514. *Submicro-methods for the Analysis of Organic Compounds. Part VI.* The Determination of Carbon.*

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The carbon content of organic compounds can be determined on samples of only 18—50 μg. with a precision equal to that obtained by micro-methods. The carbon dioxide is separated from the water vapour by freezing and is measured by means of a McLeod gauge. The interference of sulphur and the four halogens is eliminated by means of magnesium oxide at 500°, and that of nitrogen oxides by manganese dioxide at room temperature.

GRAVIMETRIC methods similar to those used in microchemistry are not suited to the submicro-determination of carbon and hydrogen in organic compounds. Hence, although titrimetric methods have not met with approval in microchemistry,[1,2] we have examined several such procedures on the submicro-scale. These methods involved absorption of the carbon dioxide in barium hydroxide, strontium hydroxide, ammoniacal barium chloride, lead acetate, and organic solvents; none was sufficiently reliable to provide the necessary precision (±0·3% absolute). A modified Unterzaucher method was also examined,[3] but difficulties were encountered owing to incomplete separation of carbon dioxide and water, and because of reduction of the iodine pentoxide by hydrogen. Another procedure, based on conversion of carbon into cyanide by fusion with sodium and a nitrogenous compound, was unsatisfactory owing to incomplete recoveries.

Compounds (M.A.R. Standards)	Range of sample wts. (μg.)	No. of detns.	Carbon (%) Calc.	Carbon (%) Found	Error Min.	Error Max.
Sucrose	25·00—71·00	12	42·10	42·07 ± 0·24 *	0·00	0·43
Benzoic acid	19·19—51·29	6	68·84	68·73 ± 0·2 *	0·08	0·39
Glucose	38·86—53·63	3	40·00	40·13	0·00	0·30
Penta-acetylglucose	24·02—35·62	3	49·23	49·44	0·07	0·32
Benzyl disulphide	18·50—48·40	4	68·30	68·18	0·20	0·30
Sulphonal	25·01—46·51	3	36·80	36·93	0·10	0·30
p-Fluorobenzoic acid	20·79—51·01	3	60·00	60·00	0·20	0·50
p-Chlorobenzoic acid	15·75—51·06	4	53·70	53·58	0·10	0·50
p-Bromobenzoic acid	18·05—48·51	4	41·80	41·60	0·20	0·60
p-Iodobenzoic acid	26·76—49·99	4	33·90	33·90	0·10	0·40
m-Dinitrobenzene	25·97—50·00	3	42·85	42·86	0·31	0·58
Phenacetin	25·16—44·91	3	67·02	67·06	0·16	0·22
Acetanilide	35·79—47·04	2	71·04	70·94	0·06	0·15
S-Benzylthiuronium chloride	21·38—53·87	10	47·40	47·27 ± 0·21 *	0·10	1·00
Compounds from Research School						
1,4-Di-iodo-2,3-di-*O*-tosylbutane	28·17—36·08	2	33·30	33·55	0·20	0·30
Ethylmethylglyoxime	20·97—50·00	2	46·14	46·45	0·26	0·36
Di-*S*-benzylthiuronium difluoromalonate	35·28—53·87	2	48·30	48·00	0·10	0·50
p-Fluorophenylhydrazone of pyruvic acid	27·24	1	55·10	55·10	0·00	0·00

* Standard deviation.

As a result of this work [4] it was concluded that physical rather than chemical methods of separating and estimating carbon dioxide and water were required.

The method finally selected involves combustion at 500—600° in a limited volume of pure oxygen on a platinum catalyst. The carbon dioxide and water vapour are separated under reduced pressure by freezing traps at −183° and −100°. The excess of oxygen is pumped off, and the carbon dioxide is allowed to expand into the evacuated apparatus

* Part V, *J.*, 1958, 4054.

[1] Belcher, Thompson, and West, *Analyt. Chim. Acta*, 1958, **19**, 309, and references therein.
[2] *Idem, ibid.*, p. 148, and references therein.
[3] Unterzaucher, *Mikrochem. Mikrochim. Acta*, 1951, **36**, **37**, 706.
[4] Ayers, Ph.D. Thesis, Birmingham, 1958.

where it is measured on a McLeod gauge. Attempts to determine the hydrogen simultaneously by a similar procedure failed because of variable adsorption on the walls of the combustion tube and the glassware. Magnesium oxide at *ca.* 500° is used to remove the halogens (including fluorine) and the oxides of sulphur, since silver wool does not retain these interfering elements quantitatively under vacuum. Manganese dioxide is effective in removing nitrogen oxides, but it must not be allowed to become hot.

The Table shows that the method is precise and widely applicable. The time required to complete all operations including weighing is 30—45 minutes.

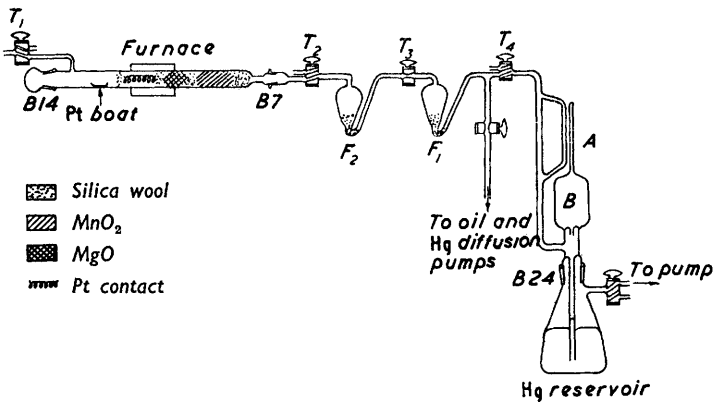
An attempt was made to devise a combined carbon and hydrogen method, and promising results were obtained on a limited range of compounds. However, the method is not of universal application. Briefly, it consists of combusting the sample *in vacuo* with nickel oxide and decomposing the water vapour to hydrogen by means of a film of magnesium metal on the walls of the combustion tube. The hydrogen and carbon dioxide are separated by freezing and are determined manometrically as above.

This problem of the simultaneous determination of carbon and hydrogen is still being investigated. Meanwhile, there appears to be no reason why the halogens and sulphur should not be determined simultaneously with carbon.

Since the present work was begun Kirsten [5] and Unterzaucher [6] have described small-scale methods for carbon and hydrogen, but both are decimilligram rather than submicro-procedures.

Experimental

Apparatus (see Figure).—(a) *Pressure gauge.* The McLeod gauge had a capillary *A* of 0·5 ml. volume and a bulb *B* of 200 ml. A 50 μg. sample containing 50% of carbon gives a pressure



in the apparatus of *ca.* 0·1 mm. of mercury, so the measured pressure is 4 cm. Consequently, the gauge must be read to within ±0·006 cm. to obtain the overall accuracy of ±0·3% of carbon. A cathetometer reading to 0·001 cm. was used to measure the mercury levels.

(b) *Freezing traps.* Two inverted pear-shaped traps, F_1 and F_2, were loosely packed at the narrow ends with silica wool. The dimensions were 30 mm. at the widest point, *ca.* 65 mm. long, constricted to *ca.* 8 mm., external diameter, at the narrowest point.

(c) *Combustion tube.* Silica tubing 300 mm. long and 8 mm. in diameter with side arm and standard joints was used as shown in the Figure. The use of a cap rather than a cone at the entrance to the combustion tube minimises the possibility of contamination of the combustion boat with stop-cock grease.

(d) *Furnace.* This was a wire-wound, refractory-lined, split furnace 75 mm. long, operating in the range 500—900°, controlled by a Variac unit.

(e) *Purification train.* Organic impurities were removed from the cylinder oxygen by passing the gas through a silica tube packed with platinised asbestos and kept at 450°. The

[5] Kirsten, *Chim. analyt.* 1958, 253.
[6] Unterzaucher, *Mikrochim. Acta*, 1957, 448.

emerging gas was passed through anhydrone, soda-asbestos, phosphoric oxide, and finally a liquid-oxygen trap.

(f) *Vacuum pump.* A two-stage oil pump (Edwards Speedivac 2Sc 20) was used. Pressures less than 10^{-2} cm. were avoided to prevent loss of carbon dioxide.

(g) *Other apparatus.* Submicro-balance,[7] platinum micro-combustion boat, platinum submicro-weighing vessel, platinum contact.

Reagents.—(1) Anhydrone, (2) phosphoric oxide, (3) soda-asbestos, (4) liquid O_2, (5) liquid N_2, (6) ethanol. The liquid oxygen (−183°) was used to trap the carbon dioxide, and a slurry of ethanol and liquid nitrogen (−100°) to trap the water vapour. A large boiling-tube full of liquid nitrogen was immersed in the −100° freezing mixture to prevent over-rapid evaporation of nitrogen from the mixture.

Procedure.—The sample (18—50 μg.) was weighed into the platinum submicro-weighing vessel which was then placed in the platinum microcombustion boat, for introduction into the combustion tube.

First the whole apparatus was evacuated. The freezing baths, one of liquid oxygen (F_1), the other a slurry of ethanol and liquid nitrogen (F_2), were placed in position. The combustion tube was isolated from the rest of the apparatus by turning tap T_2, and purified oxygen was introduced into the combustion tube by opening tap T_1. The cap of the combustion tube was removed with oxygen still passing to minimise diffusion of the atmosphere into the tube. The platinum boat, containing the sample, was quickly introduced into the combustion tube, the cap replaced, and the supply of oxygen turned off.

The platinum boat was heated with a Bunsen burner, gently for 30 sec. followed by 30 sec. at a red heat. After this, the combustion tube was connected to the freezing traps by opening taps T_2 and T_3 for 5 min. The excess of oxygen was then pumped off; 5 minutes' pumping sufficed. Tap T_4, connecting the gauge to the rest of the apparatus, was opened, and the initial pressure measured. The liquid-oxygen bath was removed, and freezing trap F_1 allowed to come to room temperature, the pressure again being measured. The difference in these two pressures gave the pressure due to carbon dioxide. The whole apparatus was then evacuated for the next determination.

Blank determinations. These were carried out in precisely the same way as an actual determination with an empty boat. It is necessary that the temperature of the laboratory should be steady to ±1°.

Determination of Carbon in Presence of Halogens and Sulphur.—Reagents and apparatus were as above, with the addition of a packing of magnesium oxide in the combustion tube. Pure magnesium oxide [8] was made into pellet form (20—40 mesh) and packed into the combustion tube in a short zone (25—30 mm.) which was kept at about 500°. Before use, the magnesium oxide was completely degassed by heating under vacuum. The procedure was then as above.

Determination of Carbon in Presence of Nitrogen.—Reagents and apparatus were as in the carbon determination with the addition of a packing of manganese dioxide in the cold section of the combustion tube. The manganese dioxide was specially prepared, for even commercial samples stated to be suitable for microanalysis contained carbonate. It was washed with 0·001M-sulphuric acid, water, *ca.* 0·001M-ammonia, and then thoroughly with distilled water, then dried at 120° overnight. It was then packed into the far end of the combustion tube, near to the B7 cone, so that it would not be heated. (Manganese dioxide decomposes when heated under vacuum.[4]) The sample was degassed by keeping it under vacuum.

Procedure. As above, except that after combustion, the excess of oxygen was pumped off for 3 min. Tap T_2 was then closed, and the pumping continued for an additional 2 min. The last traces of oxygen were removed in this way so that decomposition of the manganese dioxide did not affect the results.

Standardisation of Apparatus.—The pressure gauge can be standardised in the usual way but as this is laborious the gauge was standardised by combusting accurately weighed amounts of sucrose of primary-standard purity.

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[7] Asbury, Belcher, and West, *Mikrochim. Acta*, 1956, 598.
[8] Throckmorton and Hutton, *Analyt. Chem.*, 1952, **24**, 2003.