it does with manipulations of aliquot portions and titrations.

Combined with the previous data at 35°, the new rate constant permits an estimate of the activation energy for the dissociation in 0.835 molar sulfuric acid, $\Delta E = 17$ kcal./mole at 30°.

Summary

The partial pressure of isobutene over dilute solutions in dioxane containing 0.835 molar sulfuric acid was measured by a static method in a Van Slyke instrument at 25°. In its dependence on the concentration, the pressure adheres closely to the ideal law.

The data, applied to the acid-catalyzed reversible dissociation of *t*-butyl benzoate into benzoic acid and isobutene in dioxane solution, provide a basis for manometric kinetic studies and give a first-order rate constant for this reaction. This is in good agreement with the values obtained acidimetrically under reversible and under irreversible conditions.

The activation energy for this reaction is approximately 17 kcal./mole at 30°.

BRONXVILLE 8, NEW YORK

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[CONTRIBUTION NO. 50 FROM THE CHEMISTRY DEPARTMENT OF THE UNIVERSITY OF TENNESSEE]

The Salts of Perrhenic Acid. I. The Alkali Metals and Ammonium[1]

BY WM. T. SMITH, JR., AND SELMA HARMON LONG

Although the perrhenates of many of the metals have been prepared, in most cases the physical properties of these salts have been determined only approximately if at all. Of the perrhenates of the alkali metals and ammonium the potassium salt has been most thoroughly characterized, apparently due to the fact that this salt is precipitated in a common method for the recovery of rhenium[2] and that it is easily purified by recrystallization from water. The melting point,[3,4,5,6] boiling point,[6] solubility,[6,7,8,9] heat of solution[9,10] and crystal structure[11] have been reported. The density of the potassium salt was calculated from X-ray measurements of the crystal structure.[11] The density of no other perrhenate of this family has been reported. The only other melting point reported is for sodium perrhenate.[3] The solubilities at room temperature have been reported for all of these salts except lithium perrhenate.[7] The crystal structures of all of the salts except lithium perrhenate have been reported.[11,12,13,14]

Preparation of the Salts.—Rhenium was burned in a stream of oxygen at atmospheric pressure. The finely divided metal ignites under these conditions at approximately 300°. The heptoxide formed was dissolved in water to form perrhenic acid. The acid solution was then neutralized with the C. P. carbonate or hydroxide of the metal.[3] Litmus paper was used as an indicator. The salts so obtained were purified by several recrystallizations from water. They are all white in appearance. Evaporation of water from lithium perrhenate solutions was carried out at room temperature. The vapor from heated solutions of this salt was acidic and contained rhenium which indicated hydrolysis of the salt at elevated temperatures.

After each recrystallization the freezing point of the salt was determined. This was repeated until the same freezing point was observed after successive recrystallizations. The freezing points so obtained are listed in Table I.

The freezing points were obtained by cooling molten samples of 20 to 40 g. at a rate of approximately 2° per minute. The temperatures for the cooling curve for the dihydrate of lithium perrhenate were read on a mercury in glass thermometer while all others were read to 0.2° (0.01 millivolt) on a Brown Portable Potentiometer, Model 1117, using a Chromel–Alumel thermocouple which had been calibrated by direct comparison with a calibrated Iron–Constantan couple furnished by the Brown Instrument Company and guaranteed to give standard voltages corresponding to within 3°F. over the range 0–1200°F. Distinct "arrests" in the cooling curves were noted for each salt which could be melted at atmospheric pressure. The dihydrate of lithium perrhenate does not completely melt at 87.5° but undergoes a transition in which the hydrate decomposes to form a liquid and a white solid which will not completely dissolve in the liquid at 100°. Some of this white solid was separated from the liquid by decantation. The loss in weight of this wet solid on complete dehydration was not so great as the theoretical loss for the monohydrate to anhydrous salt. Apparently the equilibrium is $LiReO_4 \cdot 2H_2O(s) \rightleftarrows$ saturated solution(l) + $LiReO_4(s)$.

The purified salts were analyzed gravimetrically for rhenium as a check on the formula for the salt and its purity. The procedure outlined by Willard and Smith[15] was followed in general. It was found in washing the precipitate of tetraphenylarsonium perrhenate with ice water that precipitation occurred when the wash water was allowed to mix with the original filtrate. Whether this was due to the solubility of the precipitate in the wash water or to the decrease in solubility of the rhenium compound as the salt water filtrate was diluted was not definitely established. The procedure was modified by preparing the reagent, tetraphenylarsonium chloride, already mixed with the specified concentration of sodium

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[1] This paper is based on a thesis presented by Selma Harmon Long to the Graduate School of the University of Tennessee in partial fulfillment of the requirements for the M.S. degree, May 26, 1947.

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TABLE I

Salt	F. p., °C.	% of theoretical Re found	Density, g./cc.	Solubility (moles/100 g. H_2O × 10^2) 0.0°	30.0°	50.3°	Heat of soln. ΔH (kcal./mole ± 0.02)
$LiReO_4 \cdot 2H_2O$	87.5		3.69	100	140	140	+1.6 approx.
$LiReO_4 \cdot H_2O$	Dec.						
$LiReO_4$	426	99.8	4.61				
$NaReO_4$	414[a]	99.8	5.24	37.8	53.2	63.6	1.83
$KReO_4$	555[b]	100.0	4.38[d]	0.124	0.508	1.11[e]	7.68[f]
NH_4ReO_4	[c]	99.5	3.53	1.03	3.25	5.99	6.21
$RbReO_4$	598	99.8	4.73	0.116	0.468	1.05	7.70
$CsReO_4$	616	100.4	4.76	0.0861	0.287	0.640	7.69

[a] Melting point of 300° previously reported.[3] [b] Melting points previously reported 518°[6] and 552–553°.[5] [c] Decomposes around 365°. [d] Density of 4.887 previously reported.[11] [e] The values for the solubility of potassium perrhenate are calculated from previously published data.[9] The solubility of this salt in the 50.3° column is the solubility at 49.78°. [f] Roth and Becker[10] report 13.80 ± 0.03 kcal./mole at 21.7°.

chloride and saturated with tetraphenylarsonium perrhenate. The unknown samples were digested with this solution until they were completely converted to the tetraphenylarsonium perrhenate precipitate. During this digestion water was added, if necessary, to maintain the original volume. The precipitate was caught on a Gooch filter, sucked dry and then washed with a saturated solution of tetraphenylarsonium perrhenate. Excellent checks on duplicate samples were obtained in this manner and carefully purified samples of potassium perrhenate agreed with the theoretical rhenium content. The results of these analyses are presented in Table I.

The percentage of water removed from the hydrate of lithium perrhenate in a vacuum desiccator over anhydrous calcium chloride corresponded to the composition of a dihydrate. No further loss of water occurred even above the melting point of the dehydration product. The composition of the monohydrate of lithium perrhenate was established by following the loss in weight of a sample of the dihydrate and the gain in weight of an anhydrous sample of the lithium salt on standing over a 60% sulfuric acid solution in a desiccator at room temperature. After two months both samples were within 0.5% of the theoretical weight for the monohydrate.

Water Vapor Pressures of the Hydrates of Lithium Perrhenate.—In order to determine the conditions under which the hydrates of lithium perrhenate are stable, vapor pressure determinations on saturated solutions and on the solid–vapor systems were made over the approximate temperature range of 25 to 45°. A glass tube containing the sample was sealed to a closed mercury manometer and to a two-way stopcock through which the system was evacuated. The temperature of the sample tube was maintained by a water-bath which was controlled to ±0.05°. When the equilibrium pressure was established readings were made with a depth gage which could be read to 0.05 mm. Water vapor was pumped out of the apparatus after several pressure readings and new pressure readings were made. The pumping was continued periodically until no measurable pressure was found over the sample. The data so obtained are plotted in Fig. 1. Curve I is for the system which contained saturated solution in contact with solid salt. Curve II was obtained on the same samples after pumping off water vapor until lower equilibrium pressures were found. This system contained the two solid hydrates. Curve III was obtained by pumping off more water vapor from the above samples and also by admitting water vapor to anhydrous lithium perrhenate. The two solids in this system were anhydrous salt and monohydrate. The slopes of curves II and III give the following heats of hydration. $LiReO_4(s) + H_2O(v) \rightarrow LiReO_4 \cdot H_2O(s)$, $\Delta H = -13.7$ kcal. $LiReO_4 \cdot H_2O(s) + H_2O(v) \rightarrow LiReO_4 \cdot 2H_2O(s)$, $\Delta H = -12.4$ kcal. $LiReO_4(s) + 2H_2O(v) \rightarrow LiReO_4 \cdot 2H_2O(s)$, $\Delta H = -26.1$ kcal. Using 10.4 kcal. as the molar heat of vaporization of water at the mean temperature of the vapor pressure measurements, the heats of hydration using liquid water instead of vapor in the above reactions are $\Delta H = -3.3, -2.0$ and -5.3 kcal., respectively.

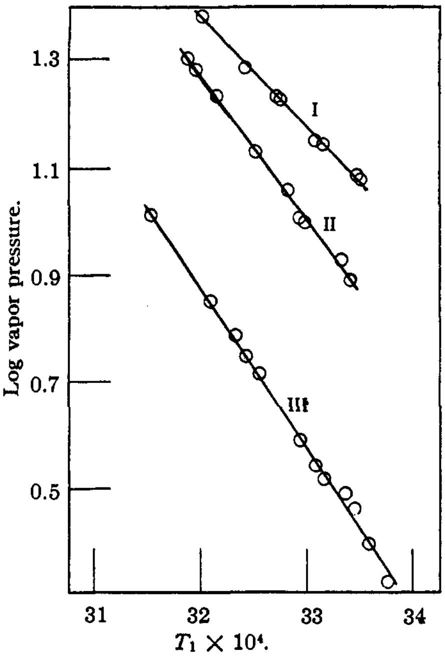

Fig. 1.—Plot of log vapor pressure against the reciprocal of the absolute temperature.

Density Determinations.—The densities of the solid salts were determined at room temperature in a pycnometer using benzene since it does not dissolve these salts and is volatile enough to be effectively removed from the sample without washing. The method was checked using sodium chloride and copper sulfate pentahydrate. The results were 1.4 and 3.5% lower, respectively, than published values[16,17] but duplicate determinations checked within 0.3% of the mean in all cases. Table I lists the densities of these salts.

Solubility Measurements.—The solubilities were determined at 0, 30 and 50.3 ±0.05°, using a method previously described.[9] Lithium perrhenate is so soluble that its solubility was measured only approximately by adding the minimum amount of water necessary to completely dissolve a weighed amount of the salt. Table I includes

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(16) Baxter and Wallace, THIS JOURNAL, **38**, 259 (1916).

(17) Andreae, *Z. physik. Chem.*, **76**, 491 (1911).

the solubility of the salts and the heat of solution calculated from the slope of the line obtained when the logarithm of the solubility is plotted against the reciprocal of the absolute temperature. The method of least squares was used to find the best slope.

Microscopic Examination of Crystals.—All of the solid salts prepared were examined with a microscope using polarized light. They are all anisotropic. This has been previously reported[11,12,13,14] for all of these salts except anhydrous lithium perrhenate and its hydrates.

Freezing Point of Ammonium Perrhenate.—Although it was found that ammonium perrhenate decomposes rapidly at 365° at atmospheric pressure, its freezing point can be estimated from other properties. Plots of solubility, molar volume and radius of the cation against the freezing points of the alkali metal perrhenates indicate freezing points of 550, 605 and 580°, respectively, for ammonium perrhenate. The average value, approximately 580°, is predicted as the freezing point of this salt.

Summary

The perrhenates of the alkali metals and ammonium have been prepared and the formulas verified. The densities, freezing points and solubilities from 0 to 50° have been measured. The heats of solution have been calculated. The freezing point of ammonium perrhenate has been predicted.

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CONTRIBUTION FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY. No. 1072]

Studies on the Denaturation of Antibody. III. Kinetic Aspects of the Inactivation of Diphtheria Antitoxin by Urea

BY GEORGE G. WRIGHT AND VERNER SCHOMAKER

Evidence has been presented[1,2] that the inactivation of antitoxin which takes place in urea solutions, as shown by the change in toxin-neutralizing activity, is a first-order reaction in antibody, since the specific rate of inactivation is independent of the initial concentration of antibody. This indicates that a polymolecular complexing reaction of the protein molecules[3-6] is not the mechanism of the inactivation under consideration, even though the occurrence of complexing as a result of the urea treatment could be inferred from the abnormalities of the quantitative flocculation reaction with toxin of the denatured samples.[2] The course of the reaction is not entirely typical of a classical first-order process, however, since the rate decreases more rapidly as the reaction proceeds than is predicted by the first-order law. Similar results have been obtained in the inactivation of antitoxin by heating in the neighborhood of 65°.[7,8] An understanding and a quantitative formulation of these deviations from first-order behavior are essential if the experimental results on antibody inactivation are to yield the maximum information regarding the chemical nature of the reactions and the structure and stabilizing forces of the combining region of the antibody.

In the present investigation we have studied the effects of *p*H, urea concentration, and temperature on the inactivation reaction, in order to explore the influence of these factors on its abnormalities. The experimental results are shown to be compatible with a simple kinetic mechanism.

Materials and Methods

The diphtheria antitoxin was the same globulin preparation used in previous work,[2] and consisted of the water-soluble portion of the protein precipitated from crude horse antitoxin plasma between 1.38 and 1.65 M ammonium sulfate. It contained 16% protein and had an activity of about 4000 units per ml. The urea was twice recrystallized from 70% alcohol. The denaturation reactions were carried out in buffered solutions at ionic strength about 0.1; acetate was used in the acid range and borate in the alkaline. The buffers were prepared by mixing various proportions of 0.2 N sodium hydroxide and a solution 0.2 N in the acid and 0.2 M in sodium chloride, so that the ionic strength remained approximately constant at different *p*H values. The *p*H measurements were made with a glass electrode. The buffers were effective in keeping the *p*H change during the experiment to less than 0.1 *p*H unit in practically every case; the recorded value represents the average of the initial and final *p*H measurements. The reactions were carried out in a thermostat constant to within a few hundredths of a degree, at 25.0° except where otherwise indicated.

The solutions for denaturation were prepared by weighing out the calculated amount of urea (using the density data of Dunstan and Mussel[9]), adding

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(1) G. G. Wright, *J. Exptl. Med.*, **79**, 455 (1944).

(2) G. G. Wright, *ibid.*, **81**, 647 (1945).

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