ORNL-746, Series A, Nov. 13, 1950.

(3) "Gmelin's Handbuch der anorganischen Chemie," 8 Auflage, Verlag Chemie, Berlin, 1938.

(4) H. Debray and A. Joly, *Compt. rend.*, **106**, 1494 (1888).

(5) H. Sainte-Claire Deville and H. Debray, *ibid.*, **83**, 926 (1876).

(6) D. M. Yost and R. J. White, THIS JOURNAL, **50**, 81 (1928).

The reduction of RuO_4^- by water under the above conditions was too slow to affect the above results. It is conceivable however that this reduction is induced by the peroxide–RuO_4^- reaction. That such is not the case is proven by the third experiment where, even with an excess of peroxide, not all the RuO_4^- was reduced.

A stock solution approximately 0.1 M in sodium hydroxide, 4.3 × 10^{-4} M in ruthenate and 0.7 × 10^{-4} M in perruthenate (the solution was slowly converting to 100% ruthenate) was treated with 0.45 mole of ClO^- per mole of $RuO_4^=$. The spectrum was measured immediately after mixing, 14 minutes later and 53 minutes later. During the first 14 minutes perruthenate was forming. In the interval 14 to 53 minutes all the hypochlorite was used up and reduction of perruthenate by water was noticeable. This necessitates correcting for the reduction by water to obtain the total amount of ruthenate oxidized. However, even without correction, the observed ruthenate oxidized, 76%, is greater than can be obtained for a two electron oxidation, *i.e.*, 45%, thus indicating that the reaction involves a one electron change. Similar results were obtained in 0.25 M Na_3PO_4 solution and in phosphate solutions of *p*H 11.5. The *p*H measurements were made with a Beckman type E glass electrode. The reduction of RuO_4^- by water is possibly a heterogeneous reaction under the conditions used here and thus irreproducible.

Potential of the Ruthenate–Perruthenate Couple.

—The potential of the $RuO_4^=$–RuO_4^- couple was found by direct measurement at 25° to be $E^0 = -0.59$ volt. This confirms the value −0.6 volt estimated by Latimer[7] from the chemical behavior of ruthenium and agrees exactly with the value measured polarographically by Silverman and Levy.[2] Assuming ruthenate ion to be $RuO_4^=$, the absence of acidity dependence between *p*H 11.4 and 12.6 indicates that RuO_4^- is the correct formula of perruthenate ion in this acidity region. Silverman and Levy reached a similar conclusion.

Experimental.—Alkaline solutions of ruthenate ion were partially oxidized by hypochlorite ion to perruthenate ion and the potential measured using a Beckman *p*H meter and calomel reference electrode. A shiny platinum electrode in the ruthenium half-cell appeared to give better results than gold or tantalum. The potential changed several millivolts when the electrode was agitated, but quickly returned to a steady reading when the motion was stopped.

The perruthenate concentration increased to a maximum as the oxidation by hypochlorite took place, and then decreased slowly because of reduction by water. A portion of the cell solution was measured spectrophotometrically immediately following the cell reading.

The data are presented in Table I. The quantity E_f is the observed e.m.f. minus the saturated calomel potential (−0.246 volt) and corrected to equal concentrations of $RuO_4^=$ and RuO_4^-. The symbol $E°$ indicates the standard potential for the $RuO_4^=$–RuO_4^- couple, obtained by correcting E_f to zero ionic strength. Kielland's[8] equation (1) and a_i parameter for individual ionic activity coefficients were used, assuming $RuO_4^=$ and RuO_4^- to be similar to $SO_4^=$ and ClO_4^-, respectively.

Table I

Potential of the $RuO_4^=$–RuO_4^- Couple at 25 ± 1°

$RuO_4^=$, moles/ l. × 10^4	RuO_4^-, moles/ l. × 10^4	*p*H	μ^a	$-E_f$	$-E°$
4.8	1.00	11.50	0.34	0.618	0.589
2.21	3.8	11.43	.34	.614	.585
2.16	2.80	12.60	.15	.609	.586

^{a} μ = ionic strength. The first two solutions contained NaOH, Na_3PO_4 and K_2HPO_4; the third contained NaOH and Na_2CO_3.

The values of $E°$ agree well considering the experimental accuracy and the uncertainty in correcting for ionic strength. If it is assumed that the measured potential referred to a two electron change, the values of E_f found for the first two experiments at the same ionic strength are −0.598 and −0.621. The disagreement is outside experimental error and shows the oxidized species is the +7 state, not +8.

The Disproportionation of $RuO_4^=$ into RuO_4^- and $RuO_2 \cdot xH_2O$, and the Ru(IV)–Ru(VI) Potential.

—An alkaline solution of ruthenate ion, when acidified, reacts according to the equation

$$3RuO_4^= + (2 + x)H_2O = 2RuO_4^- + RuO_2 \cdot xH_2O + 4\ OH^- \quad (3)$$

This equilibrium was studied in order to determine the "stability" of $RuO_4^=$ and to obtain a value for the Ru(IV)–Ru(VI) potential.

The equilibrium was approached from both "sides." In one case an alkaline ruthenate solution was acidified with potassium dihydrogen phosphate to a *p*H where ruthenium dioxide and perruthenate formed; in the other case a perruthenate solution containing some dioxide was made more alkaline with sodium phosphate, and ruthenate appeared. On standing all solutions showed a steady decrease in the ruthenate and perruthenate concentrations, owing to reduction by water. It is assumed that this relatively slow reaction did not greatly disturb the equilibrium being studied. The concentrations of ruthenium species were determined by spectrophotometric analysis of the solutions after centrifugation.

The solid formed in the reaction is reported to be a hydrated dioxide.[9] A sample was thoroughly washed and dried for 3 hours at 140°. The X-ray diffraction pattern[10] was not that of RuO_2.[11] The sample was then heated to 700° in a stream of dry oxygen. The water which was collected amounted to 22% of the original weight; for conversion of $RuO_2 \cdot 2H_2O$ to RuO_2 the weight loss is 21.2%. The X-ray pattern of the strongly heated sample was that of RuO_2. From the above results it would appear that the solid formed in the equilibrium experiments is $RuO_2 \cdot 2H_2O$ or a higher hydrate.

The experimental data are given in Table II. In most cases more than one measurement was made on the same solution. The concentrations changed with time because of the reduction by water and the decrease in *p*H, presumably caused by carbon dioxide picked up from the air. In the last column is tabulated K' defined by

$$K' = \frac{(RuO_4^-)^2[OH^-]^4}{(RuO_4^=)^3}$$

where parentheses indicate concentrations in moles per liter and the brackets indicate the activity of OH^- calculated from the *p*H meter reading. The exponents of the ion concentrations are high, thus making K' sensitive to small errors in concentration and activity. The greatest uncertainty probably arose from the error in the *p*H measurements, caused by carbon dioxide and the absence of a

---

[7] W. M. Latimer, "The Oxidation States of the Elements and Their Potentials in Aqueous Solutions," Prentice-Hall, Inc., New York, N. Y., 1938.

[8] J. Kielland, This Journal, **59**, 1675 (1937).

[9] L. Wohler, P. Balz and L. Metz, *Z. anorg. Chem.*, **139**, 205 (1924).

[10] We are indebted to Professor D. H. Templeton and Dr. A. Zalkin for the X-ray analyses.

[11] R. W. G. Wyckoff, "Crystal Structures," Interscience Publishers, New York, N. Y., 1948.

buffer. The true equilibrium constant for reaction (3) is

$$K = \frac{K' \gamma^2{}_{\mathrm{RuO_4^-}}}{\gamma^3{}_{\mathrm{RuO_4^=}}\, \gamma^{2+x}_{\mathrm{H_2O}}}$$

It was assumed that $\gamma_{\mathrm{RuO_4^-}}$ and $\gamma_{\mathrm{RuO_4^-}}$ are equal to the individual activity coefficients of perchlorate and sulfate ions, respectively. Approximate values for the latter were estimated by using Kielland's[8] equation (1) and his a_i parameters. Taking $\gamma_{\mathrm{H_2O}} = 1$, it was calculated that the values of K' for the first three experiments of Table II should have been 1.7 times greater than for the last three. Averaging all the values corrected to zero ionic strength, except the second reading of the third experiment, gives $K = 6 \times 10^{-9}$ at 25°.

TABLE II

MEASUREMENT OF THE RuO_4^-–$RuO_4^=$–$RuO_2 \cdot xH_2O$ EQUILIBRIUM AT 25°

Solution no.[b]	RuO_4^-, moles/l. × 10^6	$RuO_4^=$, moles/l. × 10^5	pH	μ	Time from mixing (hr.)	K' × 10^{10}
1	0.78	0.98	10.55	0.20	19	1.0
	.77	0.48	10.40	.20	41	2.1
2	.62	1.45	10.87	.21	17	3.8
	.47	1.02	10.82	.21	35	3.9
3	.51	1.94	10.90	.21	17	1.4
	.48	0.86	10.82	.21	35	7
4[a]	.27	.43	10.50	.34	56	0.9
5[a]	.80	.46	10.31	.32	56	1.2
6	.24	1.15	10.83	.36	56	0.8
	.175	0.79	10.82	.36	74	1.2

[a] Solution measured twice previously, but $RuO_2 \cdot xH_2O$ suspended as colloid. [b] Equilibrium approached from $RuO_4^=$ side in first three experiments and from $RuO_2 \cdot xH_2O$–RuO_4^- side in last three. The dioxide in the latter case was formed by reduction of RuO_4^- by water.

The standard basic[7] potential for the following couple is obtained from the above equilibrium constant and the $RuO_4^=$–RuO_4^- potential

$$RuO_2 \cdot xH_2O + 4OH^- = RuO_4^= + 2e^- + (2 + x)H_2O$$
$$E^0_B = -0.35 \text{ volt}$$

Spectra.—Most analyses for ruthenium in the various oxidation states were made spectrophotometrically. The molar extinction coefficients, ϵ, as a function of wave length are given for Ru(VI), -(VII) and -(VIII) in Fig. 1. Data for the wave lengths used in the analyses are given in Table III.

TABLE III

MOLAR EXTINCTION COEFFICIENTS OF Ru(VI), -(VII) AND -(VIII) AT WAVE LENGTHS USED FOR ANALYSIS

Oxidn. state	4600 Å.	3850 Å.	3100 Å.
VI	1820	1030	..
VII	283	2275	2445
VIII	..	930	2960

Experimental.—The values of ϵ were measured with a Beckman quartz spectrophotometer, using cells thermostated at 25°.

Ru(VIII).—Anhydrous RuO_4 was prepared by distillation in a stream of nitrogen from a ruthenium sulfate solution containing potassium permanganate and dilute sulfuric acid. The volatile oxide was passed through a trap of anhydrous magnesium perchlorate and collected in a U-tube immersed in liquid nitrogen.[12] Two standardized solutions of RuO_4 were made up by dissolving weighed portions in dilute perchloric acid. To do this, capillaries were drawn from eight-inch Pyrex test-tubes and sealed on one end. The other end was drawn down to a much finer capillary for filling. The capillary was dried and weighed on a micro balance to ±5 micrograms. It was then heated slightly and the open end inserted into the RuO_4 which had been melted by warming in the U-tube. Cooling of the capillary caused RuO_4 to be drawn in. After reweighing, the capillaries were placed inside glass stoppered bottles nearly filled to the top with 200 ml. of 0.0114 *M* perchloric acid and were pulverized by shaking with glass stoppers inside. Volatility losses in the weighing and dissolving should have been negligible.

Samples of the solution were quickly pipetted into 1-cm. quartz cells and the light absorption measured. No change occurred during ten minutes. Rough tests of the transfer procedure indicated that the volatility loss should have been less than 1%.

The results from the two different RuO_4 stock solutions (1.957×10^{-4} and 2.947×10^{-4} *M* RuO_4) agreed to 1.1% at 310 mμ and 0.1% at 385 mμ. The molar extinction coefficients should be good to 2%. Our results appear to be approximately 5% higher than those of Wehner and Hindman[12] and 2% higher than those of Silverman and Levy.[2]

The above experiments show there is no large deviation from Beer's law. Another series designated to test this more stringently gave deviations ranging from 0 to 7.5% for tenfold dilution of *ca.* 3×10^{-4} *M* ruthenium tetroxide solutions. In every case the deviation was in the direction of a lower ϵ for the more dilute solution. The apparent deviation is probably not real but is caused by partial reduction of the RuO_4 to the colloidal Ru(IV) hydroxide. Increased absorption at 6000 Å. by the solutions which showed the largest deviation supports this view. The above experiments were performed in 0.08 *M* acetic acid or 0.011 *M* perchloric acid. Wehner and Hindman[12] reported that ruthenium tetroxide in 1 *M* perchloric acid does not strictly obey Beer's law, but they gave no data. Silverman and Levy[2] found no deviation at 310 and 385 mμ for 0.4 to 8 × 10^{-4} *M* ruthenium tetroxide in water.

The peak at 310 mμ is sharp and therefore may not have been completely resolved at the large slit widths used (*ca.* 0.3 mm.). To test this, the same solution was measured in a 10-cm. and a 2-cm. cell. The apparent molar extinction coefficient was several per cent. less for the shorter cell, which effect is in the direction opposite to that expected for incomplete resolution. The difference probably arose from volatility or reduction of the ruthenium. The effectiveness of resolution was further checked by varying the slit widths. For a solution with optical density 0.9, the reading changed less than 1% in the range 0.3 to 0.8 mm.

Ru(VII).—The same general procedure was used for determining the molar extinction coefficient of RuO_4^- as for RuO_4 (Table III). The capillary containing the RuO_4 was broken in a glass stoppered bottle containing 200 ml. of 0.001 *M* trisodium phosphate plus *ca.* 7×10^{-5} *M* sodium hypochlorite. The latter was added to remove any reducing impurities. In addition the bottle contained 10 mg. of powdered ruthenium metal which catalyzes the reduction of water by RuO_4 to produce RuO_4^-. The hypochlorite is destroyed by oxidation of RuO_4^- to RuO_4, while the RuO_4 rapidly oxidizes water in the presence of the metal catalyst to reform RuO_4^-. The concentration of hypochlorite was too small to give a measurable light absorption. A blank experiment showed that the hypochlorite did not attack the metal.

The solution was centrifuged to remove the powdered metal before measuring the absorption. The optical density remained constant over a period of several days, showing that the hypochlorite had been destroyed within a few minutes after mixing and that a solution of RuO_4^- is comparatively stable under these conditions. There is of course no volatility loss from perruthenate solutions.

Values of the molar extinction coefficient from two separate solutions (3.652×10^{-4} and 3.416×10^{-4} *M*) agreed to 0.6% at 310 and 385 mμ. The spectrum shown in Fig. 1 agrees in general shape with that obtained by Silverman and Levy[2] by dissolving potassium perruthenate in alkaline solution, but their curve is considerably lower because of partial reduction.

Ru(VI).—Three samples of one of the RuO_4^- solutions used in the molar extinction coefficient measurements were

---

[12] P. Wehner and J. C. Hindman, THIS JOURNAL, **72**, 3911 (1950).

made 1 *M* in sodium hydroxide. To these were added a 300-fold, 150-fold and 75-fold excess of hydrogen peroxide, respectively, over the amount required to reduce a 3.40 × 10^{-4} *M* RuO_4^- solution to $RuO_4^=$. The absorption spectrum of the $RuO_4^=$ formed by reduction was measured on the Cary Spectrophotometer in the first two cases and at selected wave lengths on the Beckman in the third case. The absorption spectra were the same within experimental error when corrected for differences in dilution. Hydrogen peroxide in alkaline solution has an absorption band which becomes significant at 370 mμ at the concentrations used in these experiments. This is below the wave length measured (Table III). The values given in Table III are believed to be accurate to within 4%. The spectrum of $RuO_4^=$ shown in Fig. 1 was obtained with solutions containing no peroxide.

To verify that the spectrum of only a single oxidation state was being measured, powdered zinc was added to one solution and the spectrum followed for several days. A black precipitate formed and the absorption of the solution decreased, but the shape of the curve remained the same.

Marshall and Rickard[13] and Silverman and Levy[2] have reported the molar extinction coefficient of $RuO_4^=$ at 465 mμ to be 1742 ± 12, and 1735, respectively. Their spectra show a distinct maximum at 375 mμ, indicating the presence of some RuO_4^- in their $RuO_4^=$ solutions. This may account for deviations from Beer's law and the lower molar extinction coefficients found by the former authors.

It has been reported[13] that ruthenate solutions could not be kept without change in composition and precipitation of hydrated ruthenium dioxide, even in 2 *M* potassium hydroxide solutions. We have had orange solutions of potassium ruthenate in approximately 1 *M* sodium hydroxide which have shown no signs of precipitation in more than one year.[14] Here, as with the +7 and +8 oxidation states, the reduction of ruthenium appears to be very sensitive to minute traces of impurities.

---

(13) E. D. Marshall and R. R. Rickard, *Anal. Chem.*, **22**, 795 (1950).

(14) See also ref. (2), p. 5.

BERKELEY, CALIFORNIA

[CONTRIBUTION FROM THE GIBBS CHEMICAL LABORATORY, HARVARD UNIVERSITY]

The Effects of Electrolytes on Urease Activity[1]

BY G. B. KISTIAKOWSKY, PAUL C. MANGELSDORF, JR., ARTHUR J. ROSENBERG AND WILLIAM H. R. SHAW

RECEIVED APRIL 4, 1952

A sensitive quantitative analytical procedure for the determination of ammonia has been developed. The ammonium ion is separated from interfering substances by ion-exchange, and its concentration is determined photometrically after nesslerization. The effect of enzyme concentration, *p*H and added electrolytes on the catalytic activity of urease has been studied. Measurements were made in the presence of various added salts utilizing a number of buffer systems. Over a range of more than 10^3, the activity of the enzyme is strictly proportional to its concentration. Below *p*H 7, over the thousand-fold concentration range studied, sodium maleate buffers and other added salts are without effect on the activity of urease. At *p*H 7 and 7.5 the enzymic activity decreases slightly with increasing electrolyte concentration. This effect is not specific for a large number of ions. Its interpretation as a salt effect is consistent with the data. Specific inhibition of the enzyme was caused by several other salts. It is suggested that the real inhibitory substances in phosphate buffers may be complexes formed by the alkali cations with the various buffer species. This explains the different behavior of the enzyme in sodium and potassium phosphate buffers as observed by others.

It is well known that the activity of urease is sensitive to changes in *p*H and that several electrolytes are inhibitory. No conclusive evidence exists[2] that urease requires activators for its functioning, as do certain other enzymes.

Harmon and Niemann[3] have shown that phosphate buffers inhibit urease competitively. In a more recent publication Fasman and Niemann[4] concluded that phosphate ions activate this enzyme, and that the inhibition is due to sodium and potassium ions. Their concept of a complex pattern of simultaneous activation and inhibition by the several components of a buffer, including the alkali ions, makes a quantitative treatment of urease kinetics most difficult if not impossible. A kinetic study, undertaken in this Laboratory, made it necessary to determine whether or not the components of the buffers used affect the activity of urease in the complex manner suggested by Fasman and Niemann.

We are not concerned here with the behavior of urease in the total absence of ions, nor with the effects on it of traces of ions. The objective of these experiments is to demonstrate whether or not there exists a "plateau" of ureolytic activity in electrolyte solutions. If the specific activity is virtually unaffected by large changes in concentration and kind of ions present, as well as by changes in the concentration of the enzyme itself, then the effects of *p*H, substrate and inhibitor concentrations, temperature, etc., should be interpretable by the standard kinetic procedures.

Experimental Details

The procedure consisted of rapidly mixing in a thermostat at 25° a small volume (0.5 cc.) of buffered urease and a buffered solution of urea (25 cc.), adding strong acid after a known time interval, and determining the ammonia formed. The thermostat was controlled to 0.01° and extremely fast mixing was obtained with the aid of glass-enclosed magnetic stirrers, so that reaction times as short as 60 sec. were reproducibly obtained. The analytical procedure (developed by A. J. R.) consisted of the following steps: (1) neutralization of the acidified reaction mixture; (2) absorption of the ammonium ion on Dowex 50 resin contained in an ion-exchange column; (3) washing the resin with ammonia-free water; (4) elution of the ammonium ion with 0.02 *N* sodium hydroxide; (5) Nesslerization of the resultant solution; and (6) determination of the optical density of the Nesslerized samples with a Lumetron photoelectric colorimeter after a 15-min. waiting period. The absolute amount of ammonium ion was obtained by comparison with a calibration chart derived from the Nesslerization of known quantities of ammonium ion. Thus the method is similar to that

---

[1] This work was made possible by grants from the American Cancer Society and from the Rockefeller Foundation, to both of which the authors wish to express their sincere gratitude.

[2] J. B. Sumner and K. Myrback, "The Enzymes," Academic Press, Inc., New York, N. Y., 1951.

[3] K. M. Harmon and C. Niemann, *J. Biol. Chem.*, **177**, 601 (1949).

[4] G. D. Fasman and C. Niemann, THIS JOURNAL, **73**, 1646 (1951).