An Improved Procedure for the Determination of Carbon in Organic Compounds in Aqueous Solution

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(Received 5 April 1951)

The rapid semi-micro determination of carbon by wet combustion of organic compounds (Van Slyke & Folch, 1940) has been subjected to various modifications, principally in the apparatus used (McCready & Hassid, 1942; Hockenhull, 1950).

The apparatus is shown in Fig. 1, and is essentially that described by Hockenhull (1946; and personal communication), using the spiral bubbler modified from that of Pearce & Robertson (1936).

Fig. 1. Apparatus for the determination of carbon in compounds in aqueous solution. Scale = $\frac{1}{2}$. Constructed in thick-wall Pyrex glass. The ground joint connecting the combustion flask (A) to the rest of the apparatus is size B19. (A) is the combustion flask, (B) is the dropping funnel, (C) is a water condenser. Bubbler (D) contains pure H₂SO₄. Bubbler (E) contains water acidified with H₂SO₄. The spiral bubbler (F) contains barium hydroxide. The sections of the apparatus are joined together by rubber tubing.

The application of this procedure to aqueous samples presents a difficulty, since the combustion is then incomplete (Van Slyke & Folch, 1940). Friedeman & Kendal (1929) had previously overcome this difficulty by working on a macro scale and using large volumes of concentrated sulphuric acid to absorb the excess water. In this way the carbon of ethanol could be directly estimated. The need for a semi-micro method for the rapid analysis of total carbon in fermentation liquids prompted the following modification of the method of Hockenhull (1950).

Water is removed from the reaction mixture by excess phosphorus pentoxide in the combustion flask before combustion.

METHOD

Reagents. Oxidizing mixture (Van Slyke & Folch, 1940); P₂O₅; KIO₃ (A.R.); 0.1 N-HCl; 0.05 N-Ba(OH)₂; phenolphthalein (1% w/v in ethanol); *n*-butanol (redistilled).

Apparatus and procedure. In the combustion flask (A) is placed P₂O₅ (1.5 g./ml. of liquid in the sample) and 200 mg. of KIO₃. The joints of A and B are moistened with syrupy orthophosphoric acid and the flask is fitted to the apparatus. The oxidizing mixture (5 ml.) is then added and CO₂-free air is aspirated for 5 min. The tip of the dropping funnel (B) must be below the surface of the oxidizing mixture at this stage, otherwise when the sample is added loss will occur due to volatilization of labile constituents. After 5 min. the air flow is stopped and 40–50 ml. of 0.05 N-Ba(OH)₂ (exactly measured), together with 2–3 ml. of *n*-butanol and 0.5 ml. of

Table 1. Carbon content of various substances determined in aqueous solution

(Glucose was British Drug Houses Analar dextrose. *iso*Butyric acid was supplied by Mr K. el Shazly; uric acid was a specimen from Kahlbaum. The purity of the ethanol (Distillers Company Ltd.) was estimated by dichromate oxidation.)

Substance	Vol. of solution (ml.)	Wt. of substance taken (mg.)	Carbon content				P ₂ O ₅ (g.)
			Calculated (mg.)	Found (mg.)	Loss or gain (mg.)	Recovery (%)	
Glucose	1	14.99	5.99	5.94	-0.05	99.4	1.5
	1	14.99	5.99	6.07	+0.08	100.5	
	1	14.99	5.99	6.13	+0.14	101.2	
	4	14.99	5.99	5.94	-0.05	99.4	5-6
	4	14.99	5.99	5.87	-0.12	98.6	
	4	14.99	5.99	6.07	+0.08	100.5	
<i>iso</i> Butyric acid	1	8.626	4.70	4.95	+0.25	105.0	1.5
	1	8.626	4.70	5.01	+0.31	106.1	
	1	8.684	4.73	4.68	-0.05	98.9	
	1	8.684	4.73	4.81	+0.07	100.5	
	1	8.684	4.73	4.62	-0.11	98.3	
	1	8.684	4.73	4.62	-0.11	98.3	
Ethanol	1	9.145	4.77	4.62	-0.15	96.8	1.5
	1	9.145	4.77	4.55	-0.22	95.4	
	1	9.145	4.77	4.62	-0.15	96.8	
	4	9.145	4.77	4.75	-0.02	99.5	5-6
	4	9.145	4.77	4.58	-0.09	98.1	
	4	9.145	4.77	4.58	-0.09	98.1	
Uric acid	2	11.98	4.26	4.42	+0.16	103.7	3-4
	2	11.98	4.26	4.35	-0.09	102.1	

phenolphthalein solution, are added to the spiral bubbler. The *n*-butanol prevents foaming, assists aggregation of particles of BaCO₃ and further forms a protective surface layer during the final titration. The aqueous sample (containing about 4 mg. C) is then carefully added and washed in with 2 ml. of oxidizing mixture. CO₂-free air is again aspirated through the apparatus while the contents of the flask (A) are heated until refluxing freely. After 15 min. the heating is stopped and the contents of the spiral bubbler are run into a 250 ml. flask. A three-way stopcock on the aspirator bottle allows the apparatus to be open to the air during this operation. The spiral is rinsed out by filling with CO₂-free water and again running out; the total liquid is then titrated with 0.1 N-HCl. A blank is run under similar conditions. 1 ml. 0.1 N-HCl $\equiv$ 0.60 mg. C.

RESULTS

The results of the analysis of four compounds are shown in Table 1. For samples containing about

5 mg. carbon the recoveries range from 95 to 106 %; the procedure is, therefore, sufficiently accurate for many biological purposes, for example, the determination of carbon in fermentation liquids. In this latter respect the high recoveries obtained with ethanol are of particular interest.

SUMMARY

1. A method is described for the rapid determination of carbon in aqueous solutions of organic compounds.

2. For samples containing about 5 mg. carbon, the method is accurate within ± 5 %.

I wish to thank the Faculty of Technology, The University, Manchester, for a gift of the apparatus, Mr K. Bartholomew, glass-blower, for constructing it, and Mr K. el Shazly, Rowett Research Institute, for providing a highly purified sample of *isobutyric* acid.

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