A VOLUMETRIC METHOD FOR DETERMINATION OF POTASSIUM IN BIOLOGICAL MATERIALS

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During the past 3 years we have carried out analyses of tissues for potassium in this laboratory using the separation by chloroplatinic acid. For 1 year potassium has been estimated by a Volhard titration of the chlorine in K₂PtCl₆ separated by the usual procedure. Because the method seems more accurate and convenient than previous titrimetric methods, a description is warranted.

**Solutions**—

- Solution of chloroplatinic acid in 1 N HCl. 26.5 gm. of chloroplatinic acid (H₂PtCl₆·6H₂O) dissolved in 100 ml. of 1 N HCl.
- Acid 80 per cent ethyl alcohol saturated with K₂PtCl₆. Add a small amount of K₂PtCl₆ to 15 ml. of approximately 1 N HNO₃. Shake for a few minutes until no more K₂PtCl₆ dissolves. Add 80 ml. of stock 95 per cent alcohol. Filter just before using.
- 15 per cent solution of NaHSO₃.
- 0.05 N AgNO₃ in concentrated HNO₃.
- 0.02 N NH₄SCN.
- Ground ferric alum.

**Procedure**

Most biological material will require a preliminary removal of phosphate from the ash. To a suitable amount of ash which is dissolved in water or dilute HCl add sufficient chloroplatinic acid to combine with all the K and Na. Evaporate until the salts separate out but still look moist on cooling. Add a small amount of 80 per cent acid alcohol and filter,¹ using preferably an automatic transfer.

¹ The alcoholic solutions are so difficult to pour that some form of automatic transfer is almost essential. Fritted glass filters may be used but asbestos filters made as recommended by Pregl are more rapid and give equally satisfactory results.
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transfer by suction as recommended by Pregl (1). Wash in the usual fashion until the filtrate is free of chloride. The separated K₂PtCl₆ is now dissolved with small amounts of hot water.

To a suitable aliquot of the solution of K₂PtCl₆, add 0.4 to 0.5 ml. of 15 per cent NaHSO₃. Boil for 1 minute. Add 3 ml. of 0.05 N AgNO₃ (a smaller amount of AgNO₃ should be used when very small amounts of K are being determined) and then 2 to 4 drops of 30 per cent H₂O₂. Boil, cool in ice water, and titrate by the usual Volhard technique.

The titration of chloride should be carried out on 0.004 to 0.04 mM of K (0.156 to 1.56 mg. of K).² The end-point of the titration is best when the volume of solution does not exceed 10 ml.

Calculation

\[ \text{mM K} = \left(7.54 - \frac{x}{7.54}\right) \times \frac{0.02}{3} \]

where \( x \) = ml. of 0.02 N NH₄SCN used.

Accuracy

Known mixtures of KCl and NaCl varying from 0.004 to 0.15 mM of KCl in the presence of 0.15 mM of NaCl give theoretical results within 1 per cent. Since the sensitivity of the Volhard end-point is not greater than 0.02 ml. of 0.02 N NH₄SCN, this accuracy is all that can be expected.

To test the method of precipitation of chlorine further, gravimetric estimations of AgCl formed from K₂PtCl₆ isolated from known amounts of KCl were carried out. Reduction of the K₂PtCl₆ was carried out by boiling in the presence of NaHSO₃. With 29.82 mg. of KCl, the AgCl weighed 171.7 mg. (theoretical 172); with 18.64 mg. of KCl, 108.0 mg. (theoretical 107.5 mg.). Other analyses were of similar accuracy. Apparently the chloride can be precipitated quantitatively after reduction of K₂PtCl₆ with NaHSO₃.

The reproducibility of the results on biological material is indi-

² The lower limit of accuracy of the titration is equivalent to the potassium content of 1 cc. of blood serum, while the upper limit is equivalent to the potassium content of 400 mg. of fresh muscle. If larger quantities are to be determined, an aliquot of the K₂PtCl₆ solution must be taken.
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cated by analyses of twenty-five samples of muscle. Two samples were ashed and aliquots were used for estimation of potassium after removal of the phosphate. The average difference between the results was $1.6 \pm 1.2$ per cent of the amount determined where $\pm 1.2$ is the standard deviation of the difference. For comparison, the same calculation was made on duplicate analyses of the same material for nitrogen by the Kjeldahl method. The average difference for nitrogen was $1.5 \pm 0.8$ per cent of the amount determined. Since the Kjeldahl method is subject to few errors of manipulation, the results obtained for potassium may be considered satisfactory for the material used. The greater scattering in the case of the determination of potassium is probably more dependent on the many manipulations than on any inherent difficulty in separating $K_2PtCl_6$ or titrating the chloride.

**Comment**

In the titration of chloride formed from $K_2PtCl_6$ by reduction with NaHSO$_3$ definite limitations are found with the Volhard technique. Removal of AgCl does not do away with these limitations. Theoretical results are obtained with the Volhard titration if no more than 3 ml. of 0.05 n AgNO$_3$ are used. If larger amounts of AgNO$_3$ are required, the results are apt to be low by 2 to 5 per cent. Such low results apparently occur owing to some interference with the end-point induced by platinum in solution. The end-point fades rapidly when one attempts to titrate large amounts of $K_2PtCl_6$ and one goes past the end-point, obtaining a low figure for chloride. Adding H$_2$O$_2$ as recommended in the method gives more nearly correct values. With the smaller amounts of $K_2PtCl_6$ recommended, theoretical results are obtained with a very sharp end-point.

Sodium bisulfite reduces chloroplatinic to chloroplatinous acid and not to platinum. The solution of $K_2PtCl_6$ becomes colorless on boiling with NaHSO$_3$ and on addition of AgNO$_3$ gives a white precipitate of AgCl. If reduction is not complete, the precipitate shows a yellowish discoloration. Hydrogen peroxide also reduces chloroplatinic to chloroplatinous acid. However (in our hands), hydrogen peroxide could not be used alone.

Bullock and Kirk (2) have reported a microvolumetric method for potassium in which the separated $K_2PtCl_6$ is reduced with
powdered magnesium, filtered, and the chloride estimated by
direct titration with AgNO₃, with dichlorofluorescein as an indi-
cator. In our hands the use of agents which reduced K₂PtCl₆ to
platinum (Zn, Mg, and formic acid) gave low results when the
chloride is estimated by the Volhard titration.

The separation of K₂PtCl₆ cannot be carried out quantitatively
with large amounts of phosphate in the solution. Phosphates
form a gummy precipitate on addition of alcohol and washing
the precipitate of K₂PtCl₆ is almost impossible. A small amount
of phosphate does not interfere. Removal of phosphate with
powdered Ca(OH)₂ is satisfactory when the ash is dissolved in
little or no HCl. With large amounts of acid, considerable cal-
cium is left in solution and CaSO₄ may precipitate out in the alco-
hol in such large quantities as to make quantitative washing of the
precipitate impossible. Owing to the sulfur of organic material,
this difficulty arises even when no H₂SO₄ is added.

Acid is added to the alcohol in order to increase the accuracy of
the sodium determination on the filtrate, by Hald's method (3).
With neutral 80 per cent alcohol low values for Na are sometimes
obtained, although the determinations of potassium are accurate.
An additional advantage of acid alcohol is that the saturated
solution of K₂PtCl₆ keeps for at least 5 to 6 weeks, while, in
neutral alcohol, K₂PtCl₆ is rapidly reduced to platinum.

Before the present technique was adopted, the conditions for ob-
taining theoretical results by the Shohl and Bennett (4) titration
were examined. It was found that the concentration of KI, the
acidity, the time of heating, the temperature of heating, and the
rapidity with which the titration is carried out must all be rigidly
controlled. With a rigid technique, theoretical results may be
obtained on known solutions, but in practise many irregular
results are encountered when variable amounts of K are estimated.
Even if these difficulties can be entirely overcome, the superiority
of the end-point of the Volhard titration makes it the method
of choice.

SUMMARY

A method for quantitatively estimating potassium by the Vol-
hard titration of chlorine freed from K₂PtCl₆ is described. The
usual separation of potassium by chloroplatinic acid is utilized.
By reduction with NaHSO$_3$ the chlorine is made available for precipitation with AgNO$_3$. The method is applicable to the titration of 0.004 to 0.04 mM of K and is accurate within 1 per cent on theoretical solutions.

BIBLIOGRAPHY

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