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A Photometric Adaptation of the Zinc Uranyl Acetate Method for Sodium.

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Several colorimetric methods suitable for use with biological material have been proposed for the determination of sodium.[1,2,3] The disadvantage of these in routine biochemical analysis varies from a lack of the necessary sensitivity[1,2,3] to interference from chromogenic materials usually encountered.[3]

The proposed method is a modification of that of Butler and Tuthill.[4] All reagents used are described in that method. The precautions and stoichiometric relations are carefully preserved while the tedious weighing and washing procedures have been eliminated. In addition, a greater sensitivity is obtained which makes possible the use of a smaller volume of sample and the analysis of fluids with very low sodium content.

The procedure will be described for urine. Serum and other fluids may be prepared for analysis according to Peters and Van Slyke[5] with appropriate dilution to bring the solutions into the range of the standard curve.

Method. Add to 5 ml of urine in a 50 ml volumetric flask, 5 ml of 20% trichloroacetic acid. Mix, and dilute with water to the mark. After again thoroughly mixing, filter out any precipitated protein, if present, using a dry Whatman No. 40 or 42 filter paper and discarding the first 5 ml of filtrate.

To 10 ml of uranyl zinc acetate reagent in a 15 ml graduated pyrex centrifuge tube, add dropwise 1 ml of the urine filtrate (or prepared sample of serum, etc.) with constant stirring. Mechanical stirring is desirable though not imperative. Allow the mixture to stand for 30 to 45 minutes with occasional stirring. Remove the stirring rod, washing down with 3 ml of reagent as it is being removed. Cap the tubes (IEC No. 580 rubber caps) and centrifuge at 2000 r.p.m. for 5 to 10 minutes. Aspirate off the supernatant fluid as completely as possible. Wash the precipitate with 95% ethyl alcohol saturated with sodium zinc uranyl acetate, cap and centrifuge as above. Aspirate the supernatant and repeat the washing and aspiration. After drying the tubes in an oven or water bath at 60° to 70°C for 5 to 10 minutes, add 8 ml of hot water (60°C) to dissolve the precipitate. Cool to room temperature, make up to the 10 ml mark with water and mix. Bring the temperature to 25°C in a water bath and read at 430 mμ in a spectrophotometer or with a suitable blue filter in any photoelectric colorimeter. The zero density setting is made using a reagent blank of 1 ml water treated and washed in the same manner as the sample. The concentration of the unknown is read from a standard curve prepared with sodium chloride solutions containing 0.002 to 0.05 meq sodium/ml.

Precautions. 1. Stirring of the solution should be continued for at least 1 minute after the precipitate appears. Incomplete stirring will give erroneously low results.

2. Aspiration of the supernatant fluid in the centrifuge tube is preferable to decantation because the granular quality of the precipitate is such that it could easily be lost through rough handling. It is obvious that as much as possible of the supernatant fluid should be removed with each aspiration. This is best done if a drawn out capillary tube is used as an aspirator.

3. In the washing procedure it is best to

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1 Salit, P. W., *J. Biol. Chem.*, 1932, **96**, 959.

2 Arnold, A. E., and Pray, A. R., *Ind. and Eng. Chem.* (Anal Ed.), 1943, **15**, 294.

3 Bradbury, J. T., *J. Lab. Clin. Med.*, 1946, **31**, 1257.

4 Butler, A. M., and Tuthill, E., *J. Biol. Chem.*, 1931, **93**, 171.

5 Peters, J. P., and Van Slyke, D. D., *Quantitative Clinical Chemistry*, Baltimore, Williams and Wilkins, 1932, p. 733.

stir up the precipitate by forcing the alcohol wash solution over it in a sharp stream. The last 1 to 2 ml of the wash solution should be used to wash down the sides of the centrifuge tube.

Discussion. It will be noted that in the preparation of the urine sample, as given above, a 1:10 dilution of urine is prepared and that the phosphate has not been removed. We have found only negligible differences in comparative values obtained whether or not phosphate is removed at this dilution. An advantage offered by the extreme sensitivity of this method is that samples of very low sodium content are immediately recognized at the first step in the precipitation; *i.e.,* a minute amount of precipitate forms. In such cases, it usually suffices to repeat the precipitation using the preparation as recommended by Butler and Tuthill in which the urine is not diluted during the removal of protein and phosphate.

In the determination of serum sodium, a 1 ml aliquot of the solution of ashed sample containing the equivalent of 0.1 ml of serum is used. Here, again, the great dilution eliminates the necessity for the removal of phosphate.

Recoveries of sodium added to urines of known value are shown in Table I. Comparison of results obtained with the proposed method and that of Butler and Tuthill are presented in Table II.

The color characteristics of the water solution of the sodium uranyl zinc acetate complex were determined using both a Beckman Model DU Spectrophotometer and a Coleman Model 11A Spectrophotometer. Absorption maxima were obtained at 430 mμ on both instruments. Plots of concentration *vs* density do not give a straight line at this wave length. However, the hyperbolic curve obtained is smooth and highly reproducible.

The yellow color of the dissolved complex is stable for at least 4 hours. The intensity varies with temperature, and unknown solutions must be read at the same temperature at which the standard curve was prepared.

TABLE I.
Recovery of Sodium Added to Urine Samples.

Meq. sodium added	Meq. sodium recovered	% recovered
.0118	.0113	95.8
.0118	.0116	98.3
.0059	.0055	93.3
.0059	.0057	96.6
.0020	.0020	100.0
.0020	.0021	105.0

TABLE II.
Comparison of Results with Colorimetric and Gravimetric[4] Procedures.

Gravimetric—Meq./liter	Colorimetric—Meq./liter
36.0	36.0
69.0	68.8
28.6	30.0
63.6	65.0
82.3	81.4
89.0	87.5

In explanation of the sensitivity obtained by this method, it may be suggested that the intrinsic errors in weighing and possible imperceptible small leakage through the pores of the sintered glass filter are additive factors in the gravimetric procedure, which are eliminated in the procedure described.

Summary. A photometric method is described based on the gravimetric procedure of Butler and Tuthill in which the precipitated sodium zinc uranyl acetate is dissolved in water, giving a yellow color which is read with a suitable filter against a standard curve at 430 mμ. Phosphates in the normal urine were found to exert negligible interference. Recoveries and comparison values show the method to be more rapid and at least of equivalent accuracy with the gravimetric procedure.

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