acid. A sample made by digesting 4 g. of the oxide with the acid for three hours was analyzed for copper:

0.2256 g. gave 0.1156 g. CuO; found: Cu 50.95%.

The reactions of many other organic acids on cuprous sulfite have been tried but, in all cases, more or less green coloration has indicated the formation of cupric compounds. Apparently, the solubility of the cuprous compound first formed is chiefly responsible for the success of the experiment. The appearance of the insoluble portions obtained in some of the experiments gives encouragement to the hope that they can be used for further transformations as cuprous salts.

Conclusions.

1. Attention has been called to an erroneous statement common in text-books of general chemistry.

2. A new method for the preparation of cuprous salts of oxygen acids has been devised, and the probable conditions of its applicability have been indicated.

3. A new compound of the class has been prepared, some of its properties described, and its probable formula determined.

LEXINGTON, KY.

[HARRIMAN RESEARCH LABORATORY, ROOSEVELT HOSPITAL, NEW YORK CITY.]

A PRECIPITANT FOR AMMONIA.

(A SUBSTITUTE FOR NESSLER'S REAGENT.)

BY SARA STOWELL GRAVES.

Received March 3, 1915.

I. Introduction.

Nessler's reagent for ammonia, developed in the early part of the last century, was applied to water analysis in 1867. It has stood the test of time and has come into wide use, particularly in physiological work. But with the development of colorimetry, its disadvantages, as well as its value, have become apparent and innumerable modifications of the reagent have resulted. Its instability and tendency to produce a cloud in dilute solutions are the chief difficulties. Recently efforts have been made to apply the reagent in micro-Kjeldahl[1] work (without previous distillation) with varying degrees of success, the precipitate due to salts making the accurate matching of colors extremely hard.

A probable explanation of why the colored solution produced by Nessler's reagent becomes cloudy, especially in the presence of salts, may be found in the following considerations:

---

[1] Folin and Farmer, *J. Biol. Chem.*, **11**, 493 (1912); Gulick, *Ibid.*, **18**, (1914); Bock and Benedict, *Ibid.*, **20**, 47 (1914).

(*a*) Only the iodide complex of mercury and ammonia is highly colored and soluble.

(*b*) The other complexes of mercury and ammonia, like the chloride described in this paper, are colorless, insoluble compounds.

Therefore in the presence of other salts, the colored iodide complex is probably partially changed to, and in equilibrium with, the colorless complexes, such as the chloride or sulfate, and as only the color is estimated, appreciable error may result.

The idea of finding a precipitant for ammonia, was suggested by a research of Dr. W. G. Lyle as to the possibility of determining the non-protein nitrogen in blood, nephelometrically, and in the course of discussion, mercuric chloride was mentioned as a likely reagent.

Having in mind Nessler's solution and its disadvantages, an attempt was made to evolve an alkaline mercuric chloride solution which would be stable and colorless and which would form a white precipitate with ammonia, quantitatively. Since the salts, which interfere with the Nessler color, produce a white complex with mercury and ammonia, no probable error would be introduced by this proposed reagent.

II. Reagent.

Mercuric Chloride Complexes.—When a solution of mercuric chloride is made alkaline, immediately the yellow or red oxide forms, but it was found that by first adding sodium chloride, a complex was formed which was stable except in the presence of a large excess of alkali. As will be seen from the following equation, 2 molecules of sodium chloride unite with one of mercuric chloride:

$$2NaCl + HgCl_2 \rightleftarrows Na_2HgCl_4$$

This complex is readily soluble in water and while it has an appreciable dissociation into $HgCl_2$ and NaCl,[1] with large amounts of sodium chloride, the dissociation is practically zero. It has the further advantage that chlorine compounds are more stable than those of the other halogens. Thus, the ammonium complexes of mercuric chloride are more stable than those of the bromide or iodide. The neutral iodine complex has a noticeable vapor tension of ammonia, which the analogous complex of chlorine has not.

Components of Reagent.—The reagent used consisted of mercuric chloride, sodium chloride, and lithium carbonate. The latter was chosen because of the low atomic weight of the cation and, therefore, but slight tendency to cause agglutination of suspensoids. Each component of the reagent was varied, in order to ascertain the most favorable conditions for the quantitative precipitation of ammonia.

---

[1] T. W. Richards and Archibald, *Z. physik. Chem.*, **40**, 385 (1902); Le Blanc and A. A. Noyes, *Ibid.*, **6**, 393 (1890).

50 cc. of a cold saturated solution of mercuric chloride and 50 cc. of water were chosen arbitrarily and the sodium chloride and lithium carbonate varied to the points at which mercuric oxide formed—with the following results:

TABLE I.—PRECIPITATION POINTS OF MERCURIC OXIDE.

Sat. sol. of $HgCl_2$. Cc.	H_2O. Cc.	NaCl. Grams.	Saturated Li_2CO_3 solution. Cc.
50	50	5	5
50	50	10	30
50	50	15	50 plus
50	50	20	50 plus

50 cc. of mercuric chloride solution, 15 g. of sodium chloride, 35 cc. of saturated lithium carbonate solution, and 65 cc. of water were found to produce a satisfactory reagent. Experiments showed that it is fully as sensitive as Nessler's reagent, and it seemed likely that, owing to the sensitiveness of nephelometry, very small amounts of ammonia could be determined quantitatively more easily as a cloud than as a very faint color. The reagent precipitates ammonia from ammonium sulfate solutions as dilute as 1 part per million, producing a bluish white cloud which agglutinates in stronger solutions after a few minutes.

Before studying the reagent further it was necessary to find a protective colloid, to keep the precipitate in suspension long enough to make nephelometric readings (10–30 minutes). Soluble starch, when very dilute, proved satisfactory.

Two sets of experiments were accordingly carried out, using 10 cc. of ammonium sulfate solution (10.0 mg. per liter), 15 cc. of starch solution (5.0 mg. per liter) and 5 cc. of reagent, in which the mercuric chloride and lithium carbonate were varied. The clouds formed were compared with a fixed standard, in the nephelometer.

(1) **Mercuric Chloride.**—The mercuric chloride was varied from 25 to 75 cc. of saturated solution per 150 cc. of reagent. There was no appreciable change in the readings of the different solutions tested.

(2) **Lithium Carbonate.**—The amount of saturated solution of lithium carbonate (about 1%) was varied from 12.5 to 75 cc. 65 to 75 cc. produced mercuric oxide on standing. Solutions containing less than 65 cc. showed no marked difference in nephelometric readings.

Protective Colloid.—The effect of starch upon salt was studied in two connections: (1) When the reagent contained varying amounts of sodium chloride. (2) When the ammonia solutions contained varying amounts of potassium sulfate, thus allowing for any reasonable variation in the amount of ammonia as compared to salt found in micro-analysis.

(1) To 150 cc. of reagent, which already contained 15 g. of sodium chloride, more sodium chloride was added in varying amounts of 10,

20, 25, and 35 g., respectively. It was found that the solutions containing 20, 25 and 35 g. additional salt all allowed the use of a fairly large amount of starch. 25 g. were then decided upon, making a total of 40 g. of sodium chloride in 150 cc. of reagent. Later experiments show the effect of sodium chloride upon the constants of the reagent.

(2) The ammonium sulfate solutions were of three types: *A*, pure. *B*, with the amount of potassium sulfate found in normal Kjeldahl work. *C*, with four times the amount of salt found in normal Kjeldahl work.

Solution A contained 1.0 mg. of ammonium sulfate in 100 cc. or 2.57 mg. of ammonia per liter.

Solution B contained 1.0 mg. of ammonium sulfate, and 10 cc. of potassium sulfate solution (75 g. per liter), in 100 cc.

Solution C contained 1.0 mg. of ammonium sulfate, and 40 cc. of potassium sulfate solution (75 g. per liter) in 100 cc.

To solutions A_1, B_1, and C_1 (10 cc.) were added various amounts of starch, always diluting to 25 cc. at this point, and the time was determined from the moment the reagent (5 cc.) was added, until agglutinations were visible to the naked eye. At the same time it was observed that the nephelometric readings became slightly higher. The results obtained are seen in Table II, the minutes indicating the time during which the solutions are constant and suitable for nephelometric work, and the concentration of starch indicating the strength of the final solutions:

TABLE II.—TIME OF AGGLUTINATION.

Concentration starch. Mg. per liter.	A_1. Min.	B_1. Min.	C_1. Min.
0	14	11	5
2.5	27	15	6
5	37	25	10
10	48	40	14
15	60	60	20
20	..	..	28
25	..	..	32
40	..	..	50
50	..	..	60

It may be seen that the suspensions are fairly stable even without starch.

Completeness of Precipitation.—It was necessary to determine whether or not the completeness of precipitation was affected by the colloid.

To do this, solutions A_2, B_2, and C_2 were prepared containing 0.500 mg. of ammonium sulfate instead of 1 mg. as in A_1, B_1, and C_1. To 10 cc. of solutions A_2, B_2 and C_2 were added definite amounts of starch, diluting at this point to 25 cc. and finally 5 cc. of reagent were added with shaking, and the solutions compared with solutions A_1, B_1, and C_1, respectively, in the nephelometer. From the results the nephelometric constants (k) were calculated, using the formula[1]

---

[1] For details as to nephelometric instrument, formula and manipulations, see Kober, *J. Biol. Chem.*, **13**, 490 (1913).

$$y = s/x - (1 - x)sk/x^2$$

where y equals the reading of A_2
" s " " " " A_1
" x " " ratio of A_1 to A_2 or 0.500.

Using the reagent containing 40 g. of sodium chloride per 150 cc., the following constants were obtained:

Table III.—Effect of Starch on Nephelometric Constants.

Concentration of starch. Mg. per liter.	A/k.	B/k.	C/k.
50	0.033	0.140	0.138
35	0.075	0.135	0.132
25	0.130	0.136	0.136
15	0.133	0.138	0.136
5	0.140	0.132	0.133

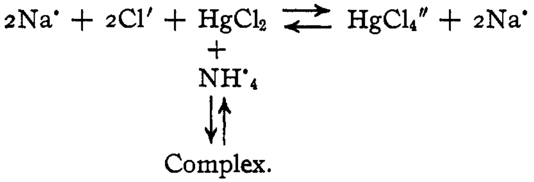
The above results show that in starch solutions containing 35 and 50 mg. per liter the precipitation of the pure solutions A, is retarded, but that in solutions containing less than 35 g. per liter the precipitation of the three solutions is apparently complete.

Table IV shows the effect of a reagent containing only 15 g. of sodium chloride per 150 cc. under similar conditions.

Table IV (Continuation of III).

Concentration of starch. Mg. per liter.	A/k.	B/k.	C/k.
15.0	Negative	Negative	0.077
5.0	0.090	0.180	0.173
2.5	0.164	0.154	0.166

Here it is evident that the precipitation is delayed in a starch solution containing 15 mg. per liter. The slightly higher constants with this reagent might be explained by the following equation:



$$\begin{array}{c} 2Na^{\cdot} + 2Cl' + HgCl_2 \rightleftarrows HgCl_4'' + 2Na^{\cdot} \\ + \\ NH_4^{\cdot} \\ \updownarrow \\ \text{Complex.} \end{array}$$

According to the Law of Mass action the increased amount of sodium chloride would have a tendency to shift the equilibrium from the ammonium complex formation and therefore the reagent containing less sodium chloride might give more complete precipitation and thus slightly higher nephelometric constants.

III. Results with Reagent.

To test the precipitability of ammonia by the modified mercuric chloride reagent, three sets of ammonium sulfate solutions A, B, and C were made, as previously described, in which the amount of ammonium sulfate was varied from 10 to 5 mg. per liter.

Starch was added, the ammonia precipitated by the reagent, and the solution compared with a known standard in the nephelometer. From the readings (usually 2) the accompanying curves were plotted. They are compared with the hypothetical or colorimetric curves. Curve No. I, with solutions A; Curve No. II, with solutions B; Curve No. III, with solutions C.

Solutions A contained 10 cc. of ammonium sulfate solutions containing 100, 90, 80, 70, 60, 50 mg. per liter, made up to 100 cc. with ammonia-free water.

Solutions B contained ammonium sulfate in the same amounts as solutions A, and in addition 10 cc. of potassium sulfate solution (75 g. per liter); the volume was then made up to 100 cc. with ammonia-free water.

Curve No. I.

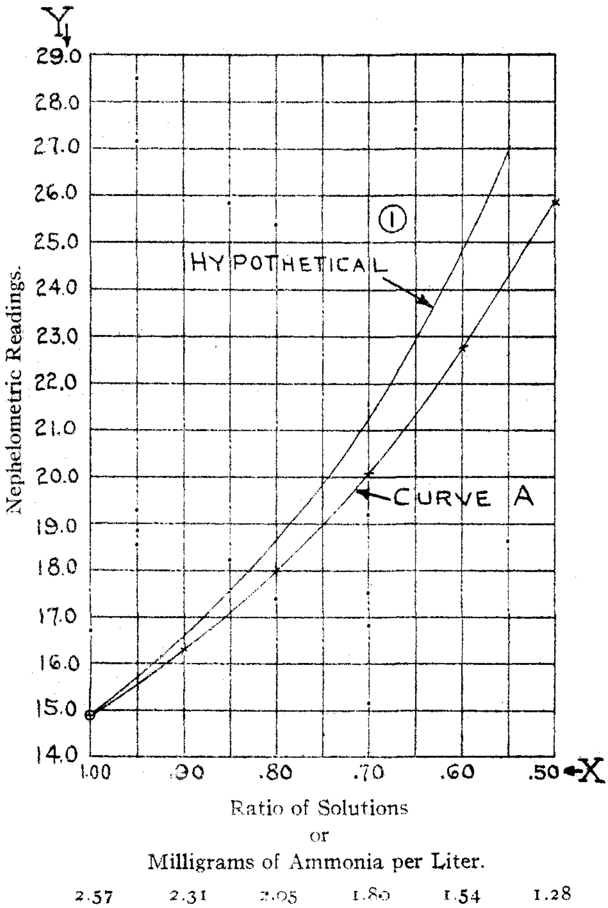


TABLE V.—NEPHELOMETRIC READINGS.

$(NH_4)_2SO_4$. Mg. per liter.	Solutions A.		Solutions B.		Solutions C.	
	Readings. Mm.	Constant. *k*.	Readings. Mm.	Constant. *k*.	Readings. Mm.	Constant. *k*.
10.0	14.90	...	14.90	...	14.90	...
9.0	16.30	0.140	16.30	0.140	16.30	0.140
8.0	17.95	0.141	17.90	0.152	17.93	0.146
7.0	20.05	0.140	20.06	0.139	20.00	0.145
6.0	22.75	0.131	22.80	0.128	22.77	0.136
5.0	25.80	0.134	25.85	0.132	25.85	0.132
	Average,	0.137		0.138		0.140

[1] The hypothetical or colorimetric curve is obtained by assuming the readings to be inversely proportional to the concentration of the substance.

Solutions C were like solutions B except that they contained 40 cc. instead of 10 cc. of potassium sulfate solution (75 g. per liter).

To 10 cc. of solution were added 15 cc. of starch solution (30 mg. per liter) and finally, while shaking in a rotary fashion, 5 cc. of reagent were run in from a pipet. The strongest solution (10 mg. per liter) of a series was used as a standard, and the others of a series compared with it in succession. The preceding readings are the average of 2 or 3 readings.

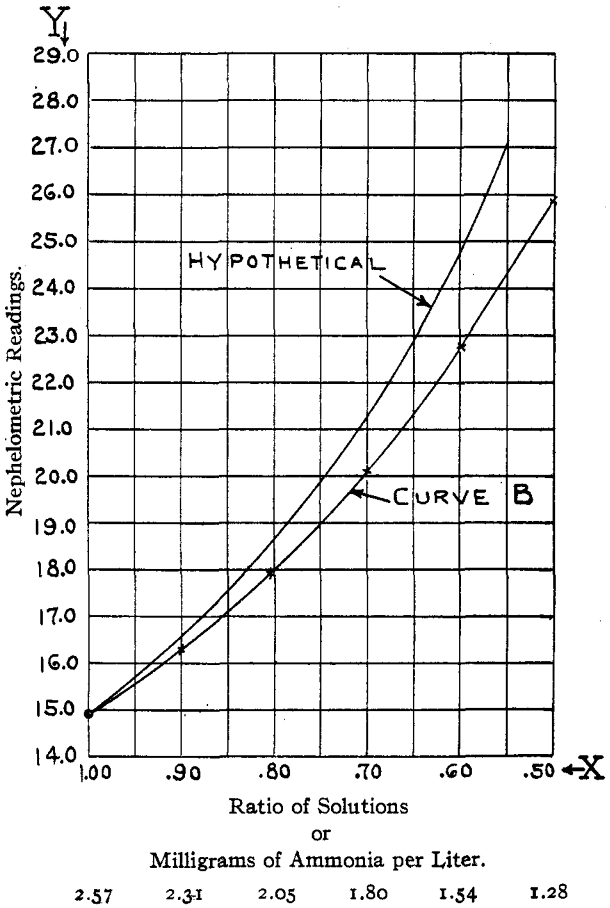
From these results it seems probable that the precipitation is nearly complete, since:

(1) The curves are well below the hypothetical.

(2) The readings are consistent.

(3) The nephelometric constants are in close agreement.

Curve No. II.

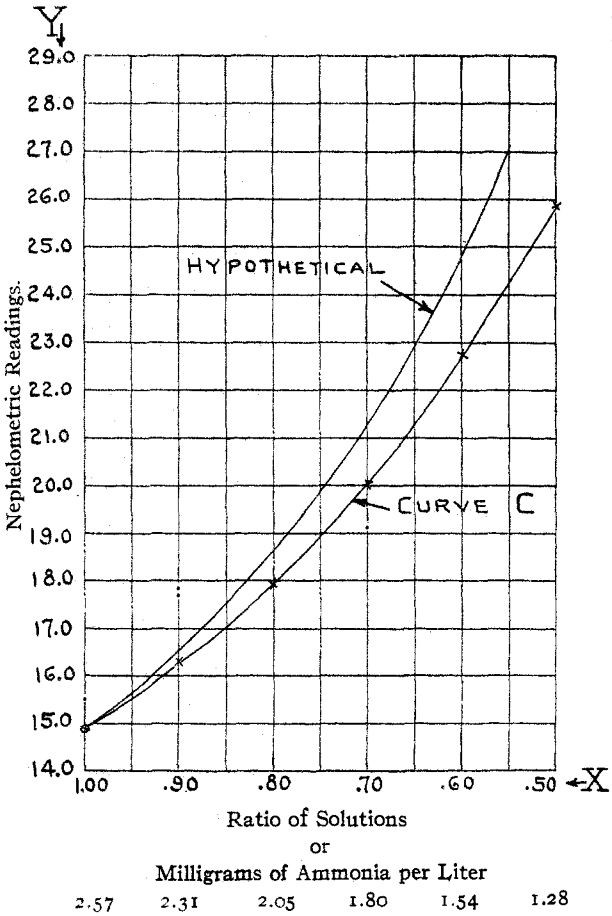


IV. Directions.

A. Solutions: Ammonia-free Water.—Ammonia-free water must be used in making up all solutions which enter into the standard solution, just as with the Nessler standard. The usual recommendation is water which contains not more than 0.005 mg. of ammonia in 100 cc. This may be easily obtained by distilling tap water which has been made slightly acid with sulfuric acid.

Reagent.—To 80 g. of sodium chloride are added 130 cc. of water and 100 cc. of a cold saturated solution of mercuric chloride, with shaking. When the salt is practically all dissolved, 70 cc. of a saturated solution of lithium carbonate (1%) is added slowly while shaking, so that no mercuric oxide forms on the sides of the flask. The solution is usually cloudy, owing to the ammonia in the reagents, but if well shaken with talcum powder (3–5 g.) it is easily filtered clear. The solution may be used at once, if carefully stoppered may be kept almost any length of time.[1] Filtering the reagent does away with errors due to impurities in its constituents.

Curve No. III.



Protective Colloid.—A stock solution of starch is freshly made each day by boiling 1 g. of starch in ammonia-free water until clear and making up to 100 cc. with ammonia-free water.

Standard Solution.—Ammonium sulfate prepared and purified according to Folin's[2] directions is used for a standard. The standard solution is made by taking 10 cc. of an ammonium sulfate solution (100 mg. per liter), adding 10 cc. of potassium sulfate solution (75 g. per liter) and making up to 100 cc. in a volumetric flask with ammonia-free water. The amount chosen for a standard cloud is usually 10 cc. of this solution, ammonium sulfate (10 mg. per liter) to which is added 15 cc. of 0.003% starch and 5 cc. of reagent. Half or even one-fourth of the above amount is ample, the object in choosing a fairly strong standard being to lessen the percentage of error due to traces of ammonia in dust or water. In all experiments controls should be made on the water as well as on the reagents.

---

[1] Solutions have been kept several weeks without noticeable change.

[2] *Loc. cit.*

Potassium sulfate is added to the standard solutions in order to reproduce as far as possible the conditions found in all Kjeldahl work. Preceding data show that large or small amounts of salt do not affect the precipitation of ammonia with the reagents as given.

(B) Kjeldahl Nitrogen Estimation.

Catalyst.—It is necessary to use mercuric oxide instead of cupric sulfate as a catalytic agent, owing to the blue color developed by copper in alkaline solution.

Dilution.—After the usual heating the solutions containing the substance are diluted to 500 cc. and the blanks to 250 cc. These solutions may be designated as solutions A_x and A_y.

Neutralization.—5 cc. or more of solution A_x and 50 cc. or more of solution A_y are neutralized with sodium hydroxide, using litmus paper as indicator and each is made up to 100 cc. in a volumetric flask with ammonia-free water. Designation, solutions B_x and B_y.

To 5 cc. of solution A_x is added about 5 cc. 1 *N* sodium hydroxide and then 1 *N* sodium hydroxide drop by drop to the neutral point. To 50 cc. of solution A_y is added about 30 cc. of 15% NaOH and then 1 *N* NaOH drop by drop to the neutral point.

Care must be taken not to add a considerable excess of sodium hydroxide, or else mercuric oxide will form upon addition of the reagent. The small amounts of ammonia in the control can be readily estimated, if the control solution is not more than one-twentieth as dilute as the solution containing the substance. If the solutions B are cloudy because of the precipitation of alkaline earths, etc., they should be filtered through a dry filter after making up to volume.

Precipitation.—To 10 cc. of solution B are added 16 cc. of starch solution (30 mg. per liter) and 5 cc. of reagent with shaking, and the cloud produced compared with a standard ammonium sulfate cloud, by means of the nephelometer.

Calculation of Results.—From the readings of the standard solution and the unknown, by means of the formula $y = s/x - (1 - x)sk/x2$ the ratio of the concentrations of the solutions can be found and the amount of ammonia or nitrogen in the substance taken for analysis readily calculated, subtracting the amount of ammonia in the control. A more convenient form of the equation when solving for x is $x = \frac{s + sk + \sqrt{(s + sk)^2 - 4sky}}{2y}$ in which $(s + sk)$ is constant throughout any series of determinations. For this reagent k is about 0.138.

V. Applications.

The preceding method of making nitrogen estimations was tried on ammonium sulfate alone, and on ammonium sulfate with organic matter (filter paper), as well as on uric acid and urine, with satisfactory results—which attest to the sensitivity of the reagent and its ability to precipitate ammonia quantitatively.

(1) 0.1001 g. of ammonium sulfate was digested with 5 g. of potassium sulfate and 20 cc. of sulfuric acid in the usual way. Duplicate estimations as well as duplicate controls were made. The solutions were diluted directly *without the usual distillation*, and the ammonia was determined according to the preceding directions, the standard solution containing 0.1001 g. of ammonium sulfate.

Solution.	Y.	S.	X.	Nitrogen found. g.	% Nitrogen found.	% Nitrogen theoretical.
I	13.75	13.96	1.016	0.02150	21.24	21.15
II	13.72	13.96	1.018	0.02155	21.29	21.15
		1/2 S				
Control[1]	25.43	13.96	0.483	0.00024	...	...

(2) The above experiment was repeated with the difference that one sheet of purest filter paper (7 cm.) was digested in each flask in lieu of organic matter.

Solution.	Y.	S.	X.	Nitrogen found. g.	% Nitrogen found.	% Nitrogen theoretical.
I	13.82	14.00	1.014	0.02145	21.18	21.15
II	13.80	14.00	1.015	0.02150	21.23	21.15
		1/2 S				
Control[1]	24.00	14.00	0.503	0.00025	...	...

(3) 0.100 g. uric acid was digested in each of four experiments with 10 g. of potassium sulfate and 20 cc. of concentrated sulfuric acid in the usual way. Two controls were made at the same time.

Two of the uric acid digestions and one control were distilled by the aeration method[2] and the ammonia was estimated by titrating the distillate. The other two digestions and a control were treated as in Experiments 1 and 2.

PERCENTAGE OF NITROGEN IN URIC ACID.

No.	Found by aeration method.	Found by new method.	Theoretical.
I	32.62	32.90	33.33
II	32.60	32.95	33.33

No attempt was made to purify or dry the substance, which may well have contained 1% of moisture.

(4) Experiment 3 was repeated, replacing the uric acid by 5 cc. of urine, with the following results: (urine was diluted with an equal volume of 0.2% tricresol).

---

[1] See solutions under *neutralization* marked A_y and B_y. 1/2 S indicates one-half as strong as standard solution (which see).

[2] P. A. Kober and S. S. Graves, THIS JOURNAL, **35**, 1546 (1913).

PERCENTAGE OF NITROGEN IN URINE.

No.	Found by aeration method.	Found by new method.	(A mixed specimen of normal urine.)
I	0.960	0.952	...
II	0.976	0.948	...

VI. Summary.

A new reagent for ammonia has been found which:

(1) Is more stable than Nessler's reagent in the presence of salts.

(2) Is fully as sensitive as Nessler's reagent.

(3) Will precipitate ammonia quantitatively.

(4) Will give accurate results nephelometrically.

(5) May be useful in water analysis.

(6) May be directly applied to normal- and micro-Kjeldahl determinations, thus doing away with the tedium and errors of distillation.

I desire to express my gratitude to Mr. P. A. Kober, for the opportunity of working out this problem.

[CONTRIBUTION FROM THE GEOPHYSICAL LABORATORY, CARNEGIE INSTITUTION OF WASHINGTON.]

THE USE OF THE INTERFEROMETER FOR THE ANALYSIS OF SOLUTIONS.

BY LEASON H. ADAMS.

Received March 29, 1915.

Chemists have long used the refractometer as an aid in analytical work, but have not made use of the interferometer to the extent that its precision and general convenience would warrant. The use of the ordinary forms of refractometer is limited by the circumstance that the change of refractive index with temperature is usually such as to require regulation of temperature to 0.01° in order to secure an accuracy of one unit in the sixth place in the measurement of refractive index. By means of the interferometer, on the other hand, it is a simple matter, requiring no special regulation of temperature, to secure an accuracy of one unit in the seventh place; this is possible because in the latter case we are comparing the refringence of one liquid (or gas) with that of another of very nearly the same composition and hence possessing almost the same temperature coefficient of refringence. In other words, with the refractometer one can determine the composition of a solution to 2 parts in 10,000 of solvent, but with the interferometer—provided that certain simple precautions be observed—to 2 parts in a million. The interferometer is adapted to the determination in any transparent mixture of a single varying component; this component may be solute or solvent, electrolyte or nonelectrolyte, indeed any substance which will not attack the