[Chem. Pharm. Bull. 20(4) 837—839 (1972)]

UDC 547.458.04 : 547.495.2.08

A New Colorimetric Method for the Determination of Urea Nitrogen in Blood with Diacetyl Monoxime-Glucuronolactone-Glucosaccharodilactone Reagent[1]

Toyoshige Kabeya and Yosuke Ohkura

Faculty of Pharmaceutical Sciences, Kyushu University[2]

(Received October 26, 1971)

In a previous paper,[3] a colorimetric method for the determination of urea nitrogen in blood and urine was presented on the basis of a color reaction of urea with diacetyl monoxime (DAM) in phosphoric acid solution in the presence of D-glucuronolactone (GL), which served to produce a photo-stable color and eliminate the sigmoidal nature of calibration curve observed usually in the DAM method.[4] The method was then modified for the microdetermination[5] and the automated determination of urea nitrogen in blood.[6] These methods, however, required a relatively long heating time (40 min) to obtain an appropriate and constant intensity.

In the course of study on the determination of urea in biological fluids, D-gluco-1,4-3,6-disaccharolactone (SL) was found to produce rapidly an intense color when added to the reaction mixture of urea with DAM in phosphoric acid solution. The developed color gradually faded in daylight, but the initial color became stable when GL was present in the reaction mixture. Then a simple, rapid and reliable method for the determination of urea nitrogen in blood was developed utilizing this finding.

Experimental[7]

Reagents[8]——Diacetyl monoxime-glucuronolactone-glucodisaccharolactone (DAM-GL-SL) reagent: Dissolve successively 5 g of DAM, 2.5 g of GL,[9] 10 g of SL[9] and 25 ml of AcOH in H_2O and dilute to 500 ml, and store in a brown bottle.

Phosphoric Acid Solution: Dilute 900 ml of H_3PO_4 (85%) with H_2O to 1000 ml.

Sodium Tungstate Solution: Prepare 10% aqueous solution of $Na_2WO_4 \cdot 2H_2O$.

Aluminum Potassium Sulfate Solution: Prepare 9.6% aqueous solution of $KAl(SO_4)_2 \cdot 12H_2O$.

Urea Nitrogen Standard Solutions: Dissolve 429 mg of dried urea in H_2O and dilute to 1000 ml. Dilute 100 ml of this solution with H_2O to 1000 ml. The resulting solution contains 20 μg/ml urea nitrogen. Using this solution, prepare dilutions corresponding to 1, 2, 3, 4, 5, 7, and 10 μg/ml urea nitrogen with H_2O.

Procedure——Dilute 0.05 ml of blood or serum with 2.95 ml of H_2O in a test-tube, add 0.5 ml of Na_2WO_4 solution and mix. Add 0.5 ml of $KAl(SO_4)_2$ solution and mix well. Transfer the mixture to a centrifuge-tube, cover with a piece of Para-film and centrifuge for 5 min at 3000 rpm. Pipet 1.0 ml of the clear supernatant solution into a test-tube, add successively 1.0 ml of DAM-GL-SL reagent and 5.0 ml of H_3PO_4 solution and mix well. Cover the tube with a piece of aluminum foil and heat it in a boiling water-bath for 20 min, then cool in running water. Measure the absorbance at 476 mμ against the reagent blank and read the value of urea nitrogen on the calibration curve, prepared as described below.

1) This forms "Organic Analysis LXXXIV." Part LXXXIII: J. Shiota, Y. Ueda, Y. Ohkura, and T. Momose, *Yakugaku Zasshi*, in press.
2) Location: *Katakasu, Fukuoka.*
3) T. Momose, Y. Ohkura, and J. Tomita, *Clin. Chem.* **11**, 113 (1965).
4) For example, see C.J. Anderson and B. Strange, *Scandinav. J. Clin. Lab. Invest.*, **11**, 122 (1959).
5) T. Momose, Y. Ohkura, and T. Imaizumi, *Yakugaku Zasshi*, **86**, 678 (1966).
6) T. Kabeya, Y. Ohkura, and T. Momose, *Chem. Pharm. Bull.* (Tokyo), **18**, 2253 (1970).
7) Absorbance and absorption spectrum were measured by a Hitachi UV-Vis 139 Spectrophotometer and a Shimadzu Recording Spectrophotometer SV-50A, respectively, with a cell of 10 mm light path.
8) All reagents used were JIS Reagent Grade unless otherwise noted.
9) Supplied from the Research Laboratories of Chugai Pharmaceutical Co., Tokyo.

Calibration Curve——Treat three 1.0 ml aliquots of each urea nitrogen standard solution (1—10 μg/ml) and of H_2O for the reagent blanks in the same way as for the deproteinized blood solution, and measure the absorbances against the pooled blank. The calibration curve thus obtained is shown in Fig. 1. The urea nitrogen value of sample calculated in mg/dl is obtained by multiplying the μg/ml of urea nitrogen standard solution by 8.

Result and Discussion

The absorption spectrum of the developed color (absorption maximum, 476 mμ) is almost identical in the shape and maximum with that of the color developed in the absence of SL[3] (absroption maximum, 475 mμ), suggesting that SL might only act as an accelerator of the reaction.

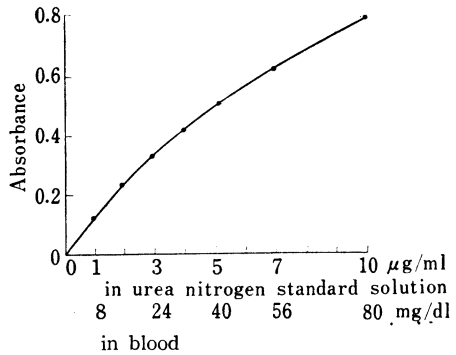

Fig. 1. Calibration Curve

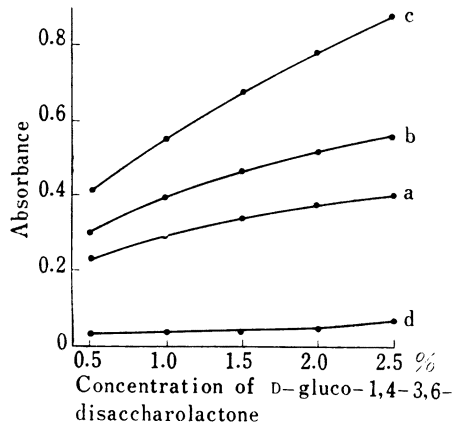

Fig. 2. Effect of the Concentration of D-Gluco-1,4-3,6-disaccharolactone on the Color Development

urea nitrogen concentration: a, 3; b, 5; c, 10 μg/ml. d was the corresponding reagent blank measured against water.

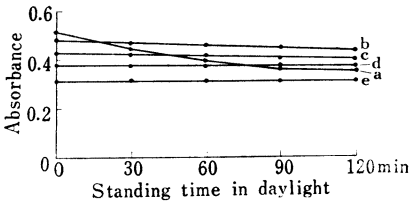

Fig. 3. Effect of the Concentration of D-Glucuronolactone on the Stability of Color Developed

urea nitrogen concentration: 3 μg/ml
D-glucuronolactone concentration: a, 0; b, 0.1; c, 0.3; d, 0.5; e, 1.0%

DAM does not affects the color development over the concentration range of 0.5—1.5%, and 1% was selected for convenience. The color intensity increases with increasing concentration of SL in the range of 0.5—2.5%, as shown in Fig. 2, and 2% was selected for lower blank. The increasing concentration of GL depresses the color development, but stabilizes the color as shown in Fig. 3. The prescribed concentration, 0.5%, gives a photostable and moderately intense color.

Phosphoric acid concentration has a relatively large effect on the color development. A 76.5% solution of the acid gave the most intense color in urea nitrogen concentration of 1—6 μg/ml (corresponded to 8—48 mg/dl of urea nitrogen in blood), and this concentration was employed as the optimum for clinical use, though a more concentated urea nitrogen required a more concentrated acid to reach the maximum intensity. With the proposed method, the color intensity reaches its maximum with the heating time of 20 min in urea nitrogen concentration of 1—6 μg/ml.

Deproteinization of blood and serum was successfully performed with sodium tungstate and aluminum potassium sulfate as in the previously established methods.[3,5]

Recovery test was carried out by adding a known amount of urea to blood or serum sample. Satisfactory results were obtained as shown in Table I.

TABLE I. Recovery of Added Urea

Sample		Initial urea nitrogen[a] (mg/dl)	Urea nitrogen added (mg/dl)	Total urea nitrogen[a] (mg/dl)	Recovery (%)
Blood	1	12.2	18.0	29.8	97.8
	2	19.8	18.0	37.1	96.1
	3	17.8	18.0	35.8	100.0
	4	16.2	18.0	34.0	98.9
	5	8.0	18.0	26.1	100.6
Serum	1	9.4	18.0	27.3	99.4
	2	11.8	18.0	29.8	100.0
	3	11.7	18.0	28.6	93.9
	4	13.7	18.0	32.2	102.8
	5	15.4	18.0	33.0	97.8
					mean 98.7

a) mean value of triplicate determinations

Substances other than urea which might be occured in blood did not influence the color development even in a concentration of 20 mg/dl in blood. Those examined were creatine, creatinine, uric acid, ammonia, histamine, choline, chondroitine, glutathione, nicotinic amide, lactic acid, 3-hydroxybutyric acid, 2-oxoglutaric acid, oxalacetic acid, pyruvic acid, ascorbic acid, inositol and 17 different α-amino acids. Glucose did not interfere with the method at a concentration of 200 mg/dl. Triptophan reduced the value of urea nitrogen by 1.6% if presented in blood in a concentration of 10 mg/dl. Citrulline and bromvalerylurea showed a positive reaction and increased the value of urea nitrogen by 2.5 and 3.8% as much, respectively, in a concentration of 10 mg/dl.

Parallel tests with the previously established micromethod[5] were carried out on 30 blood samples with the urea nitrogen values ranging from 9 to 41 mg/dl. Satisfactory coefficient of correlation for both methods, 1.01, was obtained. The precision of the method was studied with respect to repeatability, which was obtained by performing 30 analyses on blood with mean urea nitrogen value of 13.2 mg/dl. The standard deviation was 0.37 (coefficient of variation: 2.8%).

Acknowledgement We would like to acknowledge the continuing guidance of Prof. T. Momose. Thanks are also due to the staff of Central Clinical Laboratory of Kyushu University Hospital for the supply of blood samples, and to the Research Laboratories of Chugai Pharmaceutical Co., Tokyo, for the generous gifts of GL and SL.