LV. THE COLORIMETRIC DETERMINATION OF SODIUM.

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No easy, rapid and accurate method of determining sodium in foodstuffs and other biological material is at present available. Such a method should preferably be applicable to the same hydrochloric acid extract of the incinerated material in which calcium, magnesium and potassium are being determined. Many of the published methods are gravimetric [Kramer and Tisdall, 1921] and therefore are not only slow, but require relatively large amounts. Doisy and Bell's [1920] caesium method admittedly presents considerable technical difficulties. Of the other methods examined by us the most promising appeared to be those depending on the precipitation of the sodium as the sodium uranyl zinc (or magnesium) acetate [Barber and Kolthoff, 1928; Caley and Foulk, 1929], and the subsequent estimation of the uranium in the precipitate, either by reduction and titration with potassium permanganate, or colorimetrically with potassium ferrocyanide. Barrensheen and Messiner [1927] have described a colorimetric method based on this principle suitable for serum or other biological fluids, but, as described by them, the method is very unsatisfactory. If, for example, the precipitate is washed according to their directions it goes completely into solution during the second washing. Poulsson [1928] modified the method to overcome this obvious defect, but the method is still not directly applicable to acid extracts of incinerated materials. We have therefore subjected the whole technique to a critical study, and have established a method which can be applied to neutral or acid solutions of sodium salts. A further communication will shortly be made on the application of the method to blood and serum without incineration.

Principle.

This depends on the removal of free acids and phosphates with zinc acetate and hydroxide in 50 % alcohol. The sodium is next precipitated as the triple acetate with uranium and zinc. The uranium in the precipitate is estimated colorimetrically with potassium ferrocyanide. The standard colour is obtained either by submitting a standard solution of sodium to the same treatment as the unknown, or by using a standard solution of the triple acetate.

Reagents.

(1) *Alcoholic zinc acetate with zinc hydroxide.* As all the samples of zinc acetate we have examined contained small amounts of sodium we prepare the acetate as follows. To a hot strong solution of A.R. zinc sulphate add a slight excess of ammonia (sp. gr. 0·880). Filter on a Büchner funnel, wash thoroughly with hot water and finally suck as dry as possible. To 12·5 cc. of glacial acetic acid add the zinc hydroxide paste, prepared as above, in small amounts at a time until in slight excess. Filter and wash; make up the combined filtrate and washings to 100 cc.; add 3·0 cc. of ammonia (sp. gr. 0·880) and 300 cc. of 95 % alcohol.

(2) *Alcoholic uranyl zinc acetate reagent* [modified from Kolthoff, 1927]. (*a*) Dissolve 10 g. of uranyl acetate in 50 cc. of boiling water containing 2·0 cc. of glacial acetic acid. (*b*) Dissolve 30 g. of zinc acetate in 50 cc. of boiling water containing 1 cc. of glacial acetic acid.

Mix both solutions while boiling, raise the temperature again just to boiling, allow to stand overnight and filter. Mix the filtrate with an equal volume of absolute alcohol, allow to stand 48 hours at 0° and filter at 0°. The reagent is stable at room temperature.

(3) *95 % alcohol saturated with the triple acetate.* Prepare a sample of sodium uranyl zinc acetate by adding the uranyl zinc acetate reagent to some NaCl dissolved in 50 % alcohol. Filter or centrifuge and wash the precipitate thoroughly with 95 % alcohol. Suspend the precipitate in 95 % alcohol and allow to settle in the ice-chest. Use the supernatant fluid for washing the precipitate. Filter before use if not absolutely clear.

(4) *20 % potassium ferrocyanide.*

(5) *Standard sodium chloride.* Dissolve 1 g. of pure dry NaCl in water and make up to 100 cc. This forms the stock solution. For use dilute 2 cc. to 100 cc. 1 cc. of this dilute standard contains 0·2 mg. NaCl (0·0786 mg. Na).

(6) *Standard triple acetate.* (*a*) *Preparation of the stock solution.* Take 10 cc. of a 1 % solution of NaCl, add 80 cc. of water and 100 cc. of alcohol. Add 100–120 cc. of zinc uranyl acetate reagent. Stand for at least an hour. Collect this precipitate quantitatively and wash it carefully with ice-cold 95 % alcohol. Dissolve the precipitate in water and make up to 1000 cc. This forms the strong stock solution. Dilute some of this accurately 1 to 5. Take 5 cc. of this weak solution, dilute with water in a 25 cc. flask. Add 1 drop of glacial acetic acid and 0·5 cc. 20 % potassium ferrocyanide. Make up to 25 cc. The resulting colour is close to that obtained from 0·2 mg. NaCl (0·0786 mg. Na) in 2 cc. of water submitted to all stages of the method and made up to 25 cc.

In the removal of phosphate it is necessary to add alcohol to the aqueous solution containing Na originally measured, and to take an aliquot portion of the mixture. Since there is a contraction on mixing alcohol and aqueous

solutions the exact value of this aliquot is not known and therefore this triple acetate solution must now be accurately standardised against Na solutions.

(*b*) *Standardisation of the solution.* This should be done at least in quadruplicate. Take four samples of exactly 0·2 mg. NaCl (0·0786 mg. Na) in 2 cc. of water and subject them to every stage of the estimation, including the steps for the removal of phosphates. Transfer the precipitates to 25 cc. flasks. In two other flasks take 5 cc. of the dilute standard and add water to about 18–20 cc. Develop the uranium colour as described below in all six flasks. Match both standards against each of the quadruplicate flasks, setting the latter at 20 mm. Take the mean (the individual readings should all agree within 3 %). Suppose this to be 23 mm. The simplest method of using the standard is now to set it always at 23 mm. in the colorimeter. This is equivalent to the colour obtained by estimation from 0·0786 mg. Na, the precipitate being made up to 25 cc. and the colorimeter set at 20 mm. and the results must be calculated on this basis (see below). Those who prefer it may make the necessary dilution of the stock solution so that 5 or 10 cc. of the weak standard diluted to 25 cc. gives exactly the same colour intensity as that obtained from 0·0786 mg. Na.

Detailed description of the procedure.

In a centrifuge tube take an amount of the unknown solution containing 0·04–0·16 mg. of sodium. (This may be described as the normal range of the method, but as shown below the method will estimate accurately and directly amounts up to 0·8 mg. Na.) Dilute to 2 cc. with water, add 4 cc. of the alcoholic zinc acetate reagent, stir and cover with a rubber cap (10 cc. vaccine caps are suitable). Allow to stand for 2–3 hours at room temperature and leave at 0° overnight. While still cold centrifuge and take 3 cc. of the supernatant liquid into another centrifuge tube. Add 4 cc. of the uranyl zinc acetate reagent and stir with a glass rod drawn out at its end to about 1 mm. thick. The stirring should be continued until the precipitate begins to appear. Cover with a rubber cap and allow to stand for one hour at 0°. Centrifuge, pour off the liquid and drain the tubes by inverting on filter-paper; wipe the mouths of the tubes, and wash once with 5 cc. of the ice-cold alcohol saturated with the precipitate, taking care that the whole of the inside of the tube is rinsed. The precipitate should be stirred up. Centrifuge, and drain again. Dissolve the precipitate in water and transfer it quantitatively to a 25 cc. volumetric flask. If the precipitate is very bulky, indicating that the unknown contained more than 0·15 mg. Na, transfer it to a 50, 100, or even 200 cc. flask according to discretion. After a little experience it is easy to judge the size of flask required from the bulk of the precipitate. For the standard either take 1 cc. of the dilute sodium chloride solution (containing 0·2 mg. NaCl per cc.) and treat it in exactly the same way as the unknown and transfer it to a 25 cc. flask; or, take 5 cc. of the dilute standard triple acetate solution in a 25 cc. flask. To both standard and unknown add 1 drop of glacial acetic acid and

0·5 cc. of 20 % potassium ferrocyanide, make up to the mark with water, allow to stand for 3 minutes and match. The amounts of acetic acid and potassium ferrocyanide are those required for 25 cc. flasks; if larger flasks are used, proportionately larger amounts must be taken.

Calculation.

(*a*) *Using a standard NaCl solution.*

$$\text{Na mg. per 100 cc.} = \frac{20\ (\text{standard colorimeter reading})}{\text{Reading of unknown}} \times 0{\cdot}0786 \times \frac{100}{\text{Volume of unknown taken}}.$$

(*b*) *Using the standardised triple acetate solution.* Suppose the colorimeter set at 23 mm. (see p. 451) is equivalent to 0·2 mg. of NaCl submitted to all stages of the estimation made up to 25 cc. and set in the colorimeter at 20 mm. Then, although the arbitrary standard was set at 23 mm., the calculation is:

$$\text{Na mg. per 100 cc.} = \frac{20}{\text{Unknown}} \times 0{\cdot}0786 \times \frac{100}{\text{Vol. of unknown taken}}.$$

If 50 cc. or larger flasks have been used instead of 25 cc., the requisite additional factor must be introduced.

Discussion.

1. *Effect of* (*a*) *Time, and*
   (*b*) *Temperature on the precipitation.*

(*a*) Working with the same reagent, but in aqueous solution, Barber and Kolthoff [1928] found that precipitation was complete in ½ hour and directed that the mixture be left for 1 hour. We have shown that with our reagent 1 hour is sufficient to give complete precipitation.

Table I. *0·0786 mg. Na taken for estimation.*

Recovered at 0° (mg.)	
After 1 hour	After 18 hours
0·0785	0·0785
0·0782	0·0774
0·0793	0·0800
0·0762	0·0786

(*b*) We have found that from 0° to 10° the precipitation of sodium is complete, but that at 20° the precipitate is appreciably soluble and at 37°

Table II. *0·0786 mg. Na taken for estimation.*

	% recovered at			
	0°	10°	20°	37°
	100·0	100·5	92·7	79·6
	100·5	98·0	95·0	81·6
	99·5	99·2	92·7	80·3
	100·0	100·5	92·1	84·6
Mean	100·0	99·8	93·0	81·5

much more so. The precipitation therefore should be carried out at 0°. Table II shows the extent of the error introduced.

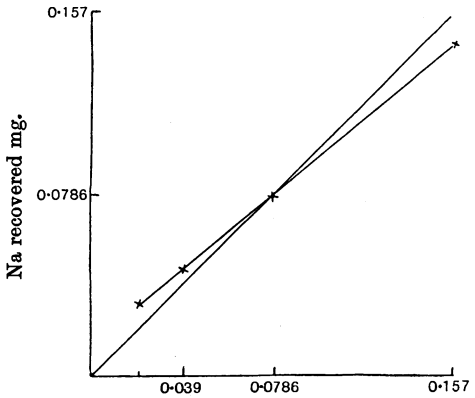
2. We have satisfied ourselves by direct experiment that the intensity of the colour developed by the potassium ferrocyanide is directly proportional to the amount of the triple acetate present. From a stock solution of the triple acetate 2 cc. of which contained about 0·039 mg. Na the following results were obtained: 1 cc., 2 cc. and 4 cc. were measured into 25 cc. flasks each in quadruplicate, 1 drop of glacial acetic acid and 0·5 cc. of potassium ferrocyanide were added to each and water added to make up to 25 cc. The flasks containing 2 cc. of stock triple acetate solution were taken as standard; they agreed with each other. The standard was then set at 20 mm. in the colorimeter and the other solutions matched against it. The results are shown in Table III.

Table III.

cc. triple acetate taken	Actual colorimeter reading mm.	Theoretical colorimeter reading mm.
1	39·4	40
1	39·4	40
1	41·0	40
1	40·5	40
4	10·0	10
4	10·1	10
4	10·0	10
4	10·0	10

The range of colour comparison is therefore correct for intensities from half to twice that of the standard.

3. When we first used this method we followed Kolthoff's [1927] directions for making up the precipitating reagent and did not add an equal volume of alcohol. On testing the method by taking known amounts of sodium we



Theoretical curve ———. Experimental curve × ——— ×.
The colour developed from 0·0786 mg. Na is taken as standard and all the other values expressed as fractions of this.

Fig. 1.

invariably obtained the results shown in Fig. 1. Such a curve would have necessitated working with the colour of the unknown very close to that of the standard and would therefore have greatly restricted the range of the method.

This deviation from the theoretical recovery would be accounted for by the presence of 0·017 mg. of sodium as impurity in both the unknown and the standard. We had great difficulty in locating this impurity. Alcohol, distilled water and acetic acid were all suspected in turn, but the contaminating sodium was finally found in solution in the actual reagent used to precipitate the sodium. In the absence of alcohol some, or all, of the sodium present originally as an impurity in the zinc acetate remains in solution, and precipitates on adding the reagent to the alcoholic solution resulting from the removal of phosphates. After modifying the reagent the recoveries became theoretical (see Table IV below).

4. *Range of the method.* The amount of sodium precipitated by 4 cc. of reagent from the 2 cc. of aqueous solution taken is strictly proportional to the amount of sodium present from 0·02 to 0·8 mg. The larger amounts must be made up in larger flasks and the smallest made up to 8 cc. in the centrifuge tube without transference (see below under potassium interference).

Table IV.

mg. NaCl taken	Recovered	% recovery	mg. NaCl taken	Recovered	% recovery
0·05	0·0482	96·3	0·2	0·204	102
0·05	0·0502	100·5	0·2	0·200	100
0·05	0·0516	103·0	0·2	0·200	100
0·05	0·0500	100·0	0·2	0·202	101
0·1	0·097	97	0·4	0·405	101·3
0·1	0·100	100	0·4	0·405	101·3
0·1	0·100	100	0·4	0·416	104
0·1	0·098	98	0·4	0·412	103
0·2	0·192	96	2·0	2·05	102·5
0·2	0·195	97·5	2·0	1·94	97
0·2	0·195	97·5	2·0	2·04	102·0
0·2	0·206	103	2·0	1·96	98

Note. All the determinations were carried out against an arbitrary standard.

5. *The standard colour.* Although much easier to prepare, a solution of uranium acetate does not make such a satisfactory permanent standard as a solution of the triple acetate. This is because the zinc present in triple acetate solutions begins to precipitate as the colloidal ferrocyanide after some minutes. If the colour developed from triple acetate is compared in the colorimeter with that from a uranium acetate solution it will be found that the triple acetate colour remains stable for about 10 minutes and then appears slowly to become more intense. This increase is accompanied by the appearance of a colloidal precipitate which can only be seen when the solution is viewed by reflected light. The change, however, is slow and we have used a uranium acetate standard quite satisfactorily by matching always between 3 and 8 minutes after developing the colour. All these difficulties are overcome by

using a solution of the triple acetate as standard. The colour comparisons are then stable for at least an hour, but we advise matching within 20 minutes of the development of the colour. The standardisation of this arbitrary standard has already been explained in detail under "Reagents."

6. *Interfering substances.* Caley and Foulk [1929] have shown that Ca, Mg, Sr, Ba and Fe do not interfere with this estimation of sodium. We can confirm their statement that Ca, Mg and Fe do not interfere. We have not tested Sr and Ba. The same authors report that lithium interferes with the precipitation, but there is practically no lithium in biological materials.

Phosphates are well known to be precipitated by uranium and must be removed. Table V shows that this is effectually accomplished by the zinc acetate in 50 % alcohol. A solution was prepared containing 9·82 mg. Na (25 mg. NaCl), 19·5 mg. K, 1·0 mg. Ca, 2·0 mg. Mg, 0·1 mg. Fe and 15·5 mg. P per 100 cc. The amount of sodium present in 1 cc. was estimated in quadruplicate. 1 cc. of a pure solution of NaCl containing 0·0786 mg. Na per cc. was also estimated in quadruplicate. All the colours were matched against an arbitrary triple acetate standard. The recoveries are given in Table V.

Table V.

Taken	Recovered mg.	%
1 cc. pure NaCl = 0·20 mg. NaCl	0·201	100·5
,, ,,	0·203	101·5
,, ,,	0·199	99·5
,, ,,	0·199	99·5
1 cc. salt mixture = 0·25 mg. NaCl	0·248	99·3
,, ,,	0·243	97·4
,, ,,	0·248	99·3
,, ,,	0·251	100·5

Arsenates also are stated by Barber and Kolthoff [1929] to interfere, but we have not deemed it necessary to investigate their removal as they are absent from most biological material.

As potassium is often present in considerable excess over sodium in biological material, we have tested the effects of large amounts and have found that the method is satisfactory if the amount of potassium present in the volume taken for analysis does not exceed 0·6 mg. Table VI shows that interference is definite in the presence of 0·9 mg. K.

Table VI. *0·0786 mg. Na taken.*

% recovered in the presence of	
0·62 mg. K	0·93 mg. K
97·0	120·5
100·7	119·2
100·0	119·2

0·6 mg. K is about 8 times the amount of Na used for the standard. In vegetable materials, *e.g.* grass, this ratio is often exceeded. When this is so the following modification of the method should be employed. Dilute the

specimen until 2 cc. contains less than 0·6 mg. K. Proceed as already described till the washing of the precipitate is completed. Now evaporate the residual drop of alcohol on a water-bath. Add 7·5 cc. of water and 0·5 cc. of 7 % potassium ferrocyanide acidified slightly with acetic acid. Match against the same standard that is always used. By this means it is possible to work with K/Na ratios of 32:1.

7. *Attention to detail.* Scrupulous care is essential when determining small amounts of Na. The element is ubiquitous so that the chances of contaminating the glassware and reagents are manifold. The authors recommend that the reagents be tested for purity from time to time in the following simple way. Take 0·04 and 0·08 mg. Na (or 0·1 and 0·2 mg. NaCl), make each up to 2 cc. and submit these to every stage of the sodium estimation. Make up the precipitates to 25 cc. Develop the colours and set the stronger at 20. The weaker should read 40. If it reads less than 40 one or more of the reagents contains sodium.

SUMMARY.

1. A method is described by which 0·02–0·8 mg. Na may be directly determined. In the absence of phosphates the range is 0·01–0·4 mg. Na.

2. The sodium is precipitated as sodium uranyl zinc acetate and the uranium is determined colorimetrically with potassium ferrocyanide.

3. The method is applicable to neutral or acid solutions.

4. Ca, Mg and Fe do not interfere. Phosphates interfere, but are removed with zinc acetate.

5. Sodium may be determined directly in the presence of 30 times its weight of potassium.

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