

DETERMINATION OF CARBONATE, ORGANIC CARBON AND TOTAL NITROGEN IN THE SAME SAMPLE.

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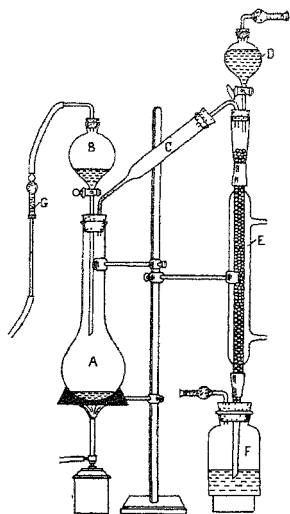
The present communication deals with an elegant and rapid method for the estimation of these three important constituents in the same sample of soil or other biological material.

Principle.—The carbonate is decomposed by excess of phosphoric acid and estimated as carbon dioxide. It has been observed by a number of previous workers that dilute sulphuric acid is not suitable for this purpose, especially when the material contains alkaline earth carbonate. In the latter case, the insoluble sulphate forms a coat around unattacked carbonate and thus protects it from reaction with acid. Neither addition of more acid nor prolonged heating is effective in completely decomposing the carbonate. Treatment with hydrochloric acid is more efficient and is generally adopted for this purpose. The residue is not suitable, however, for the estimation of organic carbon, especially by methods of wet combustion. The resulting chlorine will interfere with the accuracy of the estimation of both organic carbon and total nitrogen. Dilute phosphoric acid forms the insoluble alkaline earth salt, but on addition of excess of the acid, the soluble acid salt is formed, so that the entire quantity of carbonate is ultimately decomposed.

Organic carbon in the residue is estimated by a simplified method of wet combustion with a mixture of sulphuric and chromic acids. It has been shown by Subrahmanyam, Narayanayya and Bhagvat (*Proc. Ind. Acad. Sci.*, 1934, **1B**, 155) that as long as there is a useful quantity of water in the digesting mixture, only carbon dioxide and water pass off as vapour. According to the proposed method, the water vapour together with traces of nitric acid (which may be either present in the original material or formed during reaction) are condensed and drop back into the reaction vessel, while only carbon dioxide passes into the absorption system, where it is cooled and, at the same time, quantitatively absorbed by alkali. Interference from halides (up to 2 per cent.) present in the original material or in the reagents is eliminated by addition of a mercury salt (preferably the oxide) to the digesting mixture (Narayanayya and Subrahmanyam, *Proc. Ind. Acad. Sci.*, 1935, **2B**, 213).

It may be mentioned that in the ordinary methods of wet combustion, the oxidising agent (chromic acid) is added in the cold to the material to be digested. This procedure causes some loss of nitrogen because of the intermediary formation of ammonium dichromate (Harihara Iyer and Rajagopalan, *J. S. C. I.*, 1935, 54, 341T) which decomposes on heating. This loss can be entirely avoided by (a) adding sulphuric acid in the proportion of at least 2:1 to the water in the suspension, and (b) adding chromic acid to the boiling mixture (temperature about 170°) of the material to be digested with sulphuric acid. Ammonium dichromate is not formed at that high temperature (Harihara Iyer and Rajagopalan, *loc. cit.*). The loss of nitrogen observed by Acharya (*Nature*, 1935, 136, 644) under conditions of low temperature and reduced pressure does not take place—at any rate in the case of soils and commoner biological materials—under the conditions outlined in this paper. The residue in the digesting flask contains the entire quantity of nitrogen present in the original substance. The major part is present as ammonium sulphate, but small portions also occur (a) in combination with the chromium in the digest, and (b) as nitric acid. Both of these forms can be converted into ammonia on treatment with reducing agents. A large number of reagents have been tried for this purpose, but the best results are obtained with a combination of alkali sulphite and zinc (Harihara Iyer and Rajagopalan, *loc. cit.*). The subsequent distillation is carried out in the usual way.

Apparatus and Procedure: Carbonate.—The material to be examined (soil 10 g.; others in proportion) is weighed into a Kjeldahl flask (A) (any other long-necked, round-bottomed flask will do; capacity about 500 c.c.) and treated with mercuric oxide (red or yellow; about 2 g.) and distilled water (15 c.c.). The suspension is well shaken and the flask fitted in position. Through tap funnel B (capacity, 100 c.c.) 10 c.c. of syrupy phosphoric acid (sp. gr. 1.8) is then introduced into the flask. (Ordinarily, there is no difficulty in adding the acid. Sometimes, especially when the material is rich in carbonates, there is such a high pressure of gas inside that, after the first few drops, further addition of acid is rendered difficult. In such a case, a convenient procedure will be to blow in the acid, the rubber tube extension with soda-lime guard (G) being used for the purpose.) The contents of flask (A) are then raised to boil. The vapours (chiefly air, water vapour and carbon dioxide) pass into the air condenser (C) (length of wider part, 6-8"; internal diameter, about 1 inch) where the water is condensed and drops back into A. Carbon dioxide passes into the condenser (E) where it meets standard alkali (2 N; 25 c.c.) trickling from D (capacity about 50 c.c.). The cooling combined with



the large surface exposed by the glass beads facilitates the absorption of almost the entire quantity of carbon dioxide. Any little portion which escapes is absorbed in F. After boiling for about 15 minutes, the heating is stopped and tap D opened to equalise pressure. The alkali in D and E is washed down into F, a few drops of phenolphthalein being added from time to time to ensure complete removal of all the alkali. (The washing is greatly facilitated by using fairly big-sized—diameter, about 5 m.m.—glass beads. Very small beads are rather difficult to wash.) The contents of F are then treated with excess of barium chloride (20 c.c.; 10 per cent.) and back-titrated against standard acid (2 N) in the usual way. The end-point is sharpened by either finishing the titration against weaker acid (N/10) or, after the phenolphthalein colour is discharged, back-titrating against weaker alkali (N/10) until the colour is just regained.

Organic Carbon.—A new reservoir is introduced in place of F and fresh alkali (2 N: 25 c.c.) kept slowly dropping from D. Concentrated sulphuric acid (40 c.c.) is introduced into A through B. The last portions of acid adhering to the funnel are rinsed out with 1-2 c.c. of water and discharged into A. (This is to facilitate the introduction

of chromic acid which is to follow: otherwise, there will be precipitation of chromic anhydride which will choke up the funnel.) The contents of A are then heated and just raised to boiling. The flame is momentarily removed and a saturated aqueous solution (5 c.c.) of chromic acid (CrO_3) introduced into A through B. (By skilfully managing tap B, chromic acid can be generally introduced without much difficulty. If the reaction is too violent or the interior pressure otherwise too high, the acid may be blown in, the procedure being the same as that already described.) The boiling is then resumed, the height of flame being so adjusted that the water vapour just condenses in C. The absorption of carbon dioxide in E and back-titration of unused alkali are carried out in the manner described already.

Total Nitrogen including Nitrates.—The procedure is similar to that outlined by Harihara Iyer and Rajagopalan (*loc. cit.*). The contents of A are transferred to the distilling flask (capacity about 1,500 c.c.) with frequent washings (200–300 c.c.). They are then treated with pure sodium sulphite and raised to boil. (If the sulphite is fairly pure, 5–7 g. is generally sufficient to completely reduce all the unused chromic acid. The sulphite need not be weighed, but may be added in small instalments until the smell of sulphur dioxide is just pronounced. The end-point is also indicated by the change of colour from emerald green to a pale blue.) After boiling for about 15 minutes, pure zinc (powdered, 2 g.) is added and the boiling continued for a further period of 10 minutes. The contents of the flask are then cooled, diluted with more water, if necessary, and distilled with excess of alkali in the usual way for nitrogen. The estimate thus obtained includes not only the organic carbon but also the nitrogen present in the original material. It has been found that upto 300 p.p.m. of nitrate nitrogen can thus be included.

It may be mentioned that even the purest specimens of zinc contain traces of nitrogen. For eliminating the error due to this and other reagents, it may be useful to carry out a blank determination and apply the necessary correction.

The following are some of the results obtained:—

TABLE I.

Estimation of known quantities of calcium carbonate.

Acid used	Carbonate as mg. of carbon					
	As taken	As estimated	As taken	As estimated	As taken	As estimated
H ₂ SO ₄ (1: 40) ..	28.0	22.8	48.4	33.2	62.4	48.2
H ₂ SO ₄ (2: 1) ..	25.1	23.1	43.2	37.4	65.6	54.2
H ₂ CrO ₄ + H ₂ SO ₄ (2: 1)	20.8	20.3	43.2	37.4	66.9	59.0
HCl (1: 15) ..	20.0	20.0	40.2	40.2	60.0	60.0
H ₃ PO ₄ (Syrupy) ..	29.4	29.4	42.4	42.0	62.8	62.6

It may be seen from the above that correct estimates of carbonate can be obtained by treatment with either hydrochloric or phosphoric acid. Sulphuric acid, either by itself or combined with chromic acid, gives low estimates especially with increasing quantities of carbonate.

TABLE II.

Known quantities of carbonate added to soil and the carbonate and organic carbon contents of the mixtures estimated by different methods.

Treatment	Carbon in mg. (as estimated)								
	Soil alone (10 g.)			Soil (10 g.) + 48 mg. of carbonate carbon (as CaCO ₃)			Soil (10 g.) + 80 mg. of carbonate carbon (as CaCO ₃)		
	Carbo- nate	Organic	Total	Carbo- nate	Organic	Total	Carbo- nate	Organic	Total
Dil. H ₂ SO ₄ (1: 40) followed by CrO ₃ + H ₂ SO ₄ (2: 1) (same sample)	Nil	57.0	57.0	39.4	60.8	100.2	56.3	64.5	120.8
Dil. HCl (1: 15) for carbonate CrO ₃ + H ₂ SO ₄ (2: 1) for total carbon (separate samples)	"	57.6 (by dif- ference)	57.6	48.8	53.0 (by dif- ference)	101.1	80.0	42.0 (by dif- ference)	122.0
H ₃ PO ₄ (conc.) followed by CrO ₃ and H ₂ SO ₄ (2: 1) (same sample)	"	57.0	57.0	48.2	57.2	105.4	79.4	57.4	136.8

The first method did not yield correct estimates of either carbonate or organic carbon : the latter determination included some of the carbonate and the results were obviously too high. The second procedure gave accurate estimates of added carbonate but yielded inaccurate values for total carbon. The estimates of organic carbon (obtained by difference) were consequently too low. The last method yielded the most accurate estimates of both carbonate and organic carbon and hence the most reliable.

TABLE III.

Carbonate carbon, organic carbon and total nitrogen contents of some Indian soils (as determined by the new method).

Description of Soil	Percentages		Total Nitrogen (in parts per million)
	Carbonate Carbon	Organic Carbon	
Kalar Plot—Willingdon Cattle Farm Sandy Loam	1.196	0.542	522
Sweet Land—Sindh	1.081	0.304	375
Alluvial—N. Bihar	1.252	0.534	648
Black Cotton—Nagpur	1.045	0.309	428
Heavy Black—Sholapur	0.683	0.578	240
Khamba Village—Ahmedabad	0.479	0.214	392
Red Loam—Bangalore	Nil	0.570	552
Paddy Lands—Kandy	„	2.276	1768
Upland Farm—Cuttack	„	0.510	410
Alluvial—Travancore	„	2.280	1960
Barani Area—Punjab	„	0.384	537
Surface Soil—Estate Area—Nilgiris..	„	1.812	1669
Cultivated Highland—Dacca	„	0.621	680

TABLE IV.

Carbon and nitrogen contents of some biological materials.

Material	Percentages			
	Total Carbon		Total Nitrogen	
	Expected*	Found	Expected†	Found
Urea	19.84	19.82	46.3	46.26
Cyanamide	20.80	20.82	15.09	15.00
Yeast	34.80	34.40	5.62	5.67
Dried Blood	44.00	44.20	12.30	12.40
Lantana Leaf	43.00	43.50	2.75	2.77
Paddy Husk	34.40	34.20	0.260	0.265

* Subrahmanyan, Narayanayya and Bhagvat, *J. Indian Inst. Sci.*, 1934, 17A, 197.† Harihara Iyer and Rajagopalan, *J. S. C. I.*, 1935, 54, 341 T.

It is not always necessary that the material should be treated with phosphoric acid for the estimation of either carbonate or total carbon. Many substances, including a large number of biological materials, do not contain any carbonate. In such cases, as also in those of substances containing only traces of carbonate, pre-treatment with even dilute sulphuric acid will suffice. It may be useful, therefore, to conduct some preliminary tests before undertaking the examination. If there is marked effervescence on treatment with hydrochloric acid, then the phosphoric acid method may be followed not only for the estimation of carbonate but also as a pre-treatment for the determination of organic carbon. If, on the other hand, there is no effervescence or only a faint evolution of gas, then boiling with dilute sulphuric acid followed by digestion with sulphuro-chromic acid mixture may be adopted.

Extension of Technique.—The method—especially the estimation of organic carbon and total nitrogen—can be extended to a variety of organic substances. In some cases, a preliminary reduction is needed to include all the nitrogen. Attempts are also being made to devise a micro modification particularly suitable for the analysis of biological materials.

SUMMARY.

An elegant and rapid method for the estimation of carbonate, organic carbon and total nitrogen in the same sample of soil or other biological material has been developed.

The procedure consists in decomposing carbonates with excess of phosphoric acid (which includes alkaline earth carbonates) followed by wet combustion of organic carbon. A simple apparatus for the purpose, as also a modified procedure for the digestion, have been described.

The residue contains the major part of the nitrogen as ammonium salt. A small part occurs in combination with the chromium in the digest and a further quantity as nitric acid. These forms can be converted into ammonia by boiling with alkaline sulphite followed by reduction with zinc in acid medium. The subsequent distillation is carried out in the usual way.

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