CIII. THE PHOTOMETRIC ESTIMATION OF COBALT

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VARIOUS colorimetric methods have been employed for the estimation of cobalt in soils, animal organs, etc., notably that of Stare & Elvehjem [1932], recently modified by Kidson *et al.* [1936]. This method depends on the development of a red-coloured complex of nitroso-*R*-salt $[\text{C}_{10}\text{H}_4.\text{OH}.\text{NO}.\text{(NaSO}_3)_2]$ and cobalt, the intensity of the colour being proportional to the amount of cobalt present. These workers used a Lovibond tintometer to measure the final colour and Kidson claims to have obtained reproducible results with as little as 0.0001 mg. cobalt.

In such material as grasses and animal organs in which the cobalt content is minute, a very accurate method of measuring the quantity of cobalt complex is essential. The present work was undertaken primarily to see whether the Pulfrich photometer could be successfully substituted for the tintometer, since an efficient photometer would give considerably more accurate readings. With a tintometer there is no means of compensating for colours, notably yellow and green, developed in the solution by elements other than cobalt. Copper and iron in particular interfere seriously if present in any quantity and must be removed. With the photometer, on the other hand, provided that the appropriate colour filter is used, it is found that removal of copper is not necessary if present in small amount and iron need not be removed at all.

The extinction coefficient, which could be read directly on the instrument, was used as a measurement of the depth of colour of the solution. With pure cobalt solutions it was found that the extinction coefficient was directly proportional to the amount of cobalt present, and very small quantities of cobalt could be accurately estimated, the error being approximately ± 0.0001 mg. Any colour filter could be used for these solutions, a convenient range of readings being given by a blue filter (S 47).

The effect of other metals was then investigated. Using a cobalt solution containing varying amounts of iron, readings were obtained which depended on the quantity of iron present and were therefore of no value in estimating the cobalt. It was found, however, that if a green filter (S 53) was substituted for the blue, a constant reading was obtained for a particular cobalt solution, no matter what quantity of iron was present. Iron, therefore, does not interfere if this filter is employed. In an attempt to retain the blue filter for the estimation of cobalt, since it gave considerably higher readings than the green, removal of iron with ether as directed by Kidson *et al.* [1936] was carried out. This was unsatisfactory, since the iron was not completely removed even after several washings. Precipitation of iron as hydroxide by means of ammonia was unsuccessful, since some cobalt was removed even in presence of excess ammonium chloride: the basic acetate method also removed part of the cobalt. A very satisfactory method was found in the precipitation by means of zinc oxide

[Scott, 1917]. In numerous experiments with this method it was found that no cobalt was lost, whilst very complete removal of iron was effected. Using this method of precipitation, under the conditions described later, cobalt can be accurately estimated with either blue or green filters. The green was eventually employed, since it cuts out traces of other elements which may be present.

Since it would be convenient if estimations could be carried out without removal of iron, cobalt solutions containing large amounts of iron were examined. It was found that by a slight modification of Kidson's method, cobalt could be accurately estimated in presence of iron, provided that a green filter was used.

The effect of copper was then investigated. When present in very small amount, copper does not interfere, but if much of it is present, it interferes seriously with the estimation even when the green filter is employed. In quantity, it appears to give rise to a yellowish brown colour in the final solution and must therefore be removed. Precipitation by means of H_2S is satisfactory, if carried out in a solution sufficiently acid to prevent co-precipitation of cobalt. A more convenient and equally effective method of removing the copper is displacement from solution by means of a more electropositive element such as aluminium. The method is described later.

Manganese did not interfere, even in very large amount.

This paper is concerned chiefly with the estimation of cobalt in grasses. The methods, however, could be equally well applied to the analysis of soils, animal organs or blood samples. In none of the normal bloods examined was there any detectable amount of cobalt, but the method would be of use in analysis of blood from cobalt-feeding experiments.

The preliminary treatment of the sample and subsequent estimation of cobalt will be described according to the nature of the substance under examination.

(1) In grasses, neither copper nor iron is, as a rule, present in sufficient quantity to require removal.

(2) In soils, animal organs and bloods, it is generally advisable to remove the considerable amount of iron present, more for convenience in manipulation of the final solution than for any possible interference with the colour development. Since complete removal of iron is not essential in the photometric method, washing with ether in acid solution would also serve, though not so satisfactorily as a precipitation method.

(3) In soils, minerals or other samples containing appreciable amounts of both copper and iron, copper is first removed, either by means of aluminium or precipitation with H_2S , and the iron then precipitated with zinc oxide.

PROCEDURE

5–10 g. of the sample are ashed by igniting for 30 min. at dull red heat. The ash is then taken up with 10–15 ml. concentrated HCl and slowly evaporated, the subsequent procedure depending on whether copper and iron are to be removed. The method of developing the cobalt complex is based on that of Kidson *et al.* [1936].

Reagents required:

HCl : conc., $N/2$ and sp.gr. 1.12 (7.3 N).

HNO_3 : conc. and 1 : 1.

KOH : 20% aqueous solution.

Sodium acetate (A.R.).

Zinc oxide (A.R.).

Nitroso-*R*-salt: 0.1 % aqueous solution.

Phenolphthalein: 0.2 % alcoholic solution.

H₂S generator or pure aluminium foil.

(1) When neither copper nor iron is to be removed, as in the case of grasses, evaporate the contents of the basin to complete dryness and take up the residue with 1 ml. HCl (sp.gr. 1.12), 6 drops 1 : 1 HNO₃ and a little hot distilled water. Filter into a 250 ml. conical flask, washing with hot water and bringing the volume to about 25 ml. Boil for 2 min., cool, add 2 ml. nitroso-*R*-salt solution and 2 g. sodium acetate. Heat to about 70°, add 5 drops phenolphthalein and make just alkaline with the KOH solution. Then make just acid with the *N*/2 HCl and boil for 2 min., keeping just acid to phenolphthalein. Add 5 ml. concentrated HNO₃ and boil for a further 2 min. Cool and make up to 25 ml. in a graduated cylinder. Find the extinction coefficient by means of the photometer, using filter S 53 and a 20 mm. cell, with distilled water in the compensating cell. The cobalt present is then found from the standard graph, obtained by plotting the figures given in Table I.

Note. It is advisable to filter all final solutions through a fine-grain paper before taking a reading, since unless they are perfectly clear, a serious error will be introduced. Filtration does not affect the intensity of the colour due to the cobalt.

(2) When iron is to be removed, evaporate the contents of the basin almost to dryness, add 10 drops concentrated HNO₃ and after evaporating almost to dryness, take up the residue with 2 ml. HCl (sp.gr. 1.12) and a little hot water. Filter and wash with a little hot dilute HCl; the volume should be about 25 ml. Boil for 2 min. and cool. Add a slight excess of an aqueous suspension of zinc oxide, boil for 1 min. and filter hot. Wash thoroughly with hot water. Evaporate filtrate and washings to about 20 ml., add 0.5 ml. HCl (1.12) and 6 drops 1 : 1 HNO₃, boil for 2 min. and proceed with the estimation of cobalt as directed above.

Notes. During neutralization with KOH, a precipitate is formed due to the zinc in solution. This, however, does not interfere with the adjustment of the reaction and disappears on addition of the concentrated HNO₃.

Concentrated HNO₃ is preferable to 1 : 1, since without affecting the cobalt complex, it more readily destroys colours formed by other elements, and is necessary in this case to destroy that due to the zinc itself.

(3) When copper is to be removed, evaporate the acid in the basin to about 2 ml., add a little hot distilled water and rinse the contents into a 150 ml. flask. Make the volume up to about 25 ml., warm and saturate with H₂S. Filter and wash with H₂S solution containing HCl.

To remove the copper by means of aluminium, evaporate the contents of the basin almost to dryness, add a little hot distilled water and rinse into a 150 ml. flask, making up to about 25 ml. Add a small piece of pure aluminium foil and heat on the steam bath for 1 hr. The copper, if present in sufficient quantity, forms a spongy deposit on the surface of the metal. The flask should be agitated occasionally to loosen the deposit. Filter and wash carefully with hot water.

In either case, evaporate the filtrate to 20 ml. If no removal of iron is required, proceed with the estimation of cobalt as in (1) above; if iron is to be removed, add 1 ml. HCl (1.12) and 6 drops 1 : 1 HNO₃, boil for 2 min., cool and proceed with addition of zinc oxide as in (2) above. Again all final solutions should be filtered. The cobalt is in all cases found from the standard graph.

It is to be noted that the graph passes through the origin. This shows that there is no interference from any of the other elements present in the solution.

EXPERIMENTAL RESULTS

Standard cobalt solution: 0.4034 g. $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ (A.R.) is dissolved in a litre of water, giving a solution containing 0.1 mg. cobalt per ml. Dilute 50 ml. of this solution to 1 litre, giving 0.005 mg. per ml. Varying amounts of this dilute standard solution are measured by means of N.P.L. standard pipettes into 150 ml. flasks. To each flask is then added distilled water to about 20 ml., 0.5 ml. HCl (1:12) and 6 drops 1:1 HNO_3 . The solutions are boiled for 2 min., cooled, 2 ml. nitroso-*R*-salt solution and 2 g. sodium acetate added and the cobalt estimated as in (1) above using filter S 53 and 20 mm. cells. The values so obtained (Table I) are found to lie on a straight line.

Table I

ml. standard cobalt solution	mg. cobalt present	Extinction coefficient (<i>k</i>)
0.5	0.0025	0.045
1.0	0.0050	0.088
1.5	0.0075	0.125
2.0	0.0100	0.175
3.0	0.0150	0.263
4.0	0.0200	0.350
5.0	0.0250	0.438
6.0	0.0300	0.525

All readings were taken at 15°.

Experiments were then carried out to test the validity of the graph so obtained after copper and iron had been removed by the methods described. 1.0 and 2.0 ml. of the same standard cobalt solution were used. To the solutions varying amounts of copper and iron were added and these elements then removed, copper by H_2S and by aluminium, and iron by means of zinc oxide. It was found that the extinction coefficients of the final solutions after development of the cobalt complex were respectively 0.088 and 0.175 ± 0.003 .

Cobalt in grasses. Cobalt estimations were carried out on numerous samples, the quantity found ranging from a mere trace to a figure of 1.4 parts per million. There did not appear to be sufficient copper in any of the samples to affect the result, since treatment with H_2S or aluminium made no difference to the figures.

Recovery of cobalt. This was applied as a further check on the method. To a known weight of a grass there was added a measured volume of standard cobalt solution, and the two ignited together. The cobalt content of the grass was

Table II

Sample no.	Weight taken g.	Added cobalt mg.	Extinction coefficient	Co found mg.	Co calculated mg.	% recovery of Co
5	5	—	0.014	0.0008	—	—
	5	0.0050	0.102	0.0059	0.0058	101.7
12	5	—	0.052	0.0030	—	—
	5	0.0025	0.092	0.0053	0.0055	96.4
	5	0.0050	0.135	0.0078	0.0080	97.5
	5	0.0100	0.215	0.0124	0.0130	95.4
101	10	—	0.065	0.0038	—	—
	10	0.0050	0.150	0.0086	0.0088	97.7

already known, so that the difference between the figures is a measure of the added cobalt. The recovery figures are very satisfactory as can be seen from the results in Table II. Incidentally, on no occasion was any loss of cobalt noted after ignition at low red heat.

It is to be noted that the cobalt present in grass is considerably less than that in the parent soil. For example, two grasses showed cobalt contents of 0.2 and 0.6 p.p.m., whereas the corresponding soils gave figures of 8 and 9 p.p.m. respectively.

SUMMARY

The application of the Pulfrich photometer in place of the tintometer for the estimation of cobalt has been investigated. The instrument is found to be eminently suited for this purpose, since not only does it give rapid and accurate readings, but the colour filters provide a simple means of eliminating the interference of other elements such as iron without having to remove the metal from solution.

Estimation of the cobalt depends on the formation of a complex between it and nitroso-*R*-salt. The method used for the development of the complex in the solution under examination is similar to that given by Kidson *et al.*

The grasses examined showed a very small cobalt content, in the majority of cases less than 1 p.p.m. By means of the photometric method described above, results can be reproduced to an accuracy within 0.1 p.p.m.

Several normal blood samples examined showed no trace of cobalt, nor was this element found in a dried sample of sheep's liver.

REFERENCES

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