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RADIO-GAS CHROMATOGRAPHIC ANALYSIS OF MOLECULES CONTAINING LABILE TRITIUM

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SUMMARY

A simple method for the radio-gas chromatographic analysis of species that contain labile tritium is described. The method can be also applied to mixtures that contain, in addition to other tritium-labelled compounds, one labile tritium species. The procedure consists of the use of an exchangeable carrier or a mixture of an inert carrier with an exchangeable compound.

The analyses of some test mixtures are reported and the applicability of the method is briefly discussed.

INTRODUCTION

In many fields of chemistry and biochemistry where radioactive tracers are used, gas chromatography (GC) greatly simplifies the separation techniques that are necessary in order to determine specific activities. However, it is still common to collect numerous minute samples from the separation device, each requiring a separate analysis for radioactivity.

Such a procedure often requires a relatively large amount of valuable sample and complicated and time-consuming handling techniques. Radio-GC simplifies the analysis by providing simultaneously GC and continuous radioactivity analyses from which specific activity data can be calculated. With this technique, any isotope used as a label on a suitable molecule can be detected and measured. The radio-GC method, because of its high sensitivity, permits the separation and the analysis of trace amounts of substance, *i.e.*, of the carrier-free components of a mixture. Many papers dealing with this subject have been published (*e.g.*, refs. 1–3).

Unfortunately, there are some important compounds to which the method cannot be applied, *viz.*, tritiated molecules that contain N–^{3}H, O–^{3}H and halogen–^{3}H bonds. When the tritium atom in a tritiated molecule is bonded to a labile position, the radioactivity detected by radio-GC does not correspond to the actual activity of the sample, owing to the isotopic exchange between the labile tritium of the sample and the exchangeable hydrogen atoms that are inevitably present in the chromatographic system. Hence, while the mass of the compound, if detectable, is determined exactly, the main part of its activity sticks on the apparatus.

Some workers[4] used a plug of highly exchangeable material (*e.g.*, cotton) at the inlet of the column in order to trap the labile tritium, avoiding column contamination. The determination of labile tritium can then be performed on the cotton plug by a suitable technique. However, the results should be more reliable if the labile and non-labile activities are measured by the same technique, *i.e.*, by radio-GC. This can be done, in principle, by displacing the activity exchanged in the column with a set of injections of a compound containing the same exchangeable atom [as is commonly done for some labeling process (*e.g.*, refs. 5 and 6)]. Then the radioactivity is eluted as a set of peaks, the heights of which follow a Maxwellian distribution, and the true activity of the exchanged tritium is the sum of the activities of these peaks. In spite of its apparent simplicity, the method is tedious and requires thorough cleaning of the system between successive analyses.

A more useful and less difficult technique for solving the problem is a continuous extraction of the activity by using a continuous stream of the exchangeable compound for the recovery of the exchanged activity. This can be achieved by using an exchangeable carrier (*e.g.*, ammonia) or a common carrier (helium, nitrogen, etc.) mixed with an exchangeable compound (ammonia, water, alcohol, etc.).

In this paper, we report some results on this useful technique for determining, by radio-GC, the activities of samples that contain labile tritium.

EXPERIMENTAL

Apparatus

The apparatus includes a Loenco Model 70 High-Flex gas chromatograph, a Cary Model 31 vibrating reed electrometer, a 270-ml high-temperature ionization chamber and a dual pen recorder. The gas chromatograph is equipped with a 2 m × 1/4 in. O.D. stainless-steel column filled with Chromosorb 101 (Johns Manville, Denver, Colo., U.S.A.), 60–80 mesh.

Compounds

The labelled compounds used were $NH_2{}^3H$, H^3HO, $PH_2{}^3H$, CH_3O^3H, $(CH_3)_3$-CO^3H and their mixtures with H^3H, $CH_3{}^3H$ and $C_4H_9{}^3H$. Argon (S.I.O., Milan, Italy) was used as the purge gas for the ion chamber. Commercial ammonia (S.I.O.) and helium–alcohol mixtures were used as carriers. When the carrier was ammonia, the soap film flow meter could not be used and the ammonia flow-rate was measured by titration with 1 *N* sulphuric acid with methyl red as indicator. The helium–alcohol carriers were obtained by bubbling helium through a gas-washing bottle containing methanol or ethanol.

RESULTS AND DISCUSSION

In the ammonia carrier experiments, the activity of the exchangeable sample is rapidly eluted with a very short retention time, which is not significantly affected by either the nature of the analyzed compound or the column temperature. Indeed, the exchanged radioactive atoms are eluted with a retention time slightly longer than the dead time of the apparatus, because of the rapid exchange with ammonia molecules present on the column, whereas the no longer radioactive compound gives the mass signal at its own retention time. Fig. 1 shows a chromatogram for H^3HO.

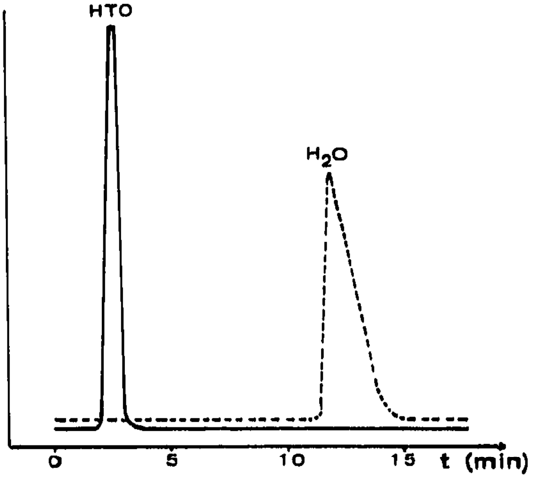

Fig. 1. Chromatogram of H^3HO sample. Ammonia carrier gas, 40 ml/min. Temperature programmed from 40° to 150° at 20°/min. ———, Activity; – – –, mass.

For any radioactivity ionization detector, the efficiency is strictly correlated with the nature of the counting gas and therefore, for a quantitative measurement, the influence of its ionization energy cannot be neglected.

The ionization potentials for helium, argon and ammonia are 24.48, 15.75 and 10.2 eV, respectively[7]. Thus a change in counting efficiency could be expected whether helium or ammonia carrier is used[8]. This change may be considerable for ion chambers operating at a low purge to carrier gas flow ratio. The Cary ion chamber used in this work has an optimum purge flow-rate of about 42 l/h, *i.e.*, 5–15 times higher than the usual GC carrier gas flow-rates; hence the average ionization potential of the gas mixture flowing through the chamber does not change significantly from helium–argon to ammonia–argon pairs. However, experiments to evaluate the influence of the gas composition on the chamber performance have been carried out using a non-exchangeable compound (H^3H) as test gas. Table I shows some of the results.

From the data in Table I, it appears that on changing the argon to ammonia ratio from 2.2 to 41, the activity peak area does not differ substantially from that obtained when using helium carrier.

With ammonia as carrier, in spite of the good performance of the ion chamber for the ammonia–argon mixture, the very short retention time of the exchanged activity is a severe limitation to the method. In fact, it will not be possible to resolve any mixture that contains, in addition to a labile tritium compound, other molecules that have short retention times (for example, H^3H or $CH_3{}^3H$) because all of the activities will be superimposed at the beginning of the chromatogram.

TABLE I

IONIZATION CHAMBER RESPONSE *VERSUS* COMPOSITION OF FLOWING GAS

Carrier gas	*Flow-rate (l/h)*	*Purge: Ar (l/h)*	*H^3H peak area (± 5%)*
He	3.0	42	3850
NH_3	1.2	20	4020
		30	3975
		40	3980
		50	3795
NH_3	9.0	20	4150
		30	4050
		40	3920
		50	3820

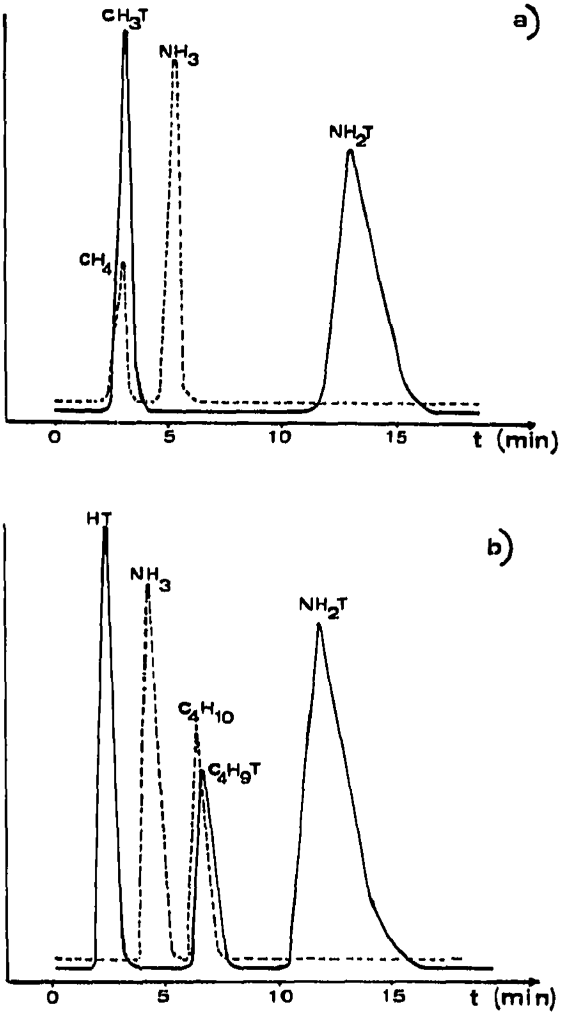

Fig. 2. (a) $CH_3{}^3H + NH_2{}^3H$. Carrier gas, He–C_2H_5OH, 40 ml/min. Temperature programmed from 20° to 155° at 20°/min. (b) $H^3H + C_4H_9{}^3H + NH_2{}^3H$. Carrier gas, He–$CH_3OH$, 30 ml/min. Isothermal at 125°. ———, Activity; – – –, mass.

The use of helium–methanol or helium–ethanol carrier mixtures gives a satisfactory solution to such a problem. The resolving power of these mixed carriers was tested for some gaseous samples. Fig. 2 shows the chromatograms for $CH_3{}^3H$–$NH_2{}^3H$ and H^3H–$C_4H_9{}^3H$–$NH_2{}^3H$.

The mechanism of the peak resolution for the double carrier experiments depends on the different flow-rates through the column of the main carrier and the co-carrier (helium and alcohol, respectively). The non-exchangeable components of the mixture are eluted, mainly by the main carrier, according to their physicochemical behaviour on the column used, and the same happens to the mass of the exchangeable component, whereas its activity, transferred to the co-carrier, is eluted with a fixed retention time that is longer than that of the co-carrier itself.

As reported in the examples in Fig. 2, the peak of the exchanged activity is broad and asymmetrical owing to two main causes: firstly, the exchange rate between the labile tritium molecule and the co-carrier, and secondly, the continuous exchange between co-carrier molecules along the column (a phenomenon similar to mass transfer resistance).

If two or more highly exchangeable compounds are present in the mixture, their activities will be eluted at the same time and possible differences in their exchange rate with co-carrier molecules will contribute to the broadening and asymmetry of the activity peak. On the contrary, however, the activity of a component with an exchange rate that is very low compared with its stay in the column, is eluted at the same time of mass as a more or less tailed peak. Fig. 3 illustrates this behaviour for $PH_2{}^3H$.

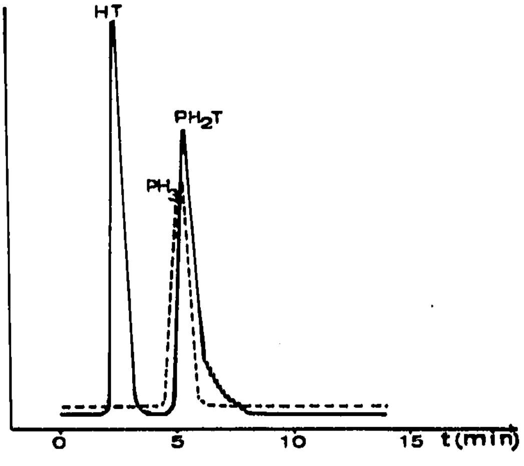

Fig. 3. $H^3H + PH_2{}^3H$. Carrier gas, He–C_2H_5OH, 40 ml/min. Isothermal at 55°. ———, Activity; — — —, mass.

CONCLUSIONS

The method proposed permits the complete radio-GC analysis of mixtures that contain, in addition to other labelled compounds, one labile tritium species. In the absence of highly volatile compounds, the choice of an exchangeable carrier, such as ammonia, is satisfactory. If one or more highly volatile components are present in the sample, a mixed carrier is necessary for a complete analysis. The choice of the co-carrier, among any exchangeable gas or liquid (ammonia, water, alcohols, etc.), and the experimental conditions should be made in accordance with the particular mixture to be analyzed.

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