Radio Gas Chromatography of $^{14}$C-Labelled Compounds by Measuring the Radioactivity of the FID Combustion Products After GLC Analysis

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Summary

A simple method of radio gas chromatography, which avoids the necessity for an effluent gas stream splitter and a special reactor after the GLC column, has been described. The system uses the FID as a combined mass detector and combustion furnace for the conversion of $^{14}$C-labelled compounds into $^{14}$CO$_2$ and operates the FID in series with the $^{14}$CO$_2$ detection system. Specific activity values of weakly $^{14}$C-labelled compounds such as organic methyl esters and TMS sugars can be determined precisely with the standard error of the mean less than 3%.

Introduction

Radio gas chromatography is an important tool for measuring the mass as well as the radioactivity of labelled compounds after separation on a gas chromatographic column. In most simple systems the effluent from the column is first passed through a non-destructive mass detector (e.g. thermal conductivity) and subsequently monitored for radioactivity (for reviews see [1, 2, 3, 4]).

Before entering the radiation detector, the organic compounds emerging from the GLC column are often converted to a gas which is stable at normal ambient temperature and suitable for the analysis of carbon-14, e.g. into $^{14}$CO$_2$ by oxidation or into $^{14}$CH$_4$ by reduction [4].

For many gas chromatographic applications in organic chemistry, biochemistry, and physiology, the non-destructive thermal conductivity detector was not sensitive enough for organic compounds and has been replaced by the far more sensitive flame ionization detector (FID) [7, 8, 9, 10, 11]. Since the organic substances in the effluent gas from the column are destroyed during analysis, the effluent is divided by means of a gas stream splitter; one portion of the gas passing to the FID for destructive mass detection, in the other the organic compounds are burned in a furnace to $^{14}$C-labelled carbon dioxide or reduced to methane before passage through the radiation detector [3, 4].

However, in our experience, the split ratio tends to change from one sample to another because of changing back pressure in the furnace, the absorber needed to remove water produced in combustion and the anthracene filled flow-cell of the $^{14}$CO$_2$ detector. Therefore the stream splitter is a serious source of error in the determination of radioactivity and mass of compounds.

In an attempt to simplify our radio gas chromatographic procedure for the determination of radioactivity in $^{14}$C-labelled organic acids and sugars we investigated ways of dispensing with the stream splitter. The total effluent from the column was passed into the FID, the combustion products from the FID were dried, and the dry gas was continuously pumped through an ionization chamber for visual $^{14}$C peak detection. Subsequently, the $^{14}$CO$_2$ was absorbed in ethanolamine, and assayed for radioactivity in a liquid scintillation spectrometer (LSC).

Apparatus and Experimental Methods

1. Radio Gas Chromatographic Systems

System 1: Packard) gas chromatograph (series 7300) with flame ionization detector (FID), linear temperature programmer, and Kipp and Zonen$^2$ electronic integrator BC 1;

*) counting yield = \( \frac{c}{dpm} \times 100 \)

\( c \) = counts without background during residence time in detector; \( dpm \) = disintegrations per minute

$^\star\star$) GM = Geiger-Müller counter

$^\star\star\star$) GLC = gas liquid chromatography

$^1$) Downers Grove, Illinois, U.S.A.

$^2$) Delft, Holland
Packard instrument combustion furnace, model 325; Berthold-Frieske 3) liquid scintillation spectrometer, BF 5020, equipped with anthracene filled plexiglass gas flow-cell (void volume = 6 cm³, manufactured in the university workshop);
Berthold ratemeter LB 2232;
Kienzle printer D 44 EN as integrator for radioactive peaks;
Two-pen recorder (Honeywell, Electronic 194).

**System 2:**
Packard gas chromatograph (series 7300) with FID, linear temperature programmer, and Kipp and Zonen electronic integrator BC 1;
150 cm³ continuous flow-through ionization chamber (milled in the university workshop) coupled to a vibrating capacitor-electrometer system (RFT Meeselektronik 5), model VA-J-51.1-S with a measuring leak resistor of 10¹¹ ohms and a storage capacitance of 25 x 10⁻¹² farads;
Two-pen recorder (Honeywell, Electronic 194).

**System 3:**
Varian 5) gas chromatograph (series 1800) with FID and temperature programmer;
150 cm³ continuous flow-through ionization chamber (milled in the university workshop) coupled to a Nuclear-Chicago 9) “Dynacon” vibrating capacitor-electrometer system (model 6000) with a measuring leak resistor of 10¹² ohms and a storage capacitance of 2.5 x 10⁻¹² farads;
Two-pen recorder (Siemens, Kompensograph III).

**System 1** (Fig. 1a) is a commercial radio gas chromatographic system with a gas stream splitter for the column effluent. Two stainless steel splitters with fixed split ratio were used with nominal split ratio of 85 % (but delivery between 82 and 89 % because of back pressure) to direct the gas through the flow cell. The amount of radioactivity eluted in each peak was determined by means of a planimeter or from the printed scaler readings [9]. The ¹⁴CO₂ was quantitatively absorbed in ethanolamine (1 cm³ ethanolamine + 1 cm³ ethylene glycol monomethylether on glass wool in 20 cm x 1 cm ID glass tube) for scintillation counting (Table 1).

The 150 cm³ flow-through ionization chamber had a height/diameter ratio of 0.44 and the potential applied was 85 V (system 2) or 135 V (system 3), respectively. The “equilibrium voltage” method was applied to determine the ion current. The ionization chamber response was shown to be a linear function of the ¹⁴CO₂ in the chamber by use of ¹⁴CO₂-containing gas streams delivered from precise gas mixing pumps [12, 13]. The calibration constant for the ionization chamber was 3.52 x 10⁻¹² A curie⁻¹ (system 2) or 3.64 x 10⁻¹³ A curie⁻¹ (system 3) with detector efficiencies [14] of 43 % and 44 % and counting yields of 9.2 % and 9.4 % respectively at a flow of 700 cm³ min⁻¹.

**2. Preparation of Derivatives**
Methyl esters of standard organic acids (100 µg C per organic acid and sample) and of the organic acid fraction from leaves (acidic-1 fraction, according to Atkins and Conwin [15]) were dried and esterified with 75 mg of methanol washed and dried cation-exchange resin AG-50* (H⁺, 200–400 mesh) in 300 mm³ dry methanol [16, 17] overnight at 80 °C in tubes sealed with teflon lined screw caps. Standard sugars (20 µg C of glucose or fructose or sucrose per sample) were chromatographed as trimethylsilyl (TMS) derivatives. The samples were dried, redissolved in 2 x 20 mm³ dry methanol, dried again and reacted with 100 mm³ TRISIL “Z”** in 0.3 cm³ conical vials for 10 min at room temperature.

**3. Gas Chromatographic Conditions**
Methyl esters of most organic acids detectable in leaves were separated with 10 % polyethylene glycol 4000 on Gaschrom Q, 60–80 mesh (column A, systems 1–3) or in 3 % polyethylene glycol 20,000 on Teflon 6, 40–60 mesh (column B, system 2), 300 cm X 0.4 cm ID glass column; carrier gas (N₂) 50 cm³ min⁻¹; H₂ 25 cm³ min⁻¹; synthetic air 350 cm³ min⁻¹. Excellent separations, especially for...