

THE PERFORMANCE OF A COMMERCIAL HIGH-TEMPERATURE IONIZATION CHAMBER FOR RADIOISOTOPE ANALYSIS OF GAS CHROMATOGRAPHY VAPORS*

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The technique of gas liquid chromatography as a tool for chemical research has found numerous applications in recent years. In biological research it is often desirable to separate a sample into its components and to determine the amount of radioactivity that might have been incorporated into the various components. A common procedure now used is to trap the effluent as it emerges from the column and count it separately or, as reported recently, continuously as it bubbles into liquid scintillator¹. The disadvantages of these procedures are discussed by JAMES AND PIPER².

Methods have been developed in which the detector is an integral part of the system. Proportional counters have been used in which the effluent from the gas chromatograph is first combusted to CO₂³. Proportional counters have been designed for use at temperatures up to 200°³. WINKELMAN AND KARMAN⁴ used a 275 c.c. ionization chamber to determine the activity in the products of the combusted effluent.

CACACE AND INAM-UL-HAQ⁵ used a 100 ml ionization chamber which did not require heating for compounds that boiled under 150°. These authors used nitrogen as a purge gas. MASON *et al.*⁶ constructed an ionization chamber with a working volume of 9 ml which was insulated with teflon. The ionization chamber was not included in the oven but was heated separately to 240°.

GANT⁷ included a teflon insulated ionization chamber in the gas chromatographic oven but found that noise greatly increased at temperatures above 125°.

Sapphire insulated high temperature ionization chambers have been developed⁸ that are suitable for inclusion in gas chromatographic ovens. This ionization chamber is used in the commercially available Loenco-Cary Model 70.

We wish to describe a complete system which employs a Cary high temperature ionization chamber and has proved useful in the detection of small amounts of radioactivities in the system.

EXPERIMENTAL

Apparatus and reagents

The gas chromatograph was constructed by the authors for inclusion of an ionization chamber. Both the injection and exit blocks had provision for heating and the oven temperature was maintained by an Assembly Products controlling pyrometer.

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Brooks rotameters model 1100 were used to control the gas flows. For helium the capacity was 14.4–144 c.c./min and for argon 110–1103 c.c./min. Five foot copper columns were packed with 16% LAC 296 (Chrom Line Laboratories, Kansas City, Missouri) on acid washed fire brick 42/60, 84% (Wilkins Instrument and Research, Inc.).

The gas chromatograph bridge circuit was similar to that used in the Aerograph A-90 and the detector cell was an XA-222 Aerograph stainless steel detector cell. Better stability was obtained with the bridge circuit separated from the oven. The electrometer was a Cary Model 31 Vibrating Reed Electrometer with critical damping. The high resistance leak method for measuring ionization current was used with a single $10^{12} \Omega$ resistor. The ionization chamber was a Cary No. 3295700 High Temperature Ion Chamber Assembly with a 12 in. stainless steel rigid line and adaptor. The output from the gas chromatograph and the electrometer was fed into a Weston Model 6807 two pen recorder. Twenty-five mV was used for the Vibrating Reed Electrometer and 1 mV for the gas chromatograph. The carriage speed on both channels was 1 sec and the chart speed was 30 in./h. The first channel was connected to the electrometer and the second to the gas chromatograph bridge. Connected in this manner the two peaks were made to coincide even though the signal reached the gas chromatograph channel first.

A 90 V DC battery was used to provide the polarizing voltage for the ionization chamber. Insulation from the rest of the system was provided by Kovar to glass seals. The glass seals were protected from strain by the use of a coiled $1/8$ in. copper tubing in the system.

Benzene- ^{14}C uniformly labelled (Nuclear-Chicago Corporation) was diluted with reagent grade benzene such that final activities of 20,700, 2060 and 206 disintegrations/min/ μl were obtained. A Packard-tri Carb Model 314EX Automatic Liquid Scintillator Spectrometer was used to check the radioactivity of the injected and recovered samples.

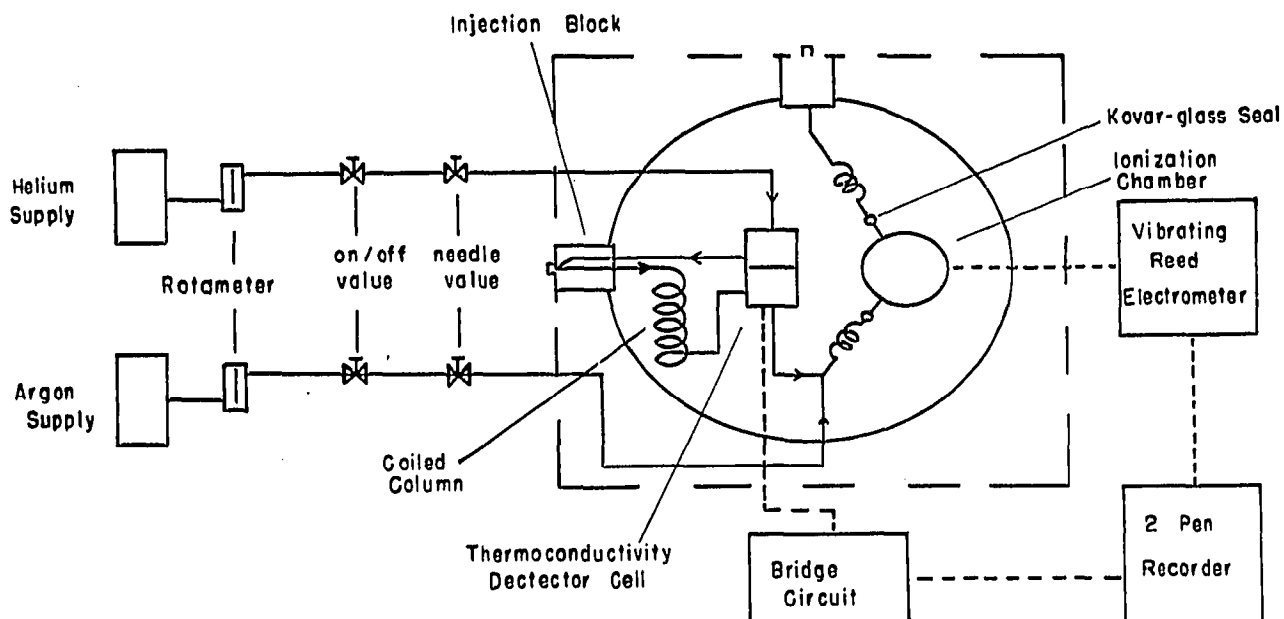


Fig. 1. System flow diagram.

The components were arranged according to the diagram in Fig. 1. With a helium valve pressure of 15 p.s.i., the full range of the gas chromatograph flow rates could be obtained (28–140 c.c./min). With the argon valve set at 10 p.s.i. flow rates of from 97 to 940 c.c./min were obtained. The helium flow rate was set for 95 c.c./min. Both the injection and exit block heaters were set at 150°. Injections of from 0.5 μ l to 3 μ l of the samples were made using a 10 μ l syringe. The gas chromatograph was run at an attenuation of 32–128 times.

An impingement type trap in dry ice-ethanol was used to trap the vapor from the system. The vapor was passed from the trap to a secondary glass coil which was also in the coolant. The trap was constructed with a cone at the bottom such that the condensate could be withdrawn with a capillary pipet. Increased recoveries were obtained if 0.5 ml of toluene was added to the bottom of the trap. Where liquid scintillation counting of the condensate was desired, 3.5 ml of toluene in portions was used to rinse the trap. This arrangement allowed a recovery of better than 95 % of the activity added to the system.

RESULTS

In order to determine the optimum flow rate for the purge gas, a series of injections of 20,700 d.p.m. of benzene-¹⁴C in 1 μ l volumes was made. The series was started with 197 c.c./min of helium and argon and the latter gas was increased in step-wise manner until a final flow rate of 1041 c.c./min was reached. These flow rates represent chamber constants of from 84 to 15.8 sec. At the lower flow rates the ionization chamber required 4 min to return to the background level. As the argon flow rate was increased the base of the peak narrowed. At flow rates above 689 c.c./min (600 on the argon rotameter) the shape of the base of the curve was changed only to a slight degree. The ratio of radioactivity to thermoconductivity peak heights remained constant up to 689 c.c./min. With increased flow rates above 689 c.c./min the ratio was found to decrease slightly. A flow rate of 689 c.c./min represents a chamber constant of 23.9 sec, under these conditions 1.5 min were required for the ionization chamber to return to the background level.

Fig. 2 shows a simultaneous measurement of radioactivity and thermoconductivity. The three peaks represent activities of 515, 1030 and 2060 d.p.m. respectively.

Fig. 3 shows that a straight line relationship exists between the area under the peaks and the amount of radioactivity injected. Fig. 4 shows the results of a plot of peak height *versus* d.p.m. of ¹⁴C injected. At the higher activities, peak height is proportional to the amount of ¹⁴C injected. At the lower activities, however, there is no linear relationship between peak height and the amount of ¹⁴C injected. Although each point in Figs. 3 and 4 represents the average of five determinations, peak heights particularly at low activities would be higher than expected due to "bursts" of activity. WINKELMAN AND KARMAN⁴ also found that peak area was more linearly related to the amount of radioactivity injected than was peak height.

Fig. 5 shows the excellent resolution that can be obtained with the system. A mixture of benzene-¹⁴C and toluene were injected into the system (oven temperature 70°). Although the benzene and toluene were not completely separated all of the radioactivity was shown to be associated with the benzene peak.

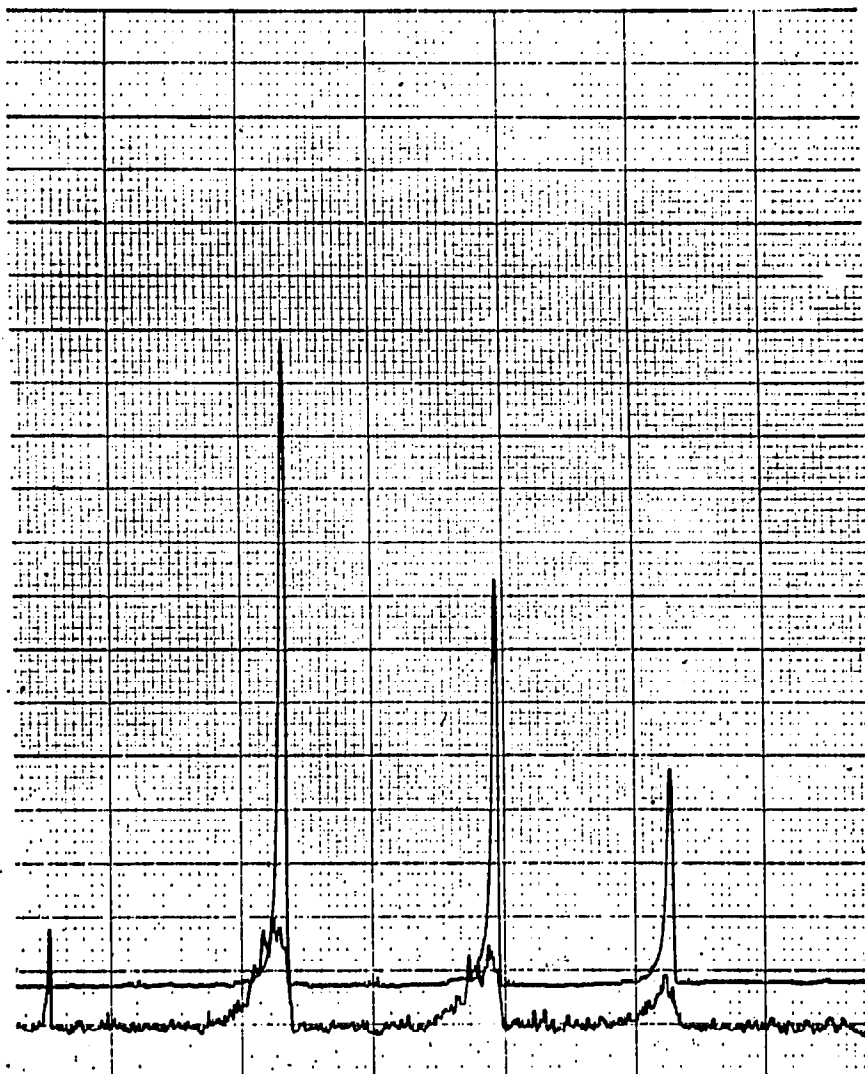


Fig. 2. Simultaneous measurement of radioactivity (lower curve) and thermoconductivity (upper curve) of benzene- ^{14}C .

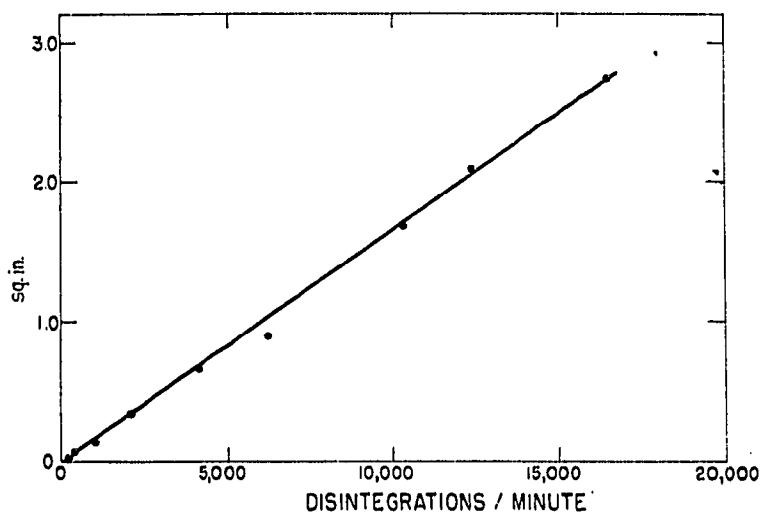


Fig. 3. Relation of d.p.m. injected of ^{14}C to peak area.

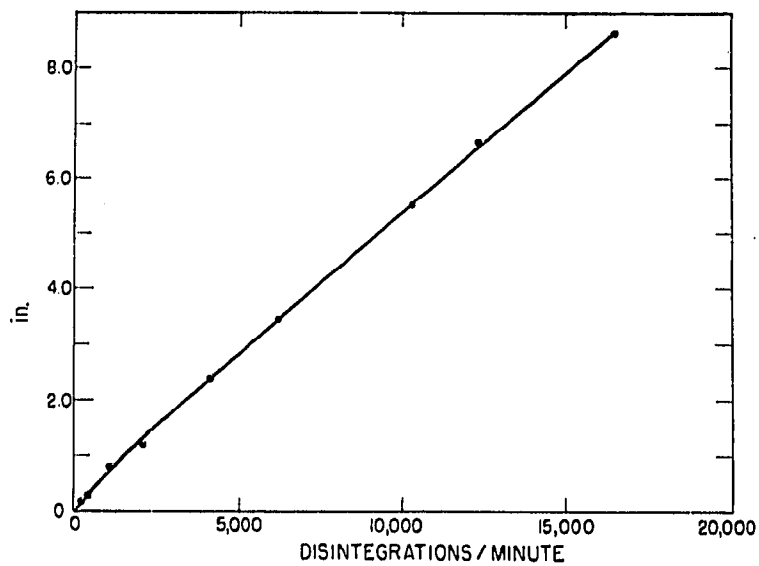


Fig. 4. Relation of d.p.m. injected of ^{14}C to peak height.

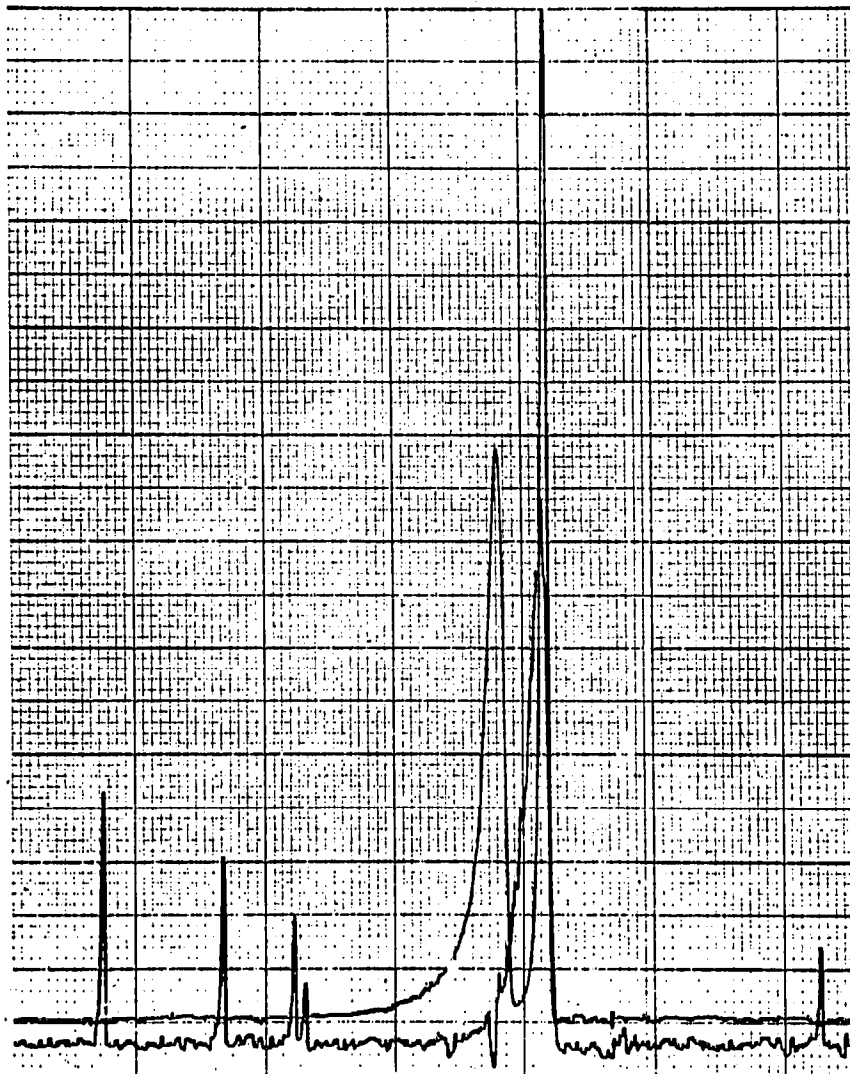


Fig. 5. Separation of benzene- ^{14}C and toluene.

DISCUSSION

A number of factors are involved in the ability to detect radioactivity in a sample injected into a system as described in this paper. The ionization chamber should be of sufficient size such that most of the energy from a disintegration is absorbed by the gas rather than the walls of the chamber⁴. A 275 c.c. ionization chamber without the use of a purge gas would give very poor resolution. Argon has been shown^{1,9} to be a better ionization gas in this application than helium.

It is important that the noise level of both the ionization chamber and the electrometer be low particularly if one desires to detect small amounts of radioactivity. Although benzene-¹⁴C was used in the experiments reported here, similar results were obtained at temperatures above 200° using lauric acid-¹⁴C methyl ester. Benzene-¹⁴C was used because of its short retention time.

Both peak height and peak area are affected by the manner in which the peak reaches the ionization chamber and the chamber constant. The ideal situation is one in which the peak reaches the ionization chamber in a very small volume and is quickly purged from the chamber. Where the sample enters the chamber in a larger volume, *i.e.* over a longer period of time, the peak height is lower and the area is spread over a longer time interval even under an optimum chamber constant. The sensitivity of the system is lowered under these conditions. Thus a sample with low activity could pass through the ionization chamber undetected.

Under the conditions employed in these experiments it was possible to detect as little as 200 d.p.m. although at this level of activity the normal fluctuations of the background excluding α -disturbances nearly equaled the peak height. At 400–500 d.p.m. the peak representing the radioactivity could be distinguished from the background noise. It would of course be desirable to use larger amounts of radioactivity in order to use less sensitive electrometer settings. In biological research the experimenter is often required to analyze samples with very low activities.

The problem of trapping the effluent from this system is much greater than those experienced with conventional gas chromatograph flow rates. The trap system that was used was found to be very efficient at the flow rate used. With the use of a trap, the experimenter is able to recover samples from the system. This represents a real advantage over methods in which the samples are combusted or otherwise changed in detection.

The main problem in using this system for quantitative work is one of being able to inject accurate amounts into the system. The ratios of the peak heights (thermoconductivity to radioactivity) were nearly constant above 2060 d.p.m. in the data from which Figs. 3 and 4 were derived. However, in this series the individual thermoconductivity peaks varied by $\pm 2-3\%$. In preliminary work several syringes were used to inject the same volume, however, when the charts were compared there was a greater variation than should be expected. The most consistent results were obtained by using one syringe and, by the use of dilutions, injecting similar volumes.

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SUMMARY

A system is described for the simultaneous analysis of the effluent of a gas chromatograph column for thermoconductivity and radioactivity. A gas chromatograph was constructed for inclusion of a 275 ml high temperature ionization chamber. The effluent from the thermoconductivity cell passes directly into the ionization chamber providing coincident peaks on two pen recorder. Argon is used as a purge gas for the system. The sensitivity of the system was such that 400 disintegrations per minute of carbon-14 could be detected. Under ideal conditions 206 d.p.m. could be distinguished from background noise.

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