NOTE ON THE ESTIMATION OF POTASSIUM IN URINE

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The purpose of this note is to direct attention to the method of estimating potassium by precipitation as the double cobalti-nitrite of sodium and potassium, $K_2NaCo(NO_2)_6 \cdot H_2O$ —a method useful, but apparently little used, especially in physiological work. Its advantages over the platinic chloride process are the greater rapidity, the shorter working time involved, and the very much lower cost. In point of accuracy it may, in ordinary practice, be made equal to the platinum method. Its special feature lies in the fact that the presence of sodium does not interfere, since the precipitant is itself a sodium salt. Salts of other metals do not in general interfere, and hence a troublesome preliminary separation is avoided. Further, the difficulty of separating the mixed chloroplatinates of sodium and potassium by washing with alcohol is well known, and the results obtained by the platinum method as usually conducted may well be high, owing to occlusion of Na_2PtCl_6 , which cannot be separated by extraction with alcohol, however long continued. The method of obviating this error by re-dissolving the K_2PtCl_6 in water and re-precipitating by evaporation is troublesome.

The first reference to the cobalti-nitrite reaction appears to be the discovery by Fischer¹ in 1849 that on adding a solution of potassium nitrite to a cobalt salt, an orange coloured double compound is precipitated. Fischer found that a cobalt salt of concentration so dilute as 1 in 3,000 was apparently completely precipitated on the addition of potassium nitrite. Amongst other workers who have investigated the reaction may be mentioned Erdmann², Sadtler³, de Koninck⁴, Curtman⁵, and Bülman⁶. Macallum⁷ in 1905, in a paper upon 'Potassium in cells,' gives a full

1. *Poggen, Annalen*, LXXIV, p. 124, 1849.
2. *J. pr. Chem.*, XCVII, p. 385, 1866.
3. *Amer. J. Sci.*, 2, XLIX, p. 189, 1870.
4. *Zeit. für anal. Chem.*, XX, p. 390, 1881.
5. *Berichte d. ch. G. Jahrg.*, XIV, p. 1951, 1881.
6. *Zeit. für anal. Chem.*, XXXIX, p. 284, 1900.
7. *J. Physiol.*, XXXII, p. 95, 1905.

discussion of the reagent, and uses the method with satisfactory results for histological purposes. The fact that ammonium compounds react with sodium cobalti-nitrite suggested the possibility of amino and amido compounds reacting similarly. On investigation, however, Macallum finds that neither glycine, leucine, tyrosine, aspartic acid, glutamic acid, sarcosine, nor glucosamine, precipitate and that therefore their presence in tissues presents no difficulty. Neither do urea, asparagine, alloxan, allantoin, guanidine, and the purine bodies react. Although creatinine does not react, creatine does so readily, giving a precipitate similar to that of potassium.

The first quantitative method of importance appears to be that of Adie and Wood⁸ in 1900. The reagent as prepared by them, and now in general use, is made as follows:—113 grams of cobalt acetate are dissolved in 400 c.c. of water, and 100 c.c. of glacial acetic acid added. 220 grams of sodium nitrite are separately dissolved in 400 c.c. of water, and the two solutions are then mixed with stirring, the last of the NO_2 evolved being removed by evacuation over night or by blowing air through the mixture. After standing at least twenty-four hours any sediment is filtered off and the solution made up to a litre. It will be found that the bulk is approximately a litre without the addition of water, and it is perhaps best not to dilute further. The reagent prepared in this manner is of a dark plum colour. It should be stored in the dark and decanted for use as required.

Adie and Wood describe both volumetric and gravimetric methods of determining potassium in soils and manures.

The precipitation is accomplished by adding 10 c.c. of the reagent and 1 c.c. of glacial acetic acid to 10 c.c. of the solution to be determined, which must be so arranged as to have a concentration of 0.5 to 1 per cent. K_2O . Adie and Wood found the most important factor in the precipitation to be the state of dilution—at 0.1 per cent. concentration of K_2O , only two-thirds of the theoretical amount of potassium was recoverable. They determine the formula of the precipitate as obtained by them under standard conditions as $\text{K}_2\text{NaCo}(\text{NO}_2)_6 \cdot \text{H}_2\text{O}$, and its solubility in water as 1 in 20,000.

Autenreith and Bernheim⁹ in 1902 described a method for the determination of potassium in urine, and so far as I have ascertained, this represents the only instance in which the use of the cobalt method

8. *J.C.S.*, II, p. 1076, 1900.

9. *Zeit. physiol. Chem.*, XXXVII, p. 29, 1902.

in the analysis of physiological products is recorded. Their method consisted in treating 50 c.c. of the filtered urine direct with 6 to 10 c.c. of the reagent, shaking, allowing to stand, and determining the potassium in the precipitate by subsequent conversion into the perchlorate. This method, however, is tedious, and it was determined to seek a shorter method. A direct treatment of the precipitate without conversion into the perchlorate does not give satisfactory results.

Drushel¹⁰ in 1907 published a modification of Adie and Wood's method in which the reagent was added to the solution to be estimated, and the mixture evaporated almost to dryness. The precipitate was then extracted, washed with water, and decomposed directly with standard potassium permanganate. The reaction involved in treating the precipitate, $K_2NaCo(NO_2)_6 \cdot H_2O$, with permanganate, is one of oxidation of the nitrite to nitrate and concomitant reduction of trivalent cobalt to the divalent form.

The method of dealing with the precipitate which I have adopted for physiological purposes is in essence that of Drushel. The process as carried out on urine is as follows:—

25 c.c. of the urine is evaporated to dryness in a platinum dish, and gently ignited at a dull red heat. The ash is then moistened with nitric acid and reignited until free from organic matter and *ammonium salts*. The use of nitric acid allows of easy ignition at a temperature sufficiently low to prevent any loss of potassium by volatilisation. The ash is then dissolved up in water containing a few drops of hydrochloric acid and washed into a porcelain basin (200 c.c. capacity). The solution is then neutralised or rendered slightly alkaline with a few drops of caustic soda, acidified with acetic acid, and evaporated to between 5 c.c. and 10 c.c. 1 c.c. of glacial acetic acid, and 10 c.c. of the reagent (prepared according to the recipe of Adie and Wood, *loc. cit.*) are then added, and the evaporation is continued until the resulting syrup is of such consistency as to set to a stiff hardish mass on cooling. The time taken for evaporation is rather less than an hour, but the stage of evaporation is readily fixed after a little practice.

The cold mass, consisting of the yellow precipitate, $K_2NaCo(NO_2)_6 \cdot H_2O$, imbedded in a dark purplish-brown matrix, is then worked up with about 50 c.c. of 10 per cent. acetic acid and allowed to soak (stirring up occasionally with a glass rod) until the yellow precipitate is

10. *Amer. J. Sci.*, XXIV, p. 433, 1907.

disentangled and the rest of the mass *completely* dissolved up to give the clear tawny colour of the dilute reagent. The yellow precipitate is then washed by decantation with dilute acetic acid, the washings being poured through an asbestos filter. Finally, the precipitate is transferred completely to the filter, washed with cold water, and sucked dry. In general, six washings by decantation are necessary, using in all about 300 c.c. of wash-liquid. From a mechanical point of view, the precipitate is easily washed and filtered, but it is somewhat difficult to completely wash free from the excess of reagent used. A funnel with a Gooch disc well packed with clean coarse Gooch-asbestos, makes a convenient filter. If any trouble is experienced by sucking through of the precipitate taking place, a second packed disc above the first ensures complete retention.

While the filtering of the precipitate is in process, about 300 c.c. of water is raised to boiling and the requisite excess (30 c.c. to 50 c.c. N/5) of potassium permanganate run in from a burette. The pad of asbestos with the precipitate is picked off the disc and dropped into the hot permanganate, any precipitate adhering to the funnel being also carefully washed in. The precipitate is stirred up, and after a few minutes dilute sulphuric acid, in amount required to react with the permanganate, is added (20 c.c. of 25 per cent. sulphuric acid). The contents are well stirred, replaced over the bunsen and kept at, or just below, the boiling point until the yellow precipitate is completely decomposed (about five minutes). Darkening of the liquid with formation of manganese hydroxide begins before the addition of sulphuric acid and continues on heating. Standard oxalic acid is then run in from a burette, with stirring, until the manganese hydroxide is dissolved and the hot liquid is clear and of a very faint rose pink colour. Care must be taken that any manganese hydroxide adhering to the glass and asbestos is completely dissolved up. The excess of oxalic acid is then titrated back to colour with permanganate, the end-point being sharply marked. The difference between the total permanganate and oxalic acid burette readings gives the amount of permanganate used, from which the amount of K_2O is calculated. The theoretical factor for N/5 potassium permanganate is 1 c.c. = 0.001714 grams K_2O . It is, however, preferable to standardise the permanganate accurately against a solution of pure fused potassium chloride, using the equivalent so found—by this means any slight constant errors are embodied in the standard, if the procedure adopted in standardisation be rigidly adhered to in all subsequent determinations.

Calculation is obviously saved if the standard permanganate and oxalic acid be made up so that 1 c.c. = 0.001 gram K_2O ; or, if more convenient, 1 c.c. = 0.002 gram K_2O . This latter concentration is a little over N/5 and 10 c.c. of a 1 per cent. solution of KCl used in standardisation then requires 31.6 c.c. of permanganate—a convenient burette reading on a convenient working quantity of precipitate.

In order to investigate the influence of other factors which have been said to interfere with the accuracy of the results, a series of a hundred determinations was made in which various foreign salts were added to the same quantity of potassium chloride solution. Quantities of sodium chloride, sodium phosphate, calcium chloride, calcium nitrate, magnesium chloride and magnesium sulphate, in amounts varying between wide limits were found not to seriously influence the results—in most cases the error was below 0.4 c.c. in a titrate of 32.6 c.c. of permanganate, i.e., the determination was accurate to about 1 per cent. The general conclusion is that it is unnecessary to first remove such foreign salts unless they are present in amount many times greater than the potassium itself. For practical purposes, the amount of salts other than those of potassium likely to be present in the ash of urine, may be ignored. Potassium was found to be estimable with equal accuracy in the form of chloride, sulphate, nitrate and acetate, provided that no free acid other than acetic was present during evaporation with the sodium cobalti-nitrite reagent. The quantity of reagent used was found to be of little consequence provided that large excess was taken. Theoretically 2 c.c. of the reagent is rather more than sufficient to precipitate the 10 c.c. of 1 per cent. KCl (= 0.0632 gram K_2O) used in standardisation. The 10 c.c. of reagent used therefore represents the necessary large excess. The colour of the filtrate from the washed precipitate should be the characteristic colour of the dilute reagent—a pink filtrate indicates decomposition, and estimations showing such should be repeated, using a larger excess of the reagent. The concentrated reagent itself, if stored in the dark, may be kept for a year, but the fresh reagent is always preferable. In sunlight, decomposition is more rapid. If diluted and exposed to light it quickly goes pink, indicating rapid decomposition. After the 'thick paste' stage of evaporation in the estimation, further heating should be avoided as the precipitate then becomes more difficult to extract, and results may be accordingly vitiated.

To test the accuracy of the method exactly as it is conducted upon

urine, an artificial urine (free from potassium) was made up of the following composition per litre:—

Urea	30.0	grams	
NaCl	12.0	„	
NH ₄ · Cl	3.0	„	
MgSO ₄	0.8	„	
CaCl ₂	0.5	„	
Na ₂ HPO ₄	5.0	„	
Glucose	5.0	„	(to simulate difficulty of ignition)

—the solution being acidified to keep the phosphates in solution.

20 c.c. of this solution plus 10 c.c. of a one per cent. solution of KCl were evaporated to dryness, ignited until free from ammonium salts, and treated exactly as in the process described for urines. 10 c.c. of the KCl solution alone required 32.4 c.c. of the standard permanganate used. The following table shows, to the first decimal place, six successive determinations in which the artificial mixture was present:—

No.	K ₂ Mn ₂ O ₈ burette	Oxalic acid burette	Difference = K ₂ Mn ₂ O ₈ used	c.c. error	Per cent. KCl recovered
1	42.6 c.c.	10.0 c.c.	32.6 c.c.	+ 0.2 c.c.	100.6
2	41.6	9.3	32.3	— 0.1	99.7
3	42.3	10.0	32.3	— 0.1	99.7
4	42.9	11.0	31.9	— 0.5	98.5
5	42.3	10.0	32.3	— 0.1	99.7
6	42.5	10.0	32.5	+ 0.1	100.3

Two estimations, adopting the method of wet combustion with sulphuric acid, removing the excess of sulphuric acid by evaporation over a low flame to avoid spattering, and subsequently removing the ammonium sulphate by ignition, gave 100.3 per cent. and 99.4 per cent. of potash recovered. The cobalti-nitrite method is obviously applicable to all physiological products after careful preliminary ashing to remove organic matter and ammonium salts.

Where sodium as well as potassium is to be estimated, the ash is dissolved up in dilute hydrochloric acid, and the foreign metals along with sulphate and phosphate removed by treatment with barium chloride and ammonium carbonate as in the ordinary preliminary treatment in the platinic chloride process—the 'NaCl+KCl' being weighed as such, and the sodium found by difference after the estimation of potassium.

Even at this stage great economy in time, labour and cost of material is effected.

The cobalt method, as here described, has been used with satisfactory analytical results (checked by comparison with the platinum method) in a series of experiments upon the mineral metabolism of the dog—a discussion of the results of which is reserved for a future date.

NOTE.—Since the work in this paper was completed, I have discovered a third paper by Drushel (*Amer J. Sci.*, December, 1908) which had unfortunately been previously overlooked. He adapted his method for use on physiological fluids, and found it satisfactory.

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