The Quantitative Determination of Methenamine*

By Edmund F. Slowick† and Ray S. Kelley‡

Since the discovery of methenamine, many methods have been suggested for its quantitative determination. These methods depend on one or both of the following properties: (1) methenamine, when hydrolyzed with acids, yields formaldehyde and the ammonium salt of the acid used for the hydrolysis. (2) Methenamine forms addition products with certain chemicals as the halogens, picric acid and uranyl sulfate.

The methods suggested in the literature are slow, tedious and not particularly accurate. In this study an attempt was made to devise a more rapid and accurate method for the assay of methenamine.

The samples used in the work were obtained from reputable manufacturers and were labeled, "Methenamine, U. S. P." In each instance several preliminary assays were performed previous to the determinations reported.

EXPERIMENTAL

The experimental work was divided into two classes: (1) work on certain methods, or on modifications of certain methods, previously reported in the literature; (2) work on methods of assay not previously reported in the literature.

CLASS 1. WORK ON METHODS AND MODIFICATIONS OF METHODS REPORTED IN THE LITERATURE

I. *U. S. P. XI Method.*—About 1 Gm. of methenamine, accurately weighed, is placed in a 400-cc. beaker, 40 cc. of normal sulfuric acid is added and the mixture is heated on a water bath, distilled water being added from time to time as necessary. Heating is continued until the odor of formaldehyde is no longer perceptible. The mixture is cooled, 20 cc. of distilled water is added and the excess acid is titrated with normal sodium hydroxide, using methyl red T.S. as the indicator. Each cc. of normal sulfuric acid is equivalent to 0.03503 Gm. of $(CH_2)_6N_4$.

Table I.—Results Obtained with U. S. P. XI Method

Sample	Wt. of Sample	1*N* H_2SO_4, Cc.	$(CH_2)_6N_4$, %	Time, Min.
1	1.0173	29.15	100.29	550
2	1.0042	28.67	99.92	550
3	1.0059	28.72	99.92	550
4	1.0141	28.98	100.01	550
5	1.0078	28.80	100.01	550

II. *U. S. P. XI Method, Modification A.*—Instead of heating the methenamine and acid on a water bath, direct heat is used with the temperature kept slightly below the boiling point of the mixture. The time required for the assay is thus materially reduced over the time required in the official method of the U. S. P. XI.

Table II.—Results Obtained with U. S. P. XI Method, Modification *A*

Sample	Wt. of Sample	1*N* H_2SO_4, Cc.	$(CH_2)_6N_4$, %	Time, Min.
1	1.0365	29.69	100.37	260
2	1.0209	29.21	100.26	250
3	1.0153	29.09	100.36	250
4	1.0070	28.83	100.29	220
5	1.0138	29.08	100.48	230

III. *U. S. P. XI Method, Modifications B, C, D and E.*—The sample is heated as in Modification *A*, but a change is made in the strength of the sulfuric acid used and the amount of sample is varied to correspond to the strength of the acid. Double-normal, half-normal, fifth-normal and tenth-normal sulfuric acid are used. The results show that decrease in concentration of acid tends to increase the accuracy of the determination and decrease the time required for the assay.

Table III.—Results Obtained with U. S. P. XI Method, Modification *B*

Sample	Wt. of Sample	2*N* H_2SO_4, Cc.	$(CH_2)_6N_4$, %	Time, Min.
1	2.0228	29.37	101.14	390
2	2.0023	29.43	102.88	380
3	2.0055	29.25	102.00	405
4	2.0164	28.91	100.36	405
5	2.0049	29.02	101.32	380

Table IV.—Results Obtained with U. S. P. XI Method, Modification *C*

Sample	Wt. of Sample	0.5*N* H_2SO_4, Cc.	$(CH_2)_6N_4$, %	Time, Min.
1	0.5039	28.81	100.05	210
2	0.5077	29.04	100.09	210
3	0.5071	29.05	100.25	195
4	0.5023	29.18	101.66	180
5	0.5003	28.64	100.17	180

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Table V.—Results Obtained with U. S. P. XI Method, Modification *D*

Sample	Wt. of Sample	0.2*N* H_2SO_4, Cc.	$(CH_2)_6N_4$, %	Time, Min.
1	0.2005	28.58	99.77	150
2	0.2005	28.62	99.92	140
3	0.2005	28.52	99.77	135
4	0.2005	28.50	99.50	135
5	0.2005	28.59	99.81	145

Table VI.—Results Obtained with U. S. P. XI Method, Modification *E*

Sample	Wt. of Sample	0.1*N* H_2SO_4, Cc.	$(CH_2)_6N_4$, %	Time, Min.
1	0.10063	28.60	99.55	120
2	0.10063	28.62	99.62	120
3	0.10063	28.60	99.55	135
4	0.10063	28.65	99.73	120
5	0.10063	28.65	99.73	120

IV. *U. S. P. XI Method, Modification F.*—This Modification differs from the U. S. P. XI Method in that the mixture of sample and acid is placed in a 400-cc. beaker and actively boiled to expel the formaldehyde. This lessens the time required for the assay.

Table VII.—Results Obtained with U. S. P. XI Method, Modification *F*

Sample	Wt. of Sample	1*N* H_2SO_4, Cc.	$(CH_2)_6N_4$, %	Time, Min.
1	1.0290	30.22	100.19	50
2	1.0027	29.47	100.36	50
3	1.0040	29.50	100.34	50
4	1.0041	29.54	100.51	50
5	1.0255	30.10	100.42	55

V. *U. S. P. XI Method, Modification G.*—Tenth-normal sulfuric acid and about 0.1 Gm. of sample are used in this method. In all other respects it is the same as Modification *F*.

Table VIII.—Results Obtained with U. S. P. XI Method, Modification *G*

Sample	Wt. of Sample	0.1*N* H_2SO_4, Cc.	$(CH_2)_6N_4$, %	Time, Min.
1	0.10049	28.59	99.66	35
2	0.10049	28.59	99.66	35
3	0.10049	28.65	99.87	45
4	0.10049	28.62	99.76	35
5	0.10049	28.60	99.69	30

VI. *Method of Brown and Otten (1).*—About 0.7 Gm. of methenamine is transferred to a Kjeldahl flask and 50 cc. of normal sulfuric acid are added. The flask containing the mixture is connected to a distilling apparatus and, by means of steam, the formaldehyde is distilled into a mixture of 50 cc. of normal potassium hydroxide containing 50 cc. of 3 per cent hydrogen peroxide which is previously rendered neutral to phenolphthalein T.S. After distillation is complete, the receiving flask and contents are heated on a water bath for three hours. The excess alkali is then titrated with normal sulfuric acid, phenolphthalein T.S. being used as the indicator. Each cc. of normal potassium hydroxide is equivalent to 0.0233 Gm. of $(CH_2)_6N_4$. Table IX shows the results so obtained are inconsistent.

Table IX.—Method of Brown and Otten (1)

Sample	Wt. of Sample	1*N* KOH, Cc.	$(CH_2)_6N_4$, %
1	0.7241	30.63	98.56
2	0.6824	28.47	96.91
3	0.7005	28.86	95.99
4	0.7104	31.50	103.03
5	0.7271	33.45	107.17

VII. *Sugiura and Falk (2) Method, Modified.*—Transfer about 0.1 Gm. of methenamine, accurately weighed, to a 100-cc. volumetric flask containing about 10 cc. of distilled water. Add 50 cc. of tenth-normal alcoholic iodine solution and enough distilled water to make 100 cc. Mix, and allow to stand for five minutes. Filter, and titrate 50 cc. of the filtrate with tenth-normal sodium thiosulfate solution, using starch T.S. as the indicator. Each cc. of tenth-normal iodine is equivalent to 0.0035 Gm. of $(CH_2)_6N_4$. Not more than 95.45 per cent of the theoretical amount of methenamine was found in any of the determinations by this method.

Table X.—Method of Sugiura and Falk (2)

Sample	Wt. of Sample	0.1*N* I_2, Cc.	$(CH_2)_6N_4$, %
1	0.0546	12.50	80.19
2	0.0665	14.82	78.00
3	0.0594	16.20	95.45
4	0.0594	15.92	93.63
5	0.0572	14.28	87.37

VIII. *Method of Korostishev'ska (3).*—Dissolve about 1 Gm. of methenamine, accurately weighed, in enough water to make exactly 100 cc. Transfer 5 cc. of this solution to a 150-cc. beaker and, drop by drop with constant agitation, add 30 cc. of tenth-normal iodine solution containing 18 Gm. of potassium iodide per liter. Let stand for five minutes and filter through cotton, discarding the first 5 cc. of the filtrate. Titrate 25 cc. of the subsequent filtrate with tenth-normal sodium thiosulfate, using starch T.S. as the indicator. Each cc. of tenth-normal iodine is equivalent to 0.0035 Gm. of methenamine.

The original work of Korostishev'ska (3) was not available to the writer, and it was, therefore, necessary to follow the directions given in the abstract. It was observed that the first two drops of iodine solution failed to produce a precipitate, and would lead one to suspect that the results obtained would be low due to the slight solubility of the precipitate. However, it will be noted that in the determinations made, the amount of methenamine found exceeds 100 per cent. Thus, the 99.6 to 99.8 per cent accuracy claimed for the method is not realized.

Table XI.—Method of Korostishev'ska (3)

Sample	Wt. of Sample	0.1*N* I_2, Cc.	$(CH_2)_6N_4$, %
1	0.03648	10.92	104.78
2	0.03648	11.09	106.40
3	0.03648	11.06	106.11
4	0.03606	11.07	107.43
5	0.03606	11.01	106.86

IX. *Kjeldahl Determination.*—Accurately weigh about 1 Gm. of methenamine and transfer to a 500-cc. Kjeldahl flask. Add 10 Gm. of powdered potassium sulfate, 0.5 Gm. of powdered cupric sulfate and 20 cc. of sulfuric acid. Digest for thirty minutes, allow the mixture to cool, add 150 cc. of distilled water and then cool again. To the mixture add 150 cc. of 30 per cent aqueous solution of sodium hydroxide in such a way as to cause the solution to flow down the inner side of the flask to form a layer under the acid solution. Add a few pieces of granulated zinc and connect the flask by means of a Kjeldahl connecting bulb with a condenser, the delivery tube from which dips beneath the surface of a mixture of 40 cc. of half-normal sulfuric acid and 25 cc. of distilled water contained in a 400-cc. beaker. Distil and, after distillation is complete, add 1 drop of methyl red T.S. to the contents of the receiving flask and determine the excess acid by titration with half-normal sodium hydroxide. Each cc. of half-normal sulfuric acid is equivalent to 0.007 Gm. of nitrogen or 0.0175 Gm. of $(CH_2)_6N_4$.

Table XII.—Kjeldahl Determination

Sample	Wt. of Sample	0.5*N* H_2SO_4, Cc.	$(CH_2)_6N_4$, %	Total N_2, %
1	0.5149	29.28	99.51	39.85
2	0.5469	31.10	99.51	39.85
3	0.5168	29.32	99.28	39.69
4	0.5304	30.22	99.70	39.88
5	0.5039	28.68	99.60	39.83

CLASS 2. WORK ON METHODS NOT REPORTED IN THE LITERATURE

I. *Decomposition of Methenamine with Acid and Oxidation of the Formaldehyde Formed with a Solution of Hydrogen Peroxide.*—Procedure: Dissolve about 0.1 Gm. of methenamine in 15 cc. of normal sulfuric acid and add 50 cc. of 3 per cent hydrogen peroxide, previously rendered neutral to phenolphthalein T.S. Warm the mixture and allow to stand for ten minutes at about 90° C. Now add 40 cc. of normal sodium hydroxide and gently boil the solution until all of the ammonia is expelled, adding distilled water from time to time to keep the volume constant. Titrate the excess of sodium hydroxide with normal sulfuric acid. Each cc. of normal sodium hydroxide is equivalent to 0.0233 Gm. of $(CH_2)_6N_4$.

As will be noted from the determinations reported in Table XIII, the results of this assay were consistently low and, after numerous attempts, the method was abandoned.

Table XIII.—Oxidation Method with Hydrogen Peroxide

Sample	Wt. of Sample	1*N* NaOH, Cc.	$(CH_2)_6N_4$, %
1	0.1193	3.2	62.50
2	0.1155	4.4	88.80
3	0.1140	4.4	89.90
4	0.1095	3.9	82.90
5	0.1364	5.9	101.07

II. *Argentometric Method.*—Transfer from 0.1 to 0.2 Gm. of methenamine to a 250-cc. Erlenmeyer flask containing about 20 cc. of distilled water. Add about 1 Gm. of potassium chlorate, 50 cc. of tenth-normal silver nitrate and 2 cc. of nitric acid T.S. Heat the mixture on a water bath for thirty minutes. Filter the solution and wash the precipitate with distilled water until free of silver nitrate. Titrate the combined filtrate and washings with tenth-normal ammonium thiocyanate, using ferric ammonium sulfate as the indicator. Each cc. of tenth-normal silver nitrate is equivalent to 0.007 Gm. of $(CH_2)_6N_4$. Results of this assay, as indicated in Table XIV, were not satisfactory and the method was discontinued.

Table XIV.—Argentometric Method

Sample	Wt. of Sample	0.1*N* $AgNO_3$, Cc.	$(CH_2)_6N_4$, %
1	0.2232	38.31	123.28
2	0.2286	42.10	128.91
3	0.1136	21.92	135.07
4	0.1075	20.53	133.68
5	0.1010	18.33	127.03

III. *Potassium Bromate Method.*—Spitzer (4) determined formaldehyde using a solution of potassium bromate. An attempt was made to apply the same method to the formaldehyde formed by the hydrolysis of methenamine. The results were so unsatisfactory that this method was discarded.

IV. *Alkaline Hypobromite Method.*—Ammonium compounds, treated with sodium hypobromite, liberate nitrogen. Methenamine, digested with sulfuric acid, decomposes and ammonium sulfate is one of the products of decomposition. A combination of these two reactions seemed to point to a method for the assay of methenamine.

Preparation of sodium hypobromite solution: Dissolve 125 Gm. of sodium bromide in 200 cc. of distilled water in a 1000-cc. volumetric flask, add 125 Gm. of bromine and enough distilled water to make 1000 cc. Mix thoroughly. Add 10 cc. of the bromine solution to 10 cc. of sodium hydroxide, containing 25 Gm. of U. S. P. sodium hydroxide in each 100 cc., and dilute the mixture to 100 cc. with distilled water.

Assay: Gently boil 1 Gm. of methenamine with 25 cc. of sulfuric acid T.S. contained in a 250-cc. beaker until the odor of formaldehyde is no longer perceptible. Cool, transfer to a 200-cc. volumetric flask, add distilled water to the mark and mix thoroughly. Pipette 10 cc. of this dilution into a 250-cc. glass-stoppered bottle containing 25 cc. of the sodium hypobromide solution and allow to stand for fifteen minutes, shaking intermittently. Now add 3 Gm. of potassium iodide and an excess of acetic acid (about 15 cc.). Titrate the liberated iodine with tenth-normal sodium thiosulfate, using starch T.S. as the indicator. At the same time carry out a blank test using exactly the same quantities of reagents. The difference in the number of cc. of tenth-normal sodium thiosulfate consumed in the

actual test and in the blank test, multiplied by 0.1167 and divided by the weight of sample taken, gives the per cent of $(CH_2)_6N_4$ in the sample.

Table XV shows that the results obtained by the Alkaline Hypobromite Method exceeded 100 per cent of methenamine. This may be explained by stating that the iodine color consistently returned after an apparent end-point was reached in the titration. In an attempt to overcome this difficulty, the titration was carried on in an atmosphere of carbon dioxide but the same difficulty was still encountered.

Table XV.—Alkaline Hypobromite Method

Sample	Wt. of Sample	0.1*N* NaBrO, Cc.	$(CH_2)_6N_4$, %
1	0.05025	43.29	100.53
2	0.05025	43.47	100.95
3	0.05025	43.15	100.20
4	0.05025	43.26	100.46
5	0.05025	43.15	100.20

V. *Calcium Hypochlorite Method.*—In the presence of sodium bicarbonate and bromide, ammonia is oxidized quantitatively to nitrogen and water by calcium hypochlorite (5).

$$2NH_3 + 3OBr^- = N_2 + 3Br + 3H_2O$$

After the conversion of methenamine to ammonium sulfate, a method of assay was tried which was based on the reaction between the ammonium sulfate and calcium hypochlorite. The results were accurate and the assay could be performed in about thirty minutes.

Preparation of calcium hypochlorite solution: Mix about 10 Gm. of calcium hypochlorite with about 200 cc. of water and allow to stand for five minutes. Filter the solution into a flask and add enough distilled water to the filtrate to make about 1000 cc. The solution prepared should be at least tenth-normal when titrated with sodium thiosulfate as directed in the assay.

Assay: Accurately weigh from 0.95 to 1.05 Gm. of sample, transfer it to a 200-cc. volumetric flask and add distilled water to the mark. When the sample has dissolved, pipette exactly 10 cc. of the dilution into a glass-stoppered flask, and add 10 cc. of distilled water and 10 cc. of sulfuric acid T.S. Boil the solution vigorously, adding a little distilled water from time to time, until the odor of formaldehyde is no longer perceptible. Cool, add one drop of methyl red T.S., neutralize with potassium bicarbonate, 1 : 10 and then add 3 Gm. of potassium bromide. While gently agitating the flask, slowly add 45 cc. of calcium hypochlorite solution. Stopper the flask and let stand for five minutes with occasional shaking. Remove the stopper and add 3 Gm. of potassium iodide, previously dissolved in 15 cc. of 36 per cent acetic acid. Titrate the liberated iodine with tenth-normal sodium thiosulfate, using starch T.S. as the indicator. Run a blank, omitting the sulfuric acid and potassium bicarbonate but using exactly the same quantities of the other reagents. The difference in the number of cc. of tenth-normal sodium thiosulfate consumed in the actual test and in the blank, multiplied by 0.1167 and divided by the weight of sample used, gives the per cent of $(CH_2)_6N_4$ in the sample.

Notes on the assay: The time necessary for the hydrolysis of the sample is fifteen to twenty minutes. Potassium bicarbonate is added until the solution is neutral to methyl red indicator because, if strongly acid, free bromine will be liberated and, if too alkaline, the end-point is indefinite and high results are obtained.

Table XVI.—Calcium Hypochlorite Method

Sample	Wt. of Sample	0.1*N* $Ca(OCl)_2$, Cc.	$(CH_2)_6N_4$, %
1	0.05025	42.98	99.81
2	0.05025	42.88	99.57
3	0.05025	43.03	99.92
4	0.05025	42.98	99.81
5	0.05025	42.98	99.81
6	0.05025	43.04	99.95
7	0.05025	43.07	100.01
8	0.05025	42.92	99.78
9	0.05025	43.01	99.88
10	0.05025	43.03	99.92

VI. *Tablets Containing 5 gr. of Methenamine and 5 gr. of Sodium Acid Phosphate Were Assayed by the Calcium Hypochlorite Method.*—About 1 Gm. of the powder, representing about 0.5 Gm. of methenamine, was accurately weighed and dissolved in enough water to make 200 cc. A portion of this dilution was treated in the same manner as in the previous (V) method. Since there is only about half as much of methenamine, a total of 20 cc. was used. Table XVII shows the accuracy of this method.

Table XVII.—Assay of Tablets

Sample	Wt. of 10 Tabs.	Wt. of Sample	0.1*N* $Ca(OCl)_2$, Cc.	Gm. of $(CH_2)_6N_4$ per Tablet
1	6.1888	0.10022	45.31	0.3265
2	6.1888	0.10022	45.15	0.3253
3	6.1888	0.10022	45.31	0.3265
4	6.1888	0.10022	45.41	0.3271
5	6.1888	0.10022	45.40	0.3270

CONCLUSIONS

1. Assay of methenamine by the U. S. P. XI method is slow and unsatisfactory. Greater accuracy may be obtained by using less sample and tenth-normal solutions.

2. The results obtained by the precipitation methods that were tried were inaccurate.

3. The use of hydrogen peroxide and potassium bromate as oxidizing agents gave poor results.

4. The alkaline hypobromite method was rapid but, possibly due to excess alkalinity, the method was not entirely satisfactory.

5. Of the methods tried, the calcium hypochlorite method seems best suited for the assay of methenamine and certain mixtures containing methenamine. The method is simple, comparatively rapid and accurate.

REFERENCES

(1) Brown and Otten, Merck's Report, 17 (1908), 257–260.

(2) Sugiura and Falk, *Biochem. Bull.*, 5 (1916), 18.

(3) Korostishev'ska, *Farm. Zhur.*, 13, No. 2 (1940), 23–27; *Chem. Abstr.*, 35 (1941), 848.

(4) Spitzer, *Chem.-Ztg.*, 57 (1933), 224.

(5) Kolthoff and Sandell, "Textbook of Quantitative Inorganic Analysis," New York (1936), page 611, The Macmillan Company.

Electrophoretic Separation of the Blood Pressure Principles of Hog Kidney Extracts*

By Raymond Jonnard and Marvin R. Thompson

It has been shown by various authors that the ischemic kidney of various animals yields, under suitable conditions, a vaso-pressor substance called "renin" (1, 2, 3, 4, 5). This substance is also present in the normal organ (4, 6) and is somewhat specific (8, 10). It is neutralized by a vaso-depressor substance extractable under certain conditions (7, 9). The work done to date indicates that the chemical properties of these substances have some similarity, so that their complete separation is difficult. Thus they are usually obtained together in solution during the preliminary stages of the extraction of the kidneys.

Much stress has been laid upon the biological properties of these two antagonistic principles, but the literature contains only very limited information on the physical and chemical properties of the substances involved. Most of the alleged properties of these substances have been inferred from solubility data and from the conditions of their extraction. Although it can be safely assumed, to date, that "renin" is a protein-like substance, little can be said as yet of the nature of the depressor principle.

In view of the findings by du Vigneaud and co-workers (11) that the vaso-depressor factor extracted from the hypophysis gland could be isolated in the catholyte compartment of an electrophoretic apparatus, and that this substance seems to have an iso-electric point far in the alkaline range, it was thought desirable to submit certain kidney extracts to a similar electrophoretic analysis.

In view of the uncertainty in the identification of the various kidney depressor principles claimed by various authors (6, 12, 13, 14), we have used several different types of kidney extracts.

EXPERIMENTAL

METHOD

I. Electrophoresis.—Due to the minute amount of active material contained in the hog kidney, this active material is usually obtained in the form of dilute solutions as will be discussed later. Therefore, a rather large electrophoretic cell had to be designed in order to separate the components of a volume of solution large enough to yield material for repeated bioassays. The apparatus was made of large flanged Pyrex glass fittings (15); it was somewhat similar to that of Russell and Stauffer (16). The clamps holding the various parts were also used to squeeze removable diaphragms of either paper or cellophane. Both anodic and cathodic compartments had a capacity of 90 cc. The medium cell held 200 cc. The electrode compartments held 300 cc. The anodic and cathodic compartments were separated from the central cell by paper diaphragms and from the electrode compartments by cellophane diaphragms preventing the escape of the practically non-dialyzable vaso-motor principles in the present series of experiments. The whole apparatus was held on a "flexaframe" and could be immersed in a bath of running water at any given temperature. The assembly is shown in Fig. 1.

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