Kohr, they probably agree within the limits of error, considering the individual variability, which is characteristic of the results both of Noyes and Kohr, and of the author. While the difference of the equilibrium ratio from that of Noyes and Kohr does not justify a recalculation of the free energy of water based in part on this equilibrium, it may be noted that the free energy calculated from the above results agrees with that obtained by other methods somewhat better than that calculated from the results of Noyes and Kohr.

Summary

The equilibrium of AgCl, Ag_2O, KOH, KCl and H_2O, previously studied by Noyes and Kohr, has been redetermined, using Ag_2O prepared in a variety of ways, and determining the chloride by electrometric titration. While the individual values are somewhat variable, the method of preparation has no significant effect on the equilibrium ratio of chloride and hydroxide. Within experimental error the equilibrium ratio is the same in 0.05 molal solution as in 0.1 molal solution.

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A HIGHLY ACCURATE METHOD FOR THE ANALYSIS OF UREA

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There are many problems in physical and colloid chemistry which require for their experimental solution the use of a reference substance capable of analysis to a high degree of accuracy. Such a reference substance must also be chemically inert and a non-conductor of electricity. These problems include methods of indirect analysis, determination of solvation by the method of ultrafiltration[1] and the determination of the hydration of the ions by measurement of the displacement of the reference substance during electrolysis, as suggested by Nernst in 1900. In Washburn's[2] well-known work on this latter subject, only one substance, raffinose, was considered to possess the requisite properties and it is subject to the drawback that a highly sensitive polarimeter of a type not generally accessible is required to estimate it.

The high molecular weight of raffinose is also a disadvantage. The decimolar solution contains more than 50 g. to the liter, a quantity sufficient to cause considerable increase in the viscosity of the solution and consequently to affect the migration velocities of the ions. Also, it is

[1] McBain and Jenkins, *J. Chem. Soc.*, **121**, 2325 (1922).

[2] Washburn, THIS JOURNAL, **31**, 322 (1909); Washburn and Millard, *ibid.*, **37**, 694 (1915).

conceivable, in view of Coehn's[3] observation of the Tyndall cone in sucrose solution and of his statement that sucrose moves in the electric field, that the large molecules of raffinose may tend to become colloidal and to adsorb charged ions in the presence of a considerable quantity of electrolyte. Thus doubt is thrown on the value of raffinose as a reference substance in migration experiments.

The possibility of using urea for this purpose does not appear to have been considered, yet it has properties which render it almost ideal for the purpose. Its equivalent weight is low, it is easy to obtain pure and its aqueous solution is stable at room temperature in the absence of ferments. Moreover, its basic dissociation constant,[4] $[CO(NH_2)_2H][OH]/[CO(NH_2)_2\text{-}HOH]$, at 25° is 1.5×10^{-14}, the conductivity of its solution is approximately equal to that of water and the effect of its presence in potassium chloride solution is merely a slight lowering of the conductivity, which can be accounted for by the increased viscosity of the solution.

It only remained to find a suitably accurate method of estimating it in solution. A search through the literature revealed the fact that no method has afforded results which would establish an accuracy exceeding 1% of the urea present. The method favored by physiologists at the present time consists in the fermentation of urea by urease[5,6] in the presence of potassium dihydrogen phosphate and subsequent liberation and distillation of the ammonia formed. This method appears to be satisfactory for small quantities of physiological fluids for which an accuracy of 1% is sufficient, but it presents difficulties when a degree of accuracy of the order of 0.1% is desired. As is well known, the removal of the last traces of ammonia by boiling and aerating an aqueous alkaline solution is a matter of some uncertainty, even when no colloidal organic matter is present to induce frothing and bumping. In distilling off the ammonia produced by urease fermentation, the frothing is very marked and, though it can be decreased to some extent by the addition of wax or oil, the escape of ammonia is checked and the titration of the excess of acid in the absorber is rendered less accurate, owing to the fact that a small quantity of the wax distils over with the steam and removes the indicator from the aqueous layer. A further drawback is the long boiling required for the complete expulsion of the ammonia from the mixture, resulting in the extreme and variable dilution of the acid in the absorption flask.

In view of these disadvantages, it was decided to revert to the method of Benedict and Gephart,[7] which depends on the hydrolysis of urea by acid

[3] A. Coehn. *Z. Elektrochem.*, **15**, 622–624 (1909).
[4] Walker and Wood, *J. Chem. Soc.*, **83**, 484–491 (1903).
[5] E. K. Marshall, *J. Biol. Chem.*, **17**, 351 (1914).
[6] Plimmer and Skelton. *Biochem. J.*, **8**, 70 (1914).
[7] Benedict and Gephart. THIS JOURNAL, **30**, 1760 (1908).

under a pressure of four atmospheres. The authors state that the results obtained with pure urea solutions leave no doubt that the urea is completely hydrolyzed. The one experiment quoted consists in the hydrolysis of 5 cc. of a 2.5% urea solution which yielded 0.0575 g. of nitrogen, whereas the theoretical yield is 0.0583 g., an experimental deficit of more than 1% of the total urea. Wolf and Osterberg,[8] using the same method and a 2% solution of urea, also obtain values lower than the theoretical by 1%. It is obvious that these investigators, having in view the composition of physiological fluids, are not concerned with accuracy exceeding 1%.

Experiments by the writer with larger quantities of urea than those used by Benedict and Gephart have indicated that for the purpose of estimating urea unmixed with other hydrolyzable nitrogen compounds, the autoclave method gives a high degree of accuracy. This accuracy depends on the facts that the reaction goes to completion in the presence of very slight excess of hydrochloric acid, and that the acid does not vaporize under the conditions in the autoclave. Thus the distillation with soda can be avoided by mixing a known quantity of standard acid with a known quantity of the solution to be analyzed and directly titrating the excess of acid with dilute soda. By making the excess of acid small, the effect of any slight error in the concentration of the soda becomes insignificant, and consequently the possible accuracy of estimation of the urea depends only on the accuracy of weighing out the urea and acid solutions concerned, and on the accuracy of making up the hydrochloric acid solution.

Experimental

The digestion is carried out in a 500-cc. stoppered conical flask made of Pyrex glass or silica and provided with an exit tube of an inverted U-shape sealed in near the top of the flask. The flask is weighed and the urea solution and standard acid are pipetted (see p. 3264) into it, the weight being noted after each addition. A Pyrex test-tube containing a little water into which the side tube dips acts as scrubber to the escaping carbon dioxide. The latter is absorbed by the distilled water in the autoclave, to which a few cc. of soda solution has been added. This precaution renders unnecessary any subsequent aeration or boiling of the reaction mixture for the expulsion of carbon dioxide.

The flask and scrubber tube are covered with tinfoil in the autoclave and after displacing all the air, heating for half an hour under two atmospheres, and for four hours under four atmospheres, the apparatus is allowed to cool. The autoclave is then opened, the glass stopper of the flask is removed, and replaced by a rubber bung carrying a tube, by means of which the water in the scrubber tube is sucked back and the scrubber tube rinsed several times into the flask. The titration is carried out in the presence of two drops of 0.02% methyl red indicator.

In the experiments described below, the standard hydrochloric acid was prepared by the distillation method of Hulett and Bonner,[9] and the soda was standardized against the acid to three tints of the indicator, giving three different concentrations of the soda of which the extremes differed by less than 0.02%. Thus it was unnecessary always to titrate the soda to the same tint.

[8] Wolf and Osterberg, THIS JOURNAL, **31**, 425 (1909).

[9] Hulett and Bonner, *ibid.*, **31**, 390 (1909).

The first experiments were carried out volumetrically using calibrated instruments; the later ones gravimetrically, using weight burets. The results are corrected for the buoyancy of the air.

The solutions of urea and hydrochloric acid contained about 0.2 and 0.4 equivalents per liter, respectively, and each experiment was carried out with about 50 cc. of urea solution. The soda solution used for back titration was about 0.2 N. The urea was Kahlbaum's purest material, m. p. 132.2–133.2°, which had been kept in a desiccator for some days before use. Results are recorded in Tables I to III.

In the experiments recorded in Table I, the urea was hydrolyzed by ordinary concentrated hydrochloric acid and the ammonia estimated by distillation with soda and absorption by standard hydrochloric acid. In each of these two experiments, 50.28 cc. of 0.22509 N urea was digested with 5 cc. of pure concentrated acid.

TABLE I

RESULTS OF EXPERIMENTS

Expt.	HCl concn. in abs., N	HCl soln., cc.	Concn. of soda	Vol. of soda, cc.	Equiv. of urea per liter of soln. Found	Taken
1	0.51606	25.03	0.21790	7.41	0.22478	0.22508
2	.41671	30.03	.13591	8.87	.22490	.22508
				Mean =	.22484	

In the second set of experiments (Table II), 50.28 cc. of the same urea solution was digested each time with varying quantities of 0.41671 N hydrochloric acid and titrated directly with 0.13584 N soda.

TABLE II

RESULTS OF EXPERIMENTS

Expt.	Acid, cc.	Soda, cc.	Equiv. of urea per liter of soln. Found	Taken
3	50.28	71.00	0.22489	0.22508
4	50.28	71.02	.22482	.22508
5	30.03	8.94	.22474	.22508
		Mean =	22482	

The method was then applied gravimetrically to determine the concentration of three urea solutions, A, B and C, each of unknown concentration but approximately 0.2 N_w. In all cases, 0.40756 N_w hydrochloric acid was used. The results are recorded in Table III.

Of all the solutions analyzed only one failed to give concordant results. In this case one sample of the urea solution was poured, not pipetted, into the digestion flask, and though excess of hydrochloric acid was added, the water in the scrubber tube became alkaline. The sample of the solution which was pipetted in the usual way was found to contain 0.20147 gram equivalents of urea per 1000 g. of solution, while the second sample gave only 0.20103 gram equivalents, a deficit of 0.2%. It seems probable

TABLE III

RESULTS OF EXPERIMENTS

Expt.	Urea soln., g.	HCl soln., g.	Concn. of soda soln., N_{10}	Soda soln., g.	Equiv. of urea per liter of soln.
			Solution A		
6	51.2844	50.5605	0.2383 (33)	44.544	0.194870
7	51.2857	35.4112	.2383 (33)	18.614	.194901
8	51.2511	25.1701	.2383 (54)	1.1379	.194794
				Mean =	.194855
			Solution B		
9	51.2300	25.9771	.2383 (54)	2.4635	.195196
10	50.8790	26.0405	.2383 (13)	2.8615	.195208
				Mean =	.195202
			Solution C		
11	51.0923	25.9704	.2383 (33)	1.8615	.198482
12	50.9424	26.6487	.2383 (33)	3.2235	.198526
				Mean =	.198504

that urea is rapidly hydrolyzed by water alone at the autoclave temperature of 150° and, consequently, since it is not convenient to shake up the mixture in the digestion flask, some of the ammonia escaped combination with acid.

With this exception, the extreme divergence between any values for the same solution is 0.05%, and if those results which were obtained gravimetrically and by the use of only slight excess of acid are considered exclusively, the agreement throughout is within 0.02%.

No special precaution other than the use of calibrated weights has been observed and it is probable that using more dilute solutions of acid and alkali, and titrating to a larger number of intermediate indicator tints, a considerably higher degree of accuracy could be obtained. The difference of 0.12% shown in Table I between the urea weighed out and that obtained by analysis is undoubtedly real, owing to the fact that no extreme precautions were taken to dry and purify the urea. The method is now being employed in this Laboratory in experiments on the hydration of the ions, and is found to be extremely convenient owing to the short time required for actual manipulation, the ease of removal of the ammonium chloride and to the fact that the process is at no stage precarious.

Summary

Urea is an excellent reference substance for use in physico-chemical and colloidal problems, being a non-electrolyte of low equivalent weight and chemically indifferent. A simple method of analysis whereby it is converted into ammonium chloride and carbon dioxide is described. The accuracy obtained is at least 0.02% of the urea used.

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