TECHNICAL METHODS

A PHOTOMETRIC METHOD FOR THE ESTIMATION OF SODIUM AND ITS APPLICATION TO SERUM SODIUM DETERMINATION

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Most methods for the colorimetric estimation of sodium depend upon the precipitation of the metal as sodium zinc uranyl acetate, a reaction first proposed by Barber and Kolthoff (1928). The precipitated salt, after washing, is treated in various ways to give coloured solutions. There are thus three distinct stages which have to be considered in the estimation: (1) precipitation, (2) washing of the precipitate, and (3) final colour.

Precipitation of Sodium Zinc Uranyl Acetate

Among the precipitating reagents used are a 50% alcoholic solution (McCance and Shipp, 1931), and aqueous solutions (Barber and Kolthoff, 1928; Butler and Tuthill, 1931; Weinbach, 1935) of zinc uranyl acetate, all in the presence of acetic acid. The triple salt is precipitated in a number of ways. McCance and Shipp (1931) treated 1 part of aqueous sodium solution with 10 parts of alcoholic zinc uranyl acetate at 0° C. for one hour, while Albanese and Lein (1948) used 1 to 4 parts of aqueous sodium solution and 10 parts of alcoholic reagent at 4° C. for 1 hour. Of the aqueous reagents, that of Weinbach has been used most frequently. In his original method Weinbach treated 1 part of aqueous solution and 5 parts of aqueous reagent with 3.5 parts of absolute alcohol over a period of half an hour. Bradbury (1946) used 1 part of aqueous sodium solution, 4 parts of aqueous reagent (Weinbach), and 2 parts of 95% alcohol, and allowed the mixture to stand at room temperature for 20 to 30 minutes. Fowweather and Anderson (1948) found the method of Bradbury unsatisfactory and used 1 part of aqueous solution, 2 parts of aqueous reagent, 3 parts of absolute alcohol, and 2 parts of water at 3° C. for 2 hours. In the present method a mixture of 1 part of aqueous sodium solution, 2 parts of absolute alcohol, and 3 parts of alcoholic zinc uranyl acetate (prepared according to McCance and Shipp) has been used for the precipitation at room temperature over a period of two hours.

Washing the Precipitate

In many methods a saturated alcoholic solution of sodium zinc uranyl acetate is used as washing liquor, although saturated solutions of the triple salt in acetone and

glacial acetic acid have also been used. In 1938, however, Hoffman and Osgood showed that saturated solutions of the triple salt in alcohol, acetone, or glacial acetic acid produced precipitates of uranium salts when brought into contact with zinc uranyl acetate. Since the intensity of the colour reactions of sodium zinc uranyl acetate is dependent on the amount of uranium present, high results will be obtained if any of these washing liquors is used with the alcoholic and aqueous reagents previously mentioned. This difficulty was, apparently, overcome by Noyons (1939), using an aqueous solution of zinc uranyl acetate but containing a greater proportion of acetic acid than the other aqueous reagents. The triple salt, however, is not precipitated completely under 24 hours. A further disadvantage of this method is that the precipitate, consisting of larger crystals than those obtained using alcoholic zinc uranyl acetate, is more difficult to pack on centrifuging, with a consequent greater possibility of mechanical loss when the supernatant solution is removed. Darnell and Walker (1940) used ethyl acetate-acetic acid mixture for washing. This is somewhat unpleasant, and, in addition, two extra washings with ether are necessary in order to remove the acetic acid, thus greatly lengthening the procedure. A saturated alcoholic solution of magnesium acetate has been found satisfactory as a wash liquor in the method to be described. This solution (5 ml.) on adding to alcoholic zinc uranyl acetate solution (0.5 ml.) gives no precipitate, and on shaking with sodium zinc uranyl acetate and filtering gives no colour with sodium salicylate.

Final Colour

McCance and Shipp (1931) dissolved the precipitate of sodium zinc uranyl acetate in water, added acetic acid and potassium ferrocyanide, and obtained a plum-red solution. Owing to the ease with which this solution becomes cloudy, fallacious results may be obtained using photometric technique for colour measurement. Hoffman and Osgood added ammonium thiocyanate to the dissolved triple salt, but the colour does not obey Beer's law and is not very sensitive.

Fredericq (1942) treated a solution of the triple salt with alizarin and obtained a violet solution; he reported an error of 1–8% for 400–25 $\mu\text{g. Na}$.

In 1946 Bradbury measured the residual colour of the zinc uranyl acetate after the precipitation of the triple salt, and in 1948 Albanese and Lein dissolved the triple salt in water and measured the intensity of the resulting yellow solution. Both methods, however, lack sensitivity and are liable to large experimental errors when used on the microscale in routine work. In the method of Darnell and Walker the precipitate of sodium zinc uranyl acetate is dissolved in water and treated with sulphosalicylic acid and sodium acetate. The deep yellow colour obtained, although stable, does not follow exactly Beer's law, and a calibration curve must be used.

The method developed in this laboratory produces an orange colour. The triple salt, dissolved in water, is treated with 1% sodium salicylate. This solution must be prepared accurately, since the colour varies slightly with the concentration of salicylate. The colour obtained is stable for at least 18 hours and is linear over a range 57.5–287.5 $\mu\text{g. Na}$. If a trichloroacetic acid filtrate equivalent to 0.05 ml. serum is used, this range corresponds to 115–575 mg. Na/100 ml. The curve obtained, although linear, cannot be extended through the origin, so that colorimetric readings are not exactly proportional to the concentration of sodium present. The use of a

calibration curve can easily be avoided by reading the colours against an arbitrary blank instead of water, when exact proportionality of colorimetric readings and concentration is obtained. Uranyl acetate (0.2%) was chosen as an arbitrary blank, but any stable yellow solution of suitable strength can be used.

Experimental

Reagents.—The following reagents were used.

Zinc Uranyl Acetate.—Uranyl acetate, 50 g., $\text{UO}_2(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$, is dissolved in 250 ml. boiling water containing 10 ml. of glacial acetic acid. In another flask 150 g. zinc acetate, $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$, is dissolved in 250 ml. boiling water containing 5 ml. glacial acetic acid. The boiling solutions are mixed and again heated just to boiling. After standing overnight at room temperature, the solution is filtered and mixed with an equal volume of absolute alcohol. Some triple acetate is added to ensure saturation, and the reagent is kept at room temperature. A portion is filtered or centrifuged before use.

Alcohol.—Absolute.

Sodium Zinc Uranyl Acetate, $(\text{UO}_2)_3 \text{Zn} \cdot \text{Na}(\text{CH}_3\text{COO})_9 \cdot 6\text{H}_2\text{O}$.—Fifty ml. of the zinc uranyl acetate reagent is added to about 100 ml. of 50% alcohol saturated with sodium chloride. After standing, the precipitate is filtered by suction through a sintered glass filter, washed thoroughly with alcohol, and finally with ether. The last traces of ether are removed in a desiccator. A quantity of 1.537 g. of this triple salt is dissolved in water and the volume made up to 200 ml. This is a stock solution and 1 ml. is equivalent to 0.115 mg. Na.

Standard Sodium Chloride Solution A (containing 2.30 mg. Na/ml.)—Analytical dry sodium chloride, 0.585 g., is dissolved in water, and the volume made up to 100 ml.

Standard Sodium Chloride Solution B (containing 0.75 mg. Na/ml.)—Analytical dry sodium chloride, 0.191 g., is dissolved in water and the volume made up to 100 ml.

Magnesium Acetate.—Saturated alcoholic solution, which is filtered before use.

Sodium Salicylate (1%).—A quantity of 1.0 g. is dissolved in water and the volume made up to 100 ml.

Trichloroacetic Acid (7%).—A quantity of 7.0 g. is dissolved in water and the volume made up to 100 ml.

Uranyl Acetate (0.2%).—A quantity of 0.2 g. is dissolved in water and the volume made up to 100 ml.

Method

Principle.—Precipitates of triple acetate from standard sodium chloride solutions, after washing with a saturated alcoholic solution of magnesium acetate, are treated with sodium salicylate. The colours produced are compared with those obtained using standard solutions of triple acetate and sodium salicylate.

Procedure.—Dilutions of the standard NaCl solution A are made and 0.2-ml. volumes, containing 57.5–287.5 μg . Na, are precipitated with the zinc uranyl acetate reagent in the proportions 1 part NaCl solution, 2 parts absolute alcohol, and 3 parts of reagent in pyrex centrifuge tubes. The tubes are allowed to stand at room temperature for two hours with occasional mixing of the contents; they are then centrifuged for 10 minutes. The supernatant solutions are decanted and the tubes allowed to drain on filter paper. Absolute alcohol, 5 ml., saturated with magnesium acetate is added down the sides of the tubes, and the precipitates are very thoroughly mixed with the wash liquor by means of a fine glass rod. After centrifuging for 10 minutes, the supernatant solutions are decanted and the tubes allowed to drain as before. The washed precipitates are dissolved in 5 ml. water and the colour developed by the addition of 5 ml. sodium salicylate. Dilutions of the stock

triple acetate solution (1/10, 1/5, and 1/2) are made in volumetric flasks and volumes of 5 ml. treated with 5 ml. sodium salicylate.

Reading of Colour.—The orange colour is read in a Spekker absorptiometer using Ilford blue filter 602 and 0.5-cm. cells against comparison cells containing 0.2% uranyl acetate. With an "EEL" photo-electric colorimeter the colours can be read directly using filter 622 and micro-tubes (2 ml.).

The results are given in Table I and the absolute linearity of the method is readily seen.

TABLE I
COMPARISON OF RESULTS FROM PRECIPITATED SODIUM CHLORIDE SOLUTIONS AND
STANDARD SOLUTIONS OF SODIUM ZINC URANYL ACETATE

Sodium concentration ($\mu\text{g.}$)	57.5	115.0	172.5	230.0	287.5
Equivalent serum Na. (mg./100 ml.)	115.0	230.0	345.0	460.0	575.0
Spekker readings triple acetate*	0.138	0.278	0.416	0.558	0.689
Spekker readings NaCl Sol. A.†	0.137	0.278	0.415	0.556	0.686
Range of readings Minimum	0.132	0.275	0.411	0.550	0.679
Maximum	0.140	0.283	0.419	0.559	0.693
Standard deviation $\pm$	0.0023	0.0027	0.0031	0.0028	0.0050

*Mean of 4 readings (0.2% uranyl acetate in comparison cell). †Mean of 12 estimations (0.2% uranyl acetate in comparison cell).

Serum Sodium Estimation.—Serum or plasma, 0.5 ml., is treated with 1.5 ml. trichloroacetic acid, mixed well, and filtered, and 0.2 ml. of the clear filtrate (= 0.05 ml. serum) is precipitated in the same way as the standard sodium chloride solutions. For ease of calculation the colour is compared with that produced by 0.2 ml. of standard NaCl solution B treated in a similar manner.

$$\text{Serum sodium is given by } \frac{\text{Reading of unknown}}{\text{,, ,, standard}} \times 300$$

Recovery Experiments.—The results obtained when known quantities of sodium are added to plasma are given in Table II.

TABLE II
RECOVERY OF ADDED SODIUM FROM PLASMA

Plasma No.	Sodium Added (mg./100 ml.)	Sodium Found (mg./100 ml.)	Sodium Calculated (mg./100 ml.)
1	0	342	—
	10	353	352
	20	360	362
	40	382	382
2	0	330	—
	90	425	420
	90	423	420
	90	416	420
	120	444	450
	120	446	450
3	0	335	—
	100	435	435
	100	441	435
	150	480	485
	200	529	535

Summary

A simple, accurate method for the photometric estimation of sodium, depending on the interaction of sodium zinc uranyl acetate with sodium salicylate, is described. The colour obeys Beer's law, when read against an arbitrary blank, over a range 57.5–287.5 μg . Na, corresponding to a serum sodium range of 115–575 mg./100 ml.

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