Journal of Chromatography, 127 (1976) 163–201
Chromatographic Reviews
© Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHREV. 96

GAS CHROMATOGRAPHY OF ^{3}H- AND ^{14}C-LABELLED COMPOUNDS

MIROSLAV MATUCHA
Institute for Research, Application and Production of Radioisotopes, 170 04 Prague 7 (Czechoslovakia)
and
EVA SMOLKOVÁ
Faculty of Science, Charles University, 128 40 Prague 2 (Czechoslovakia)
(Received September 7th, 1976)

CONTENTS

1. INTRODUCTION

The application and production of labelled compounds are nowadays closely related to the use of chromatographic methods. Gas chromatography (GC) and the preparation and application of labelled compounds are relatively new fields of research; the first basic paper on GC appeared in 1952[185], while the preparation of labelled

compounds began seriously after the second world war, although tritium and carbon-14 were discovered in the 1930s and their convenience for biochemical investigations was recognized at that time[124,197]. GC has contributed to modern research as a result of its separation efficiency and high sensitivity, while the use of labelled organic compounds has made possible investigations of transformations of substances during such complicated biochemical processes as photosynthesis. GC was used for the first time in the analysis of labelled compounds (^{14}C-labelled hydrocarbons) in 1955 by Kokes *et al.*[223], but soon afterwards many papers appeared on "hot-atom" chemistry and on biochemical research, accompanied by many others on methodological developments. The advantages of "radio-gas chromatography" (RGC), the GC of radioactive substances combined with a radioassay, have been described in more than 1000 papers on applications in the fields of biochemistry, clinical biochemistry, organic chemistry, chemical processing, "hot-atom" chemistry and the production of labelled compounds. This review attempts to give a complete survey of RGC methods used for analysis of ^{3}H- and ^{14}C-labelled compounds; special attention is paid to the chemical problems involved in RGC. Methods of detection of radioactivity form the main part of the review, and types of records and their evaluation and a survey of important applications of RGC are included.

2. GENERAL

2.1. Labelled compounds and their analysis

Organic molecules in which one of the atoms, larger structural units or all of the atoms are substituted partially or wholly by radioactive isotopes are called labelled compounds (the use of stable isotopes, because other types of detection are involved, will not be considered here). The methods used for their preparation[71,124,130], the incorporation of radioactive isotopes by chemical synthesis, biosynthesis[71,389], chemical exchange or by physical methods, determine the type of labelling: specific, general (non-specific, random) or uniform. The physical properties of ^{14}C and ^{3}H are similar: their long half-lives are more advantageous than the low energy of the beta-radiation (Table 1). The low energy of the beta-particles emitted by ^{14}C and ^{3}H has led to the same or a similar manner of detection of the radionuclides themselves and also in the GC of their compounds.

In recent years, increasing attention has been paid to the analysis of labelled compounds[71–73,124,160,262,343], including the determination of total activity, radionuclidic, radiochemical and chemical purity, specific activity (from the content of the labelled compound) and, in certain instances, the pattern of labelling[73,346]. Chroma-

TABLE 1

BASIC RADIOCHEMICAL CHARACTERISTICS OF CARBON-14 AND TRITIUM

Isotope	*Main nuclear production process*	*Half-life (years)*	*Max. β-energy (keV)*	*Max. attainable specific activity (mCi/milliatom)*
^{14}C	^{14}N(n,p)^{14}C	5730	159	62.6
^{3}H	^{6}Li(n,α)^{3}H	12.35	18	29,120

tographic methods play a particularly important role in checking purity[72,160,262,274], they are often suitable for the determination of contents by using a convenient mass detector and, in connection with degradation methods, they are sometimes appropriate for the control of the pattern of labelling. Another major use of RGC is in chromatographic methods for the separation of individual species in practical applications of labelled compounds, the distribution of radioactivity after chemical, biochemical or nuclear reactions being followed.

2.2. Gas chromatography of labelled compounds

The role of GC in the analysis of compounds labelled with ^{14}C and ^{3}H has been surveyed several years ago[72,152,262]. GC is virtually the only convenient method for the analysis of radioactive gases and volatile substances such as hydrocarbons, but the development of derivatization methods has introduced applications for polar substances, including carboxylic acids, steroids, sugars, nucleotides and amino acid enantiomers. In connection with chemical reactions and pyrolysis, GC can also be helpful in questions of specificity of labelling[107,110,191,346,391]. The recently developed liquid chromatography and isotachophoresis have overlapped the applications of some GC methods, but there is one property of GC that can hardly be matched, namely its sensitivity in mass detection, and in many instances also in activity measurements because of the high counting efficiency of the weak β-emitters ^{3}H and ^{14}C in the gas phase.

The application of GC to the analysis of labelled compounds enables several types of information to be obtained at the same time: it is possible to identify the compounds and to determine their content, specific activity and radiochemical and chemical purity. RGC was often used only for qualitative or semi-quantitative work in metabolic studies for tracing pathways via the label, and applications of RGC for quantitative purposes in chemical processing and in investigations of chemical and recoil reactions are well known. Suppliers of labelled compounds use RGC during production processes and in the final quantitative analysis of preparations. Relatively few papers have described all aspects of quantitative work and little attention has been paid to chemical problems in RGC.

The technique of RGC and its range of applications have been reviewed many times[5,51,83,90,108,168,182,204,205,208,210,246,270,279,292,321,326,337,345,385–387,401,414], and applications of RGC for special purposes such as in lipid analysis[80,116,192,228,275,356], "hot-atom" chemistry[6,421] and the analysis of pesticides[244] and organometallic compounds[388] have also been surveyed.

2.3. Methods used in radio-gas chromatography

The methods used to monitor the effluent stream from a GC column for radioactivity can be divided into two main groups: those which use the intermittent trapping of the effluent and subsequent separate measurement of the radioactivity of the fractions and those which measure the radioactivity continuously by means of a flow-through detector. A survey of systems used in RGC is given in Table 2.

The simplest and probably the most commonly used approach to assaying the radioactivity of labelled compounds separated by GC is a combination of a trapping

TABLE 2

TYPICAL SYSTEMS USED FOR THE RGC OF ^{3}H- AND ^{14}C-LABELLED COMPOUNDS

No.	Part	Reference												
		223 (1955)	*186 (1956)*	*418 (1957)*	*302 (1959)*	*54 (1959)*	*187 (1961)*	*211 (1962)*	*108 (1963)*	*372 (1966)*	*347 (1965)*	*251 (1968)*	*336 (1973)*	*8 (1974)*
1	Column outlet*	+	+	+	+	+	+	+	+	+	+	+	+	+
2	Splitter*	–	–	–	–	–	–	+	–	+	+	–	+	+
3	Mass detector*	TCD	GDB	TCD	GDB	TCD	TCD	AID	TCD	FID	FID	TCD	FID	FID
4	Effluent conversion*	–	–	–	–	CMB	CMB	CMB	HC	CMB	HC	CMB	–	–
5	Counting*	GM	TR	IC	SC	IC	PC	SC	PC	PC	PC	PC	PC	SC + TR
	Flow scheme**	1,3,5	1,3,5	1,3,5	1,3,5	1,4,5,3	1,4,5,3	$1,2<^{3}_{4,5}$	1,3,4,5	$1,2<^{3}_{4,5}$	$1,2<^{3}_{4,5}$	1,3,4,5	$1,2<^{3}_{4,5}$	$1,2<^{3}_{5}$
	Used for	^{14}C	^{14}C	^{3}H	^{14}C	^{3}H, ^{14}C	^{3}H, ^{14}C	^{3}H, ^{14}C	^{14}C	^{3}H, ^{14}C	^{3}H, ^{14}C	^{14}C	^{14}C	^{3}H, ^{14}C

* Abbreviations: + = used; – = avoided; TCD = thermal conductivity detector or microthermistor; FID = flame-ionization detector; GDB = gas-density balance; AID = argon ionization detector; CMB = combustion; GM = Geiger–Müller tube; HC = hydrocracking; IC = ionization chamber; SC = scintillator; PC = proportional counter; TR = trapping.

** The code system used is directly connected with the numbers in the first column of the table.

procedure for the collection of the separated components and subsequent counting of the collected fractions. In the first application of this discontinuous method for labelled compounds, described by James *et al.*[186], ^{14}C-labelled fatty acid methyl esters were detected with a gas-density balance detector and then collected in tubes filled with cotton-wool moistened with methanol, but "off-line" detection has also been used for non-radioactive substances in connection with other types of detection, such as mass spectrometry[12,70,95,203] and ultraviolet[417], infrared[48,87,88,221,393,417] and nuclear magnetic resonance[393] spectroscopy; a review[240] has also been published.

There are two principial modes of fraction collection: (1) the use of an automatic fraction collector (*e.g.*, a Packard Model 830) permits trapping at regular intervals; (2) when the mass detector indicates the appearance of a desired substance, the trap is inserted and the labelled compound collected. Mass detectors include non-destructive thermal conductivity detectors (TCD), argon ionization detectors (AID) and gas-density balances (GDB) and, for the smaller portion of a split effluent, flame-ionization detectors (FID). The use of radioactivity detectors for the preparative GC of ^{14}C-labelled compounds has been reported[397]. The often discussed risk of not detecting of substances of high specific activity when using a less sensitive mass detector[205,208,213,401] seems to be more of theoretical than of practical value, and the problem studied by means of RGC is usually known to some extent. The reported[251] sensitivity of a microthermistor of about 100 ng is sufficient for ^{14}C-labelled substances in many instances, while ^{3}H-labelled compounds of high specific activity (1–25 Ci/mmole or higher) can usually be detected only with a radioactivity detector (sub-nanogram sample sizes). The convenience of the discontinuous method for low total and low specific activities is apparent.

Many systems have been described for the continuous monitoring of radioactivity in the effluent. Early approaches involved systems with mass and heated radioactivity detectors in series[223,281,312,418]; one of these systems[281] is demonstrated in Fig. 1. They are used nowadays only in exceptional circumstances, when the counting efficiency is not influenced by the composition of the substance passing through the counter[66]. This factor, applicable to flow-through proportional counters[108,233,238], ionization chambers[212] and liquid scintillation instruments[345], led to the development

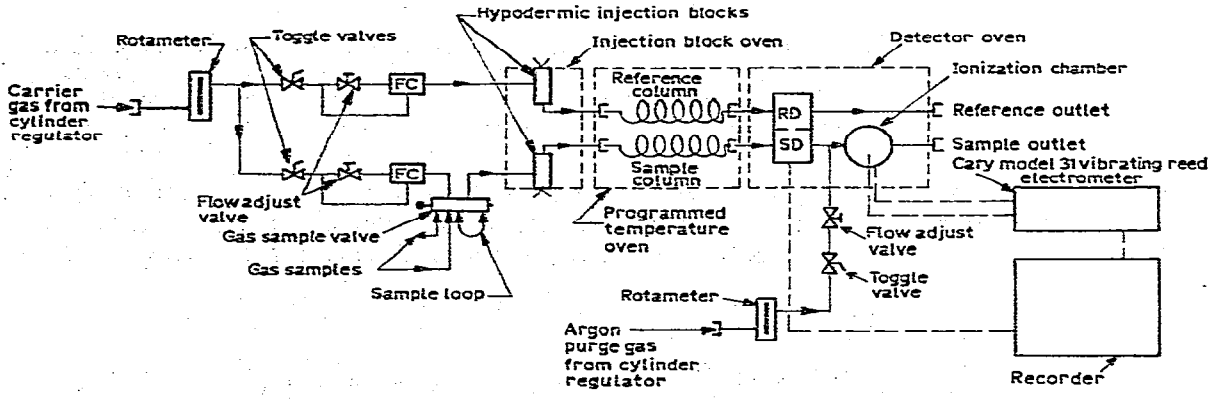


Fig. 1. Flow diagram of RGC system using heated ionization chamber. Reproduced from *Anal. Chem.*, 35 (1963) 1576 (ref. 281).

of more sophisticated systems involving combustion[54,133,142,167,187], as used previously in non-radioactive GC[150,250] and hydrocracking, applied by Zlatkis and Ridgway[431] for detection with TCD. Combustion of the effluent also permits the absorption of $^{14}CO_2$ in ethanolamine[40,122,163], hyamine[170,390] or sodium hydroxide solution[84,91,92] for discontinuous counting. Most flow-through systems use the more sensitive FID after splitting of the effluent (the AID has low linearity). The larger part (usually 80–90%) is led into the radioactive branch. The use of an additive gas for the accurate functioning of the splitter has been reported[211]; because of the non-stability of the splitter operation[135], the exact splitting achieved was determined by comparison of the mass responses of the runs with and without the splitter[372]. Several applications of the FID as a combustion element have been described[91,92,135,310]; in this way, the use of a splitter is avoided. The electron-capture detector (ECD) has probably not yet been used in RGC.

Another possible combination in RGC is absorption of the effluent in a flowing liquid scintillator[336] (Fig. 2), which permits the collection of fractions. Several further methods combine flow-through and "off-line" detection: interrupted-elution GC in conjunction with a static ionization chamber has been reported[62,63,65]; radioactive substances issuing from a column have been collected on a moving strip of paper[429] for TLC plates[100a] for subsequent radioassay[429]; and "buffer storage" (absorption of $^{14}CO_2$ in a flow of sodium hydroxide solution, which is then led through PTFE tubing, the combusted effluent is divided into sections by gas bubbles and these fractions are stored for counting) has been used[209,214]. All of the combined methods increase the time available for counting and enhance the precision and sensitivity of the radioassay, which are the advantages of discontinuous methods.

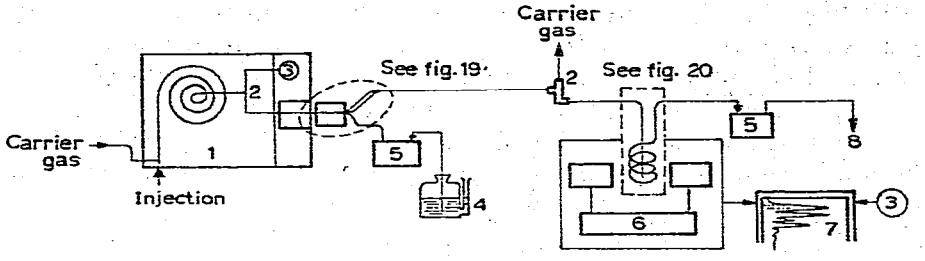


Fig. 2. Scheme of RGC system with continuous detection of radioactivity by liquid scintillation. 1 = oven of gas chromatograph; 2 = splitter; 3 = flame-ionization detector; 4 = container with scintillator solution; 5 = peristaltic pump; 6 = liquid scintillation spectrometer; 7 = two-pen recorder; 8 = fraction collector. Reproduced from *J. Chromatogr.*, 76 (1973) 14 (ref. 336).

It is obvious that many methods are possible in RGC. Those given in Table 2 are selected methods from existing combinations, depending on the type of problem to be solved, on the experience of the analyst and on the instrumental capabilities.

3. CHEMICAL PROBLEMS

3.1. Preparation of the sample for radio-gas chromatography

Only a few applications of RGC do not require prior preparation of the sample,

which can be directly injected; examples are the analysis of radioactive gases (H_2, CO, CO_2 (refs. 104 and 105), CH_4, C_2H_2, C_2H_4 (ref. 3), etc.), hydrocarbons[142] and other volatile substances that do not contain non-volatile components. If the latter are present they are retained on the column and can interact with the solutes, thus changing the retention characteristics[389a]. Also in the preparation of polar substances for GC by derivatization is it desirable and advantageous to remove substances that do not form volatile derivatives and to prevent the column packing from contamination, *e.g.*, by sugars from lipid extracts of algae and their decomposition products after methylation in the case of the analysis of higher fatty acids[260] (vacuum sublimation of methyl esters). The purification step is often accomplished by liquid chromatography[194a] or thin-layer chromatography. The sample clean-up prevents unknown radioactivity or background enhancement being recorded.

Many reviews on derivatization in GC[47a,113,311a], silylation[266], GC of amino acids[176] and fatty acids[228,80], etc., have appeared. The following summarized criteria for the derivatization step were given[139a]: (1) the derivative should be formed simply, without rearrangement or structural changes; (2) the derivatization reaction should go 95–100% to completion; (3) the derivative should have a suitable volatility, retention time and ability for concentration; (4) the derivative must be stable with respect to time, temperature and column packing. The yield of the reaction is a critical point, depending on the purpose of the analysis, *e.g.*, for checking radiochemical and chemical purity a 100% yield is desirable and no side-reactions should occur[255]. Obviously, it is not easy to satisfy all of these requirements in every case.

Control of the derivatization by means of thin-layer chromatography was useful in the RGC of ^{14}C-labelled fatty acid methyl esters and amino acids[259] (Fig. 3). The example of the RGC of ^{14}C-labelled amino acids demonstrates the problems of derivatization. Side-reactions in the formation of trifluoroacetylated *n*-butyl esters[309a], especially of carrier-free preparations, were caused by impurities (probably aldehydes) at concentrations of *ca.* 10^{-3}% and led to "radiochemical impurities" of 20–30% (Fig. 4) in spite of a good reagent blank, proposed as a control of contamination[306a]. The extraneous peaks could be suppressed by using larger amounts of car-

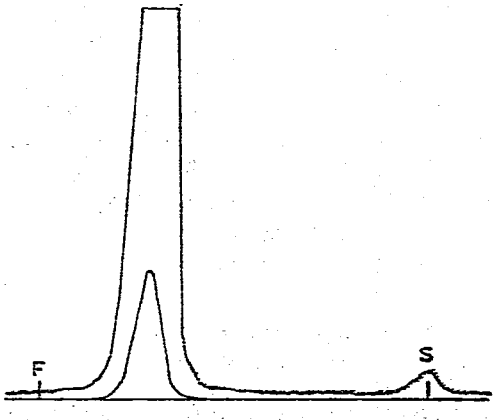


Fig. 3. Thin-layer chromatogram of [^{14}C]alanine as bis(chlorodifluoromethyl)-1,3-oxazolidinon-5-one. Solvent, chloroform; plate, Silufol; scanner sensitivities, 300 and 3000 c.p.s. From Matucha[255].

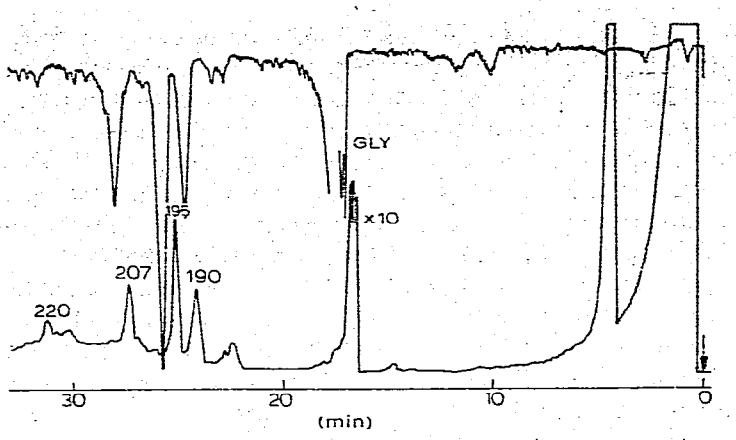


Fig. 4. Radio-gas chromatogram of carrier-free preparation of [^{14}C]glycine after butylation and acylation[309a]. The main peak corresponds to N-TFA-butyl ester. Programmed-temperature GC on OV-225 column. From Matucha[255].

rier[255]. Correct results were obtained after the choice of an appropriate derivative (Fig. 5).

Attention should be paid to the choice of solvents and derivatization reagents; their volatility should be markedly higher than that of the derivatives, as the tailing influences the evaluation of the mass record and could make reagent and solvent venting, sparing copper oxide in the combustion unit[251], impossible.

Historically, RGC is closely connected with reaction gas chromatography (loosely defined as a combination of chemical reactions with GC). Kokes *et al.*[223] investigated the hydrocracking of hydrocarbons mixed with [^{14}C]ethylene on a pre-column with various catalysts. The chemical reaction can be carried out at any of four positions: ahead of the injection port, in a pre-column reactor, within the chromatographic column or in a post-column reactor. Reaction GC involves pyrolysis, hydrogenation, subtraction, esterification, silylation, saponification and other reac-

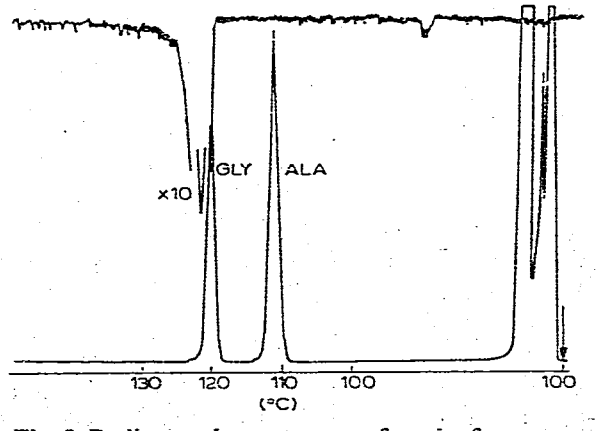


Fig. 5. Radio-gas chromatogram of carrier-free preparation of [^{14}C]glycine in the form of bis(chlorodifluoromethyl)-1,3-oxazolidinon-5-one. Non-radioactive alanine added as internal standard. Programmed-temperature GC on OV-225 column. From Matucha[255].

tions. This technique can help in many difficult separations and identifications as well as in establishing structures. For the GC of labelled compounds, interesting applications of the pyrolysis of [^{3}H]hexene-1 and [^{14}C]hexene-1[342], nucleosides and nucleotides[394,395], degradation of fatty acids[276] and investigation of the degree of uniformity of labelling[191] have been reported and the work of Drawert and co-workers[107,110,391] is of interest.

3.2. Reactions in the gas chromatographic system

One of the potential errors in RGC and GC in general are losses due to retention or decomposition of the solute in the chromatographic system, which can be divided into three parts: the vaporizer, chromatographic column and detector. The breakdown of N-TFA-*n*-butyl esters of amino acids occurred in a heated metal injection port[231a,360a]. Injection-port reactions in the analysis of alkylsilyl derivatives of nucleosides have been described[305], and some trimethylsilyl (TMS) derivatives were decomposed on column[170,354a] or by metal parts of the apparatus[139b,216a,278a,343a]. Therefore, on-column injections and all-glass systems can be recommended. Attention must be paid to the carrier gas, as trace amounts of oxygen and moisture can be sources of errors[228,389a]; the breakdown of TMS-histidine by moisture in a freshly installed septum has been reported[354b].

Interactions with column packings have been reported many times; the stationary phase selected should not be capable of reacting with the compounds being analyzed, and impurities and breakdown products must be also considered. Reactions with solutes, if complete, result in the disappearance of peaks, which may remain unnoticed. If the reaction is slow, a poorly shaped peak can be obtained. Impurities in the sample can also cause reactions of the solute[389a].

Meinertz and Dole[261] estimated the pattern of dispersion of chromatographically pure methyl [^{14}C]palmitate; in Apiezon and EGA columns the distribution of activity was not Gaussian and the background remained elevated for several days. The results indicated that a pure substance emerges from a column over a wider interval than is shown from the record of the mass output. Tailing of fatty acid methyl esters was also observed on an SE-30 column[153]. Chemical reactions may occur during passage through a GC system; dehydration of [2-^{14}C]-2-methyl-2-undecanol and isomerization of [^{14}C]methylenecyclohexane to [^{14}C]methylcyclohexene have been reported[147]. Jansen and Baglan[190] followed the recovery of silylated ^{14}C-labelled glucose, fructose, sucrose, glycerol, cholesterol and stearyl alcohol, which was considerably less than quantitative. The recoveries from Carbowax and SF 96-50 columns of 10–80% were reproducible also after re-chromatography, and the retention of TMS-glucose was significantly higher at the injection end of the column. On the contrary, ^{14}C-labelled TMS-pyrimidine bases were found to be more stable[308]; the retention on the column was not determined, but the overall recovery of TMS-uracil and TMS-thymine was found to be higher than 96% when a sufficient excess of silylating reagent was used. Errors in the GC analysis of polyunsaturated fatty acids[228] and errors due to trans-esterifications of fatty acid esters on columns with polyester stationary phases[293], etc., are well known. Only chemical inertness of the whole chromatographic system, particularly of the column packing, can lead to sufficiently high recoveries, which should be independent of the sample size.

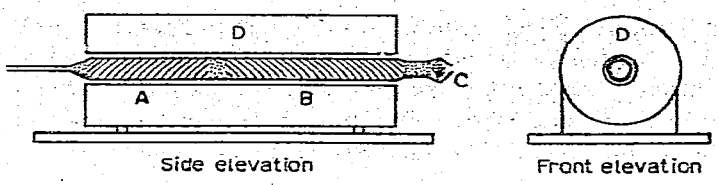


Fig. 6. Combustion tube assembly for conversion of column effluent. A = Copper oxide powder; B = iron fillings; C = spherical joint; D = furnace. Reproduced from *Anal. Chem.*, 35 (1963) 516 (ref. 188).

3.3. Conversion of the effluent for counting

The decrease in sensitivity of a TCD with increasing temperature and the possibility of condensation of substances with high boiling points led to a new approach in GC, namely conversion of the GC effluent into carbon dioxide by combustion[150,250] (used in elemental analysis[295]) or by hydrocracking[431]. Both methods have been used succesfully in RGC; they avoid the difficulties caused by changes in the composition of the gas being counted in radioactivity detection.

The combustion method was applied for the first time by Cacace and co-workers[49,58,60,64] and subsequently by James and Piper[187,188]. They used copper oxide and, in the same tube, converted water of combustion into hydrogen with iron filings or steel-wool. A scheme of the furnace is shown in Fig. 6. Hydrogen is suitable for mass detection by TCD[188] and also for monitoring tritium[47]. The combustion is performed at 650–800°, but the higher temperatures are not convenient because of reduc-

TABLE 3

HYDROCRACKING CATALYSTS USED FOR EFFLUENT CONVERSION

*Catalyst composition**	*Operating temperature (°C)*	*Compounds converted*	*Ref.*
Raney nickel	420	Esters of lower carboxylic and linoleic acids, alcohols, CO_2 (^{14}C labelled)	108, 112, 161
Zn–CoO–NiO–Fe–V_2O_5–St (60:40:20:140:10:27)	620–640	Hydrocarbons, esters of carboxylic acids, bromo, iodo and nitro derivatives, TMS-carbohydrates (^{3}H and ^{14}C labelled)	347
Zn–CoO–NiO–Fe–V_2O_5–Ch (49:16:13:114:8:22)	660	Fatty acid methyl esters (^{3}H and ^{14}C labelled)	35
Zn–CoO–G69–V_2O_5–St (30:20:20:5:8)	600	Benzene, toluene, butanol, methyl benzoate, acetone, dioxan (non-radioactive)	398
Zn–CoO–G69–V_2O_5–NiO–St (25:20:20:5:8:7)	600	Benzene, toluene, butanol, methyl benzoate, acetone, dioxan (non-radioactive)	398
G69–Ch (1:3)	500–700	Fatty acid methyl esters, hexadecane, steroids (^{3}H and ^{14}C labelled)	225

* Abbreviations: St = sterchamol; Ch = Chromosorb; G69 = zirconium-activated nickel catalyst G69, Girdler Südchemie, Munich, G.F.R.

tion of CO_2 and reaction of CuO with quartz; thus 720° was recommended as the optimal temperature[212]. Oxidation of iron caused "memory" effects with tritium (adsorption of ^3H_2O)[188,212], but the addition of hydrogen gas in the iron part of the conversion tube can eliminate this effect[212]. An auxiliary column for H_2–CO_2 separation[54,55] or adsorption of water or of CO_2 (refs. 188 and 372) for distinguishing between ^3H and ^{14}C was used. The combustion method was used in most instances for the RGC of ^{14}C-labelled compounds with flowthrough radioactivity detection[108,112,211,212,225,251,372]; Co_3O_4 (refs. 101 and 162) and an FID as the combustion chamber[91,92,135,310] have also been applied.

Hydrocracking of organic compounds is widely used in petrochemical processes. Nickel, iron, cobalt and other elements are generally used for hydrogenation, and oxides of aluminium, silicon, chromium and other compounds for cracking. This approach has often been reported in RGC[35,108,112,225,347,398]; its main advantage is its general applicability to both ^3H- and ^{14}C-labelled compounds. Exchange of the catalyst after 60–80 h of operation is recommended[347], so that the application of the catalytic procedure is not so significant an advantage. The temperature for each catalyst and class of compounds to be converted must be tested. A survey of catalysts used in the RGC of labelled compounds is given in Table 3. The hydrocracking reaction (and dead volumes) influences the peak shape (Fig. 7). Hydrocracking is recommended for smaller molecules, combustion for larger ones[398].

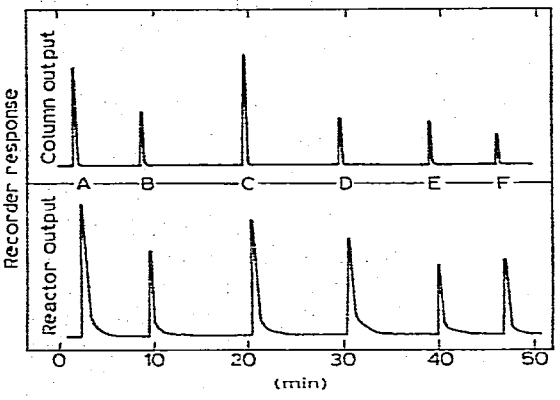


Fig. 7. Influence of the conversion of the effluent on the peak shape. Upper curve, FID response of the effluent without conversion; lower curve, FID response of the main part of the effluent after hydrocracking. A = 1.2 μl of toluene; B = 1.0 μl of butanol; C = 1.0 μl of benzene; D = 1.0 μl of methyl benzoate; E = 1.1 μl of acetone; F = 1.0 μl of dioxan. Reproduced from *Chromatographia*, 2 (1969) 7, by kind permission of R. Tykva[398].

3.4. Isotopic effects in the gas chromatography of ^3H- and ^{14}C-labelled compounds

Considerable changes in retention volumes resulting from extensive substitution of deuterium and tritium for hydrogen in organic compounds[418] (reported earlier for radioactive inert gases by Glückauf[144a]) drew attention to isotopic effects in RGC. Broadly defined as any difference in the chemical or physical behaviour between two compounds that differ only in isotopic composition, the isotopic effect in GC has the

character of a secondary effect (chemical bonds are not broken or formed during the GC process). Thus the identification of labelled compounds is rendered more difficult (isotope fractionation must also be distinguished from radiochemical impurities); however, on the other hand, the differences in retention volumes of isotopic species can be utilized for their separation[76,77], *e.g.*, of tritiated methanes[66], olefins[234] or for enrichment of tritium[256,297] and carbon-14[22]. Particular attention has been paid in GC to smaller molecules labelled with stable isotopes, the thermodynamic properties of which could easily be measured[237]. Radioactive substances of high activity can influence the chromatographic process by evolving heat.

Three categories have been considered in the GC of isotopic substances[90]: separation of isotopic molecules, separation of chemical compounds labelled with one or more radionuclides and separation of radioactive materials from other elements, compounds or other matrix materials; the first two categories are similar and related to the GC of labelled compounds. The GC of smaller isotopic molecules has been reviewed by Cram[90], while isotope fractionation during analytical separations of large molecules was discussed by Klein[220].

Two examples of the isotopic effect in the "usual" GC of labelled compounds can demonstrate the phenomenon. Sgoutas[339] observed the fractionation of a mixture of ^{3}H- and ^{14}C-labelled fatty acid methyl esters, and found that the ^{3}H:^{14}C ratio decreased during the appearance of peaks of stearic, oleic and linolenic acid on SE-30 and DEGS columns. Similar results obtained in the GC of steroids labelled with ^{3}H and ^{14}C can be explained by tritium enrichment in the first fractions of the peaks (an increase in the vapour pressure of ^{3}H molecules was predicted), but with [4-^{14}C]-testosterone [^{3}H]acetate the ^{3}H:^{14}C ratio also increased in the tail, which remained unexplained[218]. VandenHeuvel *et al.*[402] did not observe fractionation of TMS-[^{13}C]-amino acids obtained from algae grown in a $^{13}CO_2$ atmosphere, but a biosynthetic isotope effect[254,287,306] was noted (non-homogeneous distribution of ^{13}C, which can be explained, according to our experience, by precursor–product interactions as with ^{14}C-labelled fatty acids[183,256]). Preparations of high specific activity obtained by chemical reactions or biosynthesis, as they are nowadays produced, can have shifted retention volumes, however. More efficient columns for the fractionation of isotopic molecules have been used; TMS-sugars have been separated on packed columns of 40,000 plates[29] and open-tubular column chromatography, *e.g.* of methanes[46,66], has been reported.

4. METHODS OF DETECTION OF RADIOACTIVITY

Radioactivity detectors offer the advantage of high sensitivity and selectivity when used in GC; naturally, the use of radioactive compounds is a prerequisite. Exceptionally, a radioactivity detector can be used for detection of some kinds of non-radioactive compounds[245]. If the effluent fractions emerging from GC column are trapped, they can be counted by any appropriate method used for usual radioactivity measurements. Liquid scintillation counting is most commonly employed, but Geiger–Müller (GM) tubes[185] and ionization chambers[415] have also been used. The discontinuous method of analysis has the advantage that collected fractions can be counted with conventional equipment.

For continuous flow-through counting of the effluent, proportional and GM counters, ionization chambers and scintillation methods have been used and the ap-

TABLE 4

SURVEY OF RADIATION DETECTORS USED IN RGC

Type of detector	*Detector volume (ml)*	*Detection efficiency (%)*		*Background (cpm)*	*Ref.*
		^{3}H	^{14}C		
End-window GM tube	—	0	5–15	10–15	182
Internal flow proportional counter	10	30	88	35	35
	10	98.4 ± 3.5	102.4 ± 3.6	50	225
	10	—	95	3*	368
	12	64.0 ± 1.5	94.5 ± 1.0	1*	349
	20	—	78 ± 2	80	8
	20		—	40	233
	22	61.5	92.5	40–50	372
	27	—	80	60	251
Window flow proportional counter	25.5	0	20	72	413
Ionization chamber	275	75**	33**	*ca.* 700***	281
	275	—	—	*ca.* 200***	350
Plastic scintillator (coiled tube)	0.27	—	58.3	—	137
	—	—	60	—	326
Crystalline anthracene	—	11–23	62–86	30–50	211
Liquid scintillation flow cell (integral)	—	15–20§	40–50§	60	300
		25§§	80§§	60	300
Liquid scintillation flow cell (continuous)	1.4	27	85	—	336

* Gamma shielding and guard counter with the anticoincidence circuit used.
** Signal is also proportional to average radiation energy (see eqn. 1).
*** Background corresponding to the noise current measured.
§ Simultaneous measurement of both isotopes.
§§ Discriminator settings for measurement of individual isotopes.

plication of a semiconductor detector[396] in RGC has also been reported[397]. A survey of the detectors used in GC of ^{3}H- and ^{14}C-labelled compounds is given in Table 4. It is noteworthy that the sensitivity of the mass spectrometer also permits the detection of smaller amounts of radioactively labelled compounds of higher specific activities (of higher isotopic abundances)[146a].

4.1. Discontinuous methods

The approach involving radioassay after fractionation of the effluent from a column is especially convenient for low levels of radioactivity. There are similarities with the isolation of substances from a stream of carrier gas, and preparative GC has also been employed for the preparation of labelled compounds[19,147,277,298,319,401,403]. The outlet systems of preparative instruments[174] and fraction collectors[85,393,426] have been described.

The principle of the methods involves condensation of the vapour of the substance (effluent fraction) from the stream of carrier gas in an exchangeable flow-through collection device. Vapours in the GC effluent tend to form aerosols as they enter a zone of markedly lower temperature. Therefore, the safest method of trapping is the combination of condensation with sorption of aerosols on a sorbent or the use

of a short section of a GC column containing a solid support coated with a non-volatile liquid stationary phase and maintained at room temperature. Sometimes only cooled glass traps have been used[10,11,23,56,89,99,126,145,154,169,288] or PTFE tubing[27,43,288,313]. Traps containing a cooled solvent (ethanol[181], methylene chloride[222] or toluene[283]) or cotton-wool (purified, occasionally wetted)[32,146,186,261,299], cartridges with glass-wool[28,86,156], tubes containing charcoal[95,158,159], fritted filters[328], Millipore filters[153], concave folded glass-fibre paper[69], molecular sieve 5A, glass beads[81,378], Corning porous glass[198,203], uncoated GC supports[99] or coated supports[106,173,200] and cartridges with silicone-coated anthracene, convenient for scintillation measurements[141,171,213,215,411], have been described. Condensation of vapours together with carrier gas (CO_2) has also been reported[172]. GC fractions are collected in vessels containing a scintillation "cocktail"[33,117,177,227,263,265,376] or the corresponding CO_2, after combustion, is absorbed in ethanolamine[40,122,355], hyamine[390] or sodium hydroxide solution[84,92] for subsequent liquid scintillation counting (Fig. 8). An example of a simple collection device is shown in Fig. 9. Various similar traps have been used elsewhere[120,127,201,202,241,311,320,371].

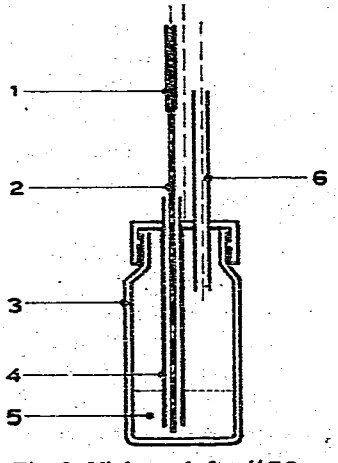
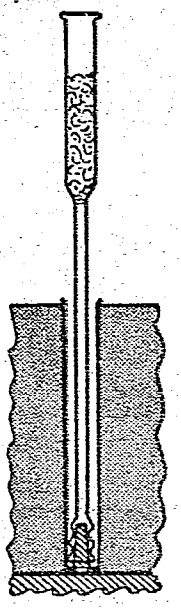


Fig. 8. Vial used for $^{14}CO_2$ trapping in ethanolamine and subsequent scintillation counting. 1 = Silicone rubber tube connecting reactor outlet with the vial; 2 = stainless-steel capillary; 3 = scintillation vial; 4 = glass mixing tube; 5 = trapping solution (3 ml of monoethanolamine in methyl Cellosolve, 2:1). Reproduced from *J. Chromatogr.*, 91 (1974) 507, by kind permission of the authors[163].

Another approach, avoiding aerosol formation, involves collection systems or devices with a temperature gradient which is useful in breaking down aerosols. Axial[226a, 235a] and radial temperature gradients[276a] have been used, while Magnusson[246a] used combined radial and axial gradients in investigating the recovery as a function of the temperature gradient and of the boiling point for substances with b.p. 151–287° at 760 mmHg. Electroprecipitation[226a] was not recommended[246a].

For some purposes, quantitative trapping and isolation of separated labelled compounds are not required (determination of specific activity, isotope dilution method, identifications), but quantitative work requires much effort and is difficult or impossible for some types of compounds or derivatives, such as some TMS derivatives, polyunsaturated long-chain fatty acids and similar labile substances that are sensitive to oxidation or hydrolysis or are otherwise reactive. A very important point in every



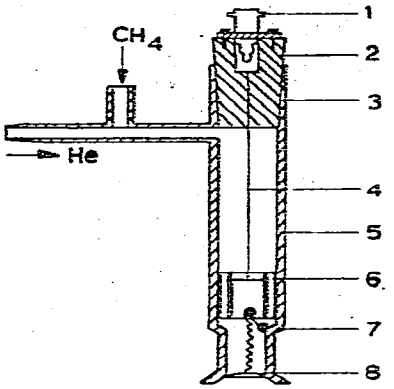


Fig. 9. Unit for collection of gas chromatographic fractions. Reproduced from *J. Lipid Res.*, 3 (1962) 141 (ref. 261).

Fig. 10. Flow-through proportional counter. 1 = Connector; 2 = PTFE plug; 3 = taper joint; 4 = 0.002-in. stainless-steel wire; 5 = brass wall; 6 = Lucite stop (grooved to permit gas flow); 7 = spring; 8 = spherical joint. Reproduced from *Anal. Chem.*, 30 (1958) 903.

discontinuous method is the verification or determination of the recovery of the overall procedure.

The main advantage of the collection method is the possibility of long-term counting, permitting work with low levels of radioactivity, and the possibility of repeating the counting. In some instances the recovery of pure substances for further analysis is possible.

4.2. Continuous methods

4.2.1. Proportional and Geiger–Müller counters

Counting tubes used in RGC for the continuous monitoring of the effluent can operate in the proportional or GM region and are constructed with or without a window. Windowless counters can be employed with advantage for both radionuclides[271], the counting efficiency for carbon-14 being as high as 100%, while for tritium it is 60% (Table 4); external counting (with window) can be used only for carbon-14. The applications of GM counters are limited by the long dead time of the tubes.

In the first applications of RGC, mostly GM counters were used[223], in connection with a flow cell[25,312] or as a flow-through detector[236,334,335]. Condensation of the effluent in a flow cell cooled by liquid nitrogen combined with a window GM counter was reported[36], and an integral record of the activity was obtained.

Because of their high detection efficiency and low background, proportional counters with internal flow have often been used in the RGC of ^{3}H- and ^{14}C-labelled compounds[66,93,94,101,178,225,251,344,359,363,372]. The windowless flow-through proportional counter was proposed by Wolfgang and MacKay[424] and Wolfgang and Rowland[425]; a similar counter had already been used previously for counting carbon-14 during

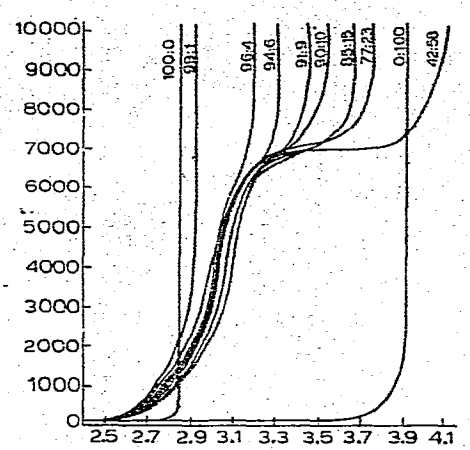


Fig. 11. Counting characteristics of gas-flow proportional counter for various hydrogen to methane ratios obtained with external source of radiation. Counting rate (ordinate) in cpm, voltage (abscissa) in kV. Reproduced from *Angew. Chem.*, 75 (1963) 720 (ref. 108).

distillation[423]. A large number of original designs have been reported[187,188,251,349,422]; they differ mostly in geometry, volume and hydrodynamic properties. An example is shown in Fig. 10. A small-volume proportional counter for open-tubular column chromatography has also been reported[9]. The sensitivity of a flow-through proportional counter changes appreciably during the passage of the effluent through the detector as a result of change in the composition of the gas being counted, *i.e.*, counting characteristics[108,233,238]. This effect is demonstrated in Fig. 11. The influence of temperature was established by Lieser *et al.*[238]; as the temperature of the counter is increased, the length of the plateau decreases and moves to higher voltages (Fig. 12) and even at 200° it is 50 V long. Coincidence losses at higher counting rates[233] can be minimized by operating the counter near the beginning of the plateau in the region of short resolution times. Composition changes in the counter with the passage of a

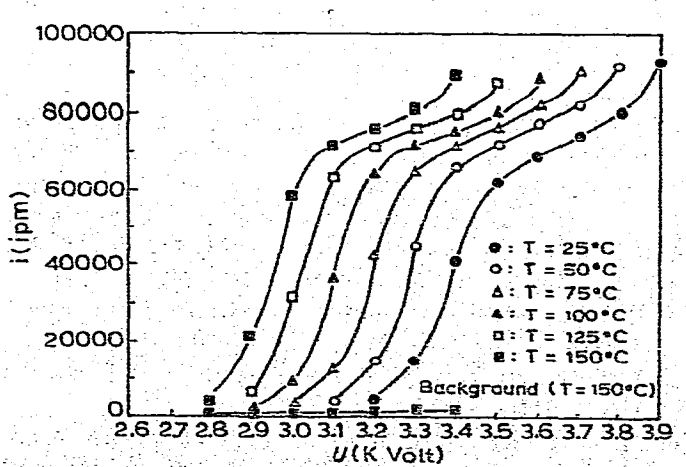


Fig. 12. Influence of temperature on counting characteristics of gas-flow proportional counter. Counting gas, methane (100 ml/min). Reproduced from *Z. Anal. Chem.*, 191 (1962) 108 (ref. 238).

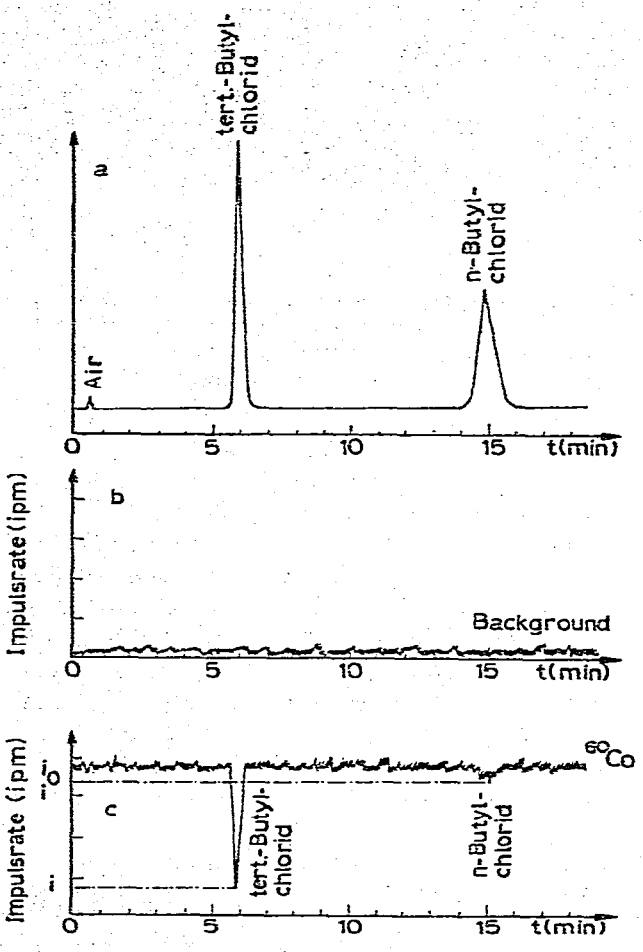


Fig. 13. Examination of influence of effluent on counting properties of gas-flow proportional counter. Injected: 30 μl of *tert.*-butyl chloride and *n*-butyl chloride (1:1). Carrier and counting gas, methane (60 ml/min). Reproduced from *Z. Anal. Chem.*, 191 (1962) 110 (ref. 238).

large amount of substance in the effluent lead to an increase in the organic to carrier gas ratio, which moves the plateau to a higher voltage, with a consequent drop in the counting efficiency. For this reason, contrary to the case with coincidence losses, operation of the counter at higher voltages makes the drop much less likely. The opposite effect, the shift of the plateau to the left due to changes in gas composition, can cause "elevated" activity or "pseudo-activity" (detection of non-radioactive substances). This effect was reported for aromatic compounds containing a halogen or a nitro group[238,347]. The correct functioning of the counter can be tested by means of an external radioactive source and a non-radioactive substance[238,256] (Fig. 13). Counting characteristics have been established for various gas mixtures, including methane–helium[425], propane–helium[233], methane–argon[9], methane–hydrogen[210] and isobutanol–argon[334,335]. The use of conversion of the effluent and of a suitable carrier gas, *e.g.*, carbon dioxide or carbon dioxide–argon[187,188,368], avoids the above dif-

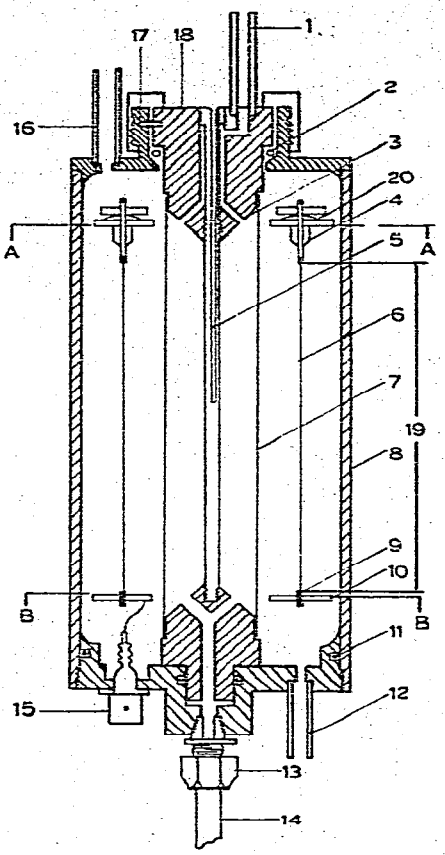


Fig. 14. Cross-section of window flow-proportional counter. 1 = Active gas inlet; 2 = O-ring; 3 = holes connecting gas inlet with active volume of the counter; 4 = spring-loaded PTFE insulators; 5 = hole for insertion of calibration source; 6 = 0.002-in. stainless-steel wire; 7 = 0.970-in. diameter Mylar tube; 8 = $2\,^{15}/_{16}$-in. O.D. brass tube (wall 0.125 in.); 9 = 0.022-in. stainless-steel capillary tube (0.005-in. I.D.); 10 = 2.5-in. I.D. disk of printed-circuit board; 11 = O-ring; 12 and 16 = counting gas inlet (or outlet); 13 = stainless-steel Swagelok connector; 14 = active gas outlet; 15 = connector; 17 = location pin; 18 = counter insert; 19 = active length of the counter; 20 = 0.010-in. U-shaped beryllium–copper tension spring. Reproduced from *Anal. Chem.*, 39 (1967) 276 (ref. 413).

ficulties due to changes in the counting characteristics. Also, a sufficiently high content of quenching gas should be added after the conversion for the same reason.

For ^{14}C-labelled compounds, window flow-proportional counters[206,363,413,424] (Fig. 14), which have the advantage of stable and reproducible counting characteristics, have also been employed. The detection efficiency is decreased to 20%[413], however, and ^{3}H cannot be counted at all.

Sensitivity due mainly to the background level, which increases with temperature, has been reported at the 10^{-11} Ci level[233,398]. Using equipment[349,368] containing a plastic scintillator, an anti-coincidence guard counter and a gamma shield (Fig. 15), the background has been measured at levels of 1 cpm[349] and 3 cpm[368]. The background is also influenced by the material used for shielding (by its age), and the quality and characteristics of a proportional counter depend on the geometry and performance of the detector; the thin anode wire is also an important component.

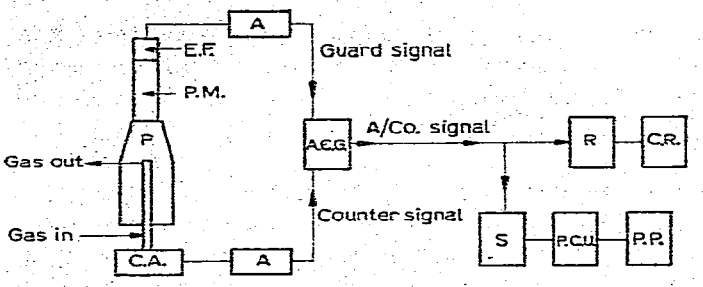


Fig. 15. Sensitive, low-background detector assembly for RGC comprising gas flow-proportional counter, plastic phosphor, anti-coincidence guard and graded gamma shield. P = Plastic scintillator; A = amplifier; R = rate meter; S = scaler; C.A. = charge amplifier; C.R. = chart recorder; E.F. = emitter follower; P.M. = photomultiplier; P.P. = parallel printer; A.C.G. = anticoincidence gate circuit; P.C.U. = printer control unit. Reproduced from *J. Chromatogr.*, 38 (1968) 26 (ref. 349).

4.2.2. Ionization chambers

For the detection of low-energy β-radiation from ^{3}H and ^{14}C, ionization chambers are convenient. The first application was reported by Wilzbach and Riesz[418], and such chambers are now in common use[46,58,59,61,119,139,309,317,415]. They can be heated, so that their application is wide[102,114,280,281,350,381–384,418,427]. Various designs have been reported[14,255,280,321,329,349], and two examples are illustrated in Figs. 16 and 17. Gas-phase counting with ionization chambers may give rise to "ghost peaks" when small amounts of unlabelled compounds pass through the detector[212,279,321]. These peaks can be partially or wholly eliminated by maintaining the chamber at a higher temperature[279], by using a relatively large-volume detector in conjunction with a large flow of diluting gas[279,350,419] or by conversion of the effluent[58,60,205,212,415,419].

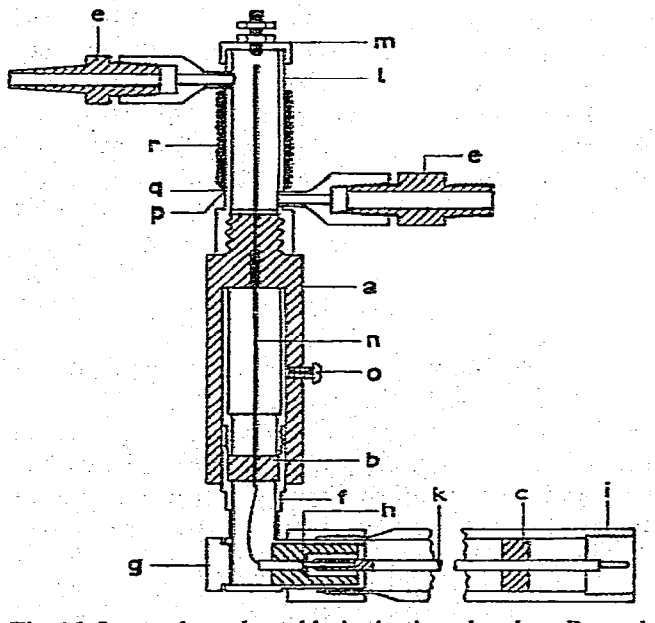
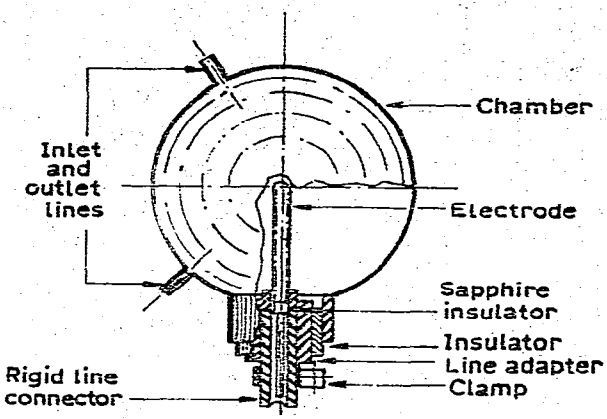


Fig. 16. Low-volume heatable ionization chamber. Reproduced from *J. Amer. Oil Chem. Soc.*, 38 (1961) 635 (ref. 114).



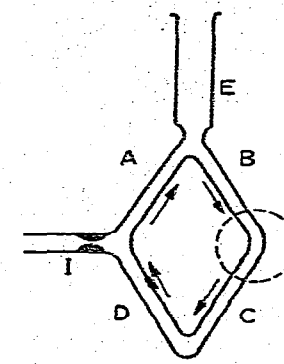


Fig. 17. High-temperature ionization chamber (volume 275 ml). Reproduced from *Anal. Chem.*, 35 (1963) 1576 (ref. 281).

Fig. 18. Liquid scintillator flow cell with integral record of radioactivity. Solution of scintillator circulates in the circuit consisting of tubes A, B, C and D. Carrier gas with radioactive vapour enters through capillary inlet I, the gas bubbles rise in tube A (the liquid circulates in the direction of the arrows) and escape through the chimney E. The dotted circle indicates the position of the photomultiplier. Reproduced from *J. Lipid Res.*, 1 (1959) 30 (ref. 303).

The current (I) measured from a radioactive source in an ionization chamber under steady-state conditions is expressed by

$$I = \frac{B N e S}{w} \tag{1}$$

where I is current in amperes, N is the number of disintegrations per second from the sample, S is the average energy per disintegration in electron volts, w is the number of electron volts required to produce an ion pair in the ionizable gas being used, e is the number of coulombs per electron and B is the chamber efficiency. However, the carrier gas containing the active fraction, on entering an ionization chamber, mixes completely with the gas already present so that, immediately after entry, exhaust of the active fraction begins and the signal E_t at any time t after $t = 0$ is given by

$$E_t = E_0 \exp\left(\frac{-f t}{v}\right) \tag{2}$$

where f ml/min is the flow-rate and v ml is the volume of the chamber[279]. The chamber response is usually measured with a vibrating reed electrometer.

Although a slightly higher sensitivity can be attained by using proportional counters, the ion chamber technique offers the advantages of being simple, stable, reproducible and usable at higher temperatures; the sensitivity to changes in composition of the measured gas is a disadvantage and conversion of the effluent is to be recommended.

4.2.3. Scintillation methods

For counting ^{3}H and ^{14}C, scintillation detection systems are extremely useful

and have been widely employed, especially in connection with the discontinuous method of activity measurement. Continuous scintillation methods involve either liquid scintillation, a flow cell from a plastic scintillator or organic crystalline scintil lators. The use of inorganic scintillation glass in the RGC of ^{3}H- and ^{14}C-labelled compounds has not been reported.

Scintillation methods have been recommended[205,246,323–325,385–387] for β- (and γ-) counting from the viewpoint of the influence of the chemical composition of the column effluent (which must be taken into account in the usual liquid scintillation[345,390]) and counting efficiency. However, a correction for quenching has not been reported in continuous methods using liquid scintillation without conversion of the effluent[243, 300–303,336]. The detection efficiency of plastic, organic and inorganic scintillators and glasses[327] is doubtless independent of the gas composition; this fact is, however, not so important when conversion of the effluent is carried out. Presumably because of the low counting efficiency, especially for tritium, chemically inert scintillating glass has not been used in GC.

Short sections of tubing containing a solid support coated with stationary liquid phase have often been used as trapping devices (see Section 4.1). Based on the work of Steinberg[362], who suggested the application of anthracene crystals for counting ^{14}C in aqueous solutions, Karmen and Tritch[215] used cartridges packed with anthracene crystals (blue-violet fluorescence grade, coated with 5% of DC 550 silicone oil). In this way, scintillation counting can be accomplished directly, without transfer to scintillation vials. Because of the absorption of radioactive material in the upper layer of the coated scintillator, the counting efficiency is dependent on the position of the cartridge in the photomultiplier compartment. This approach has been reported several times[99,141,171,212,213] and has been combined with combustion methods for continuous counting[170,211,212]. The flow cell was filled with uncoated anthracene, but other crystalline scintillators can be used (*e.g.*, terphenyl[205,212]).

In connection with trapping procedures, conventional liquid scintillation of eluted fractions has been employed many times[153,156,163,261,313,326]. It is advantageous to trap fractions directly in the scintillation solution[177,263,265,376] or in the stream of scintillator, these fractions then being collected[115,376]. When condensation of the radioactive vapour takes place in a flow cell containing scintillation solution (Fig. 18), an integral record of the activity is obtained[243,300–303]. A differential curve is recorded when the effluent is dissolved continuously in a stream of scintillator solution[336]. A

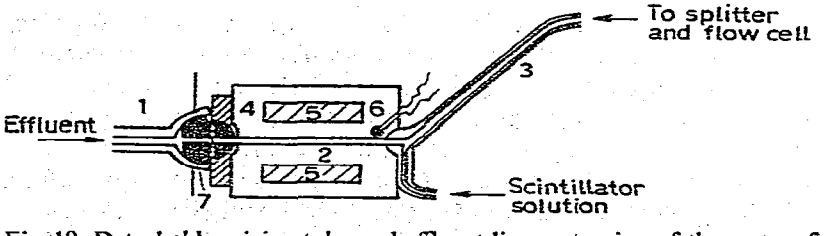
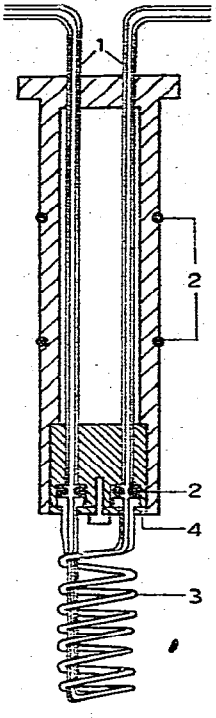


Fig. 19. Detachable mixing tube and effluent-liner extension of the system for continuous detection of radioactivity by liquid scintillation (see Fig. 2). 1 = Glass effluent liner of gas chromatograph; 2 = narrow-bore linear extension of Kovar metal, fused to glass mixing tube and to stainless-steel ball-joint; 3 = glass mixing tube; 4 = aluminium heating block; 5 = heating elements; 6 = thermocouple; 7 = O-ring. Reproduced from *J. Chromatogr.*, 76 (1973) 14 (ref. 336).



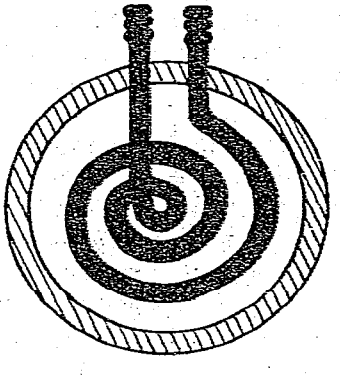


Fig. 20. Liquid scintillation flow cell for continuous measurement of effluent radioactivity. 1 = Stainless-steel pipes; 2 = O-rings; 3 = interchangeable glass cell; 4 = semi-circular collar discs (to press the cell against the O-rings). Reproduced from *J. Chromatogr.*, 76 (1973) 15 (ref. 336).

Fig. 21. Plastic scintillator flow cell. Reproduced from paper 51-47 of Proceedings of Symposium on Organic Compounds Labelled with Radioisotopes, Mariánské Lázne, May 1976, by kind permission of K.-H. Heise[162].

major difficulty of the system constitutes the mixing tube, shown in Fig. 19. Before the solution enters the liquid scintillation flow cell (Fig. 20), the major part of the carrier gas is split off (Fig. 2). The system is also suitable for distinguishing compounds labelled with different isotopes (^{14}C and ^{3}H), as in the case of liquid scintillation of collected fractions[376] or the use of an integral flow cell[300,302]. Another approach using liquid scintillation involves absorption of the combusted effluent in solutions containing sodium hydroxide[84,91,92,209] ethanolamine[122,163,310] or hyamine[170,390] in a common absorption vial (Fig. 8). This method can be combined with continuous counting[122,163].

Plastic containing scintillating substances were originally employed in liquid chromatography[137,326,366] and are not applicable at higher operating temperatures[326]. Several types of flow cells have been described, such as a plastic scintillator tubing coiled in a spiral cell[137,162,321,324,326] (Fig. 21) and a glass spiral capillary packed with spherical particles of scintillator[321]; the plastic scintillator may also form simple flow cuvette[149,322] or one of its walls[366]. The counting efficiency for gaseous $^{14}CO_2$ was reported to be 60%[137,326].

5. DATA OUTPUT AND EVALUATION

5.1. *Types of record*

In the GC of ^{3}H- and ^{14}C-labelled compounds, elution techniques are generally used. Exceptionally, frontal analysis has also been employed[189]. The simultaneous recording of mass and radioactivity is useful but is required or even possible not in every instance (at high specific activities and small sample sizes, some types of analysis follow only the distribution of radioactivity; in the preparative GC of labelled compounds, only a mass detector is commonly employed).

The discontinuous method of detection of radioactivity usually supplies a record of mass; the activity record, if produced, is received in the form of a histogram[116] or has a digital form[361]. Using continuous methods, the record from the rate meter in the form of a differential curve corresponding to the mass trace is usually obtained; sometimes in addition the integral curve is drawn (an example is shown in Fig. 22) or, for methodological reasons, only the integral record is obtained[36,243,300–303] (Fig. 23). An unusual mode of combined record, with a histogram of activity superimposed on the mass trace, has also been reported[334,335]. Development of recording techniques proceeded from the above-mentioned traditional analogue display, *i.e.*, the simultaneous recording of mass and activity curves on the chart of a two-channel recorder or by means of two single-channel recorders, to the digital mode of record[251,373], which is natural in the application of a scaler–timer, electronic integrator or microcomputer-based chromatograph. More sophisticated systems have been used: for the storage of activity data a multichannel analyzer[129] or the data acquisition system of a computer[273,376,412] have been employed, with subsequent data processing.

5.2. *Factors influencing the data output*

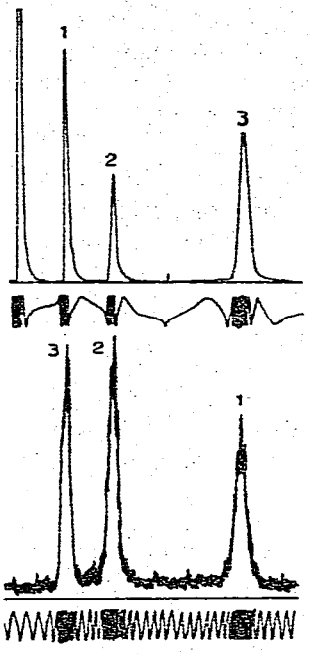
The answer to the most frequent analytical questions, *i.e.*, what substances are present in a sample and how is the activity distributed among the sample components, is based on the record and its evaluation. In RGC usually both mass and radioactivity records are obtained, the latter depending on the RGC system used. There is a time difference between the mass and activity response given by the volume of the activity-detecting part of the system that varies from fractions of a second to a minute. The activity peak is influenced by the dead volumes and also the effluent conversion (Fig. 7) and the "memory effect" (adsorption, condensation, etc.) can contribute to the distortion of the peak shape. This effect can be described by eqn. 2, which gives the perfect mixing of a "sample plug" in the detector volume. The volume of the flow-through activity detector and the flow-rate of the counted gas affect the resolution, accuracy, precision and sensitivity of the method, as expressed by eqns. 3 and 4:

$$V = V_n - V_{n-1} \tag{3}$$

$$c = \varepsilon \cdot \frac{V}{f} \cdot a + b \tag{4}$$

where

c = counts registered during the mean transit through the detector;
V = detector volume;
V_n, V_{n-1} = retention volumes of successive resolved components;



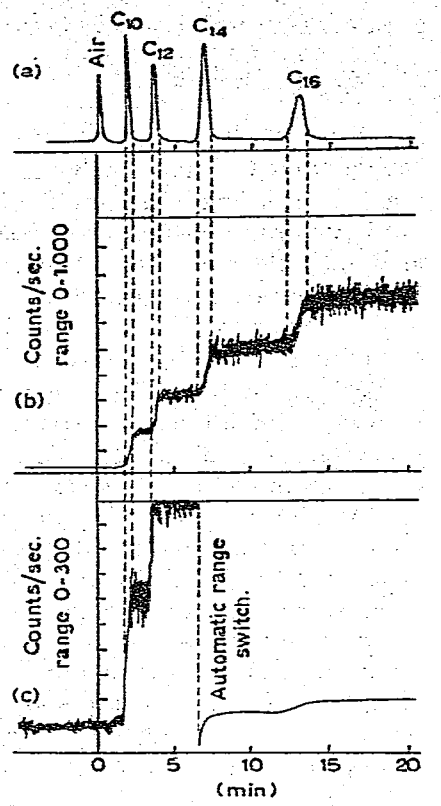


Fig. 22. RGC separation of [^{3}H]dehydroepiandrosterone (peak No. 1, 10 μg and 8000 dpm), [^{3}H]testosterone (peak No. 2, 8 μg and 9000 dpm) and [^{3}H]-Δ^4-androstene-3,17-dione (peak 3, 20 μg and 8000 dpm) on QF-1 column at 240°. Mass detection by FID (one tenth of the effluent); radioactivity detection by gas flow-proportional counter after combustion of the split effluent. Reproduced from *Anal. Biochem.*, 16 (1966) 82 (ref. 372).

Fig. 23. Radio-gas chromatogram of four ^{14}C-labelled fatty acids separated as methyl esters on a PEGA column at 197°. Sample size: 1.25 mg of esters containing 27 nCi of ^{14}C. a, Mass record by gas density balance; b and c, simultaneous records of radioactivity detected by integral liquid scintillation flow cell (Fig. 17) at two sensitivities. Reproduced from *J. Lipid Res.*, 1 (1959) 36 (ref. 303).

ε = counting efficiency;
f = flow-rate of counted gas (if quenching and/or purging gas is used, the detector volume must be correspondingly larger with respect to the resolution desired);
a = activity passing through the detector;
b = background.

The accuracy and precision of the activity determination, especially with low activities, is also limited by the statistical character of the nuclear decay. It seems sometimes to be forgotten that at least 10^4 disintegrations must be registered if a reproducibility of 1 % is required. From eqn. 4, it follows that the activity measurement depends strongly on the flow-rate of the counted gas (experimentally verified recently[217a]), which can change during peak emergence (with larger sample sizes)

and can be measured by a flow-meter[363]. The variations in the counting efficiency (pseudo-activity, quenching) were discussed in Section 4.2; the possibility of the effect of electronic circuitry has also been mentioned[244]. The background level is the limiting factor of sensitivity; it can be decreased to 1 cpm by use of gamma-ray shielding, guard counter and an anticoincidence circuit[349]. For low-level activity measurements, equipment that has not been in contact with substances of higher activity should be used. The digital output from the scaler is more sensitive than the response from a rate meter[349], the sensitivity of which can be optimized by using an appropriate time constant[349].

When a stream splitter is used, its function must be controlled[8,135,211,213,288,372]. The deviations from linearity of splitting can be explained by changes in the viscosity and density of the split gas causing the "suction effect"[259]. In addition, the changes of the hydrodynamic resistance of the branch leading to the radiation detector influence the split ratio[135]. The explanation of the observed deviation in terms of non-linearity of the FID used as a mass detector[338] must be incidentally taken into account. For nanomolar amounts of fatty acid methyl esters, a too laborious calibration of the splitter was suggested[288]. Calibration of the radiation detector[206–208] or of the entire RGC system[238] has been recommended.

5.3. Evaluation of the radio-gas chromatogram

Radio-gas chromatograms usually contain information on the course of the separation and on the contents and activities of individual components of the sample, from which further data such as the qualitative composition, distribution of radioactivity among the constituents of the mixture and their specific activities can be calculated.

A specific feature of the radioactivity record is the low signal-to-noise ratio. This property influences the evaluation and complicates computation methods (which are also used for the evaluation of related activity records, *e.g.*, in flat-bed and liquid chromatography[343,345], or records of spectroscopic measurements[345]). The measurement of low activity levels is critical owing to the poor counting statistics associated with small peak count rates, the estimation of background and its level. The reproducibility of the results increases with the activity injected[42,163]. Difficulties also arise in the resolution of peaks. Methods of quantitative RGC are the same as those of conventional GC[257]. The standard presentation of GC results involves a strip-chart recorder with interpretation accomplished by manual methods, sometimes supported by a mechanical analogue disc or electronic integrator. GC, however, has now advanced from the simple calculation of analogue or digital results to automatic data processing.

Only a few methods concerning the problems involved in the evaluation of radio-gas chromatograms have appeared. The digital recorder display of radioactive and integrated chromatograms has been discussed[373], and a schematic representation of an RGC, data acquisition and data processing system with a flow diagram of the computer program has been given[273]. The application of a multichannel analyzer in the multiscaler mode has been described[129]: the length of the time during which the response of the counter is recorded in a single channel is set by the variable time controller unit; at the end of a pre-set length of time, the contoller unit advances the

computer response to the next channel, the counts from each channel are stored in the memory of the analyzer at the end of the analysis and the results are printed out (or punched) for quantitative information or an analogue record using X–Y plotter can be obtained (the channel number is converted into time). An oscilloscope display in connection with similar systems has also been used[343].

6. APPLICATIONS OF RADIO-GAS CHROMATOGRAPHY TO THE ANALYSIS OF ^{3}H- AND ^{14}C-LABELLED COMPOUNDS

6.1. Applications in the production of labelled compounds

In addition to the preparative GC of labelled compounds[19,66,121,124,147,260,289,298,403,409], often accomplished with analytical-scale amounts, RGC is also employed for quality control of the substances prepared[71,124,343]. Important applications of RGC in the course of the production process also occur, in the control of synthesis or the analysis of intermediates and reaction mixtures, *e.g.*, the Wilzbach method of labelling [1,2,50,138,309], and in biosynthesis[140,256] (an example is illustrated in Fig. 24). The applications are concentrated on volatile substances or compounds that can be easily derivatized, such as hydrocarbons[16,57,62,66,75,143,162,219,242,269,272,318] (see Fig. 25), alcohols[162,269,358], formaldehyde[358], formic and acetic acids[269,357], fatty acids[32,117,168,249,256,258,259,275,341,364,430] and other compounds[27,405]; non-volatile and polar substances, which can be analyzed by other chromatographic techniques, are assayed only rarely by RGC, *e.g.*, amino acids[20,230,255] (Fig. 26) and pyrimidine bases[290]. Biosynthetic methods of labelling may sometimes produce non-uniformly labelled compounds, and therefore methods for the control of the pattern of labelling with subsequent RGC

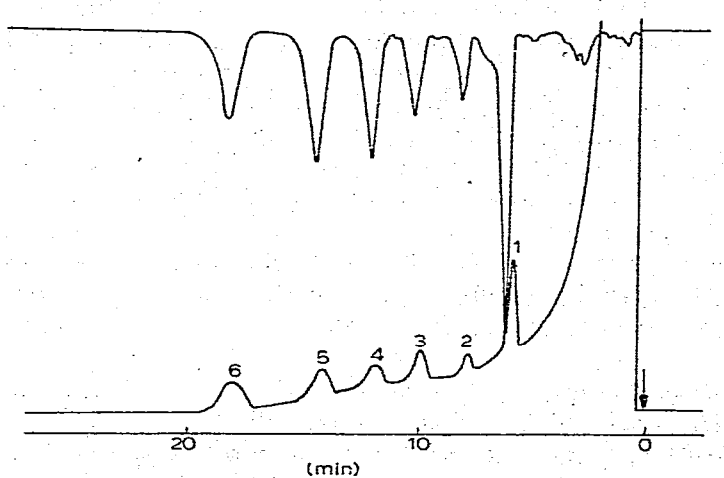


Fig. 24. Radio-gas chromatogram of ^{14}C-labelled higher fatty acids obtained by cultivation of the alga *Chlorella vulgaris* in an atmosphere of $^{14}CO_2$ separated on a BDS column. Upper curve, activity record obtained with gas flow-proportional counter after combustion of the effluent; lower curve, FID response of the effluent split as follows: 1 = 16:0; 2 = 16:2; 3 = 16:3; 4 = 18:1; 5 = 18:2; 6 = 18:3. Reproduced from *J. Chromatogr.*, 91 (1974) 501 (ref. 256).

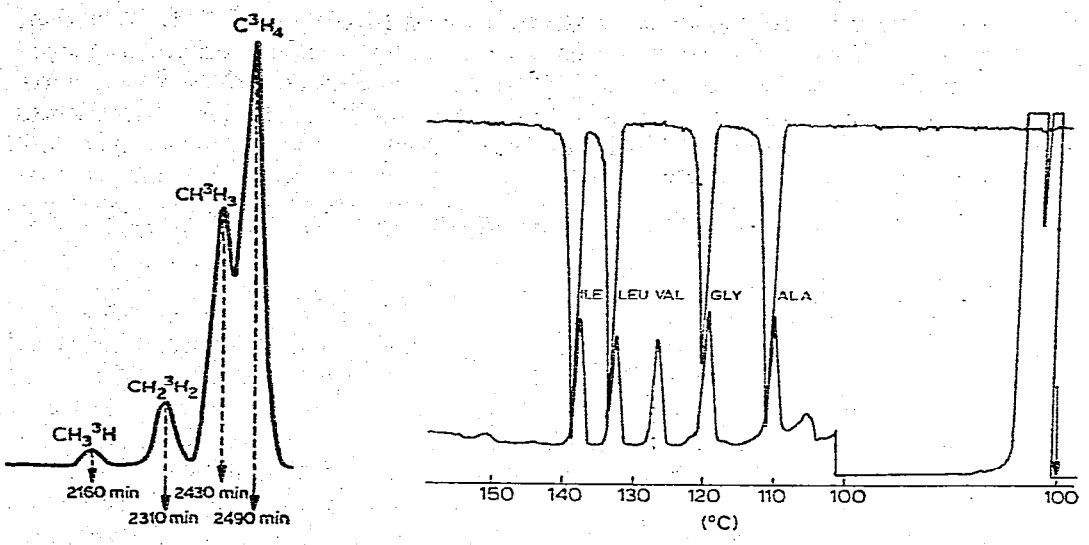


Fig. 25. Separation of tritiated methanes on a 64 m × 0.22 mm I.D. glass capillary column with a layer of active silica. Temperature, 77 °K; flow-rate of helium–nitrogen (3:7), 0.70 ml/min. Detection by 10-ml flow-through proportional counter after addition of methane as quenching gas. Reproduced from *J. Labelled Compd.*, 11 (1975) 319 (ref. 66).

Fig. 26. Determination of the specific radioactivity of carrier-free amino acids labelled with carbon-14 using non-radioactive valine as internal standard. Derivatives used, bis(chlorodifluoromethyl)-1,3-oxazolidinon-5-ones[175]; column, 1% OV-225 on Chromosorb G-HP, 100–120 mesh, 2 m × 2 mm I.D.; temperature programme, 10 min at 100° then heated to 160° at 3°/min; carrier gas, argon (40 ml/min). Upper trace, activity record of gas flow-proportional counter; lower trace, FID response. Splitting ratio, 1:1. From Matucha[255].

analysis have been developed[73,184,256,346]. A high specific activity of the preparations requires an appropriate sensitivity of the mass detector, which also gives information on the chemical purity of the sample being analyzed. The application of RGC to the analysis of molecules that contain labile ^{3}H that is exchangeable with a convenient carrier gas has been reported[407].

6.2. *Applications in "hot-atom" chemistry*

The development of RGC is closely connected with investigations of the Szilard–Chalmers effect[374]. Evans and Willard[124a] utilized GC in the analysis of a mixture of products obtained by the irradiation of *n*-propyl bromide in a reactor and detected more than 20 compounds containing radioactive bromine, formed by interaction of "hot" atoms with the medium. The recoil of ^{3}H obtained by the reactions ^{6}Li(n,α)^{3}H and ^{3}He(n,p)^{3}H has been extensively studied; ^{14}C, produced by the reaction ^{14}N(n,p)^{14}C, forms compounds with lower specific activities.

The ^{3}H recoil reactions studied by means of RGC include reactions with alkanes[79,97,123,129,304,314,354,370,379,423], alkenes[24,194,231,235,247,314], arenes[52,53,332], alkyl halogenides[232,267,286,291,353,375], methylsilanes[96–98], amino acids[348] and methyl isocyanide

[380,412]. More general problems have also been investigated[74,138,148,155,164,315,412]. The possibilities of utilizing ^{14}C recoil reactions as a labelling method, with GC as a separation technique, have been discussed[406,420,421]. Several reactions of ^{14}C with heterocyclic nitrogen-containing compounds[18,134], solid benzene[239], magnesium nitride[132] and gaseous anhydrous ammonia[428] have been reported.

6.3. Applications in organic chemistry and chemical processing

RGC has been used successfully for investigations of various organic reactions, *e.g.*, the analysis of benzoic acid nitration products[11], chlorination of benzene[56], products of methylene reactions in photolytic systems[78], kinetics of hydrogenolysis of mesitylenesulphonic acid and sulphuration of mesitylene[404], oxidation of propylene[195,415], hydrogenation of acetophenone[179], propene[307], the interconversion of cyclohexane and benzene[180] and of propenes[131]. Technological processes have also been studied, *e.g.*, mechanism of the Fischer–Tropsch synthesis[37,333], catalytic cracking[21,165] and other catalytic reactions[100,195,196,268,312] and the sequence length distribution of ethylene–propylene copolymers[125]. A radio-gas chromatogram of the aromatic products from the hydrocracking of a light catalytic cycle oil[8] (Fig. 27) illustrates an application in chemical processing.

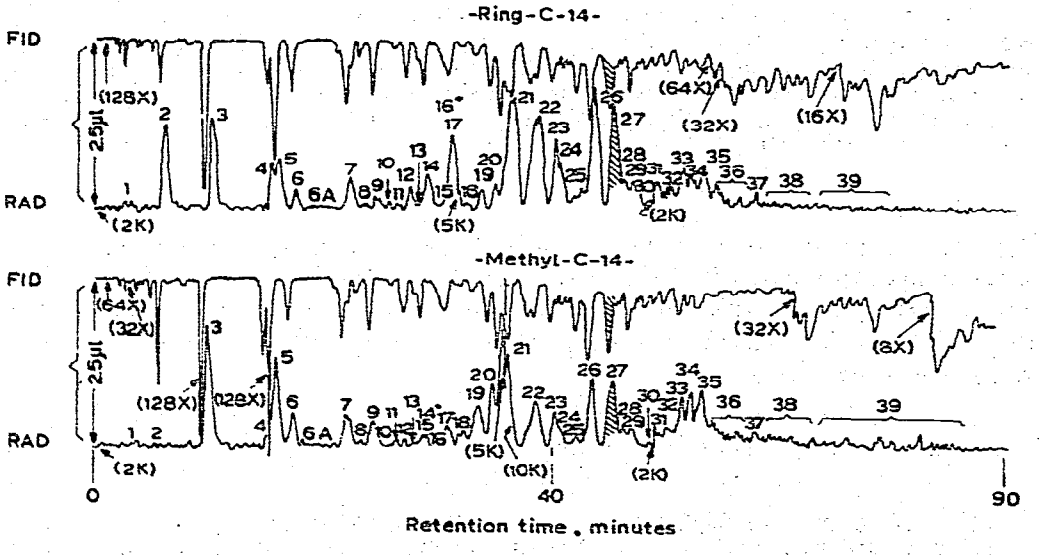


Fig. 27. Example of application of RGC to the determination of petroleum processing products. Comparison of aromatic products from ring- and methyl-labelled feeds of a light catalytic cycle oil containing 2-methylnaphthalene (labelled in the ring or in the methyl group with carbon-14). The upper curve demonstrates the FID response of one tenth of the column effluent. Column, 20 ft. × 1/8 in. O.D. stainless-steel packed with 10% OV-101 on Chromosorb W, 80–100 mesh; carrier gas, helium (30 ml/min); temperature programme, 4 min at 80°, then heated to 300° at 2°/min. Lower curve: corresponding radioactivity record obtained by gas flow-proportional counting without effluent conversion at a helium flow-rate of 60 ml/min (additional helium) with propane (5 ml/min) as quenching gas. Peak No. 27 includes 2-methylnaphthalene. Reproduced from *J. Chromatogr. Sci.*, 12 (1974) 190 (ref. 8).

6.4. Applications in biochemistry and clinical biochemistry

Compounds of biochemical interest can be analyzed by RGC without derivatization only in exceptional circumstances, *e.g.*, fermentation gases[94,282]. For investigations of biosynthesis, intermediates and metabolic pathways, appropriate derivatives must be prepared. Applications of RGC in lipid analysis are well known, *e.g.*, fatty acid methyl esters[7,13,31,34,38,44,99,118,122,140,141,146,151,156,157,161,166,171,183,186,193,199,200,216,217,224–226,256,258–261,265,273,278,284,285,294,313,331,340,365,367,377,378,399,408,416], diglycerides[17,45], triglycerides[42,82] and cholesteryl esters[163]. Many applications in steroid analysis have been described[4,23,26,33,39,41,86,126,145,218,225,229,239a,264,283,299,316,330,359,372]. RGC has also been employed in analyses of amino acids[20,43,67,128,233,248,255,256,336,352,410], pyrimidines [290,308], aroma substances[110,111,391,392], alcohols[224], pyrazine compounds[222], alkaloids[252], carbohydrates[190] and other substances[15,68,69,109,127,136,144,400]. The determination of 5-hydroxyindole-3-acetic acid in cerebrospinal fluid[30], investigations of lipid synthesis in the rat[103], the identification of hormone metabolites in tissue[330], the determination of homovanillic acid in cerebrospinal fluid[351] and of short-chain carboxylic acids in biological material[360], the effect of hormones on fat synthesis in mammary explants of pseudo-pregnant rabbits[369] and investigations of the fat metabolism of new-born domestic mammals[377] have been reported.

7. CONCLUSIONS

More than 20 years have passed since the first report on the GC of labelled compounds. During this period, RGC and labelled compounds have found valuable applications in various fields of chemical research. Investigations of many complicated problems became possible and new aspects of known processes were revealed. Moreover, the tracer technique is a powerful tool and has already contributed (and may contribute still further) to the methodological development of chromatography itself.

RGC methods have specific features, *e.g.*, requirements on derivatization, stability of the solute, inertness of the chromatographic system and problems with the handling of samples, the fractionation of isotopic species activity detection, recording and data processing. Some aspects of conventional GC that are applicable to the GC of labelled compounds have therefore been mentioned in this review. However, the main purpose was to point out the methodological problems and to summarize the current state of the art. Many problems can be solved by RGC in several different ways, but any recommendation of a universal RGC system or of a method convenient for any type of problem is questionable, and for each new application appropriate efforts must be made to work out a suitable method.

RGC plays an important role in the analysis of labelled compounds produced or transformed during various processes and its advantages, *viz.*, sensitivity, separation efficiency, possibility of simultaneous detection of mass and activity and speed of analysis, can be successfully exploited in many fields.

8. ACKNOWLEDGEMENT

The authors are indebted to Professor A. I. M. Keulemans for many helpful discussions and remarks.

9. SUMMARY

GC methods of analysis of ^{3}H- and ^{14}C-labelled compounds that have been published in the period from 1955 to mid-1976, together with important applications, are reviewed. In addition to its obvious use for identification purposes, RGC is mostly used for the determination of the distribution of radioactivity among the components of mixture under analysis and for the determination of their contents and specific activities. It can be also combined with chemical reactions (reaction gas chromatography). Applications that require derivatization have some limitations connected with the derivatization reaction and the stability of the derivatives. The separation of isotopic molecules by GC is also possible. For the measurement of radioactivity, a discontinuous method involving the collection of fractions and subsequent counting is often used, while for continuous monitoring of effluent activity flow-through radiation detectors (Geiger–Müller and proportional counters, ionization chambers and scintillation methods) are used, mainly after conversion of the effluent. The possibilities of various recording techniques with respect to quantitative evaluation are surveyed and applications of RGC in the production of labelled compounds, "hot-atom" chemistry, organic chemistry, chemical processing, biochemistry and clinical chemistry are discussed.

REFERENCES

1 H. J. Ache, W. Herr and A. Thiemann, *Chemical Effects of Nuclear Transformations, (Proceedings of Symposium, Prague, October, 1960)*, Vol. 2, IAEA, Vienna, 1961, p. 111.
2 H. J. Ache, A. Thiemann and W. Herr, *Z. Anal. Chem.*, 181 (1961) 551.
3 H. J. Ache and A. P. Wolf, *Z. Anal. Chem.*, 230 (1967) 19.
4 G. Adessi and M. F. Layle, *C.R. Acad. Sci., Ser. D*, 279 (1974) 2143.
5 J.-P. Adloff, *J. Chromatogr.*, 6 (1961) 373; *Chromatogr. Rev.*, 4 (1962) 19.
6 J.-P. Adloff, *Chromatogr. Rev.*, 7 (1965) 53.
7 R. A. Ahrens and J. R. Luick, *J. Dairy Sci.*, 47 (1964) 849.
8 D. K. Albert, R. L. Stoffer and R. D. Kaplan, *J. Chromatogr. Sci.*, 12 (1974) 183.
9 A. Ju. Alexandrov and M. I. Janovskij, *Kinet. Katal.*, 2 (1961) 794.
10 B. Aliprandi and F. Cacace, *Gazz. Chim. Ital.*, 89 (1959) 2268.
11 B. Aliprandi, F. Cacace and G. Ciranni, *Anal. Chem.*, 36 (1964) 2445.
12 J. W. Amy, E. M. Chait, W. E. Baitinger and F. W. McLafferty, *Anal. Chem.*, 37 (1965) 1265.
13 L. Appelqvist, *J. Lipid Res.*, 13 (1972) 146.
14 H. Arimoto, *Bunseki Kagaku (Jap. Anal.)*, 22 (1973) 837.
15 W. Y. W. Au, L. G. Dring, D. G. Grahame-Smith, P. Isaac and R. T. Williams, *Biochem. J.*, 129 (1972) 1.
16 I. Bally, E. Gard, E. Ciornei, M. Biltz and A. T. Balaban, *J. Labelled Compd.*, 11 (1975) 63.
17 M. W. Banschbach, R. L. Geison and J. F. O'Brien, *Anal. Biochem.*, 59 (1974) 617.
18 M. F. Barakat, L. P. Firsova and A. N. Nesmejanov, *Radiokhimiya*, 6 (1964) 626.
19 C. Baret, *Microchem. J.*, 11 (1966) 455.
20 A. V. Barooshian, M. J. Lautenschleger and W. G. Harris, *Anal. Biochem.*, 44 (1971) 543.
21 B. G. Bartley, *Doctoral Thesis*, Johns Hopkins Univ., Baltimore, 1969.
22 E. Bayer, G. Nicholson and R. E. Sievers, in A. Zlatkis (Editor), *Advances in Chromatography 1970*, Chromatography Symposium, Houston, Texas, 1970, p. 154.
23 G. H. Beastall, H. H. Rees and T. W. Goodwin, *Biochem. J.*, 128 (1972) 179.
24 J. W. Beatty, L. G. Pobo and S. Wexler, *J. Phys. Chem.*, 75 (1971) 2407.
25 S. Behrendt, *Z. Phys. Chem.*, 20 (1959) 367.
26 J. E. Belham and G. E. Neal, *Anal. Biochem.*, 45 (1972) 6.
27 J. Beneš, M. Dobiášová and K. Vereš, *Collect. Czech. Chem. Commun.*, 29 (1964) 3178.
28 M. Bennet and E. Coon, *J. Lipid Res.*, 7 (1966) 448.

29 R. Bentley, N. C. Saha and C. C. Sweeley, *Anal. Chem.*, 37 (1965) 1118.
30 L. Bertilsson, A. J. Atkinson, Jr., A. Althaus, A. Harfast, J.-E. Lindgren and B. Holmstedt, *Anal. Chem.*, 44 (1972) 1434.
31 J. Bezard, P. Boucrot and G. Clement, *J. Chromatogr.*, 14 (1964) 368.
32 C. Bishop, R. F. Glascock, E. M. Newell and V. A. Welch, *J. Lipid Res.*, 12 (1971) 777.
33 H. S. Black and W. B. Lo, *Nature (London)*, 234 (1971) 306.
34 R. Blomstrand and J. Gürtler, *Acta Chem. Scand.*, 19 (1965) 249.
35 R. Blomstrand, J. Gürtler, L. Liljeqvist and G. Nyborg, *Anal. Chem.*, 44 (1972) 277.
36 G. Blyholder, *Anal. Chem.*, 32 (1960) 572.
37 G. Blyholder and P. H. Emmet, *J. Phys. Chem.*, 63 (1959) 962.
38 I. Bojesen, E. Bojesen and K. Capito, *Biochim. Biophys. Acta*, 424 (1976) 8.
39 E. Bojesen, I. Bojesen and K. Capito, *Biochim. Biophys. Acta*, 306 (1973) 237.
40 R. E. Bosshart and R. K. Young, *Anal. Chem.*, 44 (1972) 1117.
41 P. Boucrot, *J. Chromatogr. Sci.*, 9 (1971) 32.
42 W. C. Breckenridge and A. Kuksis, *Lipids*, 5 (1970) 342.
43 W. J. Mc Bride and J. D. Klingman, *J. Neurochem.*, 19 (1972) 865.
44 R. B. Bridges and J. G. Coniglio, *J. Biol. Chem.*, 245 (1970) 46.
45 J. F. O'Brien and R. L. Geison, *J. Neurochem.*, 18 (1971) 1615.
46 F. Bruner, G. P. Cartoni and M. Possanzini, *Anal. Chem.*, 41 (1969) 1122.
47 L. Bruzzi, A. Castelli and A. Cervellati, *Nucl. Instrum. Methods*, 26 (1964) 305.
47a H. P. Burchfield and E. E. Storrs, *Biochemical Applications of Gas Chromatography*, Academic Press, New York, 1962.
48 J. Bus and T. J. Liefkens, *Z. Anal. Chem.*, 250 (1970) 294.
49 F. Cacace, *Ann. Chim. (Rome)*, 50 (1960) 931.
50 F. Cacace, *Chemical Effects of Nuclear Transformations (Proceedings of Symposium, Prague, October, 1960)*, Vol. 2, IAEA, Vienna, 1961, p. 133.
51 F. Cacace, *Nucleonics*, 19, No. 5 (1961) 45.
52 F. Cacace and S. Caronna, *J. Chem. Soc., Perkin Trans. II*, (1972) 1604.
53 F. Cacace, R. Cippollini and G. Occhiucci, *J. Chem. Soc., Perkin Trans. II*, (1972) 84.
54 F. Cacace, R. Cippollini and G. Perez, *Science*, 132 (1960) 1253.
55 F. Cacace, R. Cippollinni and G. Perez, *Anal. Chem.*, 35 (1963) 1348.
56 F. Cacace and G. Ciranni, in A. B. Littlewood (Editor), *Gas Chromatography 1966*, Institute of Petroleum, 1967, p. 337.
57 F. Cacace and A. Guarino, *Nature (London)*, 186 (1960) 632.
58 F. Cacace, A. Guarino and Inam-Ul-Haq, *Ann. Chim. (Rome)*, 50 (1960) 915.
59 F. Cacace and Inam-Ul-Haq, *Ric. Sci.*, 30 (1960) 501.
60 F. Cacace and Inam-Ul-Haq, *Science*, 131 (1960) 732.
61 F. Cacace and Inam-Ul-Haq, *Science*, 131 (1960) 150.
62 F. Cacace and G. Perez, *Anal. Chem.*, 32 (1960) 572.
63 F. Cacace and G. Perez, *Anal. Chem.*, 41 (1969) 368.
64 F. Cacace and G. Perez, *J. Labelled Compd.*, 2 (1966) 102.
65 F. Cacace and G. Perez, *Atti Accad. Naz. Lincei Cl. Sci. Fis. Mat. Nat. Rend.*, 44 (1968) 420.
66 F. Cacace and M. Schüller, *J. Labelled Compd.*, 11 (1975) 313.
67 P. Cancalon and J. D. Klingman, *J. Chromatogr. Sci.*, 12 (1974) 349.
68 R. D. Canizzaro, T. E. Oullen and R. T. Murphy, *J. Agr. Food Chem.*, 18 (1970) 728.
69 M. G. Carnes and M. L. Brenner, *Anal. Biochem.*, 64 (1975) 5.
70 M. Cartwright and A. Heywood, *Analyst (London)*, 91 (1966) 337.
71 J. R. Catch, *Carbon-14 Compounds*, Butterworths, London, 1961.
72 J. R. Catch, *RCC Rev.*, No. 8, Radiochemical Centre, Amersham, 1968.
73 J. R. Catch, *RCC Rev.*, No. 11, Radiochemical Centre, Amersham, 1971.
74 H. M. Chang and R. Wolfgang, *J. Phys. Chem.*, 75 (1971) 3042.
75 M. Cher, C. S. Hollingsworth and B. Browning, *J. Chem. Phys.*, 41 (1964) 2270.
76 V. P. Chizhkov, *Zh. Anal. Khim.*, 29 (1974) 1193.
77 V. P. Chizhkov and L. A. Sinitsina, *J. Chromatogr.*, 104 (1975) 327.
78 C. C. Chou, P. Angelberger and F. S. Rowland, *J. Phys. Chem.*, 75 (1971) 2536.
79 C. C. Chou and F. S. Rowland, *J. Phys. Chem.*, 75 (1971) 1283.
80 W. W. Christie, *Lipid Analysis*, Pergamon Press, Oxford, 1973, p. 282.

81 W. W. Christie, *Spectrophotom. Chromatogr. Anal. News*, 4 (1974) 21.
82 W. W. Christie, *J. Chromatogr. Sci.*, 13 (1975) 411.
83 D. R. Christman, *Nucleonics*, 23 (No. 12) (1965) 39.
84 H. J. Cluley and J. H. Konrath, *Analyst (London)*, 88 (1963) 761.
85 E. R. Colson, *Anal. Chem.*, 35 (1963) 1111.
86 B. A. Cooke, *Anal. Biochem.*, 32 (1969) 198.
87 H. Copier and J. H. van der Maas, *Spectrochim. Acta*, 23A (1967) 2699.
88 H. Copier and L. Schutte, *J. Chromatogr.*, 47 (1970) 464.
89 D. L. Corina and P. M. Dunstan, *Anal. Biochem.*, 53 (1973) 571.
90 S. P. Cram, *Advan. Chromatogr.*, 9 (1970) 243.
91 W. A. Cramer, J. P. W. Houtman, R. O. Koch and G. J. Piet, *Int. J. Appl. Radiat. Isot.*, 17 (1966) 97.
92 W. A. Cramer, J. P. W. Houtman, G. J. Piet and E. de Boer, *Int. J. Appl. Radiat. Isot.*, 20 (1969) 129.
93 M. L. Curtis and H. L. Rook, *Anal. Chem.*, 36 (1964) 2047.
94 J. W. Czerkawski and G. Breckenridge, *Anal. Biochem.*, 67 (1975) 476.
95 J. N. Damico, N. P. Wong and J. A. Sphon, *Anal. Chem.*, 39 (1967) 1045.
96 S. H. Daniel, G. P. Gennaro, K. M. Ranck and Y.-N. Tang, *J. Phys. Chem.*, 76 (1972) 1249.
97 S. H. Daniel and Y.-N. Tang, *J. Phys. Chem.*, 73 (1969) 4378.
98 S. H. Daniel and Y.-N. Tang, *J. Phys. Chem.*, 75 (1971) 301.
99 F. Davidoff and E. D. Korn, *J. Biol. Chem.*, 238 (1963) 3210 and 3199.
100 Yu. I. Derbentsev, G. V. Isagulyants, L. F. Rar, D. L. Pakul' and A. A. Balandin, *Dokl. Akad. Nauk SSSR*, 177 (1967) 1371.
100[a] H. J. G. M. Derks, F. A. J. Muskiet and N. M. Drayer, *Anal. Biochem.*, 72 (1976) 391.
101 B. Diehn, A. P. Wolf and F. S. Rowland, *Z. Anal. Chem.*, 204 (1964) 112.
102 H. E. Dobbs, *J. Chromatogr.*, 5 (1961) 32.
103 M. Dobiášová, *Rozpr. Česk. Akad. Věd Řada Mat. Přír. Věd*, 76 (1966) 69.
104 D. A. Dominay and F. S. Feates, *J. Sci. Instrum.*, 2 (1969) 591.
105 D. A. Dominay and A. J. Wickham, *Trans. Faraday Soc.*, 67 (1971) 2598.
106 D. R. Douglas and H. S. Black, *Chromatographia*, 6 (1973) 229.
107 F. Drawert, in M. van Swaay (Editor), *Gas Chromatography 1962*, Butterworths, London, 1962, p. 347.
108 F. Drawert and O. Bachmann, *Angew. Chem.*, 75 (1963) 717; *Angew. Chem. Int. Ed. Engl.*, 2 (1963) 540.
109 F. Drawert and J. Beier, *Chromatographia*, 7 (1974) 273.
110 F. Drawert, R. Emberger, R. Tressl and U. Prenzel, *Chromatographia*, 5 (1972) 319.
111 F. Drawert, A. Rapp and H. Ullemeyer, *Vitis*, (1967) 177.
112 F. Drawert, A. Rapp, A. Ziegler, O. Bachmann and H. Steffan, *Chem.-Ing.-Tech.*, 35 (1963) 853.
113 J. Drozd, *J. Chromatogr.*, 113 (1975) 303.
114 H. J. Dutton, *J. Amer. Oil Chem. Soc.*, 38 (1961) 631.
115 H. J. Dutton, *Advan. Tracer Methodol.*, 1 (1963) 147.
116 H. J. Dutton, *Advan. Tracer Methodol.*, 2 (1965) 123.
117 H. J. Dutton, E. P. Jones, L. H. Mason and R. F. Nystrom, *Chem. Ind. (London)*, No. 36 (1958) 1176.
118 H. J. Dutton and T. L. Mounts, *145th Amer. Chem. Soc. Meeting, Sept. 8–13, 1963, New York, Division of Colloid and Surface Chemistry*, 1963, Abstr. No. 59, p. 22.
119 H. J. Dutton and R. F. Nystom, *Advan. Tracer Methodol.*, 1 (1963) 18.
120 H. J. Dutton and R. F. Nystrom, *Symposium on Advances in Tracer Applications of Tritium*, New England Nuclear Co., New York, 1958, p. 8.
121 R. A. van Dyke, M. B. Chenoweth and E. R. Larsen, *Nature (London)*, 204 (1964) 471.
122 D. Eberhagen, B. Wittmann and W. Seitz, *Z. Anal. Chem.*, 237 (1968) 17.
123 M. F. A. El-Sayed and R. J. Wolfgang, *J. Amer. Chem. Soc.*, 79 (1957) 3286.
124 E. A. Evans, *Tritium and its Compounds*, Butterworths, London, 2nd ed., 1974, p. 555.
124[a] J. B. Evans and J. E. Willard, *J. Amer. Chem. Soc.*, 78 (1956) 2908.
125 J. Exner, M. Seeger and H.-J. Canton, *Angew. Chem.*, 83 (1971) 3723.
126 H. M. Fales, E. O. Haahti, T. Luukainen, W. J. A. VandenHeuvel and E. C. Horning, *Anal. Biochem.*, 4 (1962) 296.

127 J. W. Farquahar, W. Insull, Jr., P. Rosen, W. Stoffel and E. H. Ahrens, *Nutr. Rev.*, 17, Suppl. No. 18, (1959) 1.
128 A. del Favero, A. Darbre and M. Waterfield, *J. Chromatogr.*, 40 (1969) 213.
129 D. C. Fee and S. S. Markowitz, *Anal. Chem.*, 45 (1973) 1827.
130 L. E. Feinendegen, *Tritium-Labeled Molecules in Biology and Medicine*, Academic Press, New York, 1967.
131 J. M. Figuera, J. M. Gamboa and J. Santos, *J. Chem. Soc. Perkin Trans.*, (1972) 1434.
132 R. D. Finn, H. J. Ache and A. P. Wolf, *J. Phys. Chem.*, 73 (1969) 3928.
133 L. P. Firsova and M. F. Barakat, *Radiokhimiya*, 4 (1962) 740.
134 L. P. Firsova, M. F. Barakat, M. Forys and A. N. Nesmeyanov, *Symposium on Chemical Effects of Nuclear Transformations, Vienna, December 1964*, IAEA, Vienna, 1965, p. 373.
135 H. Fock, *Chromatographia*, 9 (1976) 99.
136 M. J. O. Francis and M. O'Connell, *Phytochemistry*, 8 (1969) 1705.
137 B. L. Funt and A. Hetherington, *Science*, 129 (1959) 1429.
138 P. L. Gant, *J. Chem. Phys.*, 31 (1959) 1589.
139 G. A. Gaziyev and M. I. Janovski, *Probl. Kinet. Katal.*, 9 (1958) 465.
139[a] C. W. Gehrke, D. Roach, R. W. Zumwalt, D. L. Stalling and L. L. Wall, *Quantitative Gas–Liquid Chromatography of Amino Acids in Proteins and Biological Substances*, Anal. Biochem. Laboratories, Columbia, Mo., 1968.
139[b] C. W. Gehrke and K. Leimer, *J. Chromatogr.*, 57 (1971) 219.
140 J. L. Gellerman and H. Schlenk, *J. Amer. Oil Chem. Soc.*, 40 (1963) 9.
141 A. Gellhorn, W. Benjamin and M. Wagner, *J. Lipid Res.*, 3 (1962) 314.
142 G. Giacomello and F. Cacace, *Sc. Azione*, 3, Pt. 1 (1963–64) 51.
143 H. A. Gillis, *Can. J. Chem.*, 49 (1971) 2861.
144 W. B. Gleason and R. Barker, *Can. J. Chem.*, 49 (1971) 1433.
144[a] E. Glückauf, in *Gas Chromatography 1958*, Butterworths, London, 1958, p. 68.
145 L. J. Goad and T. W. Goodwin, *Eur. J. Biochem.*, 7 (1969) 502.
146 H. Goldfine and K. Bloch, *J. Biol. Chem.*, 236 (1961) 2596.
146[a] G. Goldstein and W. T. Rainey, *J. Labelled Compd.*, 9 (1973) 805.
147 B. E. Gordon and M. A. Muhs, in E. D. Bransome, Jr. (Editor), *Current Status of Liquid Scintillation Counting*, Grune & Stratton, New York, 1970, Ch. 24, p. 236.
148 A. A. Gordus, M. C. Sauer, Jr. and J. E. Willard, *J. Amer. Chem. Soc.*, 79 (1957) 3284.
149 G. L. Grandy and R. C. Koch, *Rev. Sci. Instrum.*, 31 (1960) 786.
150 G. E. Green, *Nature (London)*, 180 (1957) 295.
151 M. D. Greenspan, C. H. Birge, G. Powell, W. S. Hancock and P. R. Vagelos, *Science*, 170 (1970) 1203.
152 J. P. Guermont and L. Pichat, *Bull. Soc. Chim. Fr.*, (1959) 580.
153 A. K. Hajra and N. S. Radin, *J. Lipid Res.*, 3 (1962) 131.
154 M. Halmann, *Symposium on Chemical Effects of Nuclear Transformations*, Vol. I, IAEA, Vienna, 1961, p. 195.
155 G. Harbottle, *Conference on Radioisotopes in the Physical Sciences and Industry*, Copenhagen, September 1960, Vol. II, IAEA, Vienna, 1962, p. 375.
156 W. R. Harlan and S. J. Wakil, *J. Biol. Chem.*, 238 (1963) 3216.
157 R. V. Harris and A. T. James, *Biochim. Biophys. Acta*, 106 (1965) 456 and 465.
158 W. E. Harris, *Can. J. Chem.*, 39 (1961) 121.
159 W. E. Harris, *Symposium on Chemical Effects of Nuclear Transformations, Prague, October 1960*, Vol. I, IAEA, Vienna, 1961, p. 229.
160 W. G. Harris and A. V. Barooshian, *J. Labelled Compd.*, 9 (1973) 743.
161 W. Heimann, K.-H. Franzen, A. Rapp and H. Ullemeyer, *Z. Lebensm.-Unters.-Forsch.*, 159 (1975) 1.
162 K.-H. Heise, Ch. Goerner and M. Bubner, *Symposium on Organic Compounds Labelled with Radioisotopes, Marianské Lázne, May 1976*, Paper No. 51/47.
163 O. Helmich and J. Hradec, *J. Chromatogr.*, 91 (1974) 505.
164 W. Herr, F. Schmidt and G. Stöcklin, *Z. Anal. Chem.*, 170 (1959) 301.
165 J. W. Hightower and P. H. Emmett, *J. Amer. Chem. Soc.*, 87 (1965) 939.
166 J. G. Hildebrand, *Biochemistry*, 3 (1964) 1304.
167 C. Hitchcock and A. T. James, *Kerntechnik*, 7 (1965) 5.

168 C. Hitchcock, A. T. James and A. H. Carr, in P. G. Waser and B. Glasson (Editors), *Int. Conf. on Radioactive Isotopes in Pharmacology, Geneva, 1967*, Wiley, 1969, p. 113.
169 W. den Hollander and B. van Zanten, *J. Chromatogr. Sci.*, 8 (1970) 424.
170 J. Hölzl, *Z. Anal. Chem.*, 252 (1970) 137.
170[a] M. Hori, *Steroids*, 14 (1969) 33.
171 M. G. Horning, D. B. Martin, A. Karmen and P. R. Vagelos, *J. Biol. Chem.*, 236 (1961) 669.
172 I. Hornstein and P. Crowe, *Anal. Chem.*, 37 (1965) 170.
173 M. D. D. Howlett and D. Welti, *Analyst (London)*, 91 (1966) 291.
174 K.-P. Hupe, in A. Zlatkis and V. Pretorius (Editors), *Preparative Gas Chromatography*, Wiley, 1971, p. 143.
175 P. Hušek, *J. Chromatogr.*, 91 (1974) 478 and 483.
176 P. Hušek and K. Macek, *J. Chromatogr.*, 113 (1975) 139.
177 F. A. Iddings and J. T. Wade, *J. Gas Chromatogr.*, 1 (1963) 31.
178 S. Ikeda and H. Shinya, *Radioisotopes*, 9 (1960) 231.
179 G. V. Isagulyants, N. V. Borunova, L. Ch. Freydun, S. S. Danielova and L. I. Kovalenko, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 37 (1972) 1554.
180 G. V. Isagulyants, Yu. I. Derbentsev and I. R. Khairudinov, *Neftekhimiya*, 12 (1972) 652.
181 R. M. Iyer and G. R. Martin, *Chemical Effects of Nuclear Transformations*, Vol. I, IAEA, Vienna, 1961, p. 281.
182 A. T. James, in A. T. James and L. J. Morris (Editors), *New Biochemical Separations*, Van Nostrand, London, 1964, p. 1.
183 A. T. James, *Proc. 4th Meeting FEBS, Oslo, 1967, Cellular Compartmentalization and Control of Fatty Acid Metabolism*, Academic Press, New York, 1968, p. 25.
184 A. T. James, *Methods Biochem. Anal.*, (1960) 1.
185 A. T. James and A. J. P. Martin, *Biochem. J.*, 50 (1952) 679.
186 A. T. James, G. Peeters and M. Lauryssens, *Biochem. J.*, 64 (1956) 726.
187 A. T. James and E. A. Piper, *J. Chromatogr.*, 5 (1961) 265.
188 A. T. James and E. A. Piper, *Anal. Chem.*, 35 (1963) 515.
189 M. K. Yanovskii and G. A. Gaziyev, *Dokl. Akad. Nauk SSSR*, 120 (1958) 812.
190 E. F. Jansen and N. C. Baglan, *J. Chromatogr.*, 38 (1968) 18.
191 R. W. Jenkins, Jr., R. H. Newman, K. W. Vandenbroek, R. M. Jones and T. S. Osdene, *Gas Chromatogr., Proc. Int. Symp. (Eur.)*, 9 (1972) 261.
192 A. R. Johnson, in A. R. Johnson and J. B. Davenport (Editors), *Biochemistry and Methodology of Lipids*, Wiley, New York, 1971, p. 307.
193 A. R. Johnson, A. C. Fogerty, J. A. Pearson, F. Shenstone and A. M. Bersten, *Lipids*, 4 (1969) 265.
194 A. J. Johnston and D. S. Urch, *Radiochim. Acta*, 20 (1973) 120.
194[a] F. E. Kaiser, C. W. Gehrke, R. W. Zumwalt and K. C. Kuo, *J. Chromatogr.*, 94 (1974) 113.
195 L. M. Kaliberdo and V. B. Dorogova, *Zh. Fiz. Khim.*, 44 (1970) 3071.
196 L. M. Kaliberdo, V. B. Dorogova and V. S. Pashegorova, *Neftekhimiya*, 11 (1971) 719.
197 M. D. Kamen, *Advan. Tracer Methodol.*, 2 (1965) 1.
198 D. M. Kane and F. W. Karasek, *J. Chromatogr. Sci.*, 10 (1972) 501.
199 C. G. Kannangara, B. S. Jacobson and P. K. Stumpf, *Biochem. Biophys. Res. Commun.*, 52 (1973) 648.
200 H. Kanoh and D. B. Lindsay, *Biochem. J.*, 128 (1972) 847.
201 J. N. Kaplanis, R. E. Monroe, W. E. Robbins and S. J. Louloudes, *Ann. Entomol. Soc. Amer.*, 56 (1963) 198.
202 J. N. Kaplanis, W. E. Robbins, R. E. Monroe, T. J. Shortino and M. J. Thompson, *J. Insect Physiol.*, 11 (1965) 251.
203 F. W. Karasek and R. J. Smythe, *Anal. Chem.*, 43 (1971) 2008.
204 A. Karmen, *J. Ass. Offic. Agr. Chem.*, 47 (1964) 15.
205 A. Karmen, *J. Amer. Oil Chem. Soc.*, 44 (1967) 18.
206 A. Karmen, *J. Gas Chromatogr.*, 5 (1967) 502.
207 A. Karmen, *J. Lipid Res.*, 8 (1967) 61.
208 A. Karmen, *Methods Enzymol.*, 14 (1969) 465.
209 A. Karmen and N. S. Longo, *J. Chromatogr.*, 112 (1975) 637.
210 A. Karmen, *Packard Instrument Co. Tech. Bull.*, No. 14, Packard, Downers Grove, Ill., 1965.

211 A. Karmen, I. McCaffrey and R. L. Bowman, *J. Lipid Res.*, 3 (1962) 372.
212 A. Karmen, I. McCaffrey, J. W. Winkelman and R. L. Bowman, *Anal. Chem.*, 35 (1963) 536.
213 A. Karmen, L. E. Giuffrida and R. L. Bowman, *J. Lipid Res.*, 3 (1962) 44.
214 A. Karmen, L. D. Kane, M. Karasek and B. Lapidus, *J. Chromatogr. Sci.*, 8 (1970) 439.
215 A. Karmen and H. R. Tritch, *Nature (London)*, 186 (1960) 150.
216 A. Karmen, M. Whyte and D. W. S. Goodman, *J. Lipid Res.*, 4 (1963) 312.
216[a] M. L. Kaufman, S. Friedman and J. Wender, *Anal. Chem.*, 39 (1967) 1011.
217 T. E. Kavanagh, G. A. Reineccilis, P. G. Keeney and W. Weissberger, *J. Amer. Oil Chem. Soc.*, 47 (1970) 344.
217[a] I. Kiricsi, K. Varga and P. Fejes, *J. Chromatogr.*, 123 (1976) 279.
218 M. A. Kirschner and M. B. Lipsett, *J. Lipid Res.*, 6 (1965) 7.
219 I. Kiss, G. Jakli and H. Illy, *Acta Chim. Hung.*, 71 (1972) 59.
220 P. D. Klein, *Advan. Chromatogr.*, 3 (1966) 3.
221 W. J. de Klein, *Z. Anal. Chem.*, 246 (1969) 294.
222 P. E. Koehler, M. E. Mason and J. A. Newell, *J. Agr. Food Chem.*, 17 (1969) 393.
223 R. I. Kokes, H. Tobin and P. H. Emmett, *J. Amer. Chem. Soc.*, 77 (1955) 5860.
224 P. E. Kolattukudy, *Biochemistry*, 9 (1965) 1095.
225 B. Kolb and E. Wiedeking, *Z. Anal. Chem.*, 243 (1968) 129.
226 E. D. Korn, C. L. Greenblatt, *Science*, 142 (1963) 1301.
226[c] P. Kratz, M. Jacobs and B. M. Mitzner, *Analyst (London)*, 84 (1959) 671.
227 J. Kroh, J. Hankiewicz and R. Leszcynski, *Bull. Acad. Polon. Sci., Ser. Sci. Chim.*, 20 (1972) 343.
228 A. Kuksis, *Fette, Seifen, Anstrichm.*, 73 (1971) 130.
229 B. Kuman and C. Briefer, Jr., in J. K. Grant (Editor), *The Gas–Liquid Chromatography of Steroids*, Cambridge University Press, Cambridge, 1967, p. 57.
230 A. I. Kunakovskii and V. I. Mischin, *Symposium on Organic Compounds Labelled with Radioisotopes, Mariánské Lázně, May 1976*, Paper 51/24.
231 R. Kushner and F. S. Rowland, *J. Phys. Chem.*, 75 (1971) 3771; 76 (1972) 190.
231[a] W. M. Lamkin and C. W. Gehrke, *Anal. Chem.*, 37 (1965) 383.
232 E. K. C. Lee, G. Miller and F. S. Rowland, *J. Amer. Chem. Soc.*, 87 (1965) 190.
233 J. K. Lee, E. K. C. Lee, B. Musgrave, Y.-N. Tang, J. W. Root and F. S. Rowland, *Anal. Chem.*, 34 (1962) 741.
234 E. K. C. Lee and F. S. Rowland, *Anal. Chem.*, 36 (1964) 2181.
235 E. K. C. Lee and F. S. Rowland, *J. Phys. Chem.*, 74 (1970) 439.
235[a] A. de Leenheer, *J. Chromatogr.*, 74 (1972) 35.
236 M. R. Leonov, V. A. Korsunova and I. N. Rukavisnikov, *Radiokhimiya*, 17 (1975) 509.
237 A. Liberti, G. P. Cartoni and F. Bruner, in A. Goldup (Editor), *Gas Chromatography 1964*, Institute of Petroleum, London, 1965, p. 301; *J. Chromatogr.*, 12 (1963) 8.
238 K. H. Lieser, H. Elias and F. Sorg, *Z. Anal. Chem.*, 191 (1962) 104.
239 J. Lintermans, W. Erwin and R. M. Lemmon, *J. Phys. Chem.*, 76 (1972) 2521.
239[a] B. P. Lisboa, H. T. Schneider and H. Breuer, *Acta Endocrinol. (Copenhagen)*, 82 (1976) 57.
240 A. B. Littlewood, *Chromatographia*, 1 (1968) 223.
241 B. W. Lo and H. S. Black, *J. Invest. Dermatol.*, 58 (1972) 278.
242 B. Louvale, G. Teller and J.-P. Adloff, *Bull. Soc. Chim. Fr.*, (1963) 2606.
243 A. E. Lowe and D. Moore, *Nature (London)*, 182 (1958) 133.
244 J. A. Lubkowitz, *Radiotracer Studies of Chemical Residues in Food and Agriculture, Proc. Comb. Panel and Coord. Meeting, Vienna*, IAEA, Vienna, 1972, p. 129.
245 J. A. Lubkowitz, W. C. Parker and A. F. Findeis, *Radiochem. Radioanal. Lett.*, 5 (1970) 235.
246 S. Lukáč, *Jad. Energ.*, 12 (1966) 130.
246[a] G. Magnusson, *J. Chromatogr.*, 109 (1975) 393.
247 K. I. Mahan and J. K. Garland, *J. Phys. Chem.*, 73 (1969) 1247.
248 L. R. Mandel, A. Rosegay, R. W. Walker, W. J. A. VandenHeuvel and J. Rokach, *Science*, 186 (1974) 741.
249 H. K. Mangold and D. M. Sand, *Biochim. Biophys. Acta*, 164 (1968) 124.
250 A. E. Martin and J. Smart, *Nature (London)*, 175 (1955) 422.
251 R. O. Martin, *Anal. Chem.*, 40 (1968) 1197.
252 R. O. Martin, M. E. Warren and H. Rapoport, *Biochemistry*, 6 (1967) 2355.
253 L. H. Mason, H. J. Dutton and L. R. Bair, *J. Chromatogr.*, 2 (1959) 322.

254 J. R. Mattoon, in P. Bernfield (Editor), *The Biosynthesis of Natural Compounds*, Pergamon Press, New York, 2nd ed., 1967.
255 M. Matucha, *5. Symposium Chromatographie in der klinischen Biochemie, Leipzig, 1976.*
256 M. Matucha, V. Svoboda and E. Smolková, *J. Chromatogr.*, 91 (1974) 497.
257 M. Matucha and L. Žilka, *Radioisotopy*, 10 (1969) 611.
258 M. Matucha and L. Žilka, *Euroanalysis II Conf., Budapest, 1975.*
259 M. Matucha and L. Žilka, *Symp. RVHP Organ. Znač. Slouč., Marianské Lazně, 1976.*
260 M. Matucha, L. Žilka and K. Švihel, *J. Chromatogr.*, 65 (1972) 371.
261 H. Meinertz and V. P. Dole, *J. Lipid Res.*, 3 (1962) 140.
262 E. J. Merrill and A. D. Lewis, *Anal. Chem.*, 46 (1974) 1114.
263 S. T. Michalski and E. G. Hammond, *J. Amer. Oil Chem. Soc.*, 49 (1972) 563.
264 T. A. Miettinen, E. H. Ahrens and S. M. Grundy, *J. Lipid Res.*, 6 (1965) 411.
265 S. G. Miguel and S. Abraham, *Biochim. Biophys. Acta*, 424 (1976) 213.
266 V. Miller and V. Pacáková, *Chem. Listy*, 67 (1973) 1193.
267 B. K. Min, S. H. Daniel and Y.-N. Tang, *J. Radioanal. Chem.*, 14 (1973) 29.
268 Ch. M. Minačev, M. A. Ryasentseva, G. V. Isagulyants and N. N. Rozdestvenskaya, *Izv. Akad. Nauk SSSR, Ser. Khim.*, No. 3 (1972) 705.
269 V. I. Mishin, *Primeneniye Metoda Gazozhidkostnoi Khromatografii dlya Issledovaniya Khimicheskoi i Radiokhimicheskoi Chistoty Karbonovych Kislot C_1–C_5, Spirtov C_4–C_5, Aromatocheskich Uglevodorodov Měchenych Uglerodom-14 ili Tritiem*, Atomizdat, Moscow, 1964.
270 F. L. Mitchell, *Gas Chromatography Symposium, Paisley College of Technology, May 31st, 1965; Nature (London)*, 207 (1965) 358.
271 D. Miyatani and T. Takeuchi, *Int. J. Radiat. Isotop.*, 24 (1973) 553.
272 M. Moron, G. Sundström and C. A. Wachtmeister, *Acta Chem. Scand.*, 26 (1972) 830.
273 T. L. Mounts, R. O. Butterfield, C. R. Scholfield and H.-J. Dutton, *J. Amer. Oil Chem. Soc.*, 47 (1970) 79.
274 M. A. Muhs, E. L. Bastin and B. E. Gordon, *Int. J. Radiat. Isotop.*, 16 (1965) 537.
275 K. D. Mukherjee and H. K. Mangold, *J. Labelled Compd.*, 9 (1973) 779.
276 K. E. Murray, *Aust. J. Chem.*, 12 (1959) 657.
276a R. Teranishi, J. W. Corse, J. C. Day and W. G. Jennings, *J. Chromatogr.*, 9 (1962) 244.
277 D. C. Myers and F. Schmidt-Bleek, *J. Labelled Compd.*, 3 (1967) 63.
278 M. Nakamura and O. S. Privett, *Lipids*, 4 (1969) 41.
278a J. Nekvasil, *Thesis*, Charles Univ., Prague, 1972.
279 D. C. Nelson, R. C. Hawes and P. C. Ressler, Jr., *Dev. Appl. Spectrosc.*, 4 (1965) 323.
280 D. C. Nelson and D. L. Paull, *Anal. Chem.*, 35 (1963) 1571.
281 D. C. Nelson, P. C. Ressler and R. C. Hawes, *Anal. Chem.*, 35 (1963) 1575.
282 D. R. Nelson and J. G. Zeikus, *Appl. Microbiol.*, 28 (1974) 258.
283 E. Nice and K. Williams, *Anal. Biochem.*, 59 (1974) 399.
284 B. W. Nichols, A. T. James and J. Breuer, *Biochem. J.*, 104 (1967) 486.
285 B. W. Nichols and R. Moorhouse, *Lipids*, 4 (1969) 311.
286 R. A. Odum and R. J. Wolfgang, *J. Amer. Chem. Soc.*, 85 (1963) 1050.
287 M. H. O'Leary, *J. Amer. Chem. Soc.*, 91 (1959) 6886.
288 D. T. Organisciak and J. D. Klingman, *Prep. Biochem.*, 4 (1974) 89.
289 P. Ph. L. S. Otto and B. van Zanten, *Rec. Trav. Chim. Pay-Bas Belge*, 81 (1962) 380.
290 V. Pacáková and M. Matucha, *Radioisotopy*, 12 (1971) 757.
291 G. F. Palino and F. S. Rowlands, *J. Phys. Chem.*, 75 (1971) 1299.
292 K. Pánek and K. Mudra, *Jad. Energ.*, 10 (1964) 214.
293 M. Pascaud, *J. Chromatogr.*, 10 (1963) 125.
294 M. Pascaud, *Adv. Lipid Res.*, 1 (1963) 253.
295 B. Pella and B. Colombo, *Microchim. Acta*, No. 5 (1973) 697.
296 H. Perschke, *Nature (London)*, 209 (1966) 1021.
297 H. Perschke, M. B. A. Crespi and G. B. Cook, *Int. J. Radiat. Isotop.*, 20 (1969) 813.
298 L. Pichat, C. Baret, J.-P. Guermont and M. Audinot, *Conf. on Radioisotopes in the Physical Sciences and Industry, Copenhagen, 1960*, Vol. VIII, IAEA, Vienna, 1962, p. 145.
299 G. Popjak, J. W. Conforth, R. H. Cornforth, R. Ryhage and D. S. Goodman, *J. Biol. Chem.*, 237 (1962) 56.
300 G. Popjak, A. E. Lowe and D. Moore, *Advan. Tracer Methodol.*, 1 (1963) 127.

301 G. Popjak, A. E. Lowe and D. Moore, *Biochem. J.*, 73 (1959) 33P.
302 G. Popjak, A. E. Lowe and D. Moore, *J. Lipid Res.*, 3 (1962) 364.
303 G. Popják, A. E. Lowe, D. Moore, L. Brown and F. A. Smith, *J. Lipid Res.*, 1 (1959) 29.
304 T. H. Pratt and R. Wolfgang, *Conf. on Radioisotopes in the Physical Sciences and Industry, Copenhagen, 1960*, Vol. III, IAEA, Vienna, 1962, p. 159.
305 M. A. Quilliam, K. K. Ogilvie and J. B. Westmore, *J. Chromatogr.*, 105 (1975) 297.
306 V. F. Raaen, G. A. Ropp and H. A. Raaen, *Carbon-14*, McGraw-Hill, New York, 1968, p. 83.
306* J. J. Rash, C. W. Gehrke, R. W. Zumwalt, K. C. Kuo, K. A. Kvenvolden and D. L. Stalling, *J. Chromatogr. Sci.*, 10 (1972) 444.
307 D. W. Rathburn, K. Knarr and H. C. Moser, *Trans. Faraday Soc.*, 67 (1971) 2333.
308 V. Řehák and V. Pacáková, *Anal. Biochem.*, 61 (1974) 294.
309 P. Riesz and K. E. Wilzbach, *J. Phys. Chem.*, 62 (1958) 6.
309* D. Roach and C. W. Gehrke, *J. Chromatogr.*, 44 (1969) 269.
310 J. D. Robbins and J. E. Bakke, *J. Gas Chromatogr.*, 5 (1967) 525.
311 W. E. Robbins, R. C. Dutky, R. E. Monroe and J. N. Kaplanis, *Ann. Entomol. Soc. Amer.*, 55 (1962) 102.
311* P. G. Robinson, *Chem. N. Z.*, 38 (1974) 77.
312 S. Z. Roginskii, M. I. Yanovskii, G. M. Žabrova, O. M. Vinogradova, B. M. Kadenatsi and Z. A. Markova, *Dokl. Akad. Nauk SSSR*, 121 (1958) 674.
313 H. Rothstein and P. Gotz, *Arch. Biochem. Biophys.*, 126 (1968) 131.
314 A. H. Rosenberg and R. Wolfgang, *J. Chem. Phys.*, 41 (1964) 2159.
315 F. S. Rowland and R. Wolfgang, *Radioisotopes in Scientific Research, Proc. Int. Conf. UNESCO, Paris, 1957*, Vol. II, Pergamon Press, Oxford, 1958, p. 105.
316 P. T. Russel, R. T. van Aller and W. R. Nes, *J. Biol. Chem.*, 242 (1967) 5802
317 I. Ryoichi, M. Naotake and S. Osamu, *Radioisotopes*, 10 (1961) 208.
318 S. J. Rzad and K. M. Bansal, *J. Phys. Chem.*, 76 (1972) 2374.
319 K. I. Sakodynskii, *Isotopenpraxis*, 4 (1968) 278 and 305.
320 C. H. Schaeffer, J. N. Kaplanis and W. E. Robbins, *J. Insect Physiol.*, 11 (1965) 1013.
321 H. W. Scharpenseel, *Angew. Chem.*, 73 (1961) 615.
322 H. W. Scharpenseel, *IAEA and FAO Int. Conf. on Isotopes in Organic Matter Research, Brunswick*, Pergamon Press, Oxford, 1963, p. 375.
323 H. W. Scharpenseel, E. König and E. Menthe, *Z. Pflanzenernähr., Düng., Bodenkd.*, 106 (1964) 134.
324 H. W. Scharpenseel and R. Krausse, *Z. Pflanzenernähr. Düng. Bodenkd.*, 96 (1962) 11.
325 H. W. Scharpenseel and K. H. Menke, *Tritium in the Physical and Biological Sciences*, Vol. 1, IAEA, Vienna, 1962, p. 281.
326 H. W. Scharpenseel and K. H. Menke, *Z. Anal. Chem.*, 180 (1961) 81.
327 H. W. Scharpenseel and F. Pieting, *Atompraxis*, 11 (1965) 98.
328 E. C. Schluter, *Anal. Chem.*, 41 (1969) 1360.
329 F. Schmidt-Bleek and F. S. Rowland, *Anal. Chem.*, 36 (1964) 1695.
330 H. T. Schneider, H. Breuer and B. P. Lisboa, *Z. Anal. Chem.*, 279 (1976) 161.
331 C. R. Scholfield, T. L. Mounts, R. O. Butterfield and H. J. Dutton, *J. Amer. Oil Chem. Soc.*, 48 (1971) 237.
332 J. W. Schroeder, N. M. Monroe and J. K. Garland, *J. Phys. Chem.*, 73 (1969) 1252.
333 H. Schulz, B. R. Rao and N. Elstner, *Erdöl Kohle Erdgas Petrochem. Brennstoff. Chem.*, 23 (1970) 651.
334 H. W. Schulze and H.-K. Bothe, in H. G. Struppe and D. Obst (Editors), *Gas-Chromatographie 1965*, DAW, Berlin, 1965, pp. 479–496.
335 H. W. Schulze and H.-K. Bothe, *Atomkernenergie*, 9 (1964) 363.
336 L. Schutte and E. B. Koenders, *J. Chromatogr.*, 76 (1973) 13.
337 P. G. W. Scott, *Process Biochem.*, 2 (1967) 16.
338 H. Seino, S. Watanabe, T. Nihongi and T. Nagai, *J. Amer. Oil Chem. Soc.*, 50 (1973) 335.
339 D. S. Sgoutas, *Nature (London)*, 211 (1966) 296.
340 D. Sgoutas, M. J. Kim and F. Kummerow, *J. Lipid Res.*, 6 (1965) 383.
341 D. S. Sgoutas and F. A. Kummerow, *Biochemistry*, 3 (1964) 406.
342 S. A. Shepherd, *Diss. Abstr. B*, 29 (1969) 2784.
343 G. Sheppard, *RCC Rev.*, No. 14, Radiochemical Centre, Amersham, 1972.

343[a] W. R. Sherman, S. L. Goodwin and M. Zinbo, *J. Chromatogr. Sci.*, 9 (1971) 363.
344 M. D. Silbert and R. M. Tomlinson, *Can. J. Chem.*, 39 (1961) 706.
345 H. Simon, in H. Simon, *Messung von radioaktiven und stabilen Isotopen*, Springer, Berlin, 1974, pp. 264–272.
346 H. Simon and H. G. Floss, *Bestimmung der Isotopenverteilung in markierten Verbindungen*, Springer, Berlin, 1967.
347 H. Simon, G. Müllhofer and R. Medina, *Proc. Symp. Radioisotope Sample Measurement in Medicine and Biology*, IAEA, Vienna, 1965, p. 317.
348 E. F. Simonov and A. N. Nesměyanov, *Symp. on Chemical Effects of Nuclear Transformations*, Vienna, December, 1964, Vol. I, IAEA, Vienna, 1965, p. 411.
349 T. H. Simpson, *J. Chromatogr.*, 38 (1968) 24.
350 K. L. Simpson, T. O. M. Nakayama and C. O. Chichester, *J. Chromatogr.*, 13 (1964) 354.
351 B. Sjöquist and E. Änggård, *Anal. Chem.*, 44 (1972) 2297.
352 S. V. Schlyapnikov, M. Ya. Karpeyskii, I. M. Yakushina and V. S. Oseledchik, *Biokhimiya*, 30 (1965) 457 and 226.
353 T. Smail, B. Arezzo and F. S. Rowland, *J. Phys. Chem.*, 76 (1972) 187.
354 T. Smail and F. S. Rowland, *J. Phys. Chem.*, 74 (1970) 456.
354[a] E. D. Smith, J. M. Oathout and G. T. Cook, *J. Chromatogr. Sci.*, 8 (1970) 291.
354[b] E. D. Smith, K. E. Sorrels and R. G. Swienea, *J. Chromatogr. Sci.*, 12 (1974) 101.
355 R. W. Smith and S. H. Phillips, *Int. J. Appl. Radiat. Isot.*, 20 (1969) 553.
356 F. Snyder and C. Piantadosi, *Advan. Lipid Res.*, 4 (1966) 257.
357 A. Sokolowska, *Radiochem. Radioanal. Lett.*, 17 (1974) 95.
358 A. Sokolowska, *Radiochem. Radioanal. Lett.*, 13 (1973) 287.
359 I. F. Sommerville and W. P. Collins, *Steroids*, Suppl. II (1965) 223.
360 F. Stahei, W. Leemann and P. Koehler, *Beckman Rep.*, 1 (1972) 20.
360[a] D. L. Stalling and C. W. Gehrke, *Biochem. Biophys. Res. Commun.*, 22 (1966) 329.
361 R. M. Statnik and F. Schmidt-Bleek, *Z. Anal. Chem.*, 217 (1966) 21.
362 D. Steinberg, *Nature (London)*, 183 (1959) 1253.
363 G. Stöcklin, F. Cacace and A. P. Wolf, *Z. Anal. Chem.*, 194 (1963) 406.
364 W. Stoffel, *Justus Liebigs Ann. Chem.*, 673 (1964) 26.
365 W. Stoffel, *Hoppe-Seyler's Z. Physiol. Chem.*, 333 (1963) 71.
366 D. R. Stranks, *J. Sci. Instrum.*, 33 (1956) 1.
367 C. R. Strong and R. Dils, *Biochem. J.*, 128 (1972) 1303.
368 C. Strong, R. Diels and T. Galliard, *Column*, 13 (1971) 2.
369 C R. Strong, I. Forsyth and R. Dils, *Biochem, J.*, 128 (1972) 509.
370 Y. Y. Su and Y.-N. Tang, *J. Phys. Chem.*, 76 (1972) 2187.
371 J. A. Svoboda, M. J. Thompson and W. E. Robbins, *Life Sci.*, 6 (1967) 395.
372 L. Swell, *Anal. Biochem.*, 16 (1966) 70.
373 P. A. T. Swoboda and E. H. A. Prescott, in C. L. A. Harbourn (Editor), *Gas Chromatography 1968*, Institute of Petroleum, London, 1969, pp. 284–293.
374 L. Szilard and T. A. Chalmers, *Nature (London)*, 134 (1934) 462.
375 Y.-N. Tang, E. K. C. Lee and F. S. Rowland, *J. Amer. Chem. Soc.*, 86 (1964) 1280.
376 P. U. Thomas and H. J. Dutton, *Anal. Chem.*, 41 (1969) 657.
376[a] R. Teranishi, J. W. Corse, J. C. Day and W. G. Jennings, *J. Chromatogr.*, 9 (1962) 244.
377 U. Termeulen and S. Molnar, *Fette, Seifen, Anstrichm.*, 74 (1972) 406.
378 H. van Tilborg, J. de Bruin, J. J. Gottenbos and G. K. Koch, *J. Amer. Oil Chem. Soc.*, 47 (1970) 430.
379 C. T. Ting and F. S. Rowland, *J. Phys. Chem.*, 74 (1970) 445.
380 C. T. Ting and F. S. Rowland, *J. Phys. Chem.*, 74 (1970) 4080.
381 B. M. Tolbert, *Advan. Tracer Methodol.*, 1 (1963) 167.
382 B. M. Tolbert, *Bio-Organic Chemistry Quarterly Report*, UCRL-8457, 1962, p. 3.
383 B. M. Tolbert, *Symp. on Advances in Tracer Applications of Tritium, New York, 1958*, New England Nuclear, p. 65.
384 B. M. Tolbert, *Advan. Tracer Methodol.*, 1 (1963) 167.
385 J. Tölgyessy, in J. Tölgyessy and S. Varga (Editors), *Nuclear Analytical Chemistry II*, University Park Press, 1972, pp. 320–335.
386 J. Tölgyessy and J. Hrivnák, *Chem. Zvesti*, 22 (1968) 528.

387 J. Tölgyessy, M. Šaršúnová and I. M. Hais, *Česk. Farm.*, 14 (1965) 9.
388 T. Tominaga, *Bunseki Kiki*, 10, No. 2 (1972) 88.
389 K. C. Tovery, G. H. Spiller, K. G. Oldham, N. Lucas and N. G. Carr, *Biochem. J.*, 142 (1974) 47.
389[a] J. Tranchant (Editor), *Practical Manual of Gas Chromatography*, Elsevier, Amsterdam, 1969.
390 N. R. Trenner, O. C. Speth, V. B. Gruber and W. J. A. VandenHeuvel, *J. Chromatogr.*, 71 (1972) 415.
391 R. Tressl, F. Drawert and U. Prenzel, *Chromatographia*, 6 (1973) 7.
392 R. Tressl, R. Emberger, F. Drawert and W. Heimann, *Z. Naturforsch.*, 25B (1970) 704.
393 T. Tsuda and D. Ishii, *J. Chromatogr.*, 47 (1970) 469.
394 L. P. Turner, *Anal. Biochem.*, 28 (1969) 288.
395 L. P. Turner and W. R. Barr, *J. Chromatogr. Sci.*, 9 (1971) 176.
396 R. Tykva, *Symp. on Advances in Physical and Biological Radiation Detectors*, IAEA, Vienna, 1971, p. 211.
397 R. Tykva and J. Šeda, *J. Chromatogr.*, 108 (1975) 37.
398 R. Tykva and H. Simon, *Chromatographia*, 2 (1969) 5.
399 H. Ullemeyer, A. Rapp, K. H. Franzen and W. Heimann, *Z. Lebensm.-Unters.-Forsch.*, 159 (1975) 1.
400 W. J. A. VandenHeuvel, V. F. Gruber, L. R. Mandel and R. W. Walker, *J. Chromatogr.*, 114 (1975) 476.
401 W. J. A. VandenHeuvel and W. G. Kuron, in A. Zlatkis and V. Pretorius (Editors), *Preparative Gas Chromatography*, Wiley-Interscience, New York, 1971, p. 290.
402 W. J. A. VandenHeuvel, J. L. Smith and J. S. Cohen, in A. Zlatkis (Editor), *Advances in Chromatography 1970*, Chromatography Symposium, Houston, 1970, p. 293.
403 A. P. M. van der Veek and A. C. Besemer, *J. Labelled Compd.*, 11 (1975) 149.
404 M. I. Vinnik and L. D. Abramovich, *Izv. Akad. Nauk SSSR, Ser. Khim.*, No. 4 (1972) 833.
405 V. Vlasáková and J. Beneš, *Radiochem. Radioanal. Lett.*, 14 (1973) 43.
406 A. F. Voigt, G. F. Palino and R. L. Williams, *J. Phys. Chem.*, 75 (1971) 2248.
407 P. Volpe and M. Castiglioni, *J. Chromatogr.*, 114 (1975) 23.
408 M. Vranic, D. Lau, N. Kovacevic and A. Kuksis, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, 34 (1975) 2238.
409 E. R. Wagner, W. M. Muelder, P. G. Watanabe, R. E. Hefner, Jr., W. H. Braun and P. J. Gehring, *J. Labelled Compd.*, 11 (1975) 535.
410 M. D. Waterfield and A. del Favero, *J. Chromatogr.*, 40 (1969) 295.
411 W. R. Watson and J. P. Williams, *J. Chromatogr.*, 67 (1972) 221.
412 M. J. Welch and M. G. Straatmann, *Radiochim. Acta*, 20 (1973) 124.
413 M. Welch, R. Withnel and A. P. Wolf, *Anal. Chem.*, 39 (1967) 275.
414 M. Welch and A. P. Wolf, *Abstr. Pap. 153rd Amer. Chem. Soc. Meeting, Div. of Anal. Chem., Miami Beach, Fla., April 1967*, No. 10.
415 E. R. White and H. G. Davis, *Anal. Chim. Acta*, 34 (1966) 105.
416 A. R. Whorton, T. Antalis and J. G. Coniglio, *Biochim. Biophys. Acta*, 424 (1976) 66.
417 K. Widmark and G. Widmark, *Acta Chem. Scand.*, 16 (1962) 575.
418 K. E. Wilzbach and P. Riesz, *Science*, 126 (1957) 748.
419 J. Winkelman and A. Karmen, *Anal. Chem.*, 34 (1962) 1067.
420 A. P. Wolf, in R. C. Extermann (Editor), *Radioisotopes in Scientific Research*, Vol. II, Pergamon Press, Oxford, 1958, p. 114.
421 A. P. Wolf, *Ann. Rev. Nucl. Sci.*, 10 (1960) 259.
422 R. L. Wolfgang, *Advan. Tracer Methodol.*, 1 (1963) 183.
423 R. Wolfgang, J. Eigner and F. S. Rowland, *J. Phys. Chem.*, 60 (1956) 1137.
424 R. Wolfgang and C. F. MacKay, *Nucleonics*, 16, No. 10 (1958) 69.
425 R. Wolfgang and F. S. Rowland, *Anal. Chem.*, 30 (1958) 903.
426 R. Wood and R. Reiser, *J. Amer. Oil Chem. Soc.*, 42 (1965) 159.
427 J. Y. Yang and R. B. Ingalls, *J. Amer. Chem. Soc.*, 85 (1963) 3980.
428 J. Y. Yang and A. P. Wolf, *J. Amer. Chem. Soc.*, 82 (1960) 4488.
429 H. J. Yardley and V. J. W. Long, *J. Chromatogr.*, 73 (1972) 235.
430 L. Žilka, M. Matucha and K. Švihel, *Radioisotopy*, 12 (1971) 767.
431 A. Zlatkis and J. A. Ridgway, *Nature (London)*, 182 (1958) 130.