RADIO-LIQUID CHROMATOGRAPHY FOR TRITIUM LABELLED ORGANIC COMPOUNDS USING CaF$_2$/Eu/ SCINTILLATOR

K. Shirahashi, G. Izawa, Y. Murano, Y. Muramastu, K. Yoshihara

Department of Chemistry, Faculty of Science, Tohoku University, Sendai 980 Japan

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The radio-liquid chromatography for the tritium labelled compounds was constructed with a CaF$_2$/Eu/ scintillator and applied successfully to analyze the products of recoil tritium reactions with trans-stilbene. The detection efficiency of tritium of 0.8% and the lowest detectable activity of 60 nCi were obtained for a 100-150 mesh CaF$_2$/Eu/ scintillator.

EXPERIMENTAL

In recoil tritium chemistry, the reaction mechanisms are usually elucidated by the separation and identification of tritium-labelled products resulting from reactions between energetic tritium atoms and organic compounds$^1,2$. However, these tritium-labelled products are frequently in the carrier-free state and in various
unexpected chemical forms whose carrier compounds are hard to prepare.

The gas chromatography has been successfully applied to the recoil tritium chemistry for analyzing the products from the gas-phase reactions. On the other hand, the recrystallization and the extraction methods have been employed for high boiling-point products from the liquid or solid phase reactions. In the latter two methods, however, use of carrier compounds is required and separation of products is often unsatisfactory.

The liquid absorption chromatography is suitable for complete separation of the high boiling-point products without carriers. The radioactivity of tritium in effluents from the liquid absorption chromatography is usually measured with a liquid scintillation counter, after effluents are collected fractionally and mixed with the scintillator cocktail. For rapid and continuous detection of tritium in the effluents, solid scintillators have been used in the biological fields.

Davies and Mercer used the europium-activated calcium fluoride scintillator \( \text{CaF}_2/\text{Eu} \)/ for continuous detection of the low energy \( \beta \)-particle from \( ^{14}\text{C} \) and tritium-labelled compounds in the biosynthesis\(^3\), and Schutte used lithium glass scintillator for the same purpose\(^4\).

We have constructed an apparatus of radioliquid chromatography using the \( \text{CaF}_2/\text{Eu} \)/ scintillator for the analysis of the reaction products of recoil tritium atoms with trans-stilbene. Special care was taken in this study, so that the high detection efficiency and recovery might be achieved because of the low activity of tritium-labelled products, and counting of the products which were adsorbed on the scintillator might be subtracted reasonably.
EXPERIMENTAL

The selection of a scintillator is a basis factor for our system. Since the good recovery of the effluent was required for measurement of the products after separation in the recoil tritium reaction, the scintillator must be insoluble in the organic solvent used for absorption chromatography. Moreover, the scintillator must have a high detection efficiency for tritium.

The scintillators used for tritium detection are usually liquid scintillators, plastic scintillators, cerium-activated lithium glass beads, and CaF$_2$/Eu/. The liquid scintillators must be dissolved in the effluent, so the eluent is hard to be recovered. The plastic scintillators are soluble in chloroform.

CaF$_2$/Eu/ is affected neither by organic solvents nor by water, and has relatively higher scintillation efficiency to the lithium glass scintillator$^5$. CaF$_2$/Eu/ is not available for solutions containing acids as it is soluble in acids but acids are scarcely employed as solvents in absorption chromatography. The emission spectra of CaF$_2$/Eu/ scintillator are from 385 to 450 nm$^6$ which enable to use the usual photomultiplier to detect the fluorescence.

The block diagram of the apparatus used is shown in Fig. 1. The CaF$_2$/Eu/ scintillator /Nuclear Enterprise Ltd./ is crushed to 45-100 mesh and to 100-150 mesh. The crushed scintillator is packed into a quartz U-shaped flow cell, of a capacity of 0.27 cm$^3$. The flow cell is inserted between the two photomultipliers /EMI 6097B/ which are contained in a dark box, and are cooled at 10 °C to decrease the background. The silica gel column for the absorption chromatography and the flow cell are connected with a silicon tube and a 28-cm long
Fig. 1. Diagram of column chromatographical measurement with CaF₂/Eu detector.

HV - high voltage; PM - photomultiplier; PA - preamplifier; LA - linear amplifier;
PHA - pulse-height analyzer; MCS - multi-channel scaler /CANBERRA series 40/

stainless-steel tube /1/8"/ to shield the photomultipliers from light.

The liquid scintillation spectrometer /Nuclear Chicago System/ is used for the coincidence circuit of the two photomultipliers. The multichannel analyzer /Canberra Series 40/ is used as a multichannel scaler mode for recording.

RESULTS AND DISCUSSION

The counting efficiency is evaluated by the "static" and the "flowing" methods described below. The counting efficiency and the background of the apparatus is shown in Table 1.
TABLE 1

Efficiency of the detector

<table>
<thead>
<tr>
<th>Size of scintillator, mesh</th>
<th>45-100</th>
<th>100-150</th>
<th>Ref. 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume of flow cell, ml</td>
<td>0.27</td>
<td>0.27</td>
<td>0.50</td>
</tr>
<tr>
<td>Background, cpm</td>
<td>20</td>
<td>30</td>
<td>180</td>
</tr>
<tr>
<td>Counting efficiency, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Static method</td>
<td>0.35</td>
<td>0.86</td>
<td>-</td>
</tr>
<tr>
<td>Flowing method</td>
<td>0.32</td>
<td>0.84</td>
<td>1.55</td>
</tr>
</tbody>
</table>

In the static method, the counting efficiency is constant from 10 nCi to 20 µCi tritium. The scintillator of 100-150 mesh shows two times as high efficiency as that of 45-100 mesh. The surface area of the scintillator, because of the low β-ray energy of tritium, seems to be one of the factors determining the counting efficiency. The lowest activity of tritium detected \( |A| \) at the condition of counting time \( |T| \) and the standard error \( |E| \) is estimated as follows\(^7\),

\[
A = |1 + 2E/TB|^{1/2}/|\varepsilon E^2 T|^{-1}
\]

Where \( B \) is the background, and \( \varepsilon \) is the counting efficiency. At conditions \( T = 30 \) sec and \( E = 0.05 \), the lowest detectable activity is 60 nCi, and it is sufficient for determining the yield of products of the recoil tritium reaction.

In the flow method, the n-hexane solution of the tritiated naphthalene, of which specific activity is measured beforehand, is charged on the silica gel column /10 mm i.d. x 250 mm length/. The naphthalene
Fig. 2. Chromatogram of tritiated naphthalene.
   a/ measured by radioactivity with a CaF$_2$/Eu/ detector
   b/ measured by UV absorption

is eluted with n-hexane and its activity measured with this detector in its flow path. Fig. 2a. shows the result of detection of tritiated naphthalene in the "flowing" method using the CaF$_2$/Eu/ detector. Fig. 2b. shows the chromatogram of naphthalene detected by UV absorption method, using the same column and being the same amount of naphthalene charged as before, but without the CaF$_2$/Eu/ detector. Since the half-width of the peak of Fig. 2a. is coincident with that of Fig. 2b, the band of naphthalene does not broaden in the flow cell. Not less than 98% of naphthalene was recovered chemically, when the effluent of the peak was collected.

The major products of the reaction of trans-stilbene with recoil tritium were analyzed by absorption
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Fig. 3. Elution of tritiated products from irradiated trans-stilbene, measured with a CaF₂/Eu/ detector

Chromatography and detected by this apparatus. Trans-stilbene was sealed in a quartz ampoule with $^3$He and irradiated with neutrons in the nuclear reactor, JRR-4 of the Japan Atomic Energy Research Institute. After the nuclear transformation of $^3$He/n, p/$^3$H, the recoil tritium reacts with trans-stilbene, and many kinds of tritiated products in carrier-free form are produced. The solid and liquid constituents of the products were dissolved in a small amount of n-hexane and the solution was injected on the column 11 mm i.d. x 90 cm length/, which was filled with silica gel /Wako Pure Chemical Industries Ltd./. N-hexane, chloroform, and ethanol were used successively as eluting solvents. Fig. 3. shows the chromatogram of trans-stilbene ir-
radiated with neutrons. The first peak (defined as peak A), eluted with n-hexane, was ascribed to the parent compound, trans-stilbene. The second (peak B) and third (peak C) peaks presumably correspond to polymer compounds.

In Fig. 3., the tail of peak C is observed. We have to describe how to treat this tailing in counting net radioactivity. When the effluent was collected following the flow cell in ml fractions, and its radioactivity was measured on the liquid scintillation counter, the tail of peak C was not observed. Therefore, the material of peak C could be assumed to be of high molecular weight from its chromatographical behaviour, so it was sticking to the scintillator in the flow cell. The yield of peak C was calculated omitting the part of the tail. The yields of the three peaks thus estimated as 72% for peak A, 13% for peak B, and 14% for peak C were found in good agreement with the results obtained by liquid scintillation counter after collected in fractions /72, 14 and 14%, respectively/.

REFERENCES


