[Chem. Pharm. Bull. 12 (7) 770 ~ 773]

UDC 612.461.26 : 547.495.9

108. Toshiro Murata, Shōko Etō, Kiyomi Yamatsu, and Kuniko Sugiura : Estimation of Creatinine in Urine with 3,5-Dinitrobenzenesulfonate.

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Estimation of urinary creatinine is one of the important clinical tests. Many analytical methods to determine the creatinine are known, of which the most prevailed one in this country is the modified Folin's method which is based on "Jaffe's reaction," the reaction between picric acid and active methylene group in the structure of creatinine.[1~4]

Formerly Akatsuka[5] reported that 3,5-dinitrobenzenesulfonic acid reacted with active methylene group and produced a stable colored compound. He estimated such compounds containing active methylene group in their chemical structure as digitoxin, strophanthin and ouabanin by using the reagent.

The urinary creatinine, one of active methylene compound, was satisfactorily determined with potassium 3,5-dinitrobenzenesulfonate (DNSK) by the authors in this study.

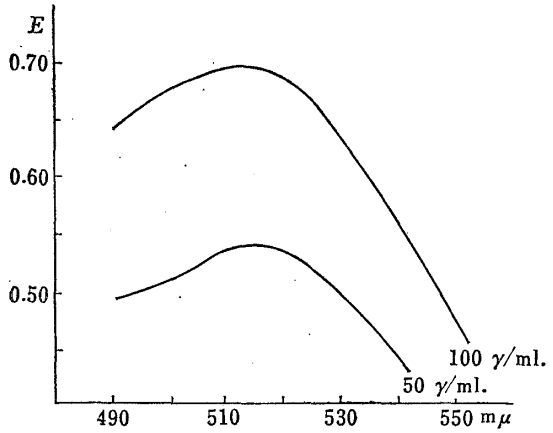

Fig. 1. Absorption Spectrum

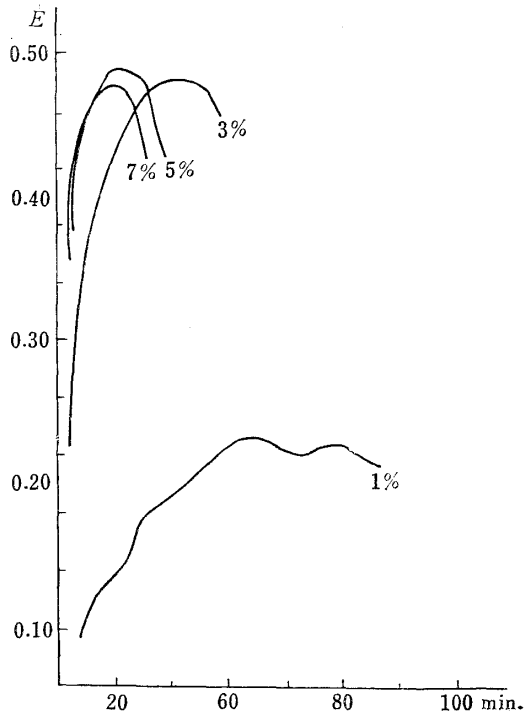

Fig. 2. Effects of the Concentration of Added Potassium Hydroxide Solution

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Experimental

Reagents

1. DNSK-reagent : DNSK[6] was recrystallized from H_2O to pale yellow needle and was dissolved in H_2O before use.
2. KOH solution : Special grade KOH was dissolved in H_2O.

Estimation of Absorption Maximum——To 4 ml. of sample solutions containing 50 γ and 100 γ of creatinine per ml. respectively, 0.5 ml. of 2% DNSK-reagent and 5% KOH solution were added, and absorption spectra were estimated. The absorption maximum was observed at about 515 mμ as shown in Fig. 1.

Effects of the Concentration of Alkali on the Color Formation——To 4 ml. of creatinine solution containing 50.48 γ of creatinine per ml., 0.5 ml. of 2% DNSK-reagent and the same volume of various concentration of the KOH solutions were added. The color developed was estimated colorimetrically at 530 mμ on every 10 min. The results are shown in Fig. 2.

After addition of the reagents, the highest extinction of the color was obtained by using 5% KOH solution. Thus the 5% KOH solution was used in the following procedures.

The Concentration of DNSK and the Stability of the Color developed——For the various concentration of creatinine, the stability of the color developed after adding 1 or 2% DNSK-reagent and 5% KOH solution were tested. It was observed that 5～20 γ per ml. of creatinine gave a stable color at 20～25 min. after addition of 2% DNSK-reagent as shown in Fig. 3.

Standard Curve of Creatinine with DNSK——The standard creatinine solution was prepared by dissolving creatinine in distilled H_2O and a few drops of toluene was added to the solution for preventing putrefaction. To 4.0 ml. of the creatinine solution, each 0.5 ml. of 2% DNSK and 5% KOH solution was added and well mixed. The reaction mixture was estimated colorimetrically at 20～25 min. after addition of the reagents. The results gave linear relationship as shown in Fig. 4.

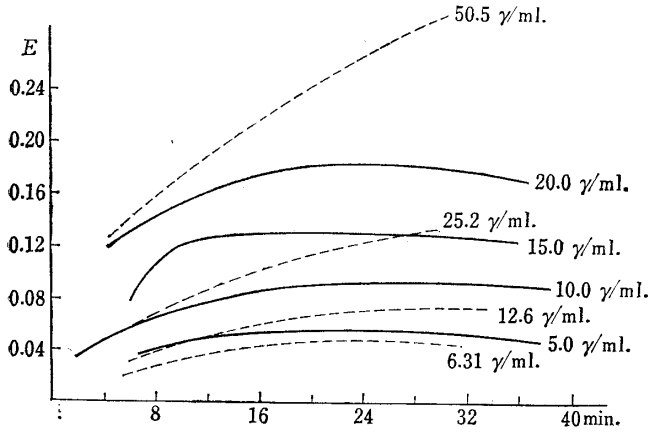

Fig. 3. Stability of the Color developed with 1 and 2% DNSK-Reagent
----- 1%, ——— 2% DNSK

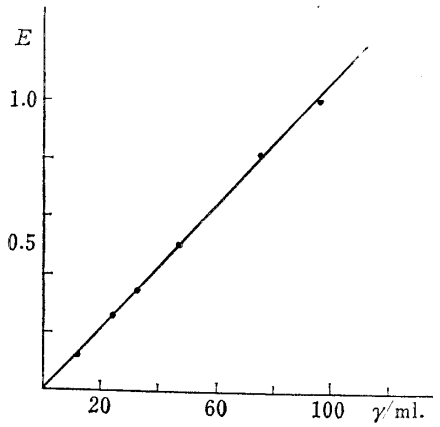

Fig. 4. Standardization Curve of Creatinine

Table I. Recovery of Creatinine Added to Urine

Sample urine	Amount of added creatinine (γ/ml.)	Found (γ/ml.)	Recovery (%)
1	9.86	9.97	101.11
2	11.46	11.93	104.10
3	18.01	18.35	101.89
4	22.81	22.65	99.30
5	19.66	19.60	99.69
6	13.58	13.75	101.25
7	10.19	10.10	99.12
8	14.84	14.45	97.31
9	14.74	14.80	100.40
Ave.			100.46±0.65

Recoveries of Creatinine contained in Urine——The urine sample was prepared by diluting normal urine until it contained 10~15 γ of creatinine per ml. Known amount of creatinine was added to the urine and the total amount of creatinine in the urine sample was estimated by the same method as described above. The recoveries thus obtained were shown in Table I. Each sample was estimated three times and average values obtained were recorded in the table.

The recoveries of creatinine added to the urine sample were also estimated by the method of using picric acid,[7] and the results were shown in Table II.

Table II. Recovery of Creatinine in Urine estimated with Picric Acid

Sample urine	Amount of added creatinine (γ/ml.)	Found (γ/ml.)	Recovery (%)
1	10.35	11.1	107.25
2	10.35	10.8	104.35
3	10.35	10.3	99.52
4	20.70	21.7	104.83
5	20.70	21.5	103.86
6	20.70	21.3	102.90
7	20.70	21.0	101.45
8	20.70	20.8	100.48
9	10.35	10.7	103.38
10	20.70	21.1	101.93
11	20.70	20.9	100.97
Ave.			102.81±0.67

Effect of Acetone on the Estimation of Creatinine——Other compounds with active methylene group may be excreted in urine, and the most probable one is so-called acetone body. In fact, 20~50 mg. of acetone bodies are daily excreted in normal urine. So the effect of Me_2CO, which is supposed to be most influential on estimating creatinine, was examined.

A control solution was prepared by adding 1 ml. of distilled H_2O to 3 ml. of creatinine solution. On the other hand, instead of the distilled H_2O, 1 ml. of Me_2CO was added to 3 ml. of creatinine solution. Those two kinds of samples were estimated and the values of creatinine were compared (Table III).

Table III. Effect of Acetone on the Estimation of Creatinine

Exptl. No.	Creatinine (γ/ml.)	Added acetone (γ/ml.)	Creatinine found (γ/ml.)
1	10.17	0	10.40
	10.17	20	10.40
2	15.26	0	15.30
	15.26	160	16.05

Discussion

Modified Folin's method for determination of urinary creatinine is based on so-called Jaffe's reaction, which is the reaction between picric acid and creatinine in alkaline medium, and the red color produced is estimated colorimetrically. In the method, however, such factors as the purity of the picric acid used, the basicity of the picric acid solution, the time lapsed for developing color after adding the reagents, and the temperature at the reaction, influence greatly on the color density.[8] Therefore, the procedure is considerably troublesome in practice and great care is required.

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The method for determination of creatinine with DNSK is comparatively simple and rapid one as shown in the experiments described above. Moreover, the recoveries of urinary creatinine gave more accurate results with DNSK than with picric acid, and standard error of the value was very small.

Acetone, which was supposed to increase the color density and be contained in normal urine, did not affect the estimation of creatinine. In the presence of a large amount of acetone, 160 γ per ml., slight increase of the recovery value (about 4%) was observed. However, such a large amount of acetone was not excreted in usual cases.

The authors express their gratitude to Dr. Akatsuka for supplying 3,5-dinitrobenzenesulfonate.

Summary

For the estimation of urinary creatinine, DNSK-reagent was used and the estimation was satisfactorily carried out under the conditions as follows;

1) Sample urine is prepared by diluting with water to contain 10~80 γ per ml. of creatinine.

2) To 4 ml. of sample urine, each 0.5 ml. of 2% DNSK-reagent and 5% KOH solution is added, and after 25 minutes the color developed is estimated at 530 mμ.

(Received February 3, 1964)

[Chem. Pharm. Bull. 12 (7) 773 ~ 778]

UDC 547.785.5.07

109. Yuichi Kanaoka, Osamu Yonemitsu, Kazutaka Tanizawa, and Yoshio Ban: Polyphosphate Esters as Synthetic Reagent. I.*[1] Synthesis of 2-Substituted Benzimidazoles.

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Research on phosphoric acid derivatives, covering an extremely wide spectrum in type and complexity, has been the subject of considerable effort.[1] The dramatic advances in the field of intermediary metabolism have been dependent upon the development of phosphorus chemistry.[2,3] In regard to organic chemical side, many effective synthetic agents have arisen based on this background. Polyphosphoric acid (PPA) may be a typical instance and its utility as an agent for acid-catalysed reactions, particularly for many condensation reactions, has been fully established.[4,5] In this laboratory, attempts have been made to find synthetic tools in phosphate ester derivatives. This first paper describes the application of "polyphosphate ester" or "metaphosphate" as reagent for benzimidazole synthesis.

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*1 Presented in part at the 83rd Annual Meeting of the Pharmaceutical Society of Japan, November 1963, Tokyo.

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