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TECHNICAL NOTE

A rapid method for precise gas phase analysis of ^{14}C-labelled compounds

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There is little doubt that the activity of weak β emitters — such as ^{14}C and ^{3}H — can be measured with the highest accuracy and sensitivity by gas phase counting methods (1).

The many advantages of this type of assay, including its outstanding precision, the absence of self-absorption and quenching problems, the comparatively inexpensive equipment required, etc., have, so far, been more than offset by the need for a quantitative chemical conversion of solid or liquid samples to suitable gaseous compounds.

In the analysis of ^{14}C, a large number of experimental procedures for the conversion of organic specimens to $^{14}CO_2$(2–15), $^{14}CH_4$(16, 17) and other compounds (18, 19) have been described. Apparently, however, despite all attempts to shorten and simplify the operations needed for gas phase assay, they still require considerable skill and take much more time than the more usual solid and scintillation counting techniques. This is understandable, since the steps involved in the quantitative conversion of organic materials into a well-defined gaseous compound have been derived from, and are closely similar to the procedures employed in elemental analysis.

Recently, however, instrumental and highly automatized methods for elemental analysis have been introduced (20, 26) and commercial apparatuses for the rapid combustion and gas chromatographic separation of the combustion products have been made available.

In the present Note we will dicuss the use of one such device, the F & M Model 185 C/H/N analyzer, coupled to a suitable ionization chamber, for the fast and accurate routine analysis of organic samples labelled with ^{14}C.

The Model 185 F & M employed in our laboratory for the elemental analysis of organic substances is connected to a 400 ml flow chamber, as shown in figure 1.

The chamber, illustrated in figure 2, has a relatively large volume, necessary in order to trap quantitatively all of the carbon dioxide produced by the combustion of the sample and diluted, at the flow rate usually employed, in a volume of carrier gas of about 80 ml.

The procedure followed for ^{14}C analysis can be described as follows: the sample (0.2-2 mg) is weighed in an aluminum boat, mixed with the catalyst and introduced into the furnace of the F &M analyzer, according to the procedure recommended for inactive samples. In the meantime, the ionization chamber is connected to the outlet of the analyzer and the electrometer, in our case a Cary

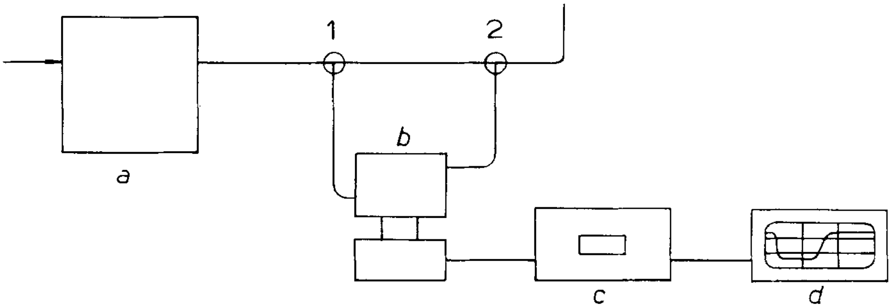

FIG. 1. — Scheme of the apparatus :
a. F & M model 185 analyzer
b. 400 ml flow ionization chamber
c. Vibrating reed electrometer
d. Recorder
1,2. Three-ways stopcocks.

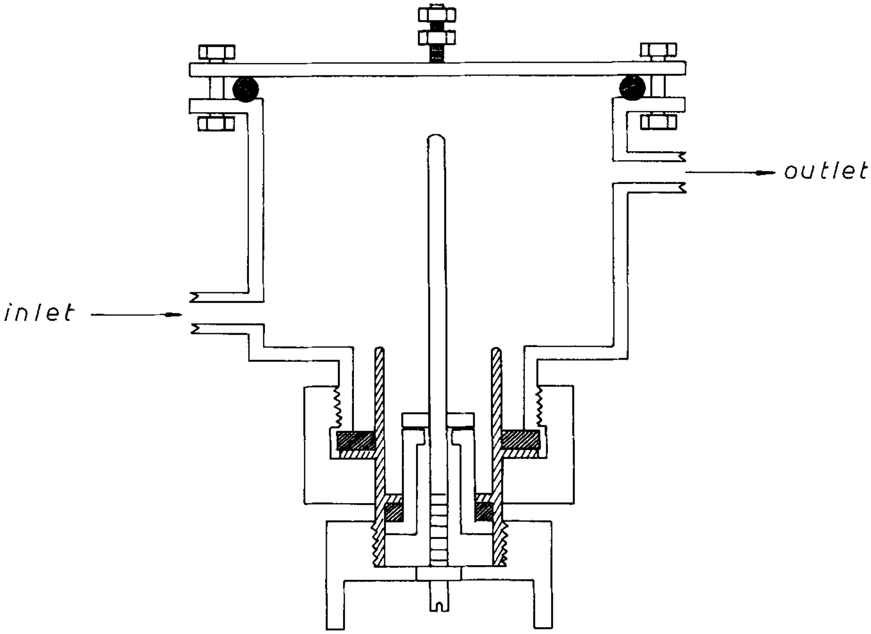

FIG. 2. — Scheme of 400 ml ionization chamber.

Model 31, is turned on to determine the background of the gaseous stream from the analyzer.

After the combustion of the sample, the radioactive CO_2 enters the chamber. Stopcocks 1 and 2 are left open until the electrometer reading reaches a constant value, indicating that the whole « plug » of radioactive CO_2 is trapped in the sensitive volume. The stopcocks are then closed and the measurement of the activity is carried out in the usual way. All the operations described can be carried out in about 20 minutes by an unskilled technician. Since the F &M analyzer provides a precise measurement of the amount of carbon contained in the sample together with its elemental analysis, weighing the sample is unne-

TABLE I. Determination of the specific activity of benzoic acid-1-^{14}C

Sample	μg Carbon [a]	μg Carbon [b]	mμC	mμC/mg [a]	mμC/mg [b]
A	673	683	106.9	159	157
A	359	366	56.6	158	155
A	449	444	69.4	155	156
A	590	581	93.1	158	160
A	361	368	57.2	158	155
A	621	623	99.4	160	160
A	207	196	31.8	154	162
A	180	176	28.3	157	161
A	190	189	30.1	158	159
A	532	533	83.8	157	157
A	429	429	69.4	162	162
A	465	458	77.5	167	169
A	140	134	22.0	157	164
A	370	383	59.5	161	155
A	1418	—	232.4	164	—
		Average value :		159 ± 3.4	159 ± 4
B	1546	1489	3.35	2.17	2.25
B	381	394	0.87	2.28	2.21
B	812	806	1.79	2.20	2.22
B	879	861	1.91	2.17	2.22
B	996	968	2.25	2.26	2.32
B	1069	1127	2.37	2.22	2.16
B	1250	1277	2.83	2.26	2.22
B	660	691	1.50	2.27	2.17
B	739	757	1.73	2.34	2.28
B	406	427	0.92	2.26	2.15
		Average value :		2.24 ± 0.07	2.23 ± 0.05

[a] From the weight of sample.
[b] From the CO_2 area given by the 185 F &M analyzer.

cessary in those cases where the specific activity is referred to the amount of carbon. The accuracy of the analysis is illustrated by the results summarized in Table I.

It may be interesting to point out that no memory effect could be found by analyzing inactive samples after the combustion of material having a specific activity of up to 1 mC/mmole. This is probably a result of the fact that the oxidizing catalyst added to each sample is discarded after the analysis. It may be pointed out that the activity measurements have been carried out, according to the standard practice in our laboratory, with the ionization chamber and a vibrating reed electrometer. However, the method could probably be applied, with a few modifications, to a proportional counting technique. In principle, this method could be also used for the simultaneous determination of ^{3}H and ^{14}C in doubly labelled compounds, since the Model 185 analyzer separates the peaks of the carbon dioxide and the water formed during combustion. Experiments on this aspect of the problem are under way in our laboratory.

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REFERENCES

1. CHRISTMAN, D.R. — Nucleonics, **23**, 12, 39 (1965)
2. VAN SLIKE, D.D. and NEIL, J.M. *J. Biol. Chem.*, **61**, 523 (1924).
3. LINDENBAUM, A. *et al.* — *Anal. Chem*, **20**, 1120 (1948).
4. GLASCOCK, R.F. — « Isotopic Gas Analysis for Biochemists » p. 66, 75 (Academic Press Inc., New York, 1954).
5. TOLBERT, B.M. — UCRL — 3499, pp. 10 (1956).
6. SIMON, H. et al. — *Angew. Chem.*, **71**, 303 (1959).
7. CHRISTMAN, D.R. — *Nucleonics*, **19**, 5, 51 (1961).
8. CHRISTMAN, D.R. et al. — *Anal. Chem.*, **28**, 1345 (1956).
9. VON SCHUCHING, S. and KARICKHOFF, C.W. — *Anal. Biochem.*, **5**, 93 (1963)
10. NAUGHTON, J.J. and FRODYMA, M.M. — *Anal. Chem.*, **22**, 711 (1950).
11. ANDERSON, R.C. et al. *Anal. Chem.*, **24**, 1298 (1952).
12. GLASCOCK, R.F. — *Biochem. J.*, **52**, 699 (1952).
13. CHRISTMAN, D.R. and PAUL, C.M. — *Anal. Chem.*, **32**, 191 (1960).
14. CHRISTMAN, D.R. et al. — *Anal. Chem.*, **27**, 1935 (1955)
15. WILZBACH, K.E. et al. — *Science*, **118**, 522 (1953).
16. JORDAN, P. and LYCOURÉZOS, Ph.A.P. — *Helv. Chim. Acta*, **48**, 581 (1965)
17. JORDAN, P. — *Nucleonics*, **23**, 11, 46 (1965).
18. AKERS, L.K. — IAEA Symposium on the Measuring Radioisotopes in Biomedical Samples Paper 5 M-61/68.

19. Arrol, W.J. and Glascock, R.F. — *J. Chem. Soc.*, (1948) 1534.
20. Cacace, F. et al. — *Anal. Chem.*, **35**, 1348 (1961).
21. Cacace, F. et al. — *Science*, **132**, 1253 (1960)
22. Cacace, F. et al. — *Gazz. Chim. It.*, **91**, 804 (1961).
23. Vogel, A.M. and Quattrone, J.J., *Anal. Chem.*, **32**, 1954 (1960)
24. Reitsema, R.M., and Alphin, N.L. — *Anal. Chem.*, **33**, 355 (1961).
25. Nightingale, C.F. and Walker, J.M. — *Anal. Chem.*, **34**, 1435 (1962).
26. Parson, M.L. et al. — *Anal. Chem.*, **35**, 842 (1963).