CHROMBIO. 2572

APPLICATION OF RADIOISOTOPE TRACER TECHNIQUES TO ANALYTICAL GAS CHROMATOGRAPHY: DETERMINATION OF GAS CHROMATOGRAPHIC PEAK YIELD

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SUMMARY

The determination of gas chromatographic peak yields using a radio-gas chromatography system, in which ^{14}C-labelled substances eluted from a gas chromatography column are burnt to $^{14}CO_2$ through a combustion tube, is described. As the first step of the study, the adequacy of the combustion tube was investigated by a radioisotope tracer technique. Consequently, it was found that almost complete combustion could be achieved by the combustion tube for the substances investigated.

INTRODUCTION

Gas chromatography (GC) is a useful technique for the identification and quantification of substances and has been widely employed because of its high sensitivity and resolution, rapidity and ease of handling. Flame-ionization detection (FID) and electron-capture detection (ECD) are usually used in GC analyses, but they suffer from the following disadvantages. The detector response differs considerably with the kind of substance involved, so calibration graphs for every component of a sample must be prepared in order to be able to quantify simultaneously many components. Further, it is impossible to know what percentage of the amount of an injected substance reaches the detector system. We define this percentage as "GC peak yield" [1].

We previously developed a radio-gas chromatography (RGC) system equipped with a new radiodetector system, synchronized accumulating radiodetection (SARD) [2], which makes it possible to improve the detection efficiency without sacrificing the resolution, and to obtain the radioactive intensity on the chromatogram in digital form. We have undertaken a series of studies of the fundamental problems in GC such as GC peak yields and the adsorption of substances on column packings, utilizing this RGC—SARD system. As the first step, an attempt to determine GC peak yields by RGC—SARD is reported in this paper.

EXPERIMENTAL

Radioactive samples and reagents

n-[1-^{14}C]Hexadecane (53.6 mCi/mmol), [4-^{14}C]testosterone (58.0 mCi/mmol) and L-[ring-2-^{14}C]histidine (59.0 mCi/mmol) were purchased from the Radiochemical Centre (Amersham, U.K.). The radioactive substances showed a single peak on thin-layer chromatography. Solutions of [^{14}C]hexadecane, [^{14}C]testosterone and [^{14}C]histidine were prepared in cyclohexane, ethanol and water, respectively. All reagents were purchased from Wako (Tokyo, Japan) and were of analytical-reagent grade.

Liquid scintillation counting

Unless stated otherwise, the radioactivity of a sample was measured in a mixture of 10 ml of toluene-base scintillation cocktail and 0.5 ml of methanol with a liquid scintillation counter (Aloka LSC 502 or 903).

Combustion furnace

The combustion tube placed in the electric furnace was a 180 x 5 mm I.D. quartz tube as described previously [2], and was packed with only copper oxide wire of dimensions 5 x 1 mm (unless otherwise stated, about 10 g). The temperature of the electric furnace during operation was about 800°C.

Injection technique

Aliquots (4 µl) from each solution were injected by the usual method with a 10-µl Hamilton microsyringe in all experiments.

Radio-gas chromatography

A glass column (1 m x 3 mm I.D.) was packed with 1.5% OV-17 on Shimalite W (80—100 mesh). The carrier gas (nitrogen) and the counting gas (methane) flow-rates were 50 and 250 ml/min, respectively.

Assembly for measurement of combustion efficiency (combustion assembly)

The injection port was connected directly to the combustion tube by a stainless-steel capillary tube (30 cm x 1 mm I.D.) placed in an oven. The sample was introduced into the combustion tube through the capillary tube with the carrier gas (nitrogen). Unless stated otherwise, the gas exhausted from the combustion tube was introduced into the gas-flow proportional counters used in the RGC—SARD after mixing with the counting gas (methane), the carrier

gas and counting gas flow-rates were set at 50 and 250 ml/min, respectively, and the injection port and the oven were heated to 250°C and 160°C, respectively.

Derivatization of [^{14}C] histidine with pentafluoropropionic anhydride (PFPA) [3]

An aqueous solution of [^{14}C] histidine (containing 5—10 μg of histidine) was lyophilized, 50 μl of PFPA were added to the residue and the solution was heated at 70°C for 1.5 h. The excess of PFPA was evaporated under a gentle stream of nitrogen, 50 μl of ethyl acetate were added to the residue and a portion of the solution was injected without any purification.

Radio-gas chromatograms of [^{14}C] hexadecane, [^{14}C] testosterone and [^{14}C] histidine derivatives

[^{14}C] Hexadecane (1.85 nCi, 0.69 mCi/mmol), [^{14}C] testosterone, (1.83 nCi, 0.67 mCi/mmol) and [^{14}C] histidine (1.79 nCi, 0.60 mCi/mmol, after derivatization with PFPA) were injected into the RGC—SARD system. The column oven and injection port temperatures for the three samples were as follows: [^{14}C] hexadecane, 160 and 250; [^{14}C] testosterone, 275 and 295; and [^{14}C]-histidine, PFPA derivative, 160 and 210°C, respectively.

Measurement of combustion efficiency

Method A. [^{14}C] Hexadecane (53.6 mCi/mmol), [^{14}C] testosterone (58.0 mCi/mmol) and [^{14}C] histidine (3.4 mCi/mmol, after derivatization with PFPA) were injected into the combustion assembly and the gas exhausted from the combustion tube was introduced into a series of two absorption traps for $^{14}CO_2$ [4] containing 6 ml of monoethanolamine—methanol (1:1) for 5 min. One twentieth of the trapping solution was taken and the radioactivity was measured in 10 ml of toluene-base scintillation cocktail with a liquid scintillation counter. The injected radioactivity was 9—20 nCi for any substance.

Method B. About 2 nCi of [^{14}C] hexadecane (5.73 mCi/mmol) and [^{14}C]-testosterone (5.87 mCi/mmol) were injected into the combustion assembly and the radioactive peak intensities were measured with the gas-flow proportional counters.

Capability of copper oxide wire for oxidation

[^{14}C] Hexadecane (about 1.8 nCi, 53.6 mCi/mmol) dissolved in cyclohexane (4 μl) and pure cyclohexane (4 μl) were injected into the RGC—SARD system alternately and the radioactive peak intensities were meausred. The radioactive peak intensities obtained by the first four injections of the radioactive sample were averaged and the mean value was taken as 100%. The injection port and the column oven were heated to 250°C and 160°C, respectively.

Relationship between carrier gas flow-rate and radioactive peak intensity

The injection port and the oven of the combustion assembly were heated to 200°C. About 2 nCi of [^{14}C] hexadecane (5.73 mCi/mmol) and [^{14}C]-testosterone (5.87 mCi/mmol) were injected into the assembly at carrier gas flow-rates of 20, 50 and 80 ml/min and the radioactive peak intensities were measured with the gas-flow proportional counters.

Relationship between temperature of injection port oven and radioactive peak intensity

About 2 nCi of [^{14}C]hexadecane (5.73 mCi/mmol), [^{14}C]testosterone (5.87 mCi/mmol) and [^{14}C]histidine (0.60 mCi/mmol, after derivatization with PFPA) were injected into the combustion assembly with temperatures of the injection port oven of 150, 200, 250, 300 and 350°C and the radioactive peak intensities were measured with the gas-flow proportional counters.

RESULTS AND DISCUSSION

In GC analyses, the kinds of substances that can be detected have increased rapidly and microanalyses below the nanogram level have been possible owing to the development of highly sensitive detectors and low-loaded column packings and to progress with derivatization techniques. However, the increase in the range of applications has been accompanied by some problems. For example, adequate accuracy cannot be obtained when analysing a very small amount of a substance because there is poor linearity between the amount of substance injected and the peak intensity. It is considered that this effect arises from incompleteness of derivatization, thermal decomposition of sample substances and adsorption of substances on the column packing and/or the injection port, etc. The response factor in GC may be represented by the following expression:

Response factor = GC peak yield (%) x detector response

It is essentially impossible to determine GC peak yields because the peak intensity shows only indirectly the amount of substance that reaches the detector and the detector response varies with the kind of substance involved.

In the RGC—SARD system, effluents from a GC column are introduced into the combustion furnace, where ^{14}C-labelled substances are burnt to produce $^{14}CO_2$, and the gas, after mixing with the counting gas (methane), is introduced into five gas-flow proportional counters arranged longitudinally in series. The response factor of the RGC—SARD system for ^{14}C-labelled substances may be represented by the following expression:

Response factor = GC peak yield (%) x combustion efficiency (%) x counting efficiency (%)

The counting efficiency is considered to be constant, irrespective of the kind of substance involved, and can be determined by using an authentic sample. Then, if the combustion efficiency can be determined, it may be possible to determine the GC peak yields of various substances from the ratio of the radioactive intensity on the chromatogram to the injected radioactivity.

Fig. 1 shows typical examples of radio-gas chromatograms that were obtained by the injection of almost identical radioactivities of [^{14}C]hexadecane, [^{14}C]-testosterone and [^{14}C]histidine (after derivatization) into the RGC—SARD system. The radioactive peak intensities varied widely with the kind of substance injected.

The counting efficiency, which was calculated from the data in Fig. 1 on the assumption that the adsorption of [^{14}C]hexadecane was negligible because of its low polarity, was 96.5%. This value is reasonable for this type of gas-flow

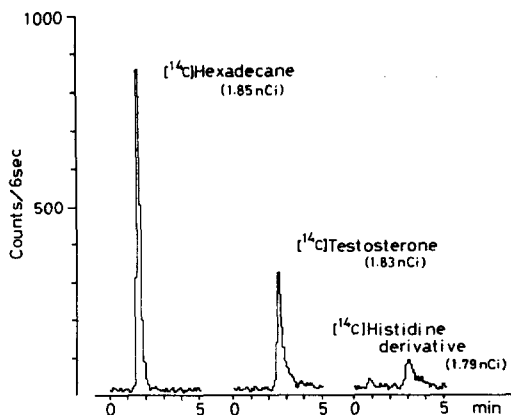Fig. 1. Radiochromatograms of ^{14}C-labelled substances. Injected radioactivity in parentheses.

proportional counter and will be used as the counting efficiency of this counting system [5, 6]. Therefore, it was concluded that all of the injected radioactivity of [14C] hexadecane reaches the detection system, that is, the GC peak yield of [14C] hexadecane is 100%. Now we can evaluate GC peak yields of various substances on the basis of [14C] hexadecane. Consequently, if the combustion efficiency is 100%, the GC peak yield of [14C] testosterone is calculated to be 74.7 ± 1.64%. When a substance is injected into a GC column after derivatization (without any purification), it was considered that the GC peak yield includes the influence of derivatization. As shown in Fig. 1, when [14C] histidine was injected after derivatization with PFPA, [14C] histidine-di-PFP appeared on the radio-gas chromatogram as the main derivative together with an unknown minor component. If the combustion efficiency is 100%, the GC peak yields are calculated to be 25.9 ± 1.18% and 7.2 ± 0.86%, respectively, and the total GC peak yield is 33.1 ± 1.89%, on the basis of the injected radioactivity.

In RGC, substances eluted from a GC column must be burnt quantitatively or at least with a constant efficiency, independent of the chemical structure. It has been reported [7, 8] that ^{14}C-labelled substances in the effluents from a GC column can be quantitatively burnt to $^{14}CO_2$ through a narrow-bore quartz tube packed with copper oxide that is maintained at 700—800°C. The investigations cited, however, dealt exclusively with readily combustible substances and the combustion efficiencies of [14C] testosterone and [14C] histidine derivatives, which may be more or less non-combustible, were compared with that of readily combustible [14C] hexadecane.

The known radioactivities of these substances were injected into the combustion assembly and the radioactivities in the gas exhausted from the combustion tube were measured by methods A and B (see Experimental). In method A, the proportions of the radioactivity recovered as $^{14}CO_2$ to that injected (recovery yield) were calculated as shown in Table I. Although the recovery yield of [14C] hexadecane was almost quantitative, those of [14C]-testosterone and [14C] histidine derivatives were low. In method B, the ratios of the radioactive peak intensity to the injected radioactivity were calculated as shown in Table II. The time course of elution of the radioactivity from the

TABLE I

RECOVERY OF ^{14}C RADIOACTIVITY FROM COMBUSTION TUBE

Compound	Recovery yield (%)*
[14C]Hexadecane	97.7 ± 1.89
[14C]Testosterone	62.7 ± 3.46
[14C]Histidine derivative	42.7 ± 3.15

*Mean ± S.D. (n = 7).

TABLE II

RATIO OF RADIOACTIVE PEAK INTENSITY TO RADIOACTIVITY INJECTED INTO COMBUSTION ASSEMBLY

Compound	Ratio (%)*
[14C]Hexadecane	100.0 ± 1.81
[14C]Testosterone	65.1 ± 3.98

*Mean ± S.D. (n = 5).
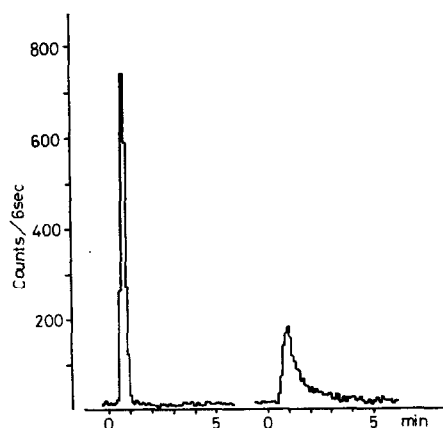
Fig. 2. Radioactive peaks obtained by injection of [14C]hexadecane (left) and [14C]-testosterone (right) into the combustion assembly.

combustion tube is shown in Fig. 2. The ratio for [14C]testosterone was lower than that for [14C]hexadecane. It is considered that this low ratio for [14C]-testosterone and the low recovery yields of [14C]testosterone and [14C]-histidine derivatives can be ascribed to the incompleteness of the combustion, and/or adsorption on the injection port and the stainless-steel capillary tube. The recovery yields in Table I were essentially identical with the ratios in Table II, and it was concluded that the recovery yield could be substituted for the ratio in order to compare combustion efficiencies.

The combustion efficiency may vary with the time during which a substance is in contact with the copper oxide wire. Consequently, the factors that influence the combustion efficiency are thought to be the amount of copper oxide wire packed in the combustion tube and the carrier gas flow-rate. Moreover, the degree of consumption of copper oxide wire, which is gradually

consumed and changed into copper with its usage, is also a factor that influences the combustion efficiency.

First, the relationship between the degree of consumption of copper oxide wire and the combustion efficiency was investigated. [14C]Hexadecane in cyclohexane and the pure cyclohexane were injected into the RGC—SARD system as described under Experimental. As shown in Fig. 3, the ratio of the radioactive peak intensity to the injected radioactivity was kept almost constant during the experiment. About four-fifths of the copper oxide wire from the inlet side of the combustion tube turned red on its surface after the injection of 200 μl of cyclohexane. The experiment revealed that the combustion tube used in this study retained a sufficient capability for combustion until the volume of the injected solvent amounted to at least 200 μl.

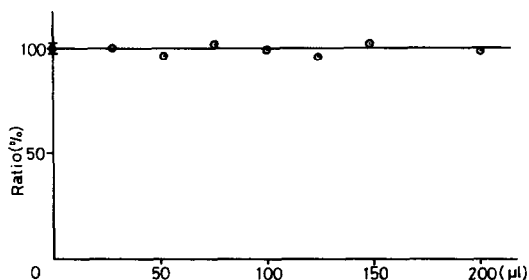Fig. 3. Capability of combustion tube. Radioactive sample: [14C]hexadecane.

Second, the relationship between the amount of copper oxide wire and the combustion efficiency was investigated. [14C]Testosterone was injected into the combustion assembly, the combustion tube of which was packed with 2.5, 5.0 and 7.5 g of copper oxide wire. Consequently, the proportion of the radio-activity recovered as $^{14}CO_2$ to that injected was more than 90% at any amount of copper oxide wire, which suggested that [14C]testosterone could be burnt almost completely in the combustion tube used. This result also means that a combustion tube packed with about 10 g of copper oxide wire can fulfil its function efficiently even if three quarters of the wire are consumed. This result agrees approximately with that in the experiment on the consumption of copper oxide wire.

Third, the relationship between the carrier gas flow-rate and the combustion efficiency was investigated. [14C]Hexadecane and [14C]testosterone were injected into the combustion assembly at carrier gas flow-rates of 20, 50 and 80 ml/min. The ratio of the radioactive peak intensity to the injected radio-activity of [14C]hexadecane was essentially constant at any flow-rate. The ratio of [14C]testosterone at each flow-rate is shown in Table III and the peak shape in Fig. 4. The times during which the substance is in contact with copper oxide wire at flow-rates of 80, 50 and 20 ml/min are calculated to be 1.6, 2.6 and 6.4 sec, respectively. The ratios at flow-rates of 80 and 50 ml/min were essentially the same. In contrast, the ratio at a flow-rate of 20 ml/min was nearly 10% lower. It seems that this phenomenon results from the increase in adsorption of the substance due to the extremely low flow-rate. In this experi-ment, the counting gas flow-rate was set so that the total gas flow-rate through

TABLE III

RELATIONSHIP BETWEEN CARRIER GAS FLOW-RATE AND RATIO OF RADIO-
ACTIVE PEAK INTENSITY TO RADIOACTIVITY INJECTED INTO COMBUSTION
ASSEMBLY

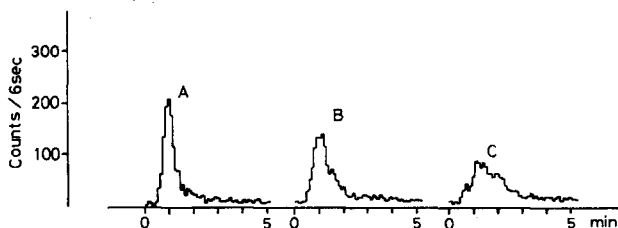|	Flow-rate (ml/min)		Ratio (%)*
	Carrier gas	Counting gas	
A	80	220	74.1 ± 0.20
B	50	250	75.4 ± 0.54
C	20	280	64.9 ± 2.29

*Mean ± S.D. ($n = 3$).

Fig. 4. Effect of carrier gas flow-rate on peak shape. A, B and C correspond to A, B and C,
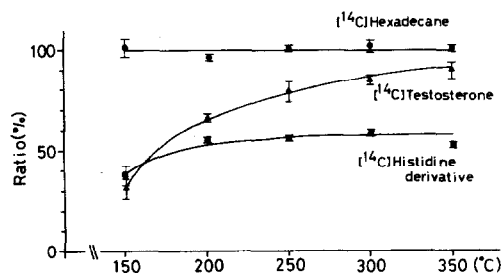respectively, in Table III.

Fig. 5. Relationship between the temperature of injection port oven and the ratio of the
radioactive peak intensity to the radioactivity injected into the combustion assembly.

the counters amounted to 300 ml/min at any carrier gas flow-rate, so the
concentration of the counting gas passing the counters varied (73, 83 and 93%).
However, it was considered that the counting efficiency did not vary because
the gas in the counters, except for the counting gas, was almost all nitrogen,
which is not a quencher. This experiment suggested that [^{14}C]testosterone,
similarly to [^{14}C]hexadecane, could be burnt almost completely in the com-
bustion tube used.

The relationship between the temperatures of the injection port and the
oven and the ratio of the radioactive peak intensity to the injected radio-
activity was investigated. [^{14}C]Hexadecane, [^{14}C]testosterone and [^{14}C]-
histidine (after derivatization) were injected into the combustion assembly. The
result is shown in Fig. 5. With [^{14}C]hexadecane the ratio was essentially
constant at any temperature. With [^{14}C]testosterone the ratio inceased with
increase in temperature and reached to 90% at 350°C. Consequently, it was

concluded that the low ratio for [^{14}C] testosterone shown in Table II resulted from adsorption on the injection port and the stainless-steel capillary tube. With [^{14}C] histidine derivatives the ratio was approximately constant (about 55%) at temperatures higher than 200°C. It is reasonable to conclude that this results from incompleteness of derivatization rather than the low combustion efficiency.

It was found that the combustion tube could burn more or less non-combustible substances, [^{14}C] testosterone and [^{14}C] histidine derivatives, similarly to [^{14}C] hexadecane, almost quantitatively and possessed sufficient capability for combustion. Consequently, it was concluded that GC peak yields can be determined by the radioisotope tracer technique using the RGC–SARD system and that [^{14}C] hexadecane is a useful substance for the standardization of GC peak yields. The derivatization yield, thermal decomposition and adsorption of substances on the column packings and/or the injection port, etc., will be investigated in subsequent studies.

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