56. THE MICRO-DETERMINATION OF POTASSIUM

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THE determination of potassium is more troublesome than that of any other biologically important cation; at the same time there are many conditions involving variations in the electrolyte content of the body fluids in which accurate estimations of potassium are desirable. It therefore appeared worth while to try to devise an analytical method for potassium which should be simpler than those at present in use; this paper describes a new method which offers certain advantages of simplicity whilst giving a degree of accuracy not significantly less than those claimed for the more time-consuming procedures.

The methods at present available for the determination of potassium fall into two groups: (1) determination as the chloroplatinate and (2) determination by one of the various modifications of the cobaltinitrite procedure [compare Peters & Van Slyke, 1932]. The chloroplatinate method is excellent for inorganic analysis when reasonably large quantities of material are available; it loses accuracy however when adapted to micro-analysis. The cobaltinitrite methods which have been most widely employed in biological work suffer from the theoretical disadvantage that there is uncertainty in the composition of the final precipitate; this means that although a series of analyses conducted by one worker under constant conditions may give mutually consistent results with a small margin of error, the basis of the method is not satisfactory.

In the search for a new procedure attention was directed to the possible use of dipicrylamine; this compound, di(2:4:6-trinitrophenyl)amine, forms a deep

orange-red potassium salt, of which the solubility in water is similar to that of potassium sodium cobaltinitrite; it has been proposed as a qualitative reagent for the detection of small amounts of potassium [Feigl, 1937] and is stated to be specific for potassium in presence of other metals of the 4th and 5th groups except rubidium and caesium and in absence of ammonium salts; no record has been found of its application to the quantitative determination of potassium.¹

Preliminary experiments showed that potassium dipicrylamine was soluble in alcohol, to give, in low concentrations, solutions of a strong orange colour; this suggested the possibility of a determination of potassium based on measurement of the colour of an alcoholic solution of the precipitate of potassium dipicrylamine obtained from the solution to be analysed.

¹ Since this was written my attention has been called to two papers on the determination of potassium with the aid of dipicrylamine by Kolthoff & Bendix [1929] and Amdur [1940]; these papers do not however describe the application of the method to the analysis of blood.

EXPERIMENTAL

Colorimetric determination of potassium dipicrylamine

It was first necessary to ascertain the possibility of accurate measurement of the colour. For this purpose a series of standard solutions of potassium dipicrylamine in alcohol was prepared as follows. Commercial dipicrylamine was recrystallized from acetic acid; a solution of the sodium salt was made by dissolving the dipicrylamine (0.44 g.) in boiling *N*/10 sodium carbonate (20 ml.) and filtering the cooled solution; a slight excess of this solution was added to one of pure potassium nitrate containing 1 mg. K/ml.; after keeping overnight in the ice chest the potassium dipicrylamine was collected, washed with ice water and recrystallized from boiling *N*/100 potassium carbonate; the recrystallized material was dried *in vacuo* over sodium hydroxide. A stock solution of the salt in alcohol was made which contained 0.02 mg. K/ml. and from this a series of dilutions was prepared.

The colours of these solutions were measured in two ways: (a) with the Zeiss step-photometer; (b) with a simple photoelectric device [compare Harington *et al.* 1940]. Using the step-photometer, the best results were obtained with filter S 53, having maximum transmission at about 530 $m\mu$; with the photoelectric device the most favourable conditions so far found have been with a combination of Wratten filter no. 61 and 2 mm. thickness of Chance's calorex glass to absorb the infra-red to which the cell is sensitive.

Fig. 1.

Results obtained by both these methods are shown in Fig. 1. It will be seen that over the range 0–0.1 mg. K/10 ml. the observations with the step-photometer are closely in accordance with Beer's law; at higher concentrations deviation from linearity became considerable. With the photoelectric device, on the other hand, a linear relationship between extinction coefficient and concentration of potassium is not obtained over any range; this is doubtless to be explained

by incomplete elimination of the infra-red and by the breadth of the transmission band of the Wratten filter employed.

It is clear that if the colour is to be measured photoelectrically it will be necessary to work from a calibration curve such as that given in Fig. 1; with the step-photometer on the other hand, provided that the solution is adjusted to give a value of $E_{1\text{ cm.}} < 0.4$, the concentration of potassium is given by the formula

$$c = 2.5E$$

where c is the concentration of potassium in mg./100 ml. in the final solution and E is the observed value of the extinction coefficient using filter S 53 and a 1 cm. cell.

The orange colour of the alcoholic solution of potassium dipicrylamine is not suitable for simple comparative colorimetry; such colorimetry can however be improved with the aid of suitable filters and fair results have been obtained with a Leitz Duboscq type of instrument using Wratten filter no. 61.

In all the analyses recorded below the colour measurements have been made with the step-photometer.

Analysis of solutions containing known amounts of potassium

The general method of analysis is as follows. The solution containing potassium at a concentration of 0.1–0.25 mg./ml. and at approximately neutral reaction is mixed with excess of the precipitating reagent (sodium or lithium dipicrylamine: see below); when crystallization of the potassium salt is complete it is collected, washed and dissolved in alcohol, the volume of the final solution being adjusted so that the value $E_{1\text{ cm.}}$ observed in the step-photometer falls in the range 0.20–0.40; the colour is then measured.

(a) *Precipitating reagent.* In the earlier experiments this consisted of a solution of sodium dipicrylamine prepared as described above; later, for reasons which will appear, this was replaced by a similar solution of lithium dipicrylamine.

(b) *Time of precipitation.* When the precipitant is mixed with a solution containing potassium at about 0.1 mg./ml. concentration, crystallization of the potassium salt is best initiated by scratching the walls of the tube with a fine glass rod; the crystallization is completed in the ice chest. An experiment was performed to determine the time necessary for complete precipitation; the results are shown in Table 1, from which it appears that 2 hr. suffice; this period was therefore adopted as a routine.

Table 1. *Effect of time of precipitation on recovery of potassium*

Sample	Time in ice chest	K present mg.	K found mg.
A	1 hr.	0.200	0.188
B	2 hr.	0.200	0.198
C	3 hr.	0.200	0.193
D	Overnight	0.200	0.198

(c) *Analysis of known solutions of potassium nitrate.* A stock solution of pure potassium nitrate was prepared, containing 2 mg. K/ml.; from this a number of dilutions were made by an independent observer. The results obtained are shown in Table 2.

Table 2. *Analyses of solutions of potassium nitrate*

Sample	K present mg./100 ml.	K found mg./100 ml.	Error %
A	20.0	19.4	-3.0
B	21.6	22.4	+3.6
C	26.8	27.6	+4.3
D	38.0	36.3	-4.5
E	42.8	40.8	-4.7

From this it appears that the error in the determination on simple potassium nitrate solutions is within $\pm 5\%$.

(d) *Effect of other blood cations.* In order to study this point a series of solutions was analysed which all contained 20 mg. K/100 ml., but which differed in the amounts of Na, Ca and Mg which were present; the results are given in Table 3.

Table 3. *Effects of other blood cations on recovery of potassium*

Solution	Present mg./100 ml.				K found mg./100 ml.
	Na	K	Ca	Mg	
A	350	20	10	3	19.3
B	700	20	20	6	23.1
C	700	20	—	—	23.6
D	—	20	20	—	19.3
E	—	20	—	6	19.3

The recovery from solution A, which contains the amounts of the various cations found in normal blood serum is satisfactory; solution B on the other hand, in which all cations save K are doubled in concentration, gives a high result; a similarly high result is obtained with solution C containing no Ca or Mg but double the normal amount of Na, whilst correct results are obtained in presence of double the normal amounts of Ca and Mg when Na is absent (solutions D and E). It is clear that the greatest danger of interference comes from Na, and extension of the above experiment showed that such danger exists at levels of Na only slightly above the normal.

The above analyses were carried out with the sodium dipicrylamine precipitant in which the sodium ion concentration is $N/10$; it was evident that the danger of interference from sodium could be eliminated if a more soluble salt of dipicrylamine could be found; the lithium salt is in fact much more soluble than the sodium salt and a precipitating reagent was therefore prepared by dissolving 0.44 g. of dipicrylamine in 20 ml. of $N/10$ lithium carbonate; with the aid of this reagent it is possible to determine potassium accurately in presence of proportions of sodium greatly in excess of those found in biological fluids (Table 4).

Table 4. *Lithium dipicrylamine as precipitant; determination of potassium in presence of increasing amounts of sodium*

Solution	Na mg./100 ml.	K mg./100 ml.	K found mg./100 ml.
A	—	20.0	19.7
B	350	20.0	20.0
C	450	20.0	20.3
D	550	20.0	19.7
E	650	20.0	20.0

In view of these results the lithium dipicrylamine solution was adopted as precipitant in all future experiments.

Determination of potassium in blood serum

The analysis cannot be carried out directly on blood serum since the precipitation of potassium dipicrylamine is apparently inhibited by the organic constituents; the method adopted in the present work has been to incinerate a trichloroacetic acid filtrate of serum with sulphuric and nitric acids and to analyse the solution of the sulphate ash. This procedure has the advantages over dry incineration of greater rapidity and freedom from danger of loss of alkali chloride.

Reagents.

1. *Trichloroacetic acid*, 20 % aqueous solution.
2. *Sulphuric acid*, Analar, 4*N* solution.
3. *Nitric acid*, Analar, concentrated.
4. *Lithium dipicrylamine*. Commercial dipicrylamine is recrystallized from glacial acetic acid, 0.44 g. is dissolved by boiling in 20 ml. of *N*/10 lithium carbonate; the cooled solution is filtered.
5. *Potassium dipicrylamine*, saturated aqueous solution. The recrystallized salt is dissolved in warm *N*/1000 potassium carbonate in the proportion of 1 mg./ml. and the solution is kept in the ice chest until crystallization is complete; the mother liquor is used as washing fluid (see below).

Procedure.

In drawing the blood precautions must be taken to avoid haemolysis. 1 ml. serum is mixed with 3 ml. distilled water followed by 1 ml. 20 % trichloroacetic acid and the mixture is filtered. Of the filtrate 2.5 ml. (equivalent to 0.5 ml. serum) are placed in a 6 × 1 in. pyrex test tube together with 0.6 ml. 4*N* H₂SO₄ and a glass bead; the water is boiled off and the charred residue treated, after partial cooling, with one drop of concentrated HNO₃; heating is then resumed until the residue is colourless; the whole process is complete in a few minutes. The residue is then transferred quantitatively with small amounts of water to a platinum dish and evaporated as far as possible on the steam bath; excess of sulphuric acid is removed by cautious heating over the free flame, the contents of the dish being finally briefly but strongly ignited.

The ash is transferred with small quantities of water to a 3 × $\frac{3}{8}$ in. test tube and the solution evaporated to 0.5–1.0 ml.; 1 ml. of the lithium dipicrylamine solution is added and crystallization of the potassium salt is initiated by scratching the tube with a fine glass rod. After 2 hr. in the ice chest the precipitate is collected on a small sintered glass funnel (1.0 cm. disk; porosity G 3 or G 4), the precipitation tube being carefully drained, and the precipitation tube and funnel are washed with two 1 ml. portions of the ice-cold saturated aqueous potassium dipicrylamine and finally with 1 ml. of ice-cold distilled water.

The receiver is then changed and the precipitation tube and funnel are washed through with warm alcohol, which dissolves the potassium salt; the alcoholic filtrate is made up to 15 ml. and the colour measured in the step-photometer. With normal samples of serum this will give a value of $E_{1\text{cm.}}$ between 0.24 and 0.32.

The results of a series of determinations on the sera of normal individuals are given in Table 5; the range observed, 17.5–24 mg./100 ml., is in accordance with the accepted values for normal serum potassium.

Table 5. *Potassium content of normal blood serum*

Sample no.	K found mg./100 ml.	Sample no.	K found mg./100 ml.
1	20.7	8	19.2
2	18.1	9	17.5
3	18.5	10	18.5
4	18.1	11	20.0
5	18.8	12	24.0
6	19.0	13	22.5
7	18.8	14	21.0

Recovery of potassium added to serum

In Table 6 are recorded the results of experiments in which analyses were made of a sample of serum before and after addition of measured amounts of potassium.

Table 6. *Recovery of potassium added to serum*

Sample no.	K added mg./100 ml.	K found mg./100 ml.	K recovered mg./100 ml.	Error %
1	0	20.7	—	—
2	10	31.3	10.6	+6.0
3	20	42.0	21.3	+6.5
4	40	59.5	38.8	-3.0

It will be seen that recoveries are satisfactory.

SUMMARY

A method for the determination of potassium is described which is based on precipitation as potassium dipicrylamine; the precipitate is collected, washed and dissolved in alcohol, and the potassium content determined by measurement of the colour of the resulting solution.

The application of the method to determination of blood serum potassium is described.

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