SOME NOTES ON THE DIACETYL MONOXIME METHOD FOR THE DETERMINATION OF UREA IN BIOLOGICAL FLUID. USE OF PERCHLORIC ACID AS OXIDIZING REAGENT

IWAO IUCHI

Department of Clinical Pathology, Yamaguchi Medical College, Ube

(Received May 1, 1958)

A variety of procedures different in principle have been invented for the the purpose of determination of urea in blood. They include the urease method,[1] the heating hydrolysis method,[2] the hypobromite method,[3] the xanthydrol method,[4] the diacetyl monoxime method[5~7] and others. Perhaps the diacetyl monoxime method will be among the simplest of all these. Urea produces a reddish brown complex when it is heated with acidic diacetyl monoxime solution. Its color is suitable for colorimetry if a concomitantly arising adverse by-product, hydroxylamine, is eliminated by an oxidizing reagent.[8] Potassium persulfate and arsenic acid have been used as oxdants in the presence of concentrated hydrochloric acid.[9,10] However, the former is an unstable and unreliable reagent which results in occasinal decolorization of the color complex of urea itself in addition to the elimination of hydroxylamine. The latter is satisfactory for the purpose if it is of excellent quality. Unfortunately an excellent commercial arsenic acid is rare.

In our laboratory a bottle of arsenic acid had been in successful use for diacetyl monoxime method until about two years ago, it was almost exhausted, necessitating another bottle to replace it. The new bottle failed to give a satisfactory result in spite of its having the same quality and same trade mark as its predecessor. Numerous bottles of arsenic acid of different trade marks were tested in vain. Finally arsenic acid was abandoned, and an attempt was made to get other oxidants. Perchloric acid was thus discovered as a suitable reagent.

The procedure to be presented in this paper is a diacetyl monoxime method using perchloric acid as an oxidant.

METHOD

Regaent

1) 10g/dl trichloroacetic acid aqueous solution
2) 1g/dl diacetyl monoxime solution
   One gram of diacetyl monoxime is dissolved in 100ml of 5% v/v acetic acid.
3) Oxidizing reagent

One volume of 60% perchloric acid (guaranteed, d: 1.55) and 4 volumes of concentrated hydrochloric acid 35%,d: 1.18) are mixed.

4) 10/3mg/dl standard urea N solution

Crystals of urea are spread on a watch glass to be dried in an electric oven (100°C) for an hour. After it has been cooled, it is kept in a desiccator. 321.5mg of this dried urea is dissolved in 300ml of water, which volume is made to 500 ml with distilled water and stored in a refrigerator. One ml of this solution is diluted with 8 ml of distillied water shortly before use.

Procedure

1) One tenth ml of serum or whole blood (urine diluted 10–30 times with distilled water, depending upon its concentration of urea) is added to 1.5 ml of distilled water in a test tube. A volume of 1.4 ml of trichloroacetic acid solution is poured into the test tube drop by drop with constant shaking to precipitate protein, and the mixture is filtered with a filter paper (Toyo-roshi No. 7 for analytical use) to obtain limpid, colorless, protein-free filtrate.

2) Standard urea solution and protein-free filtrate are pipetted into each of the test tubes (brown in color) A, B, C and S as follows.

	10/3 mg/dl Urea	Filtrate	H_2O
A	0.1		0.9
B	0.3		0.7
C	0.5		0.5
S		1.0	(ml)

One ml of diacetyl monoxime solution and 1.5 ml of oxidizing reagent are added to each, and mixed thoroughly. The test tubes are placed in a boiling water bath after having been stoppered with cork stoppers.

3) At the end of the specified time they are removed from the water bath and cooled in running water.

The absorbance of the solutions in the test tubes are measured in a photoelectric colorimeter with a blue filter which has maximum transmission at 470mμ against a blank which is composed of distilled water.

4) A calibration curve is drawn on a section paper with absorbances as ordinates and concentrations of urea as abscissas. The test tubes A, B and C are regarded as having 10, 30 and 50 mg/dl of urea N, respectively. The urea concentration of tube S is read from the absorbance of tube S by comparing it with the calibration curve. The urea concentration of blood or blood serum is identical with the urea reading (mg/dl) of tube S, while the concentration of urea in urine is given as the urea reading (mg/dl) of tube S multiplied by dilution of urine.

DISCUSSION

The maximum absorption of the colorized solution (urea-diacetyl monoxime

complex) was found at 478 mμ as shown in Figure 1. Its yellowish color obeyed Beer's law within a range of wave lengths of light from 440 to 460 mμ which were selected for colorimetry (Figure 2). The intensity of coloration was almost equal to that obtaind when arsenic acid was used as oxidant. An optical filter

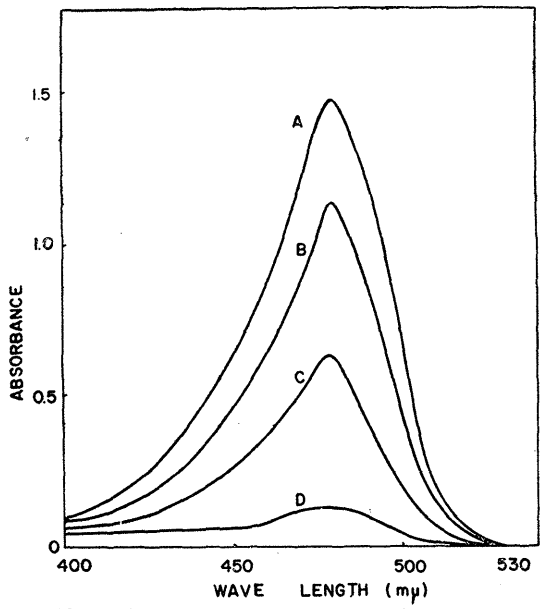

Fig. 1. Absorption spectrum of the colorized solution (Beckman QB-50 Spectrophotometer; Optical path 10mm)

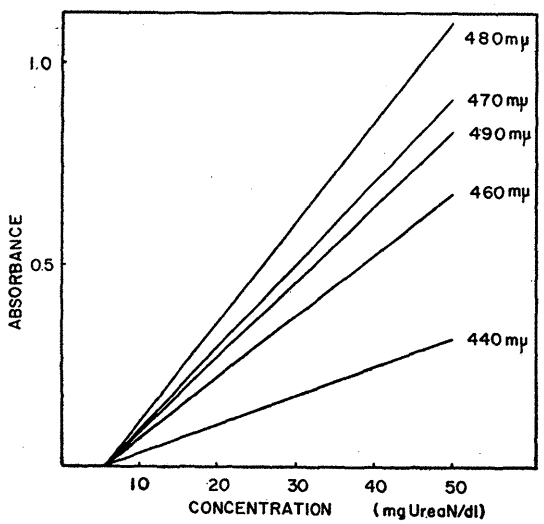

Fig. 2. Obedience of the colorized solution to Beer's law. (Beckman QB-50 Spectrophotometer, Optical path 10mm)

having its maximum transmission at 470 mμ was therefore used for colorimetry.

Since the urea- diacetyl monoxime complex was somewhat sensitive to light, it was recommended to avoid direct sun light in order to prevent the fading of the colorized solution. Brown test tubes were satisfactory for that purpose. As little as 2 per cent decrease in absorbance occurred when the colored solutions were allowed to stand for an hour after they were removed from the water bath. The fading was less distinct in this procedure than when arsenic was used as oxidant.

The coloration was distinctly more intense in this procedure than when potassium persulfate was used as oxidant. The absorbances of the colorized solutions at 470mμ varied 100 : 110 : 50 (ratio) according as perchloric acid, arsenic acid and potassium persulfate, respectively, were used as oxidants.

Arsenic acid, accordingly, seemed to produce the greatest intensity of coloration. However, it only seemed so, because arsenic acid solution was usually stained slight yellow whereas the solution of perchloric acid was always colorless. The original faintly yellow coloration of arsenic acid soloution may have resulted in apparently larger absorbance of the colorized solution of urea-diacetyl monoxime complex.

It was of primary importance to stopper the test tubes while they were in the boiling water bath to get maximum final coloration. Sixteen samples of serum (9–17 mg/dl Urea N) and 6 samples of urine (94–205 mg/dl urea N) were determined for urea concentretion by this procedure and by the conventional diacetyl monoxime method which utilizes arsenic acid as oxidant to compare their determinations. The difference of urea determination between these two methods was with in 2.4%. This was not a significant difference, because it was almost equal to the limit of technical error of these procedures.

The average discrepancy of duplicate determinations (15 samples of serums) was within 2.3%.

This procedure is therefore thought to satisfy all the requirements which qualify a routine method for the determination of urea in biological fluids.

CONCLUSION

Diacetyl monoxime method is one of the simplest procedures for the determination of urea in biological fluids. Urea-diacetyl monoxime complex which has been produced by heating the mixture of deproteinized biological fluids and diacetyl monoxime solution in the presence of a suitable oxidant is subjected to photoelectric colorimetry at 470 mμ. The successful determination depends on the quality of the oxidant. Potassium persulfate is unreliable because it fades out urea-diacetyl monoxime complex. Arsenic acid is satisfactory, but there is great difficulty in getting an excellent arsenic acid reagent on the market. This limits the use-

fulness of arsenic acid as oxidant for the diacetyl monoxime method. Perchloric acid is as satisfactory as arsenic acid, and it is a popular and stable reagent available any place.

A new diacetyl monoxime method using perchloric acid as oxidant was presented with the data of the examination on its reliability and precision.

Perchloric acid solution keeps at room temperature for several months without deterioration.

References

1) Van Slyke, D. D. & Cullen, G. E.: A permanent preparetion of urease, and its in the determination of urea. *J. Biol. Chem.* **19**: 211, 1914; The determination of urea by the urease method. J. Biol. Chem. **24**: 117, 1916.

2) Clark, E. P. & Cllip, J. B.: A procedure for the determination of urea in Folin-Wu blood filtrates by the autoclave method. J. Biol. Chem., **67**: 621, 1926.

3) Krogh, M.: Die Harnstoffbestimmung im Harn mit Natrium hypobromite. *Z. Phsiol. Chem.* **94**: 397, 1913.

4) Fosse, R.: Origine et distribution de l' urèe dans la nature. Application de nouvelles méthodes d'analyse de l' urée basèes sur l' emploi du xanthydrol. *Ann. Chim.*, **6**: 13, 1016.

5) Fister, H. J.: *Manual of Standardized Procedures for Spectrophotometric Chemistry*. Standard Scientific Supply Corporation, New York, 1950.

6) Saito, M,: *Clinical Laboratory Procedures for Spectrophotometric Chemistry*. 3rd. ed. Nanzando (Tôkyô), 1952.

7) Daiichi Pure Chemical Co. Ltd.: *Manual of Reagents Used for Clinical Diagnosis*. Daiichi Pure Chemical Co. Ltd. (Tôkyô) 1955.

8) Dickenman, R. C., Crafts, B. & Zak,B.: Use of alpha diketones for analysis of urea. Amer. J. Clin. Path. **24**: 981, 1951.

9) Ormsby, A. A.: A direct colorimetric method for the determination of urea in blood and urine. *J. Biol. Chem.*, **146**: 595, 1942.

10) Kawerau, E.: Biochemical analysis with the Spekker absorptionmeter: I. Estimation of urea, citrulline, allantoin and related compounds. Sci. Proc. Dublin. Soc., **24**: 63, 1946.