CCIX. A METHOD FOR DETERMINING ALUMINIUM IN CERTAIN ALUMINIUM PROTEIN COMPOUNDS.

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IN connection with the work which has been carried out at these Laboratories on the precipitation of diphtheria toxoid by various aluminium salts [Glenny, Pope, Waddington and Wallace, 1926; Wallace, 1927; Glenny and Barr, 1931] it became necessary to make a number of determinations of the aluminium content of the precipitates so formed.

From an investigation of various methods which have been proposed for the estimation of aluminium we came to the conclusion that precipitation of the aluminium by use of 8-hydroxyquinoline would prove most satisfactory for our purpose. This reagent was used by Berg [1926] for the estimation of aluminium, and later Pichler [1929] reported a micro-method based on its use.

The suspensions of alum-toxoid with which we were working contained aluminium in an amount varying from a fraction of a milligram to about 1 mg. per cc. and the method was adopted for convenience for amounts varying from 0.3 to 1.0 mg. Using less than 0.3 mg. the results were not reliable, and for choice the material tested should contain between 0.5 and 1.0 mg. aluminium.

A difficulty, regarding which we can find no mention in the literature, was encountered in dealing with the aluminium complex salt, owing to the fact that the precipitate is not easily "wetted" and behaves rather like finely divided sulphur. This property made it difficult to transfer the precipitate quantitatively to a filter. While this difficulty may have been due to the sample of 8-hydroxyquinoline used, it was found that repeated crystallisation of this reagent did not obviate the trouble. At the suggestion of Dr Trevan, we tried the effect of adding a little sodium taurocholate, which proved effective.

We found it more convenient to determine the amount of "complex" formed volumetrically instead of gravimetrically. Berg titrated the complex directly with standard bromine solution using an indicator, but in our experiments better results were obtained by dissolving the complex in hydrochloric acid, adding excess of potassium bromate, and then determining the excess bromine by adding potassium iodide and titrating with thiosulphate.

At first we used 2*N* ammonium acetate following the method of Pichler [1929] in order to precipitate the complex, but we found that, unless the precipitate was washed very thoroughly, trouble was experienced owing to traces of acetate remaining in the filter and reacting with bromine during the estimation. Borax solution was found to be much more satisfactory than acetate for our purpose, and it had the added advantage that it dissolved the alum-toxoid precipitates and so obviated the necessity for incineration with sulphuric acid. Further, as the solubility of 8-hydroxyquinoline was greater in borax solution than in water, the precipitate when collected on the Gooch crucible was washed with borax solution to remove the excess of this reagent.

Reagents.

Standard solution of aluminium. Potassium alum (A.R.) dried in a vacuum over calcium chloride. Dissolve 1.7509 g. in distilled water and make to 100 cc. (*Note.* In working with a solution made to contain 0.1 mg. aluminium per cc. it was found that after a day or two in a volumetric flask the solution gave no reaction for aluminium when tested with aurintricarboxylic acid, using the method of Myers, Mull and Morrison [1928]. After adding one drop of concentrated H_2SO_4 the aluminium, which may have been adsorbed on the glass of the flask, was liberated and correct values for the aluminium present were obtained. In view of this experience we have made it a practice to add one drop of H_2SO_4 when making up standards.) The standard solution contains 1 mg. of aluminium per cc.

Borax solution. A 5 % solution of sodium borate (A.R.).

8-Hydroxyquinoline. A 2 % solution in absolute alcohol. 1 cc. of this reagent is sufficient to precipitate 1.2 mg. of aluminium. When 1.0 cc. of this solution is mixed with 10 cc. of 5 % borax solution, a little 8-hydroxyquinoline may separate if the temperature is below 15°. This will not affect the results if the amount of aluminium is between 0.5 and 1.0 mg. but for amounts below 0.5 mg. it is better to use 0.5 cc. of reagent.

Hydrochloric acid. Hydrochloric acid (A.R.) diluted 1 in 3. For each test 40 cc. are used.

Bromide-bromate solution. Dissolve 1.6702 g. potassium bromate (A.R.) and 10 g. of potassium bromide in water and make to 1000 cc. This solution is *M*/100 in respect to the bromate, and 10 cc. provide sufficient bromine for 1.35 mg. of aluminium as $\text{Al}(\text{C}_9\text{H}_6\text{ON})_3$.

N/20 thiosulphate solution. Prepared and standardised in the usual manner. Each cc. of *N*/20 thiosulphate = 0.1129 mg. aluminium.

Blank titration for total bromine available.

Measure out 40 cc. of HCl reagent into a beaker and add water to about 200 cc. Add 10 cc. of bromate-bromide reagent, and after two minutes 1 g. (approx.) of potassium iodide. Titrate the liberated iodine with *N*/20 thiosulphate until almost colourless, completing after the addition of starch

indicator in the usual manner. If the bromate and thiosulphate are $M/100$ and $N/20$ respectively, 10 cc. of bromate-bromide reagent will require 12 cc. of thiosulphate.

Estimation of aluminium.

Measure out an amount of the material containing between 0.3 and 1.0 mg. (The amount present may be roughly estimated from the bulk of precipitate given with the 8-hydroxyquinoline.) Add 10 cc. of borax solution, heat to boiling-point, then add 1.0 cc. of 8-hydroxyquinoline reagent. At this stage two drops of 10 % sodium taurocholate are added to cause "wetting" of the precipitate. The tubes are now heated in a boiling water-bath to drive off the alcohol in which the precipitate is somewhat soluble. They are then cooled to room temperature.

The yellow precipitate is separated by filtration through a Gooch crucible, traces of precipitate in the tube are removed, and finally the precipitate on the crucible is washed with 5 % borax solution. The precipitate is then ready to be dissolved in HCl.

Heat 40 cc. of HCl reagent to boiling. Hold the crucible over a 400 cc. beaker and half fill it with boiling HCl. Stir gently with a small glass rod, but avoid disturbing the asbestos mat at this stage. When most of the first addition of HCl has passed through the filter, add more boiling HCl and stir gently to break up the asbestos mat. Wash through with the remainder of the 40 cc. of HCl. Finally rinse the crucible with a little distilled water, wash down the sides of the beaker and make to a total volume of about 200 cc.

Add 10 cc. of bromate-bromide solution, stir well and after two minutes add about 1.0 g. potassium iodide. Titrate with $N/20$ thiosulphate. Some caution is necessary in the titration owing to the yellow colour of the bromine derivative of 8-hydroxyquinoline, and the starch indicator should, therefore, be added earlier than usual. The amount of aluminium is obtained as follows:

$$(T_1 - T_2) \times 0.1129 \times N \text{ mg. aluminium,}$$

where

T_1 = cc. thiosulphate required for bromine blank,

T_2 = cc. thiosulphate required for excess bromine (titrated as iodine),

N = Normality factor for thiosulphate and each 1.0 cc. $N/20$ thiosulphate = 0.1129 mg. aluminium.

EXPERIMENTAL RESULTS.

The accuracy of the method using pure solution of potassium alum.

The stock solution used for the following tests was made up to contain 1.0 mg. aluminium per cc. From this there were prepared dilutions so that each fraction of a mg. used was contained in a volume of 1.0 cc. The results given below are for estimations carried out at different times, when pure solutions were included as controls in routine estimation.

Mg. Al used	Mg. Al found	Difference in mg.
0.2	0.203	+0.003
0.2	0.200	±0.000
0.2	0.216	+0.016
0.2	0.191	-0.009
0.2	0.195	-0.005
0.3	0.305	+0.005
0.3	0.302	+0.002
0.3	0.310	+0.010
0.3	0.306	+0.006
0.3	0.310	+0.010
0.4	0.409	+0.009
0.4	0.408	+0.008
0.4	0.410	+0.010
0.4	0.412	+0.012
0.4	0.404	+0.004
0.5	0.500	±0.000
0.5	0.500	±0.000
0.5	0.501	+0.001
0.5	0.506	+0.006
0.5	0.495	-0.004
0.5	0.493	-0.007
0.5	0.491	-0.009
1.0	0.995	-0.005
1.0	0.997	-0.003
1.0	0.997	-0.003
1.0	1.002	+0.002
1.0	0.998	-0.002
1.0	0.997	-0.003

Recovery of aluminium when mixed with C. diphtheriae culture filtrates.

In order to determine the accuracy of the method when organic materials were present in addition to the aluminium, the following experiment was carried out.

Known amounts of aluminium were measured into tubes, and 0.5 cc. of a *C. diphtheriae* toxin filtrate added. A precipitate formed in each. 10 cc. of borax solution were then added to each tube and the aluminium estimated in the manner described. The following results were obtained, showing that the presence of organic materials did not affect the results.

Total Al present (mg.)	Al found (mg.)	Difference (mg.)
0.3	0.303	+0.003
0.3	0.298	-0.002
0.4	0.403	+0.003
0.4	0.398	-0.002
0.5	0.501	+0.001
0.5	0.507	+0.007

In a further experiment, a typical *C. diphtheriae* culture filtrate was diluted 1 in 5 with saline and mixtures made containing varying amounts of potassium alum. In each case there was formed a precipitate, small in the low alum concentration and increasing as the alum concentration increased. From these mixtures varying volumes were measured out, precautions being taken to keep the suspensions as uniform as possible during measurement. The volumes required to give a suitable amount of aluminium for estimation varied from 25 to 2 cc. of suspension. The results show the method to be suitable for the special purpose for which it was worked out.

Potassium alum in mixture %	Vol. used for estimation cc.	Mg. Al found	Mg. Al per cc. found	Mg. Al per cc., calculated
0.05	25	0.713	0.0285	0.0285
0.1	20	1.085	0.0542	0.0590
0.2	10	1.141	0.1141	0.1140
0.3	5	0.871	0.1742	0.1710
0.4	5	1.120	0.2240	0.2230
0.5	4	1.150	0.2870	0.2850
0.5	4	1.130	0.2820	
0.6	3	1.020	0.3400	0.3420
0.7	3	1.185	0.3950	0.3990
0.8	2	0.906	0.4530	0.4560

SUMMARY.

A method suitable for the estimation of amounts of aluminium between 0.3 and 1.0 mg. present in suspensions produced by adding potassium alum or other aluminium salts to *C. diphtheriae* culture filtrates has been described. The method depends on the precipitation of the aluminium with 8-hydroxyquinoline and the estimation of the amount of the latter contained in the precipitate.

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