LXXI. A TITRIMETRIC METHOD FOR THE MICRO-DETERMINATION OF BARIUM.

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THE preparation and purification of substances of biological interest is in many cases facilitated through their isolation as barium salts. A knowledge of the percentage of barium present in these salts is often of importance in the study of their chemical nature. The determination of barium in small amounts has always, however, been attended with considerable difficulty. The well-known method of Pregl [1930] for sulphate is easily adapted to the determination of barium and gives excellent results, as also the newer Emich [1931] procedure for barium, using the filter-stick, which has proved so useful in micro-gravimetric analysis. Both these methods are thoroughly reliable, but require special apparatus, including a micro-balance, and a rather refined and specialised technique.

If the precipitate of barium sulphate be collected on a filter-paper, loss may occur through the finer particles passing through the pores of the paper. To overcome this difficulty McLaughlin [1931] proposed that the filtrate should be passed a second time through the paper to collect any particles which might have escaped during the first passage through the filter-paper. By this means he was able to show that his loss of barium sulphate did not amount to more than 0.3 % of the total, when dealing with precipitates of approximately 20 mg. in weight. Another troublesome source of error was the variable loss in weight due to reduction of some of the barium sulphate to barium sulphide in the presence of the hot reducing gases from the burner. These difficulties made it necessary to use electric heating in the final ignition of the precipitate.

An attempt was made to determine the conditions under which barium might be precipitated quantitatively as oxalate, in the hope of elaborating a procedure for barium similar to the Clark and Collip [1925] modification of Kramer and Tisdall's [1921] oxalate method for serum-calcium, in which the precipitate of calcium oxalate is collected and titrated in a small centrifuge-tube. Acidimetric titrations of the oxide formed from the oxalate on heating (similar to Fiske and Logan's [1931] method for calcium in urine), and of barium carbonate precipitated in a centrifuge-tube were also tried. Although some success was attained with these different procedures, and particularly with

barium oxalate, the results were often variable and inconsistent, and another indirect method involving the use of sulphuric acid and benzidine was eventually adopted as it was more satisfactory to apply and gave more reliable and consistent results.

A method for determining sulphate in urine in which benzidine hydrochloride was added to the diluted urine to precipitate the sulphate, and the benzidine sulphate filtered off and titrated with sodium hydroxide to a pink colour with phenolphthalein, was described by Rosenheim and Drummond [1914]. This method has been variously modified and adapted, particularly to total base determinations in serum, by several workers [Fiske, 1921, 1922; Stadie and Ross, 1925; Van Slyke, Hiller and Berthelsen, 1927], and has proved to be a useful and trustworthy procedure for the titrimetric estimation either of sulphate in solution or of sulphate in residues of total base.

The benzidine sulphate procedure may be adapted to the determination of small amounts of barium in solution. The barium is precipitated in a centrifuge-tube by an excess of standard sulphuric acid, and the excess determined by the benzidine procedure. The method has been in use in this laboratory for some time with good results. The results obtained have been repeatedly checked by comparison with gravimetric determinations (Emich) and have agreed sufficiently well to warrant the adoption of the method as a routine procedure.

METHOD.

Between 0.5 and 2 mg. of barium contained in 2 or 3 cc. of solution are measured into a conical 15 cc. centrifuge-tube, which is placed in a beaker of boiling water until hot. Exactly 4 cc. of standard 0.01 N H_2SO_4 are added to precipitate the barium and the contents of the tube thoroughly mixed. The tube is allowed to cool and 2 cc. of benzidine hydrochloride solution containing 4 g. benzidine and 50 cc. N HCl in 250 cc. are added to precipitate the excess of sulphuric acid. 3 cc. of 95 % acetone are now blown in from a fine-tipped pipette, so that the contents of the tube are well stirred up. After standing 10 minutes the sides of the tube are washed down with a little acetone, and the liquid centrifuged for 5 minutes. The supernatant liquid is siphoned off with the aid of a fine capillary tube bent up at the bottom, as in the Kramer and Tisdall [1921] method for calcium. It is best not to attempt to pour off the supernatant liquid at this stage, since some of the precipitate may be stirred up and lost.

The precipitate of barium sulphate and benzidine sulphate is washed in the centrifuge-tube by blowing in 3 cc. of the 95 % acetone, so as to impinge on the mat at the bottom of the tube, which is thus thoroughly stirred up. The sides of the tube are washed down with a further cc. of acetone, and the tube is again centrifuged for 5 minutes. The supernatant acetone layer may now be poured off carefully from the well packed precipitate and the tube inverted on a piece of clean filter-paper to drain for 2 minutes. The washing

is repeated and, after having centrifuged and drained the tube, 3 cc. of water are blown in so as to stir up the precipitate. The tube is placed in the beaker of hot water, a drop of phenolphthalein solution added and the benzidine sulphate titrated hot with 0.01 *N* NaOH to the first permanent faint pink colour. The barium sulphate does not appear to interfere in any way with the titration.

The calculation is as follows:

$$4.00 - (\text{cc. } 0.01 \text{ } N \text{ NaOH used}) = \text{cc. of } 0.01 \text{ } N \text{ H}_2\text{SO}_4$$

necessary to precipitate the barium. Each cc. of 0.01 *N* H₂SO₄ is equivalent to 0.687 mg. Ba, and therefore (4.00 – cc. 0.01 *N* NaOH) × 0.687 = mg. of barium in the sample taken.

Some examples of the results obtained with the method are given in the table.

Analysis of barium solutions by the benzidine method.

(1) Solution used	(2) cc. of 0.01 <i>N</i> NaOH to neutralise the benzidine sulphate	(3) cc. of 0.01 <i>N</i> H ₂ SO ₄ pptd. by barium [4.00 – (2)]	(4) mg. of barium found [(3) × 0.687]	(5) mg. of barium present
2 cc. of a BaCl ₂ , 2H ₂ O solution (0.687 mg. Ba per cc.)	2.02 2.09 2.11	1.98 1.91 1.89	1.36 1.31 1.30	1.37
3 cc. of a BaCl ₂ , 2H ₂ O solution (0.687 mg. Ba per cc.)	1.10 1.06	2.90 2.94	1.99 2.02	2.06
1 cc. of a BaCl ₂ , 2H ₂ O solution (0.42 mg. Ba per cc.)	3.39	0.61	0.42	0.42
3 cc. of a BaCl ₂ , 2H ₂ O solution (0.42 mg. Ba per cc.)	2.19	1.81	1.24	1.26
3 cc. of a BaCl ₂ (anhyd.) solution (0.65 mg. Ba per cc.)	1.03 1.06	2.97 2.94	2.04 2.02	1.95
Barium phenylphosphate solutions:				mg. of barium determined gravimetrically
3 cc. solution No. 1	2.29 2.20	1.71 1.80	1.17 1.23	1.16 1.17
3 cc. solution No. 2	2.50 2.48 2.43	1.50 1.52 1.57	1.03 1.04 1.08	1.04
3 cc. solution No. 3	2.20 2.24	1.80 1.76	1.23 1.21	1.23
1 cc. solution No. 4	1.11 1.16	2.89 2.84	1.98 1.95	2.03 2.02
3 cc. solution No. 5	2.16	1.84	1.26	1.24
3 cc. solution No. 6	2.18 2.17	1.82 1.83	1.25 1.26	1.27
				Calculated
Barium diphenylphosphate	2.42	1.58	1.09	1.07
Barium ethylphosphate	0.23	3.77	2.59	2.58
1 cc. approx. <i>M</i> /50 solution	0.21	3.79	2.61	
Barium diethylphosphate	1.05	2.95	2.03	2.07
3 cc. <i>M</i> /100 solution	1.03 1.05 0.95	2.97 2.95 3.05	2.04 2.03 2.09	

SUMMARY.

A titrimetric method for the micro-determination of barium is described, in which the barium is precipitated by an excess of standard sulphuric acid. The excess of sulphuric acid is determined by precipitation with benzidine and titration of the benzidine sulphate with sodium hydroxide.

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