ISSN 0378-4355

VOL. 251 NO. 1 FEBRUARY 26, 1982

AL OF
HROMATOGRAPHY
ATIONAL JOURNAL ON CHROMATOGRAPHY, ELECTROPHORESIS AND RELATED METHODS

CHROMATOGRAPHIC REVIEWS (Vol. 26, No. 1)

edited by

Michael Lederer

ELSEVIER SCIENTIFIC PUBLISHING COMPANY
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Printed in The Netherlands

VOL. 251, NO. 1 JOURNAL OF CHROMATOGRAPHY FEBRUARY 26, 1982

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(Abstracts/Contents Lists published in Analytical Abstracts, Biochemical Abstracts, Biological Abstracts, Chemical Abstracts, Chemical Titles, Current Contents/Physical, Chemical & Earth Sciences, Current Contents/Life Sciences, Index Medicus and Science Citation Index)

Electron Capture – Theory and Practice in Chromatography

edited by A. ZLATKIS, *Houston, TX, USA and* C.F. POOLE, *Detroit, MI, USA*

JOURNAL OF CHROMATOGRAPHY LIBRARY – Volume 20

Sept. 1981 xii + 418 pages
Price: US $76.50/ Dfl. 180.00
ISBN 0-444-41954-3

This book provides the first comprehensive coverage of all aspects of the theory, design, operation and applications of the electron capture detector (ECD) from the chromatographer's point of view. In addition, an up-to-date look at the ancillary techniques of selective electron-capture sensitization, atmospheric pressure ionization and plasma chromatography has been included. ECD users will find the solutions to instrumental and technical problems which arise during practice particularly valuable. These have been derived from the experiences of the internationally distinguished team of authors.

Each chapter has been prepared by experts in their field and provides an in-depth coverage of its topic. The basic theory of the mechanisms of electron capture detection is included. Practical sections form the bulk of the book and are devoted to such topics as the construction and operating principles of the detector, including the establishment of instrument design criteria, and the different methods of derivatization. A more personal touch is provided by the inventor of the ECD, J.E. Lovelock, in his review of the development of the technique. Other chapters illustrate the importance of ECD in trace analysis in environmental and biomedical research. A unique feature is the extensive tabulation of all the pertinent data concerning the use of ECD in gas and liquid chromatography.

For those analytical chemists who use chromatography in their research, this book should become a standard text.

CONTENTS: Chapter 1. The electron-capture detector – A personal odyssey *(J.E. Lovelock)*. 2. The design and operation of the electron-capture detector *(C.F. Poole and A. Zlatkis)*. 3. Theory of electron capture *(W.E. Wentworth and E.C.M. Chen)*. 4. Selective electron-capture sensitization *(F.C. Fehsenfeld, P.D. Goldan, M.P. Phillips and R.E. Sievers)*. 5. Oxygen-doping of the carrier gas in electron-capture detection *(E.P. Grimsrud)*. 6. Wide-range calibration of electron-capture detectors *(R.E. Kaiser and R.I. Rieder)*. 7. Response of the electron-capture detector to compounds with natural electrophores *(J. Vessman)*. 8. Sensitive derivatives for the determination of organic compounds by electron-capture gas chromatography *(C.F. Poole and A. Zlatkis)*. 9. The detection of inorganic and organometallic compounds by electron-capture gas chromatography *(C.F. Poole and A. Zlatkis)*. 10. Environmental applications of the electron-capture detector – pesticides *(W.P. Cochrane and R.B. Maybury)*. 11. Environmental applications of the electron-capture detector – dioxins *(F. Bruner)*. 12. The electron-capture detector as a monitor of halocarbons in the atmosphere *(P.G. Simmonds)*. 13. Biomedical applications of the electron-capture detector *(J. Vessman)*. 14. Negative ion atmospheric pressure ionization mass spectrometry and the electron-capture detector *(E.C. Horning, D.I. Carroll, I. Dzidic and R.N. Stillwell)*. 15. Electron-capture process and ion mobility spectra in plasma chromatography *(F.W. Karasek and G.E. Spangler)*. 16. The electron-capture detector as a detector in liquid chromatography *(U.A. Th. Brinkman)*. Subject index.

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Ionic Hydration in Chemistry and Biophysics

by B. E. CONWAY,
Department of Chemistry, University of Ottawa, Ontario, Canada.

STUDIES IN PHYSICAL AND THEORETICAL CHEMISTRY 12

1981 xxx + 774 pages
Price: US $131.75 / Dfl. 270.00
ISBN 0-444-41974-0

This monograph is a comprehensive coverage of all aspects of ionic hydration. Not only is the nature of the phenomenon described, but also its importance in electrochemistry, inorganic and physical chemistry, biophysics and biochemistry. The basic background of techniques and approaches (e.g. electrostatic theory) required for the study and interpretation of ionic hydration in chemistry is included.

CONTENTS: Chapters. 1. Introduction. 2. Properties of the Water Molecule. 3. Properties of Liquid Water as a Solvent for Ions. 4. Scales for Ionic Radii in Gases, Crystals and Solutions. 5. Ionic Solvation in the Gas Phase. 6. Methods for Study of Solvation of Ions. 7. Infrared Spectral Studies on Ionic Hydration. 8. Raman Spectral Studies on Ionic Hydration. 9. Studies of Ionic Solvation by Nuclear and Electron Magnetic Resonance. 10. Elements of Electrostatics for Problems in Ionic Solutions. 11. Relation between Energetics of Ion Hydration and Ionic Processes in Solution. 12. "Real" Solvation Energies: Surface Potential Effects. 13. Solvation Effects in Electrode Processes and Homogeneous Ionic Solutions. 14. Ionic Hydration and the Dielectric Behaviour of Solutions. 15. Calculation of Ionic Hydration Energies. 16. Hydration of Individual Cations and Anions: Specificity of Ion-Solvent Interactions. 17. Hydration of the Proton. 18. Hydration Effects in the Concentration Dependence of Thermodynamic Properties of Electrolyte Solutions. 19. Ion-Pairing and Ionic Hydration. 20. Salting-Out and Salting-In by Electrolytes in Solution. 21. Steric and Stereochemical Effects in Solvation. 22. Entropies of Ions in Solution, and Hydration Entropies. 23. Solute and Solvent Structure Factors in Hydration and the Entropies of Ions in Solution. 24. Hydrophobic Interactions in Ionic Hydration. 25. Electrostriction and the Partial Molal Volumes and Compressibilities of Ions. 26. H_2O-D_2O Solvent Isotope Effects in Ionic Hydration. 27. Dynamical Aspects of Ionic Solvation. 28. Solvation Effects in Conductance of Electrolytic Solutions. 29. Hydration Numbers. 30. Ionic Solvation in Terms of Lewis Acid-Base Interactions. 31. Solvation in Non-Aqueous media. 32. Hydration of Ions at Interfaces in Chemistry and Biology, and the Nature of Liquid Surfaces. 33. Hydrated Ions in Active Transport Processes at Cell Membranes. 34. Hydration of Polyions of Synthetic and Biological Origin. Subject Index.

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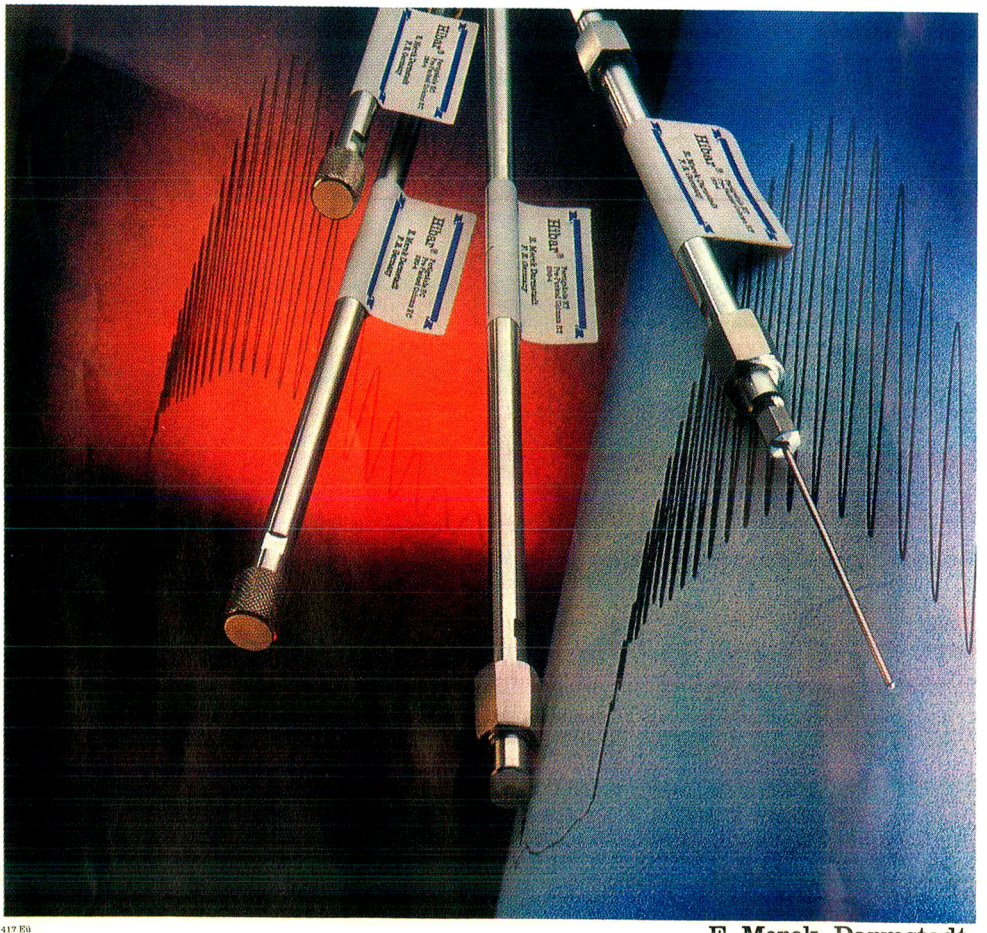

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JOURNAL OF CHROMATOGRAPHY

VOL. 251 (1982)

(Chromatographic Reviews, Vol. 26)

JOURNAL
of
CHROMATOGRAPHY

INTERNATIONAL JOURNAL ON CHROMATOGRAPHY,
ELECTROPHORESIS AND RELATED METHODS

CHROMATOGRAPHIC REVIEWS

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Michael Lederer

VOL. 26 1982

ELSEVIER SCIENTIFIC PUBLISHING COMPANY
AMSTERDAM

Printed in The Netherlands

Journal of Chromatography, 251 (1982) 1–16
Chromatographic Reviews
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHREV. 154

NEW WAYS TO INCREASE THE SPECIFICITY OF DETECTION IN LIQUID CHROMATOGRAPHY*

J. L. DiCESARE and L. S. ETTRE*
Chromatography Division, The Perkin-Elmer Corporation, Norwalk, CT 06856 (U.S.A.)
(Received August 21st, 1981)

CONTENTS

1. INTRODUCTION

Chromatography is essentially a separation technique in which the compounds of a mixture introduced at the front of the system will elute separately from the column (column chromatography) or occupy spots at different positions (plane chromatography). In order to have a visual record of the separation, various detectors are used which respond to all or a selected group of compounds. Detectors of the first category are universal while those of the second are classified as selective.

Selective detection has two basic advantages. First, it helps in the identification of the individual compounds present in a sample. In addition, in situations where compounds co-elute (*i.e.*, they could not be separated chromatographically), selective detectors may permit the detection of one compound while disregarding the other(s).

The concepts of selective detection have been established in both gas chromatography (GC) and liquid chromatography (LC) for many years and modern chromatography could not exist without it. Three years ago, one of us reviewed the

* Enlarged text of a lecture read at the *Fifth International Symposium on Column Liquid Chromatography, Avignon, France, May 10–15th, 1981*. The majority of the papers presented at this symposium has been published in *J. Chromatogr.*, Vol. 218 (1981).

0378-4355/82/0000-0000/$4.00

philosophy of selective detection, the most widely used selective detectors and their application in both gas and liquid chromatography[1,2]; the interested reader is referred to these papers and the references given therein.

In LC, all presently used detectors, except the refractive index (RI) detector, are more-or-less selective, or can be used as selective detectors. This possibility presents a great potential for liquid chromatography.

The possibilities of using the ultraviolet (UV) spectrophotometric detector for identification purposes are well established[3–5] and we shall not deal with them here. The capabilities of fluorescence spectroscopy for qualitative LC detection are also known[6], both for the analysis of naturally fluorescing compounds, or by preparing the fluorescing derivatives of other compounds[7]. In addition, there are some new techniques which enhance the applicability of the fluorescence detector. The purpose of this paper is to discuss these new techniques; in addition, we shall examine the use of infrared spectroscopy and optical activity as a means of selective detection.

2. EXPERIMENTAL

A Perkin-Elmer Series 2/2 liquid chromatograph equipped with a Rheodyne 7125 injection valve, a Model LC-100 column oven and a Sigma 15 data station were used for the measurements. Detectors included the Model 3000 and 650-10S/LC fluorescence spectrophotometers equipped with 20-μl flow cells, a Model 580 infrared (IR) spectrophotometer equipped with a 50-μl flow cell having a 3-mm path length and calcium fluoride windows, a Model 241 polarimeter equipped with a 33-μl flow cell having a 100-mm path length, a Model LC-75 variable-wavelength UV detector and a Model 25 RI detector. All of these are available from Perkin-Elmer (Norwalk, CT, U.S.A.); the flow cell used in the IR spectrophotometer was from Analabs (North Haven, CT, U.S.A.).

Post-column reactions were achieved by pumping the reacting fluid separately using a single pump (Perkin-Elmer Series 1). This fluid was combined with the column effluent using a tee and mixing was achieved using 4.3 m of tightly coiled tubing of 0.25 mm I.D.

The following columns and mobile phases were used for the investigations:

carbohydrates, apple and orange juice and cola drink: 250 × 8 mm I.D. Shodex S-801/S 10-μm columns (Part No. 0258-8886) using water as the mobile phase at 80°C;

red currant juice: 250 × 4 mm I.D. LiChrosorb NH_2 5-μm column using acetonitrile–water as the mobile phase;

phenol, aniline and polyaromatic hydrocarbons: either a 100 × 4.6 mm I.D. 3-μm (Part No. 0258-1501) or a 125 × 4.6 mm I.D. 5-μm (Part No. 0258-1001) C_{18} bonded-phase column using acetonitrile–water as the mobile phase;

polystyrene, cholesterol, diolein and phosphatidylethanolamine: 250 × 8 mm I.D. Shodex A-802/S (Part No. 0258-8286) and Shodex A-805/S (Part No. 0258-8586) columns using chloroform as the mobile phase.

All the part-numbered columns are available from Perkin-Elmer. The LiChrosorb columns were obtained from E. Merck (Darmstadt, G.F.R.).

Acetonitrile and chloroform, both HPLC grade, were obtained from Fisher

Scientific (Pittsburgh, PA, U.S.A.), while water was purified using a mixed-bed ion-exchange resin and an activated charcoal filter.

Reference chemicals were of the highest purity available and were obtained from Aldrich (Milwaukee, WI, U.S.A.), Sigma (St. Louis, MO, U.S.A.) and Pfaltz and Bauer (Stamford, CT, U.S.A.). Fruit juices and soft drinks used were obtained on the open market and were diluted and filtered through a 0.45-μm filter (Millipore, Bedford, MA, U.S.A.) prior to injection. The polystyrene standard sample was prepared from standards available from Perkin-Elmer (Part No. 0254-0074).

3. RESULTS AND DISCUSSION

3.1. Fluorescence detector

The use of fluorescence spectroscopy for selective detection in liquid chromatography is well established. The selectivity of the technique can be enhanced even further by using complementary techniques. We shall deal here with three: post-column acid–base manipulations, wavelength selection to enhance selectivity and the use of synchronous scanning for qualitative verification.

Unlike many other detection methods, fluorescence has very high sensitivity and, therefore, can be used even in trace analysis[6].

3.1.1. Post-column techniques. Usually, a fluorescing species which has a functional group will be sensitive to the pH of the mobile phase. Thus, by the proper adjustment of the pH, the selectivity of the system to certain compounds can be controlled. However, pH affects chromatography and thus the best method for manipulating this parameter is post-column before the detector. Most recently, Lee *et al.*[8] showed the application of this technique for the analysis of warfarin and its metabolites. Another important and practical example is the analysis of a mixture of phenol and aniline.

Phenol and aniline (and their respective homologues) are frequently found together, *e.g.*, in waste waters or similar samples. In many conventional reversed-phase chromatography systems, they give two very closely eluting peaks (Fig. 1) and often their separation cannot be achieved without affecting the separation of some other components in the chromatogram. However, a selective method for detecting phenol (and some of its derivatives) from aniline (and some of its derivatives) and *vice versa* is the adjustment of the pH just prior to the fluorescence detector:

(a) at high pH (*ca.* 12), phenol is converted to the non-fluorescent phenoxide ion ($C_6H_5O^-$) while aniline is strongly fluorescent at this pH;

(b) at low pH (*ca.* 2), aniline is converted to the non-fluorescent anilinium cation ($C_6H_5NH_3^+$) while phenol is strongly fluorescent at this pH.

Figs. 2 and 3 show the corresponding chromatograms. In Fig. 2, the pH of the column effluent (2 ml/min) was adjusted by the addition of a 1 *M* sodium hydroxide solution at 0.8 ml/min, thereby eliminating the response for phenol, while in the case of Fig. 3, 0.1 % orthophosphoric acid solution at 0.8 ml/min was added to the column effluent, eliminating the response to aniline.

3.1.2. Wavelength selection. Fluorescence is more specific than UV absorption because not all absorbing compounds fluoresce and the appropriate selection of both excitation and emission wavelengths often permits similar compounds to be differentiated[9,10]. In most cases, compromise wavelengths are used to provide appropriate detection for all compounds belonging to a class, but even here, if a wide-range

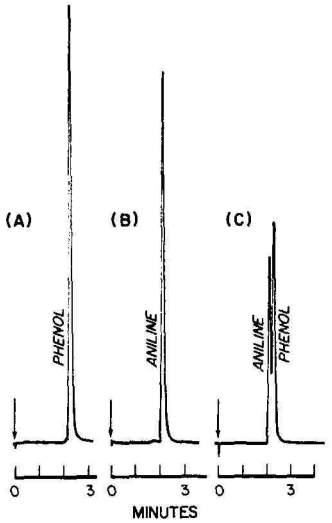
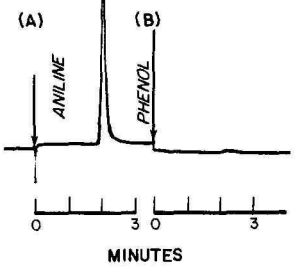


Fig. 1. Analysis of (a) phenol, (b) aniline and (c) their mixture with fluorescence detection. Column: 100 × 4.6 mm I.D., containing C_{18} bonded-phase 3-μm packing. Mobile phase: acetonitrile–water (30:70) at 2 ml/min. Wavelengths: excitation, 274 nm; emission, 325 nm.

mixture is analyzed, it is often advantageous to adjust the wavelengths during the run. For example, using 305-nm excitation and 430-nm emission wavelengths, a wide range of polyaromatic hydrocarbons from naphthalene to coronene can be analyzed with adequate sensitivity. However, as shown by Ogan *et al.*[11], better detectability for



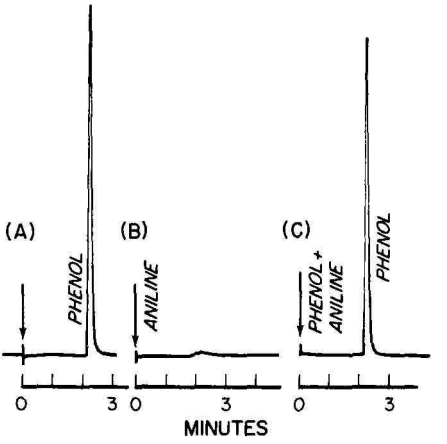


Fig. 2. Analysis of aniline and phenol with fluorescence detection at high pH. Column, mobile phase, flow-rate and wavelengths as in Fig. 1. Column effluent was mixed with a 0.8 ml/min flow of 1 *M* NaOH solution prior to entering the detector cell. (a) Sample contains only aniline; (b) sample contains only phenol.

Fig. 3. Analysis of aniline and phenol with fluorescence detection at low pH. Column, mobile phase, flow-rate and wavelengths as in Fig. 1. Column effluent was mixed with a 0.8 ml/min flow of 0.1% H_3PO_4 solution prior to entering the detector cell. (a) Sample contains only phenol; (b) sample contains only aniline; (c) sample contains a mixture of phenol and aniline.

compounds emerging earlier (naphthalene, acenaphthene, fluorene and phenanthrene) can be achieved by using 280-nm excitation and 340-nm emission wavelengths and later changing to 305/430 nm between the phenanthrene and anthracene peaks. Also, by changing the emission wavelength from 430 to 500 nm after benzo[*ghi*]perylene, a 70-fold increase in response to indeno[1,2,3-*cd*]pyrene could be realized.

By the proper selection of wavelength, not only the response to certain compounds can be enhanced, but also the response to others suppressed. As an example, Fig. 4 shows the analysis of a mixture of five polyaromatic hydrocarbons at four different wavelength combinations. At 275-nm excitation and 335-nm emission wavelengths the response to anthracene and perylene is suppressed while at 365/375 nm only anthracene and at 430/540 nm only perylene shows a response while all others are suppressed. On the other hand, at 265/305 nm, detection can be made specific to fluorene. Earlier Slavin *et al.*[10] have shown that using 338/395 nm detection is almost specific for pyrene, while at 338/450 nm detection is almost specific for coronene.

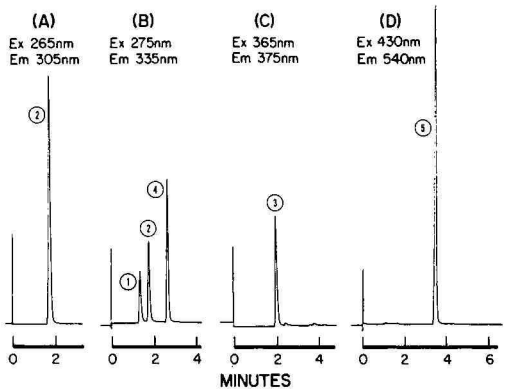


Fig. 4. Analysis of a mixture of five polyaromatic hydrocarbons with fluorescence detection. Column: 100 × 4.6 mm I.D., containing C_{18} bonded-phase 3-μm packing. Mobile phase: acetonitrile–water (65:35) at 3 ml/min. Wavelengths as given. Peaks: 1 = naphthalene; 2 = fluorene; 3 = anthracene; 4 = pyrene; 5 = perylene.

Selection of optimum wavelengths is, naturally, important not only for qualitative purposes, but also to enhance quantitative detection: by increasing the peak height for a given compound, smaller quantities can be detected extending the range of fluorescence detection. Since modern instrumentation permits the automatic change of wavelengths at any given point during an analysis, full utilization of this technique is possible.

3.1.3. Synchronous scanning. In synchronous scanning, the excitation (λ_{ex}) and emission (λ_{em}) wavelengths are varied simultaneously while maintaining a constant step, $\Delta\lambda$, between them:

$$\Delta\lambda = \lambda_{ex} - \lambda_{em} = \text{constant}$$

In this case, the fluorescence of a given compound is restricted to that excited at the wavelengths synchronously trailing the plotted emission. The result of this technique

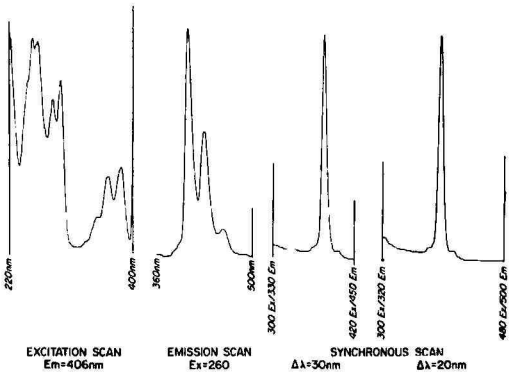


Fig. 5. Fluorescence spectra of benzo[*a*]pyrene obtained by stop-flow technique on the chromatographic peak. Chromatographic conditions: Columns: 125 × 4.6 mm I.D. containing C_{18} bonded-phase 5-μm packing. Mobile phase: acetonitrile–water (90:10) at 1 ml/min. On the scans the start and end wavelengths are indicated. Scanning speed: 120 nm/min.

is generally a much simplified spectrum where the intensity maxima are highly characteristic to a compound and thus, combined with stop-flow techniques, can be used for identification purposes. As an example, Fig. 5 shows the separate excitation and emission scans (keeping constant emission and excitation wavelength, respectively) and then the synchronous scan with two different $\Delta\lambda$ values for benzo[*a*]pyrene.

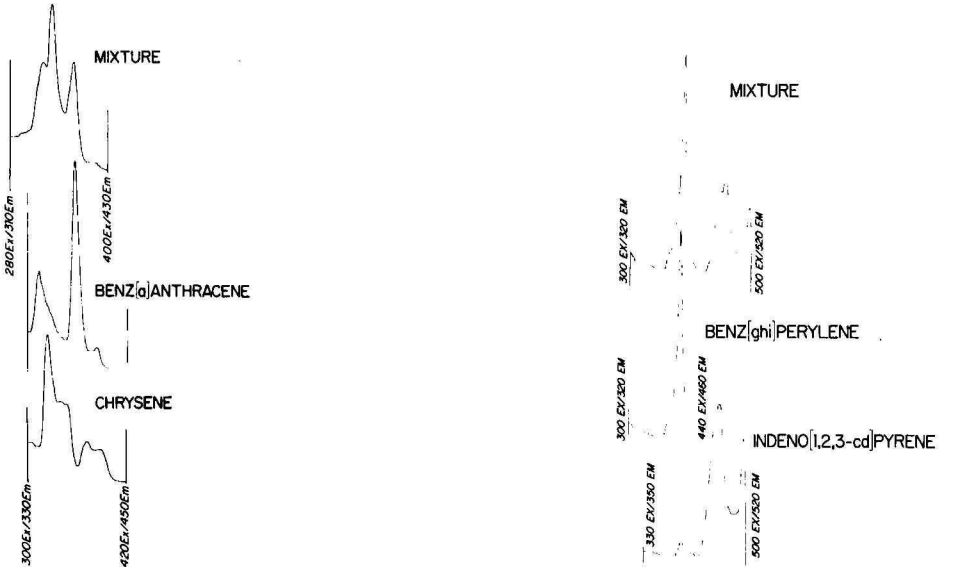


Fig. 6. Synchronous scans of pure chrysene and benz[*a*]anthracene and of their mixture, obtained by stop-flow technique on the chromatographic peak. Column: 125 × 4.6 mm I.D., containing C_{18} bonded-phase 5-μm packing. Mobile phase: acetonitrile–water (90:10) at 1 ml/min. On the scans the start and end wavelengths are indicated. Scanning speed: 120 nm/min; $\Delta\lambda$ = 30 nm.

Fig. 7. Synchronous scans of pure indeno[1,2,3 *cd*]pyrene and benz[*ghi*]perylene and of their mixture, obtained by stop-flow technique on the chromatographic peak. Column: 125 × 4.6 mm I.D., containing C_{18} bonded-phase 5-μm packing. Mobile phase: acetonitrile water (90:10) at 1 ml/min. On the scans the start and end wavelengths are indicated. Scanning speed: 120 nm/min; $\Delta\lambda$ = 20 nm.

The technique of synchronous scanning has been first described in 1971 by Lloyd[12]. Since that time, a number of papers have dealt with its theory and applications[13–25].

We have applied this technique to two pairs of polyaromatic hydrocarbons which are difficult to separate under various conditions. These are chrysene and benz[*a*]anthracene, and indeno[1,2,3-*cd*]pyrene and benz[*ghi*]perylene. Figs. 6 and 7 show the synchronous scans applied to the pure compounds and then to a peak containing the indicated compound pair. The presence of the two compounds in the respective peak can clearly be identified with help of the synchronous scans.

Synchronous scanning can be utilized with modern liquid chromatography systems with fluorescence detectors which permit stop-flow operation and wavelength scanning: when the peak of interest enters the detector cell, flow is stopped and the proper wavelength scan executed. When this is finished, flow is resumed and separation continued[10,26].

3.2. IR spectrophotometer as an LC detector

IR spectroscopy is one of our most powerful analytical tools and it has been widely used for the investigation of collected fractions, in both LC and GC; the introduction of multiple internal reflection IR spectroscopy[27,28] further enhanced this possibility. Yet, when utilized as an on-the-flow detector in a liquid chromatography system, it suffers from the lack of sensitivity and interference by the strong absorption by most of the mobile phases in use, particularly those in reversed-phase LC.

IR spectroscopy can be used in two ways for on-the-flow monitoring of column effluent: either by obtaining the spectra of each chromatographic peak and comparing these with reference spectra, or by setting the IR detector to a specific frequency characteristic to a given functional group[28]. The previous technique, *i.e.*, stopping the flow and scanning the desired wavelength range[29–31], has gained a new impetus in the last decade by the introduction of Fourier-transform IR spectroscopy[32–35].

Functional group monitoring of the column effluent has been described in the past, particularly for gel permeation[36,37] and non-aqueous reversed-phase liquid chromatography[38–41]. The essential requirements here are to find proper solvents which are transparent to IR radiation at the frequencies selected. In general, chlorocarbons and carbon disulfide fulfill these criteria. Table 1 lists the frequencies characteristic for the major functional groups and the solvents which can be used as the mobile phase[42].

We have adapted a relatively sensitive conventional IR spectrophotometer for use as an on-the-flow LC detector to explore the benefits and limitations of the technique. Here, we want to show a few applications of such a system.

Fig. 8 shows the size-exclusion separation of some polystyrene standards where ring stretching is monitored at 1604 cm^{-1}. The sensitivity of using IR as the detector as calculated from this chromatogram is about 1 μg.

Fig. 9 shows the chromatograms of a sample containing the diglyceride diolein and the phospholipid phosphatidylethanolanine at two different frequencies. Monitoring the ester bond at 1740 cm^{-1}, both compounds are detected along with some impurities, while the chromatogram obtained when monitoring the alcoholic group at

TABLE 1

PRINCIPAL FUNCTIONAL GROUP ABSORPTIONS

Compound type	*Functional group*	*Characteristic frequency (cm^{-1})*	*Solvent which is transparent*				
			CH_2Cl_2	$CHCl_3$	CCl_4	CCl_2CCl_2	CS_2
Alkanes	C–H	2850–3000	–	–	+	+	+
Alkenes	C–H	3080–3140	–	–	+	+	+
	C=C	1645–1670	+	+	+	+	+
Esters	C=O	1720–1735	+	+	+	+	+
Ketones	C=O	1665–1745	+	+	+	–	+
Alcohols:	C–H	3400–3600	–	–	+	+	+
Primary	C–O	1050	–	–	–	–	+
Secondary	C–O	1100–1125	–	–	–	–	+
Tertiary	C–O	1150–1200	–	–	–	–	+

3400 cm^{-1} reveals what appears to be a slight response to the phospholipid and a strong response to diolein.

Finally, Fig. 10 shows the application of two spectroscopic detectors, an IR and a UV spectrophotometer in series. The sample contained primarily cholesterol with some impurities present. The UV detector revealed the presence of several minor components while the IR detector, set to monitor the hydroxyl group at 3400 cm^{-1}, showed essentially only one main component.

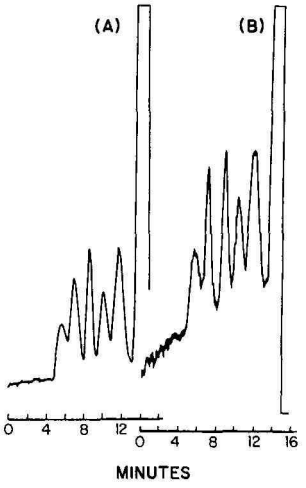


Fig. 8. Analysis of a polystyrene standard mixture using an IR detector. Column: Shodex A-805/S + A-802/S in series, each 250 × 8 mm I.D. Mobile phase: chloroform at 1 ml/min. IR detector at 1604 cm^{-1}; response time for 98% full-scale: 5.8 sec (a) 20 × ordinate expansion, 0.05 a.u.f.s., 90-μg sample. (b) 100 × ordinate expansion, 0.01 a.u.f.s., 20 μg sample. Sample composition: 1,460,000 MW (0.205%), 230,000 MW (0.206%), 19,000 MW (0.209%), 600 MW (0.336%) in chloroform solution.

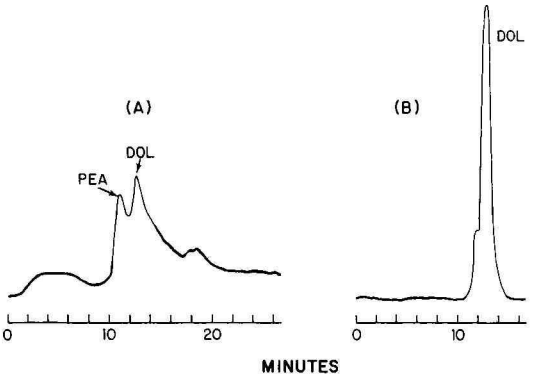


Fig. 9. Analysis of a mixture of diolein (DOL) and phosphatidylethanolamine (PEA) using an IR detector. Column: two Shodex A-802/S columns, each 250 × 8 mm I.D., in series. Mobile phase: chloroform at 1 ml/min. (A) IR detector at 1740 cm^{-1}, 10 × ordinate expansion, 0.1 a.u.f.s., injected sample contained 125 μg of DOL and 250 μg of PEA. (b) IR detector at 3400 cm^{-1}, 2 × ordinate expansion, 0.5 a.u.f.s., injected sample contained 75 μg of DOL and 150 μg of PEA.

3.3. Optical activity measurement for LC detection

The ability to differentiate between optical isomers is important since they occur frequently in nature and often only one of the isomers is active in a chemical or biological sense. However, these isomeric compounds will generally co-elute on most conventional stationary phases available today.

In liquid chromatography, great progress has been made in the development of systems in which a chiral center is present[43–46] either bonded to the stationary phase[47–61] or introduced to the mobile phase[62–73]. However, these phases and techniques are usually specific to certain classes of optical isomers. Therefore, the possibility of using a selective detector which can differentiate between the optical isomers even when no, or only partial, separation is accomplished, is of great interest. Another reason that selective determinations are important is that the compounds of interest are usually present in complex matrices where their separation from the other compounds may represent a problem.

Polarimetry, which has the capability of measuring the direction and degree of rotation of optical isomers, is a well established method in the investigation and

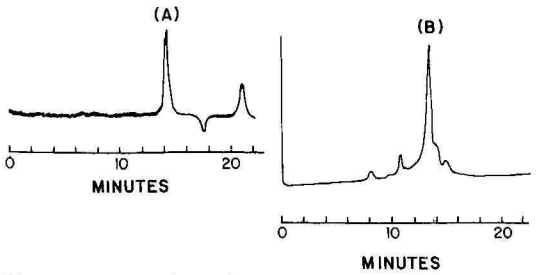


Fig. 10 Analysis of a cholesterol sample, using two spectroscopic detectors (IR + UV) in series. Column: two Shodex A-802/S columns, each 250 × 8 mm I.D., in series. Mobile phase: chloroform at 1 ml/min. Sample: 50 μg of cholesterol. (a) IR detector at 3401 cm^{-1}, 20 × ordinate expansion, 0.05 a.u.f.s. (b) UV detector at 254 nm, 0.08 a.u.f.s.

determination of optically active compounds[74–78]. However, except for a few attempts[79–82], its use as a liquid chromatographic detector has been restricted due to technical difficulties, a lack of sensitivity and the inavailability of appropriate instrumentation.

Recently, Böhme[83] demonstrated the capability of directly connecting a general-purpose polarimeter, modified with a small-volume flow cell, to monitor liquid chromatographic eluates. The polarimeter can also be used in series with other detectors*. Böhme used both a UV spectrophotometer and an RI detector, while in our work, the latter was applied. Using this approach, important detection problems can be solved, thereby extending the applications capabilities of the system.

Fig. 11 shows the separation of a carbohydrate mixture by a group-exchange mechanism, using the polarimeter and the RI detector in series. Three of the carbohydrates, raffinose, sucrose and glucose, rotate plane-polarized light in one direction (+), while fructose and ribose rotate the light in the opposite direction (−), thereby adding a degree of selectivity. The selected wavelength (365 nm) gives increased sensitivity relative to the commonly used sodium D line for all the carbohydrates present in the mixture. As discussed by Böhme[83], the specific rotation and hence detector response are strongly dependent on the wavelength and will increase for these compounds as the wavelength is decreased.

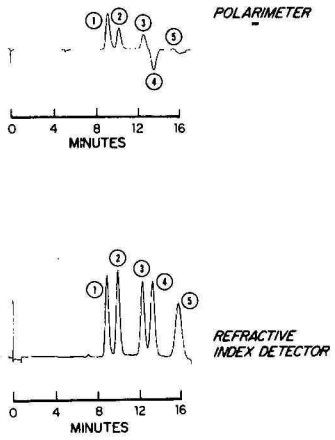


Fig. 11. Analysis of a sugar mixture. Column: two 250 × 8 mm I.D. in series, each containing Shodex S-801/S packing. Column temperature: 80°C. Mobile phase: water at 0.5 ml/min. Polarimeter at 365 nm, ± 0.5° full scale. Peaks: 1 = raffinose; 2 = sucrose; 3 = glucose; 4 = fructose; 5 = ribose.

The determination of sugars in fruit juices or similar soft drinks is an important application of liquid chromatography. In a number of instances, the separations are relatively straightforward; however, even here, one or two of the sugars present may co-elute with some other compounds. Thus, if using UV or refractive index detectors, a composite peak would be recorded. On the other hand, the polarimeter responds to the optically active compounds only and thus is blind to the other sample components which lack optical activity. As an example, Figs. 12–14 show the analysis of

* When the RI detector was used in series with another detector, it was always the last.

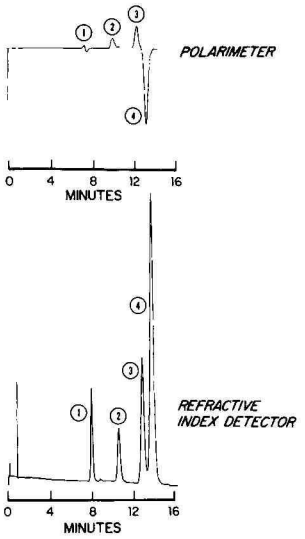
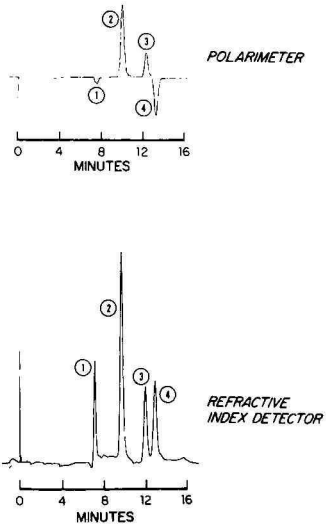


Fig. 12. Analysis of a commercial apple juice. Column, temperature, mobile phase and polarimeter conditions as in Fig. 11. The juice was diluted 10-fold and 40 μl of the diluted solution were injected. Peaks: 1 = ascorbic acid; 2 = sucrose; 3 = glucose; 4 = fructose.

commercial apple and orange juices and a cola drink, respectively. Here the sucrose peak in the RI detector chromatogram may contain at least one additional compound which is likely to give a response similar to that of sucrose. On the other hand, the



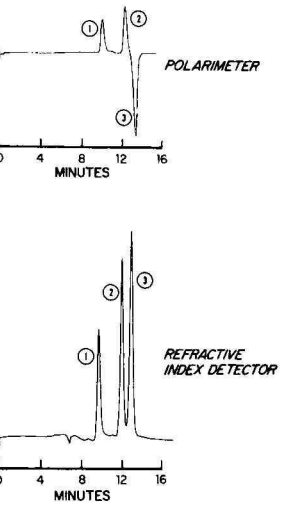


Fig. 13. Analysis of a commercial orange juice. Column, temperature, mobile phase and polarimeter conditions as in Fig. 11. The juice was diluted 10-fold and 75 μl of the diluted solution were injected. Peaks: 1 = ascorbic acid; 2 = sucrose; 3 = glucose; 4 = fructose.

Fig. 14. Analysis of a cola drink. Column, temperature, mobile phase and polarimeter conditions as in Fig. 11. A 3-μl aliquot of the filtered drink was injected without dilution. Peaks: 1 = sucrose; 2 = glucose; 3 = fructose.

possibility that the peak in the chromatogram obtained with the polarimeter corresponds only to sucrose is very likely, thereby increasing the degree of accuracy for this analysis.

In the case of more complex samples, the overlapping is quite clear, as shown in Fig. 15, presenting the chromatograms obtained when analyzing freshly pressed red currant juice[83]. Here, analysis without sample purification or same kind of pre-treatment is only possible when using the polarimeter as the LC detector.

In the chromatograms shown up to now, separation of the individual carbohydrates was adequate. Occasionally, however, one might face a situation where no separation can be observed when using a refractometric or UV detector although, if one carefully investigates the retention times of the individual compounds, it is evident that slight differences occur. If the optical activities of the two compounds are different, however, then it is quite possible that the polarimeter would indicate the presence of both compounds in spite of the apparent overlapping of their peaks on the other detectors. This is illustrated in Fig. 16 which shows the analysis of galactose and fructose both alone and as a mixture. It is clear that the polarimeter permits differentiation of these two carbohydrates, while neither UV nor RI detectors would do so.

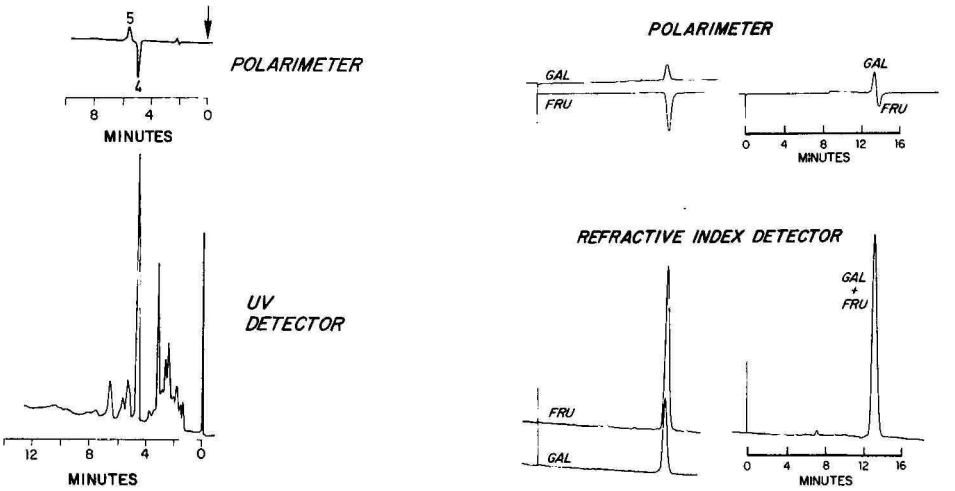


Fig. 15. Analysis of freshly pressed red currant juice[83]. Column: 250 × 4 mm I.D., containing LiChrosorb-NH_2 5-μm packing. Room temperature. Mobile phase: acetonitrile–water (80:20) at 1.5 ml/min. UV detector at 187 nm, 0.32 a.u.f.s. Polarimeter at 365 nm, ± 0.5° full scale. Sample volume: 5 μl. Peaks: 4 = fructose; 5 = glucose.

Fig. 16. Analysis of pure fructose (FRU) and galactose (GAL) and their mixture. Column, temperature, mobile phase and polarimeter conditions as in Fig. 11. Top: chromatograms recorded by the polarimeter. Bottom: chromatograms recorded by a RI detector.

3.3.1. Sensitivity. Among others, the sensitivity of the polarimeter depends on the specific rotation (α) of the compound of interest which in turn depends on the temperature and the wavelength applied. This value can vary in a relatively wide range; Table 2, after Böhme[83], lists specific rotation values of some carbohydrates at four different wavelengths. When comparing compounds of different types, the difference is even greater: *e.g.*, at 589 nm and room temperature, the value of α is +36

TABLE 2

SPECIFIC ROTATION VALUES (α) OF SOME CARBOHYDRATES AT 20°C[83]

Carbohydrate	*α (deg cm³ dm⁻¹ g⁻¹)*			
	365 nm	*436 nm*	*578 nm*	*589 nm*
Sucrose	192.0	127.8	77.8	66.1
Glucose	148.7	97.5	58.6	49.5
Fructose	−271.4	−108.7	−109.8	− 93.0
Galactose	230.3	152.9	81.9	78.3
Raffinose	354.4	235.3	142.7	120.5

for cholic acid and +223 for corticosterone (both in ethanol) or +14.5 for L(+)-alanine and +221 for D(+)-cystine (both in 1 *N* hydrochloric acid).

In general, one can see that the sensitivity of the polarimeter is in the microgram range, somewhat less than that of the RI detector.

3.3.2. Quantitative analysis. By using a concentration-sensitive detector, such as a UV or a refractive index detector, in series with the polarimeter, both the total amount and percentage of each optical isomer can often be determined. This is illustrated using the example of the analysis of penicillamine. D-Penicillamine, a derivative of penicillin, is used in medicine as a metal-chelating agent. The official method for its determination[84] is a volumetric assay where the sample is titrated with a mercury (II) acetate solution in an aqueous medium. Other methods involve a non-aqueous amine titration, a non-aqueous acid titration and a hydroxylamine assay[85]. However, none of these methods can distinguish between the D- or L-isomer.

D- and L-penicillamine cannot be separated chromatographically on a conventional stationary phase. Thus, if using an RI or UV detector, the peak obtained

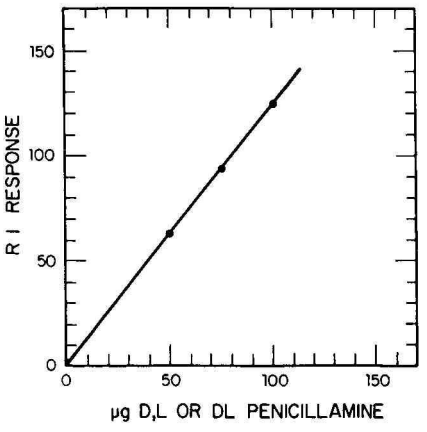


Fig. 17. Calibration curve of penicillamine (D+L) on a refractive index detector.

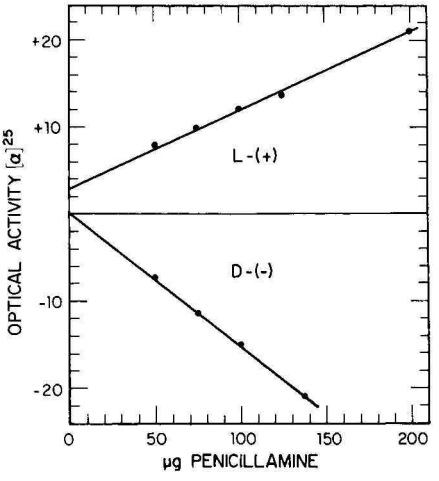


Fig. 18. Calibration curve of D- and L-penicillamine on a polarimeter detector.

corresponds to the total amount of the two optical isomers present. Using standard solutions, a concentration curve as shown in Fig. 17 is obtained. However, by analyzing different amounts of D- and L-penicillamine isomers separately using the polarimetric detector, a plot of optical activity *vs.* concentration for the D(−)- or L(+)-compounds show a linear response but with one plot having a positive slope and the other a negative slope (Fig. 18). From these calibration curves, the total concentration of penicillamine as well as the percentage of each isomer in the peak can be determined.

The following example illustrates the use of these calibration curves. A standard sample consisting of a mixture of 25 μg of L- and 70 μg of D-penicillamine was chromatographed. The peak obtained on the RI detector had an area of 115 units which, according to the calibration plot shown in Fig. 17, corresponds to 93.5 μg of penicillamine.

The polarimeter recorded a negative peak with an area corresponding to an $[\alpha]$ of 7°, equivalent to 44.5 μg of penicillamine (*cf.*, Fig. 18). This means that the mixture consisted of more than 50% D-isomer (hence, the negative peak) and the measured optical activity (or the corresponding amount) represented the difference of the two isomers. Therefore, D- + L- = 93.5 μg and D- − L- = 44.5 μg. From these data we can calculate the amounts of the two isomers as D- = 69 μg and L- = 24.5 μg and the respective deviations from the actual values are −1.4% and −2%.

4. CONCLUSIONS

In addition to the well known use of the fluorescence spectrophotometer to monitor the fluorescence of the sample components or their derivatives, its usefulness as a selective LC detector can be further enhanced either by proper wavelength selection or by additional manipulations such as post-column adjustment of the eluent's pH or simultaneous scanning.

Although their sensitivities are limited, infrared spectrophotometers and polarimeters can be successfully coupled to liquid chromatographs for on-the-flow selective detection of a wide variety of compounds or compound groups. In addition, the polarimeter can also be used for the quantitative analysis of optical isomers even if they are not separated on the column.

5. ACKNOWLEDGEMENT

We want to express our appreciation to Dr. W. Böhme (Bodenseewerk Perkin-Elmer & Co., GmbH, Überlingen, G.F.R.) for placing at our disposal the chromatogram shown in Fig. 15.

6. SUMMARY

New possibilities of selective detection in liquid chromatography are discussed. In fluorescence spectroscopy, post-column adjustment of the pH of the mobile phase permits selective quenching of the fluorescence of certain compounds making the detection more specific. Detection sensitivity can also be enhanced by wavelength selection. Finally, a special technique permitting the establishment of peak purity and

compound identification is simultaneous scanning of both excitation and emission wavelengths. On-the-flow infrared detection at pre-selected wavelengths is another possibility to enhance selective detection. Finally, analysis of the individual optical isomers can be accomplished by monitoring the optical activity of the compounds emerging from the column.

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* Davankov and co-workers published between 1971 and 1981 a large number of original papers on various aspects of ligand-exchange chromatography used for the separation of optical isomers of amino acids. Here only Parts I[47], V[48] and XII[49] are listed.

Journal of Chromatography, 251 (1982) 17–34
Chromatographic Reviews
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHREV. 155

CYCLODEXTRINS AS STATIONARY PHASES IN CHROMATOGRAPHY*

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(Received August 30th, 1981)

CONTENTS

1. INTRODUCTION

The exceptional properties of cyclodextrins (CD) have been described in many papers, books and reviews[1–10] and increasing attention has been paid to their study and use in recent years. This paper is intended to show, on the basis of the literature data and the results of our research, the possibilities of using chromatographic methods for the study of CD and the use of the selective formation of inclusion compounds (IC) of cyclodextrins for separation and analytical purposes using various chromatographic procedures.

1.1. Cyclodextrins

Cyclodextrins, which are cyclic oligosaccharides composed of D(+)-glucopyranose units interconnected by α-(1,4) bonds, are interesting chiefly for their inclusion properties, which are exhibited both in the solid state and in aqueous solutions. Inclusion complexes are formed inside the CD cavity, the geometry and chemical composition of which determine the selectivity of the inclusion process.

* Presented as plenary lecture at the International Microsymposium on Clathrates and Molecular Inclusion Phenomena, Stará Lesná, September 7–11, 1981.

0378-4355/82/0000–0000/$4.50

On laboratory and industrial scales, CD are produced by enzymatic degradation of starch, a polysaccharide containing glucose units interconnected to form a laevorotatory helix. The action of the enzyme disrupts the helix and the two ends of the fragment are connected to form a cyclic molecule (Fig. 1). As the enzymes used cut the helix not completely specifically, CD with various numbers of glucose units are formed; α-, β- or γ-CD, formed by 6, 7 and 8 glucose units, respectively, are present in the greatest amounts. The CD structure has a special arrangement of the functional groups, which has a great effect on the difference in the properties of α-, β- and γ-CD. For example, the structure of β-CD is given in Fig. 2. The secondary hydroxyl groups on the C(2) and C(3) atoms, O(2)H and O(3)H, are localized on one side of the ring, whereas the primary hydroxyl groups on C(6), O(6)H, are on the opposite side. The interior of the ring contains only a circular configuration of hydrogen atoms and glycoside oxygen atoms; therefore, the ring interior is apolar relative to water. From a side view, the shape of the ring molecule is conical. The wider side contains the secondary hydroxyl groups and the opposite opening is occupied by the primary hydroxyl groups. The glucose units assume the chair conformation, the C(6)–O(6) groups deviating out of the ring. However, during interaction with a guest (hydrogen bonding) these groups can deviate into the ring. Intramolecular hydrogen bonding, O(3)–O(2), occurs between the secondary hydroxyl groups of the neighbouring glucose units and makes the CD ring more rigid.

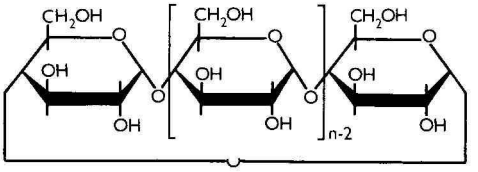

Fig. 1. Structural formula of CD.

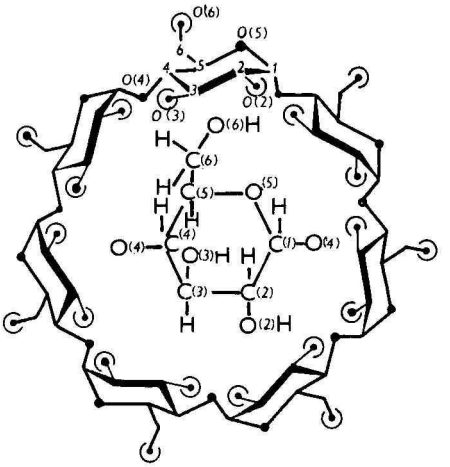

Fig. 2. Structure of β-CD.

α-, β- and γ-CD form cavities with a size of 5–8 Å that permit inclusion of molecules (or their parts) of corresponding dimensions (Fig. 3)[11]. An inclusion process between CD and a guest can be described by a dissociation constant, K_d, with values of the order of 10^{-3} mol/l characteristic of weak intermolecular interactions; there is no dependence on the guest chemical properties.

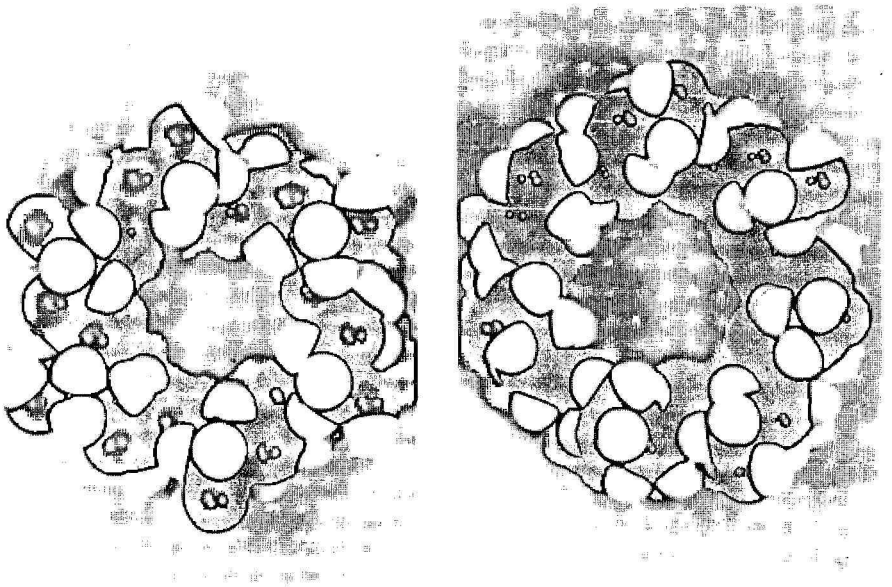

Fig. 3. Models of α-CD (left) and β-CD (right) molecules.

The thermodynamic parameters enthalphy (ΔH) and entropy (ΔS) can be found from the temperature dependence of K_d. The ΔH values are always negative, *i.e.*, the inclusion compounds dissociate on increasing the temperature, whereas ΔS can be either positive or negative depending on the force type.

The dependence of K_d on the guest polarizability indicates that Van der Waals forces predominate during the inclusion[12,13]; it can be shown from the crystallographic data that hydrogen bonding can also occur between the guest and (primarily) the O(6)H group of the CD. Moreover, hydrophobic interaction can also occur during the inclusion[14,15]. The question is which mechanism predominates in the inclusion of the guest molecule into the CD ring. The character of the interaction between CD and a guest has been studied many times, but an unambiguous picture has not yet been obtained. It seems that Van der Waals interactions often predominate, including interactions of permanent and induced dipoles and London dispersion forces. These forces are approximately proportional to the reciprocal of the sixth power of the distance between the guest and host and to the polarizability of the two components. In view of the internal dimensions of CD, where the distance between the guest and the host is small, the magnitude of these interactions can be significant.

1.2. Cyclodextrin polymers

The specific properties of CD are retained even if they are used in the form of polymers which are less soluble in water than the orginal CD. This is important, because the ability of CD to form water-soluble inclusion compounds is often a limiting factor, especially when CD is to be used as a stationary phase in liquid chromatography. Homopolymers are formed by polymerization of CD derivatives[16]. Copolymers are formed by reaction of the CD hydroxyl groups with bi- or polyfunctional molecules[17]. These polymers are often termed resins. As CD are polyfunctional molecules, cross-linking can occur and from a certain molecular weight resins with gel structures are formed, which are insoluble in water. Immobilized CD on a polymeric support has also been prepared (*e.g.*, on Sepharose), either chemically bonded[18] or obtained by mixing the CD with a water-insoluble polymeric gel [poly(vinyl acetate), polyacrylamide, nitrocellulose][19]. Block polymerization was originally used for the preparation of a water-insoluble resin and the product had to be mechanically ground[19,20]. During the pearl preparation[21,22] in which the heat is removed during spatial cross-linking, a product with a uniform grain size can be obtained. Research carried out in this direction has permitted the use of cyclodextrin polymers as chromatographic materials.

1.3. Chromatographic analysis of cyclodextrins

In addition to detection of CD by paper chromatography based on a characteristic coloration of the complexes with iodine and iodide[23] (α-CD yields a blue-black colour, whereas the analogous complexes of β- and γ-CD are brown-yellow), chromatographic procedures have been developed which are less tedious and time consuming than the classical separations. The oldest method for the separation of individual CDs based on paper chromatography still required 18 h for a single analysis. Thin-layer chromatography on cellulose has been found to be more advantageous and applicable also to preparations. Column liquid and gas chromatography can also be employed for the separation and determination of α-, β- and γ-CD. However, gas chromatography requires prior derivatization to cyclodextrin dimethylsilyl ethers. The best results in the shortest time were obtained by using high-performance liquid chromatography, when the analysis time decreased to 16 min. The methods of chromatographic separation, experimental conditions and references are summarized in Table 1.

2. CYCLODEXTRINS AS STATIONARY PHASES IN CHROMATOGRAPHY

The fact that CD can form inclusion complexes preferentially with certain types of compound, depending primarily on the molecular shape, has led to studies of their use as stationary phases in chromatography.

2.1. Liquid chromatography

The course of the inclusion process, which has been described in most papers as a process proceeding in the aqueous phase, agrees with the considerations about the

TABLE 1

CHROMATOGRAPHIC SEPARATION OF CYCLODEXTRINS

Chromatography	*Chromatographic conditions*	*Ref.*
Paper	Paper, Schleicher & Schüll 2045; mobile phase, butanol–pyridine–water (1:1:1).	24, 25
	Paper, Whatman No. 1; mobile phase, butanol–dimethylformamide–water (2:1:1); detection, alcoholic solution of I_2.	26
Thin-layer	Microchromatoplates with silicic acid; mobile phases, (a) butanol–acetic acid–water–pyridine–dimethylformamide (6:3:1:2:4), (b) butanol–acetic acid–water (6:3:1); detection, H_2SO_4–$K_2Cr_2O_7$.	26
	Microcrystalline cellulose; mobile phase, butanol–ethanol–water (4:3:3); detection, I_2 solution.	27
Liquid column	Cellulose; gradient elution, water–ethanol–butanol.	28
	Cellulose; elution, butanol–ethanol–water (42:29:29); flow-rate, 75–90 ml/h; column length, 63 cm; detection, polarimeter.	29
	Active carbon; gradient elution, butanol–water; flow-rate, 130–170 ml/h; detection, polarimeter.	30
	Sephadex G-15; elution, water–sodium azide (0.2%).	31
	Molselect G-25, G-15, cross-linked dextran; elution, water; detection, polarimeter.	32
High-performance liquid	μBondapak–carbohydrate; mobile phase, acetonitrile–25–30% water; column length, 30 cm; I.D., 1 mm; flow-rate, 2 ml/min; time of analysis, 18 min.	33
Gas	Dimethylsilyl ethers of cyclodextrins; column, 3% SXR on Chromosorb W AW DMCS (80–100 mesh); temperature, 325°C, programmed at 20°C/min up to 405°C; carrier gas, helium; flow-rate, 45–50 ml/min.	34

use of the selective character of inclusion in liquid chromatography. The requirement that the host be insoluble in aqueous media is met by cyclodextrin polymers (CDP) which, as stated above, retain the inclusion properties. These substances have become suitable chromatographic materials and have been used chiefly in gel inclusion chromatography[35].

2.1.1. Gel inclusion chromatography

Water-insoluble CD polymers in the form of gels can interact with various compounds according to various mechanisms[36]. They involve (a) interactions in the cavities, *i.e.*, the formation of inclusion compounds, (b) interactions in the internal pores of polymeric pearls and (c) interactions on the surface (which can be neglected).

These mechanisms, compared with Sephadex gels as non-inclusion analogues, have become the basis of gel inclusion chromatography (GIC).

A comparison of the interaction isotherms for two types of gel, Sephadex and a CD polymer[17] (prepared from a mixture of α-, β- and γ-CD by cross-linking with epichlorohydrin) with aniline, pyridine, benzaldehyde, butyric acid and *o*- and *p*-nitrophenol (Fig. 4), is very illustrative. Great differences have been found in all these instances, confirming the inclusion character of interactions with the CD polymer. It

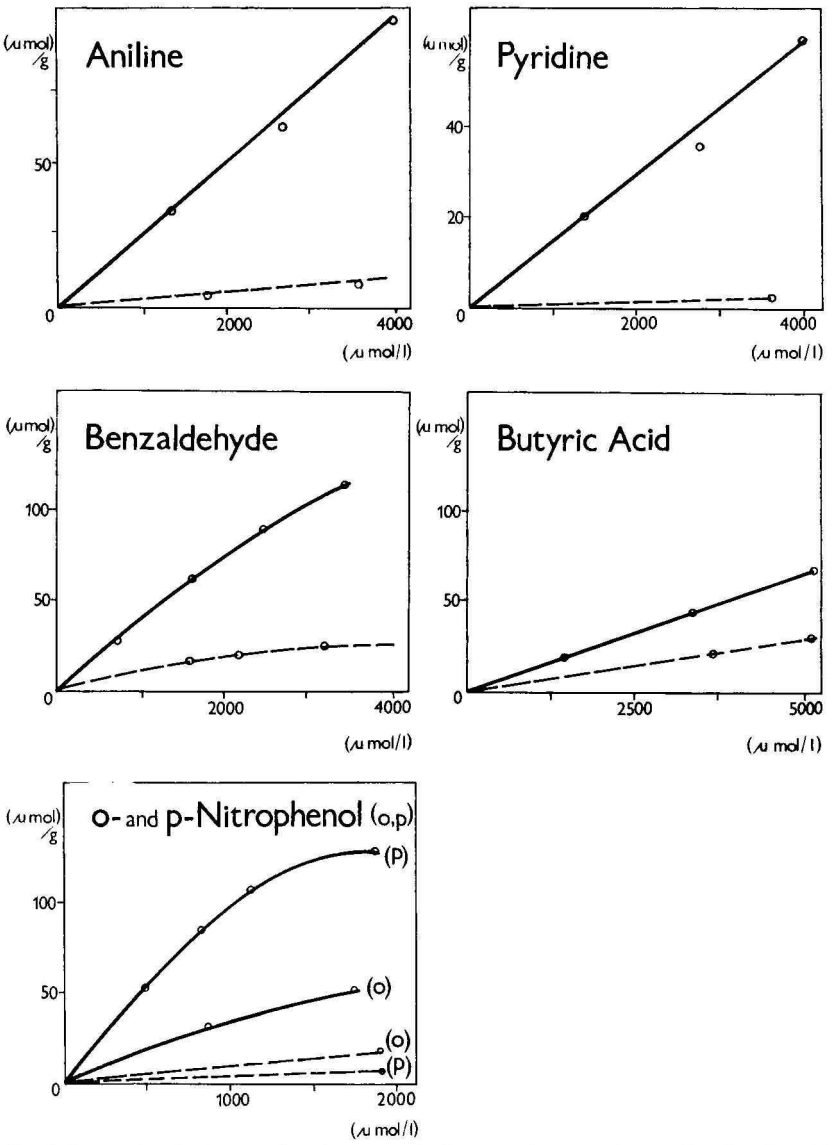

Fig. 4. Interaction isotherms of various organic compounds on a mixture of α, β and γ-CD resin (solid lines) and epichlorhydrin–dextran resin (broken lines) (from ref. 17).

follows from the shape of the isotherms of *o*- and *p*-nitrophenol that these substances cannot be separated on Sephadex, whereas the different inclusion interactions on the CD polymer can be used to advantage for their chromatographic separation[19].

Similar conclusions can be drawn from the interaction isotherms for the isomers of chlorobenzoic acid with α- or β-CD polymer (Fig. 5)[37]. It follows from the slopes of the isotherms that *m*-chlorobenzoic interacts most strongly, whereas *p*- and *o*-chlorobenzoic acids interact differently, but both substantially less. The stronger interaction with β-CD than with α-CD corresponds to the inclusion mechanism which is governed by the size of the cavity in the CD ring. The elution data given in Table 2 demonstrate the different behaviour of the substances studied on a β-CD polymer and Sephadex[35]. The data confirm a strong interaction with the β-CD polymer and the fact that *o*-chlorobenzoic acid can be separated chromatographically from benzoic acid, whereas the peaks of the two components overlap on Sephadex.

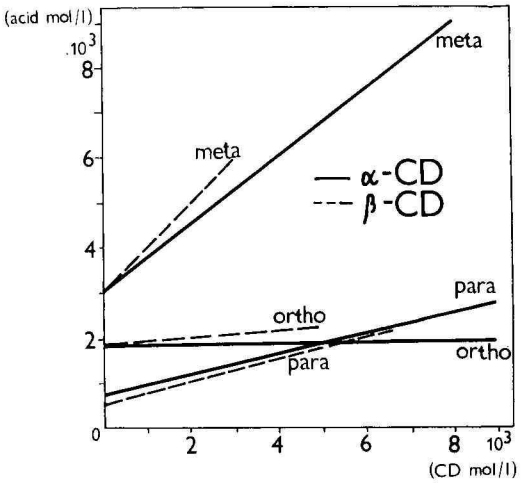

Fig. 5. Interaction isotherms of chlorbenzoic acids with α-CD (solid lines) and β-CD (broken lines) at 30°C (from ref. 35).

TABLE 2

ELUTION VOLUMES (V_e) ON SEPHADEX G-25 COARSE AND β-E25 RESIN

Compound	V_e *(ml)*	
	Sephadex G-25	*β-E25 resin*
Aniline · HCl	150	511
Benzoic acid	100	400*
Phenol	133	–**
o-Chlorobenzoic acid	83	98
m-Chlorobenzoic acid	83	400*
Dextran blue	55	40

* Tailing.

** Does not leave the column.

A general conclusion can be made on the basis of the above data about an increased affinity of CD towards aromatic molecules and a steric specificity towards their isomers. This property has been examined in detail with amino acids as model substances on CD polymers prepared for chromatographic purposes by polymerization with ethylene glycol diepoxypropyl ether in the presence of poly(vinyl acetate)[38–40]. Comparative experiments performed on α-, β- and γ-CD polymers in weakly acidic solutions (pH 5–6) showed the greatest differences in the retention data on the β-CD gel. This polymer has been found most suitable for separations of aromatic amino acids (phenylalanine, tyrosine, tryptophan). A high separation efficiency (H = 0.7–0.8 mm) was attained for these substances at laboratory temperature and a flow-rate of 10–20 ml/h. Under these conditions aromatic acids could be separated from non-aromatic acids (lysine, alanine), as well as from one another. The behaviour of these substances on α-, β- and γ-CD polymers can be demonstrated by chromatograms obtained under the same conditions (Fig. 6). The results obtained for aromatic acids, especially on β-CDP, correspond to the assumption of the extent of inclusion in the total interaction, whereas the mechanism of the separation of lysine and alanine depends primarily on adsorption. This conclusion is also confirmed by comparing the chromatograms obtained with β-CDP and Sephadex under identical conditions (Fig. 7).

Analogously, separation of indole alkaloids could be achieved on β-CDP, whereas on other carbohydrate type gels such as Sephadex, high and different retentions of alkaloids have been observed[41]. This suggests that the mechanism of the

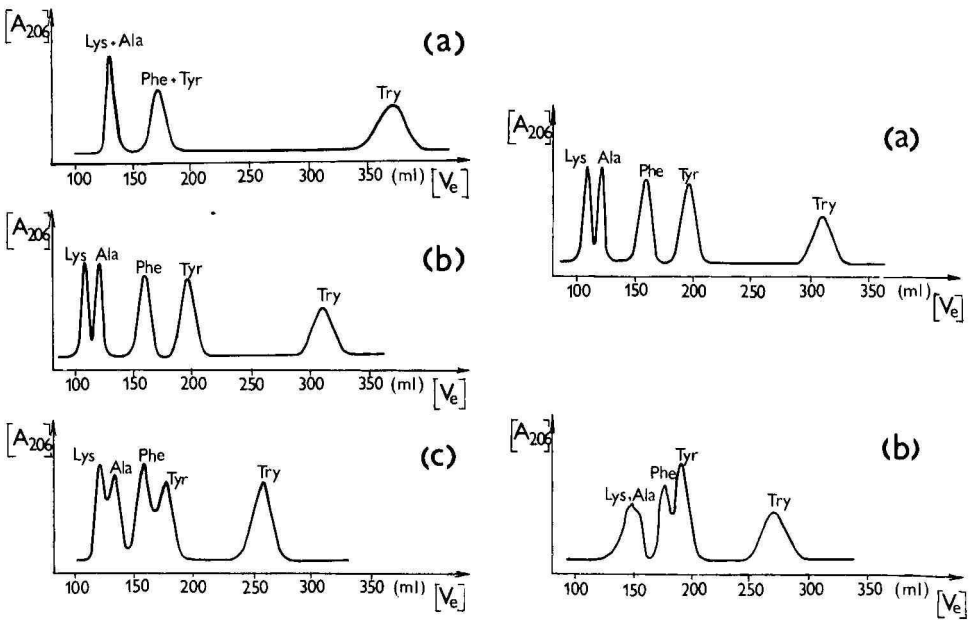

Fig. 6. Gel inclusion chromatographic separation of amino acids (a) on α-CDP, (b) on β-CDP and (c) on γ-CDP (from ref. 39).

Fig. 7. Gel inclusion chromatographic separation of amino acids (a) on β-CDP and (b) on Sephadex G-25 gel (from ref. 39).

interaction is governed by inclusion complex formation or a combined effect of inclusion and adsorption, rather than by adsorption only.

Recently separations of some aromatic amino acids on α- and β-CD polyurethane resins have been reported[42]. Good separation was achieved on the β-CD polyurethane resin, whereas no separation was observed on the α-CD resin and on polyurethane without CD. The retention behaviour on different kinds of β-CD polyurethane resin was found to be dependent upon the specific type of isocyanate cross-linking agent used. This suggests that some type of secondary interaction could be present. However, the elution order of the compounds separated confirms a host–quest interaction between the CD present in the resin and the different amino acid molecules.

The ability of some components of nucleic acids, especially those with an adenine base, to form compounds with β-CD, can also be readily used for chromatographic separations of various nucleotides and nucleosides[43,44]. In parallel experiments with solutions of CD and a CD gel it has been shown that the inclusion bond is affected by some factors, such as pH and the position of the phosphate group in the nucleotide. The latter effect with various adenosine monophosphates (AMP) and β-CD can be readily demonstrated on the shapes of the differential spectra (Fig. 8). As nucleotides with the adenine base have the same absorbance, the magnitude of the spectral shift should be proportional to the magnitude of the interaction with CD.

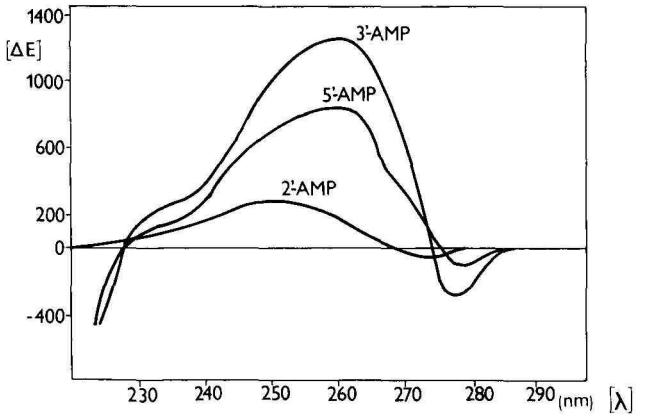

Fig. 8. Interaction of β-CD with adenosine monophosphates (from ref. 44).

The shape of the spectrum of 3′-AMP (adenosine-3-monophosphate) and 5′-AMP indicates that the strongest interaction occurs when the phosphate group remains outside the CD cavity (owing to ionization). Compared with these isomers, 2′-AMP, which contains the base and the phosphate group on the neighbouring carbon atoms of ribose, reacts substantially less with CD. From these spectral measurements conclusions can also be drawn about the chromatographic behaviour of these substances. Components with the largest change in the spectrum should be retained most on the chromatographic column. The order of elution of isomeric adenine monophosphates (Fig. 9) justifies these conclusions. The inclusion process, as stated above, is also

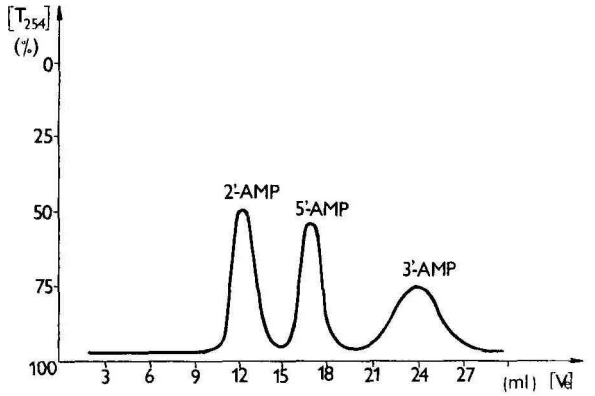

Fig. 9. Chromatogram of adenosine monophosphates on β-CD gel (from ref. 44).

affected by the pH. For successful separation the pH should be 7–8; at lower values the nucleotides–β-CD interaction decreases considerably.

With oligonucleotides containing adenine the guest–host interaction is not as strong. Therefore, anion-exchange groups were built into a CD gel (dimethylaminoethyl groups. The gel thus obtained exhibits both ion-exchange and inclusion properties and has also been used to separate tRNA[44].

CD polymers can also be used for the separation of racemic mandelic acid and its derivatives[45]. The β-CD polymer preferentially forms inclusion compounds with L-(+) isomers, permitting the separation of the DL methyl ester of mandelic acid; the first fraction contains pure D-(−) isomer. The α-CD polymer has the opposite effect and binds D-(−) isomers preferentially; however, the separation of racemates is then not complete.

2.1.2. Affinity chromatography

A competitive action of α-CD on the activity of β-amylase has been used for the solution of special analytical problems[46]. If α-CD is bound to epoxy-Sepharose 6B a gel is formed that retains β-amylase, whereas α-amylase is not retained. After elution of β-amylase by a highly specific eluent with competitive counter ligand, in this case α-CD, a highly pure enzyme with a high activity is obtained[18].

Alternatively β-CD has been used as immobilized ligand on epoxy-Sepharose 6B in biospecific affinity chromatography[47]. In this case α-amylase was selectively retained and separated from proteins and then selectively eluted by a buffer containing β-CD. By this chromatographic procedure a recovery of 90 % was obtained with a purification of up to 180 fold compared to crude extracts.

β-CD with other oligosaccharides has also been used for selective retardation of phosphorylases by affinity electrophoresis on polyacryl gel. On the base of the mobility changes of phosphorylase in dependence on the concentration of the oligosaccharides in the gel, the dissociation constants of complexes have been calculated[48].

2.1.3. Evaluation of the use of cyclodextrins in liquid chromatography

The results obtained have shown that the application of CD and CD polymers in liquid chromatography, especially in gel inclusion chromatography, has been very

successful. A number of concepts have been confirmed and the knowledge of the inclusion mechanism has been widened on the basis of data obtained by various instrumental methods. In addition, the use of CD as stationary phases has made possible many important analytical applications. The selectivity of the inclusion process is the main factor that makes these substances so attractive as chromatographic materials. However, only relatively low efficiencies have been attained so far, which also follow from the conditions of liquid chromatography. Therefore, efforts have been made in a few instances to study the character of the inclusion process with CD using guests in the gaseous phase. This process, observed, *e.g.*, during the inclusion of odorous compounds[49], does not permit more general conclusions to be drawn at present.

2.2. Gas chromatography

The present knowledge of the inclusion character has so far not enabled the inclusion of guests present in the gaseous phase into the CD cavity to be described in greater detail. In a similar manner to urea, CD also exhibit various affinities, *e.g.*, towards linear- and branched-chain alkanes[50–52]. In contrast to urea, where the inclusion structure is only formed in contact with the guest, the CD cavity is present before the inclusion process. Analogous to other inclusion compounds, molecular dimensions play a predominant role; too small guest molecules are not accepted by the relatively large CD cavity because of the short-range character of the forces operative. For example, helium, neon and argon do not form inclusion compounds with α-CD, whereas krypton and xenon do[53]. Similarly, α-CD forms very stable inclusion compounds with oxygen, carbon dioxide and chlorine at high pressure[11].

2.2.1. Cyclodextrin derivatives in gas chromatography

Acetylated cyclodextrins (α- and β-CD acetate, β-CD propionate, butyrate and valerate) have been used[54,56] as polar gas chromatographic stationary phases; however, the separation process was unaffected by inclusion. This use was based on the finding that polyesters used for chromatographic separations of fatty acids have C:O ratios similar to those of saccharide esters, the applicability of which, however, is limited by temperature. Acylated cyclodextrins have a relatively high thermal stability (220- 236°C) and a good separation efficiency for various polar compounds (α-olefins, aldehydes, alcohols, esters, aldehyde-esters and diesters).

Different results have been obtained with methylcyclodextrins phases[57], where the retention of organic compounds could be influenced by inclusion processes. The methylated CD was either deposited on silanized Chromosorb W or was part of mixed phase of 10% methylated CD in silicone oil. The elution of the hydrocarbons studied was in agreement with the stability of the inclusion compounds. Isooctane had a larger retention time on methylated β-CD than on methylated α-CD, which corresponds to the larger β-CD cavity.

The requirement of thermal stability (especially important for gas chromatography) is also met by the original CD. A thermogravimetric study of β-CD[58] (Fig. 10) shows that at 80–100°C a decrease in weight of about 8% occurs owing to the loss of moisture. A further weight decrease takes place at about 300°C, when CD begins to decompose. A necessary condition for gas chromatographic use is the presence of inclusion process even at high temperatures.

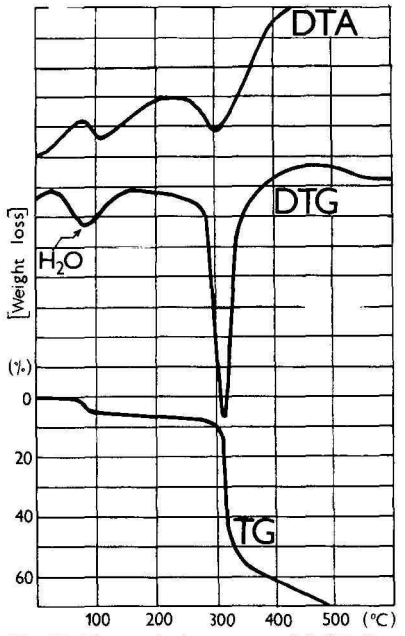

Fig. 10. Thermoderivatogram of β-CD (from ref. 58).

Recent results have shown that these requirements can be satisfied by macroporous polymers with inbuilt CD molecules[59] and by CD deposited on chromatographic supports[60].

2.2.2. Gas–solid chromatography

Cyclodextrin–polyurethane resins have been used as stationary phases in gas–solid chromatography (GSC)[59] and their sorption properties have been studied. It was found that specific interactions governed by the dimensions and configuration of the host molecules take place. The retention data for many organic substances are correlated with the inclusion phenomena, *i.e.*, with the size of the α- and β-CD cavities, and with the effect of π-electrons and heteroatoms in the guest molecule. Compared with common organic polymers (Amberlite, Porapak Q, Tenax GC), an advantage of polymers containing CD is their selectivity, which could also be utilized in trace analysis for the pre-concentration of test substances.

The formation and properties of the inclusion compounds of α- and β-CD can also be studied in the GSC system if CD (10%, w/w) is deposited on Chromosorb W from a dimethylformamide solution[60]. The sorbates were chosen to include organic molecules of various structural types and geometries (hydrocarbons, hydrocarbon halogeno derivatives, alcohols, ethers and aromatic substances).

To evaluate the character of the interaction, the dependence of the retention data on the polarizability of the sorbate was primarily followed, as a measure of the action of the dispersion forces in the interior of the cyclodextrin ring[13,60]. However, the effect of surface forces during inclusion cannot be excluded, as the sorbate molecules are in equilibrium not only with the inside of the CD ring, but also with its

surface. Therefore, the retention time can also be determined by the time of deposition on the surface prior to the interaction in the CD cavity. In Table 3 some of the measured data are summarized[60]. With aliphatic hydrocarbons only dispersion forces are operative and hence the great differences in the retention on α- and β-CD can be explained only by the inclusion process. In view of the smaller cavity of α-CD, this process is more pronounced, as demonstrated by the retention data, where for *n*-pentane the difference is *ca.* 490 sec for α- and β-CD; with higher hydrocarbons the interaction is so strong that they are completely retained in the α-CD cavity.

TABLE 3

RETENTION DATA OF VARIOUS SORBATES ON α-CD AND β-CD

Column temperature, 80°C; column length, 120 cm; I.D., 3 mm; carrier gas, nitrogen (30 ml/min); detector, FID.

Sorbate	*B.p. (°C)*	t'_R *(sec)*	
		β-CD	*α-CD*
n-Pentane	36.07	5	496
n-Hexane	68.7	25	*
n-Heptane	98.42	75	*
Cyclohexane	80.7	81	1588
Benzene	80.1	133	1628
Toluene	110.8	191	1379
1,2-Dichloroethane	83.7	154	1429
Trichloroethylene	87.0	111	1730
1,1,2,2-Tetrachloroethane	146.0	228	850
Tetrachloromethane	76.8	105	254
Chlorobenzene	132.1	501	3328
Bromobenzene	156.2	1008	*
Methanol	64.7	686	437
Ethanol	78.4	348	790
Propanol	97.8	1119	2169
Isopropanol	82.4	487	1577
Diethyl ether	34.51	914	723
Diisopropyl ether	67.8	107	232

* Does not leave the column.

A comparison of the retention data for hydrocarbon halogeno derivatives shows pronounced differences, from which it can be assumed that during inclusion into α-CD the forces inside the CD ring play a greater role, because the dispersion forces in the α-CD cavity act at a shorter distance. The especially large difference between the retention data for trichloroethylene and 1,2-dichloroethane on α- and β-CD is made even more important by the fact that the boiling points of the substances are 40–60°C lower than those for the tetrahalogeno derivatives studied, which have substantially lower retentions.

The results of measurements on aromatic and cyclic compounds again indicate

a greater interaction with α-CD. A comparison of the data for benzene and toluene is interesting; the retention order is reversed as, in agreement with the model of the α-CD inclusion compound with benzene (see Fig. 11), benzene is more retained than toluene by α-CD.

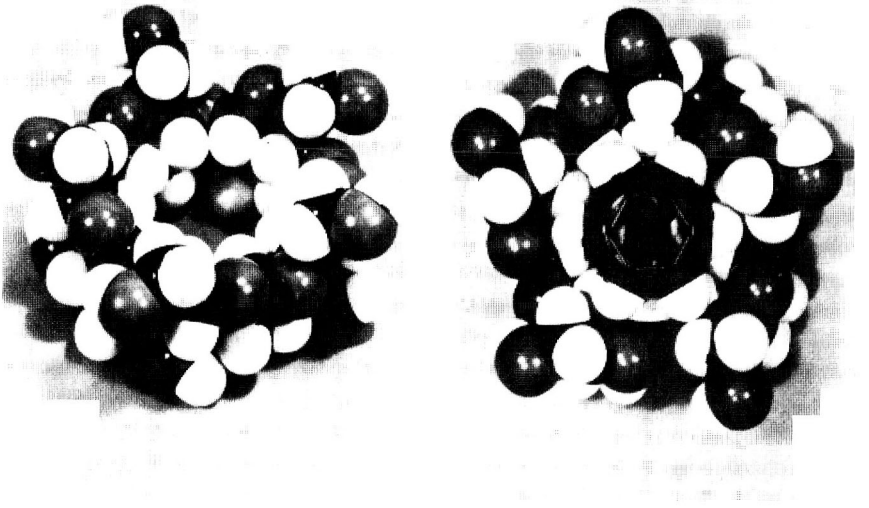

Fig. 11. Models of α-CD and α-CD inclusion compounds with benzene.

The effect of the geometric structure of the sorbates was very pronounced with diethyl ether and diisopropyl ether. The retention of the more volatile diethyl ether is more than three times higher for α-CD compared with β-CD and almost nine times higher compared with diisopropyl ether (see Table 3). This fact can be explained by the steric arrangement; analogous to urea, the branched-chain ether is less retained, in view of the cavity dimensions.

The results of the study of the interactions of α- and β-CD with various alcohols do not show great differences in retention times. This fact can be explained by the effect of the surface hydroxyl groups, which contribute significantly to the retention of polar substances on the two cyclodextrins. In addition to these polar forces, an inclusion process also takes place and is more marked for α-CD. In view of the relatively small cavity, α-CD binds the alcohols into more stable complexes, as a result of closer contact. Matsui and Mochida[61] arrived at the same conclusion and confirmed the greater stability of the α-CD inclusion compound with an aliphatic chain on the basis of the calculation of the association constants for the α- and β-CD inclusion compounds with various alcohols in aqueous solution. The difference found in the association constants again confirms the great effect of the volume of the guest molecule on the stability of the inclusion compounds of cyclodextrins with alcohols.

2.2.3. Evaluation of the use of cyclodextrins in gas chromatography

In the overall evaluation and comparison of the experimental results

TABLE 4

PHYSICAL PROPERTIES AND RETENTION DATA ON CD POLYURETHANE RESINS

Column temperature, 150°C; column length, 120 or 80 cm; I.D., 3 mm; carrier gas, nitrogen (30 ml/min); detector, FID. Data from ref. 58.

Property	*Resin**		
	β: HDI–DMF–5.5–A	*α: HDI–DMF–5.9–A*	*α: HDI–DMF–13.3–A*
Temperature limit (°C)	200	230	230
Specific surface area (m^2/g)	170	180	280
OH residues per CD molecule	13.5	10.1	0.9
Retention times of various sorbates**:			
n-Hexane	0.04	0.16	0.27
n-Heptane	0.06	0.24	0.33
Cyclohexane	0.05	0.07	0.12
Benzene	1.00 (16.68***)	1.00 (6.59***)	1.00 (4.16***)
Toluene	1.20	2.73	1.96
Methanol	0.36	0.53	1.06
Ethanol	0.78	0.83	1.53
Propanol	1.96	2.03	2.80

* Resins obtained by polymerization of CD with hexamethylenediisocyanate (HDI) in N,N-dimethylformamide (DMF). Precipitant: acetone (A) (feed composition: β-CD 10 g + 5.5 g HD; α-CD 8.6 g + 5.9 g HDI; α-CD 8.6 g + 13.3 g HDI).

** Relative to benzene = 1.00.

*** Actual retention time (min).

with CD–polyurethane resin and CD deposited on a chromatographic support as stationary phases, it is appropriate to quote some data that can help in explaining the character of the interaction. With the CD–polyurethane resin it has been found that the preparation conditions affect their physico-chemical properties (Table 4). The differences in the specific surface area can affect, as follows from the data given below, the magnitude of the retention data and make unambiguous interpretation difficult. On the other hand, the retention on the material with the lowest content of residual hydroxyl groups demonstrates that with polar substances the interaction is not markedly affected by these processes and the results can be interpreted on the basis of the inclusion process.

Although the measurements were carried out at various temperatures, *viz.*, 150–170°C with CD-polyurethane resin and 50–80°C with CD on an inert support, certain correlations can be made (see Tables 3 and 4 and Fig. 12) and some more general conclusions can be drawn, which can be supported by the results of measurements in aqueous solutions of α- or β-CD.

(1) The differences of several orders of magnitude in the retention of aliphatic hydrocarbons between α- and β-CD confirm the inclusion character of the interaction.

(2) With benzene, toluene and cyclohexane, an increased retention of benzene was found on α-CD, whereas with CD-polyurethane resin a stronger interaction with

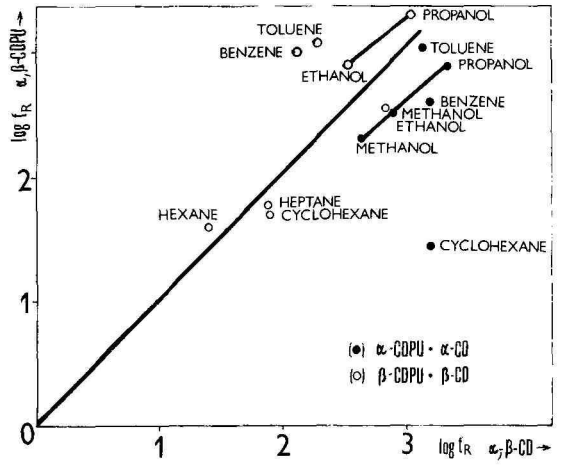

Fig. 12. Correlation diagram of retention data for various compounds measured on cyclodextrin–polyurethane resins and on cyclodextrins deposited on Chromosorb W.

β-CDP was found and explained by the existence of π-bonds. The greater interaction with α-CD, however, fully corresponds to the model in which the dimensions of the benzene molecule better match those of the cavity of α-CD as the host.

(3) The great differences in the retention of the hydrocarbon halogeno derivatives indicate that with α-CD the bonding forces can differ from those encountered with β-CD. The difference in the retention data of trichloroethylene and 1,2-dichloroethane, compared with tetrachloro derivatives, is especially marked in view of the boiling points.

(4) The experimental results for benzene halogeno derivatives, chloro- and bromobenzene, on α- and β-CD agree with the measurements in aqueous solutions. The effect of the bulky molecule of the bromo derivative is reflected in increased retention.

(5) The increased stability of the inclusion compounds of α-CD with unbranched aliphatic alcohols compared with the interaction with β-CD agrees with the association constants calculated for aqueous solutions.

(6) The interaction of unbranched and branched-chain compounds (alcohols, ethers) shows a lower retention for branched-chain compounds, the geometry of which does not correspond to the dimensions of the CD cavity.

Although the results obtained are rather qualitative, it is clear that both with macroporous polymers with inbuilt CD molecules and CD deposited on a chromatographic support, the forces operative inside the CD ring also play a role under GSC conditions, *i.e.*, the inclusion compounds are formed even when cyclodextrin as the host is in contact with sorbates in the gaseous state.

3. CONCLUSION

It can be concluded that the inclusion processes of cyclodextrins can be studied successfully by chromatographic methods and that chromatography will become an effective method for studying inclusion phenomena in general. On the other hand, it is

expected that, similar to gel inclusion chromatography, the selective formation of cyclodextrin inclusion compounds will be much more widely applied to other chromatographic techniques and will be used to solve many specific analytical problems.

4. ACKNOWLEDGEMENTS

The author thanks Dr. S. Krýsl for great help in collecting the literature data, M. Procházka for careful drawing of the figures and M. Rusová for technical help during preparation of the manuscript.

5. SUMMARY

Cyclodextrins and cyclodextrin polymers have been studied and applied in chromatography in recent years. Research carried out in this field is critically reviewed. The advantages of using the selective properties of these compounds for chromatographic separations and the possibilities of studying inclusion processes of different cyclodextrins with various types of substances, in the liquid as well as in the gaseous phase, are demonstrated and discussed.

6. NOTE

After presentation of this review, I have become acquainted with an interesting preprint[62] dealing with a similar subject, which confirms my conclusions. In addition, the possibilities of using cyclodextrins as mobile phase in TLC and HPLC are mentioned. More recently, papers have been published demonstrating that aqueous solutions of cyclodextrins have some important advantages over common mobile phases, based on the high selectivity of inclusion processes. Chromatographic separation of a variety of aromatic compounds such as monosubstituted isomers, isomers of di-, tri- and tetra-substituted phenols, nitriles, anilines and isomers of benzoic acid in TLC as well as of prostaglandins in HPLC have been reported[63–67].

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Journal of Chromatography, 251 (1982) 35–60
Chromatographic Reviews
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHREV. 153

ELEMENTAL ANALYSIS OF GAS CHROMATOGRAPHIC EFFLUENTS

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CONTENTS

1. INTRODUCTION

The qualitative analysis of organic compounds, particularly of their complex mixtures, is often carried out by gas-chromatography (GC). Retention indices[1], which express the relationship between retention behaviour and molecular structure, are commonly used for this purpose. However, as the differences between retention indices determined on two stationary phases of different polarity result from the combined effects of different structural increments of the molecules, they do not necessarily characterize the substances under analysis unambiguously so that the practical applications of this procedure remain limited.

These are the reasons why combinations of GC with UV, IR, Raman, mass and NMR spectroscopy are also significant. These combinations are expensive, however, not only with respect to the cost of the instruments but also the need for specialist operators. It is therefore desirable to supplement them with a method based on cheaper instrumentation that is less exacting as far as the interpretation of the results is concerned and commonly available. In this respect, a combination of GC with elemental analysis (EA) deserves attention.

0378-4355/82/0000–0000/$6.50

As EA is still one of the most commonly used identification methods in organic chemistry and biochemistry, its combination with GC has already been studied, particularly after the introduction of automated instrumentation. Several procedures have been suggested, but only one has found commercial realization[2–5]. A number of difficulties are involved, *e.g.*, the isolation of the separated components leaving the chromatographic column and the unacceptable precision of EA for 1–100 μg of substance with the use of different methods. The whole problem of combining GC and EA is thus worth analysing in further detail. It is justified to assume that this combination may become a useful and inexpensive tool for the identification of unknown compounds and may find a wider range of application.

2. ANALYSIS OF THE PROBLEM

The following information can be obtained by combining GC with EA: (a) the percentage composition of a compound after its separation by GC; (b) the empirical or molecular formula of the compound after its separation by GC and thus also its molecular mass; (c) the number of double bond equivalents[6]; (d) the parameter that is related to the molecular mass and Kováts retention index[1] or the molecular retention index[7,8].

2.1. Determination of percentage composition

For the determination of the percentages of various elements, the precise mass of the sample under analysis usually must be known. Even in the simplest case of the separate application of GC and EA, *i.e.*, separation of the substance by GC, its isolation, weighing and subsequent EA, the problem sometimes appears to be associated with the isolation of an amount of the sample compound sufficient for precise weighing. If a modern electronic balance is used, this level is 100 μg of the compound at the minimum. With an on-line GC–EA combination, a quantitative signal from the gas chromatographic detector can be utilized, usually with a precision substantially lower than that of weighing.

The isolation of a component represented by a peak at the outlet from the GC column assumes, except if the total effluent trapped in the loop has a sufficiently large volume, that the partial pressure of the eluate after it has entered the condensation device is much lower than that at the column outlet. Usually this is achieved by decreasing the trap temperature, by dissolving the eluate in a solvent with a low vapour tension, by adsorption or by reaction with a suitable reagent. When selecting a particular procedure, it is necessary to take into consideration that the condensation is followed by phenomena associated with the kinetic properties of the gaseous mixture at the outlet from the GC column, such as the passage of the eluate through the trap in the form of a supersaturated vapour or an aerosol. The optimal percentage efficiency of the trap, E, is given by the relationship[9]

$$E = 100\left(\frac{p - P}{p}\right) = 100\left(1 - \frac{P}{p}\right)$$

where p is the partial pressure of the eluate at ambient temperature and P that at the

temperature of the trap in the absence of the condensate. During the peak elution the partial pressure of the eluate varies with time so that the total efficiency, E, at time, t, is given by

$$E - \frac{1}{t}\int_0^t E \, dt$$

It follows that it is impossible in principle to trap the component represented by the peak merely by cooling. Further, with small peaks for which p will be smaller than P throughout the whole peak, $\bar{E} = 0$; however, this will apply even when p at the peak maximum (p_{max}) is equal to P and also when it is greater. In the last case, the isolation of the component can be performed only near p_{max}, so that re-evaporation of the condensate will not occur. In practice, the situation is further complicated by the formation of a supersaturated vapour or aerosol, as mentioned earlier. This may be avoided to a considerable extent if a trap of a suitable type is selected.

An unpacked U-trap is the simplest device, suitable for the isolation of larger amounts of sample only, being available on semi-preparative and preparative scales; all of the preceding considerations apply to it.

The formation of aerosols can be avoided by various procedures, involving temperature gradient, turbulent flow of electrostatic precipitators[9]. The formation of aerosols is suppressed considerably if the U-trap is packed with an inert material with a large surface area, *e.g.*, with glass-wool, crushed material, or deactivated support for the GC column, so that the adsorption of the isolated component may be reversible.

A suitable support coated with a stationary phase, as suggested by Desty *et al.*[10], is the most efficient GC column packing. Under suitable conditions, no loss of the isolated material occurs with these traps and, moreover, the material is concentrated. Although cooling is not applied, a considerable decrease in the vapour tension occurs as a result of the distribution of the eluate between the gaseous and the liquid phases. When the length of this trap is selected, it is necessary for the fact that the substances with short retention times and narrow peaks occupy, as consequence of low solubility in the liquid phase, a longer section on the column, to be considered. This method is suitable for trapping microgram or even smaller amounts of samples[11]; packed capillary columns have been used to trap amounts sample down to 10^{-11} mol[12].

As a rule, the procedure starts with the trapping of the component of the peak in the trap with cooling and continues with its retention, followed after a suitable period by its release for subsequent analysis by heating the trap in a stream of the carrier gas. An example of a suitable device is shown in Fig. 1 (Ref. 13).

By selecting a suitable material and with appropriate connection of the trap, an arrangement is obtained that permits differential weighing before and after the isolation of the component, *i.e.*, it makes it possible to determine its amount, which is of importance in EA.

A trap represented by a large free volume (a coiled tube of stainless steel, glass or PTFE) for trapping the entire peak together with an appropriate volume of the carrier gas or for trapping a peak section near its maximum is also of interest for EA.

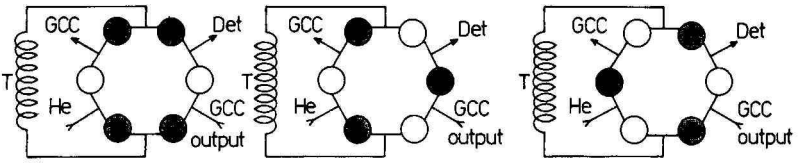

Fig. 1. Device for the isolation and sampling of GC eluates. GCC = GC column.

Isolation of this type is suitable for methods involving dilution of the products from the sample reaction after the reactor under defined conditions; it is less suitable for dynamic systems based on elution GC or selective absorption.

If there is a possibility of performing EA on all the elements contained in the molecule of a sample substance, a method in which it is not necessary to know the mass of the sample substance[14-16] can be used, but of course it requires the use of a reference substance with a known composition. As the determination of C, H, N, O and S, which represents the composition of most of the substances that can be examined by GC, can thus be carried out relatively easily, as will be shown later, this procedure is of great importance for combined GC–EA. At the same time, the determination of the empirical or molecular formula of the sample compound and of its molecular mass can also be effected.

The procedure is based on the determination of the empiricial formula $C_rH_pN_qO_uS_v$ by a common method, *i.e.*, by determining the mutual ratios of the various elements (stoichiometric coefficients r, p, q, u and v) by substituting percentage contents of the sample and the reference substances from eqn. 1.

$$\%X = \frac{K_X (E_X - e_X)}{w} \tag{1}$$

where K_X is the response factor obtained with the aid of a reference compound with a known composition:

$$K_X = \frac{\%X_{ref} \cdot w_{ref}}{(E_X - e_X)_{ref}} \tag{2}$$

E is the height of the frontal step or the peak, or is the area of the peak of the corresponding element, e is the corresponding response of a blank experiment and w is the sample weight.

In the classical procedure, the determined percentage contents of various elements are divided by the corresponding atomic weights and the results are divided by the smallest value or by one of the smallest values and, if it is necessary, they are then multiplied by a small integer (2 and 3 are the most frequent) in order to obtain stoichiometric coefficients of the empirical formula practically in the form of integers. In the present case, using eqns. 1 and 2, the sample weight, w, is cancelled out. Atomic

ratios are derived for the analysed samples directly from the corresponding signals and by means of the response factors determined with the aid of a reference substance. Hence in the analysis of carbon and hydrogen, if $r = 1$ then p is given by the relationship

$$p = \frac{\%\mathrm{H} \cdot 12.012}{1.008 \cdot \%\mathrm{C}} = 11.92 \cdot \frac{(E_\mathrm{H} - e_\mathrm{H})K_\mathrm{H}}{(E_\mathrm{C} - e_\mathrm{C})K_\mathrm{C}}$$

and because

$$\frac{K_\mathrm{H}}{K_\mathrm{C}} = \frac{\%\mathrm{H}_{\mathrm{ref}}\,(E_\mathrm{C} - e_\mathrm{C})_{\mathrm{ref}}}{\%\mathrm{C}_{\mathrm{ref}}\,(E_\mathrm{H} - e_\mathrm{H})_{\mathrm{ref}}}$$

then

$$r = 1$$

$$p = 11.92 \cdot \frac{(E_\mathrm{H} - e_\mathrm{H})\ \%\mathrm{H}_{\mathrm{ref}}(E_\mathrm{C} - e_\mathrm{C})_{\mathrm{ref}}}{(E_\mathrm{C} - e_\mathrm{C})\ \%\mathrm{C}_{\mathrm{ref}}(E_\mathrm{H} - e_\mathrm{H})_{\mathrm{ref}}}$$

Similarly, in C, H and N analysis (related to N which usually possesses the lowest stoichiometric coefficient in the empirical formula),

$$r = 1.17\,\frac{(E_\mathrm{C} - e_\mathrm{C})\ \%\mathrm{C}_{\mathrm{ref}}(E_\mathrm{N} - e_\mathrm{N})_{\mathrm{ref}}}{(E_\mathrm{N} - e_\mathrm{N})\ \%\mathrm{N}_{\mathrm{ref}}(E_\mathrm{C} - e_\mathrm{C})_{\mathrm{ref}}}$$

$$p = 13.90 \cdot \frac{(E_\mathrm{H} - e_\mathrm{H})\ \%\mathrm{H}_{\mathrm{ref}}(E_\mathrm{N} - e_\mathrm{N})_{\mathrm{ref}}}{(E_\mathrm{N} - e_\mathrm{N})\ \%\mathrm{N}_{\mathrm{ref}}(E_\mathrm{H} - e_\mathrm{H})_{\mathrm{ref}}}$$

$$q = 1$$

It is more advantageous, of course, to relate to C even in C, H, N determinations, as the programming is simplified when a mini- or microcomputer or a programmable calculator is used (this also applies to the determination of C, H and C, H, N or C, N, S and C, H, N, S). Then,

$$r = 1$$

$$p = 11.92 \cdot \frac{(E_\mathrm{H} - e_\mathrm{H})\ \%\mathrm{H}_{\mathrm{ref}}(E_\mathrm{C} - e_\mathrm{C})_{\mathrm{ref}}}{(E_\mathrm{C} - e_\mathrm{C})\ \%\mathrm{C}_{\mathrm{ref}}(E_\mathrm{H} - e_\mathrm{H})_{\mathrm{ref}}}$$

$$q = 0.86 \cdot \frac{(E_\mathrm{N} - e_\mathrm{N})\ \%\mathrm{N}_{\mathrm{ref}}(E_\mathrm{C} - e_\mathrm{C})_{\mathrm{ref}}}{(E_\mathrm{C} - e_\mathrm{C})\ \%\mathrm{C}_{\mathrm{ref}}(E_\mathrm{N} - e_\mathrm{N})_{\mathrm{ref}}}$$

As one reference compound is mostly used, the calculation is simplified con-

siderably as the parameters concerning the reference compound are transferred into a numerical constant, so that

$$r = 1$$

$$p = k_1 \cdot \frac{(E_H - e_H)}{(E_C - e_C)}$$

$$q = k_2 \cdot \frac{(E_N - e_N)}{(E_C - e_C)}$$

The same procedure is applied in C, H, S and C, H, N, S determinations:

$$r = 1$$

$$p = 11.92 \cdot \frac{(E_H - e_H)\ \%H_{ref}(E_C - e_C)_{ref}}{(E_C - e_C)\ \%C_{ref}(E_H - e_H)_{ref}}$$

$$v = 0.375 \cdot \frac{(E_S - e_S)\ \%S_{ref}(E_C - e_C)_{ref}}{(E_C - e_C)\ \%C_{ref}(E_S - e_S)_{ref}}$$

so that eventually

$$r = 1$$

$$p = k_1 \cdot \frac{(E_H - e_H)}{(E_C - e_C)}$$

$$v = k_3 \cdot \frac{(E_S - e_S)}{(E_C - e_C)}$$

and

$$r = 1$$

$$p = 11.92 \cdot \frac{(E_H - e_H)\ \%H_{ref}(E_C - e_C)_{ref}}{(E_C - e_C)\ \%C_{ref}(E_H - e_H)_{ref}}$$

$$q = 0.857 \cdot \frac{(E_N - e_N)\ \%N_{ref}(E_C - e_C)_{ref}}{(E_C - e_C)\ \%C_{ref}(E_N - e_N)_{ref}}$$

$$v = 0.375 \cdot \frac{(E_S - e_S)}{(E_C - e_C)} \frac{\%S_{ref}(E_C - e_C)_{ref}}{\%C_{ref}(E_S - e_S)_{ref}}$$

or

$$r = 1$$

$$p = k_1 \cdot \frac{(E_H - e_H)}{(E_C - e_C)}$$

$$q = k_2 \cdot \frac{(E_N - e_N)}{(E_C - e_C)}$$

$$v = k_3 \cdot \frac{(E_S - e_S)}{(E_C - e_C)}$$

As the determination of O is based on a different reaction principle, *viz.*, hydrogenation pyrolysis of the sample or its reductive conversion on a carbon packing, it is necessary, if coefficient u is to be determined, that together with O one of C, H, N or S elements is also determined simultaneously. As C and H are present in almost all sample compounds, they are of the greatest practical interest. If O/C or O/H atomic ratios and, simultaneously, C/H, C/H/N, C/H/S or C/H/N/S is known, u can be determined.

The simultaneous determination of O and C can be achieved by hydrogenation pyrolysis of the sample over a nickel catalyst, C being determined as CH_4 and O as H_2O (ref. 15); O and H are then determined by the reductive conversion of the sample pyrolysis products on a carbon packing (the best is that catalysed with nickel or platinum) and by subsequent oxidation, *e.g.*, over CuO, so that O is determined as CO_2 and H as H_2O)[16].

In the first case (related to C):

$$r = 1$$

$$u = 0.75 \cdot \frac{(E_O - e_O)}{(E_C - e_C)} \frac{\%O_{ref}(E_C - e_C)_{ref}}{\%C_{ref}(E_O - e_O)_{ref}}$$

and, as follows from the above considerations,

$$u = \left[0.75 \cdot \frac{(E_O - e_O)}{(E_C - e_C)} \frac{\%O_{ref}(E_C - e_C)_{ref}}{\%C_{ref}(E_O - e_O)_{ref}} \right] r_{(CHN)}$$

or

$$u = \left[k_4 \cdot \frac{(E_O - e_O)}{(E_C - e_C)} \right] r_{(CHN)}$$

where $r_{(CHN)}$ is the value of the empirical coefficient r determined in the C, H, N determination (it is unity if related to C).

In the second case (related to H):

$$p = 1$$

$$u = 0.063 \cdot \frac{(E_O - e_O)\ \%O_{ref}(E_H)_{ref}}{E_H\ \%H_{ref}(E_O - e_O)_{ref}}$$

so that

$$u = \left[0.063 \cdot \frac{(E_O - e_O)\ \%O_{ref}\ (E_H)_{ref}}{E_H\ \%H_{ref}\ (E_O - e_O)_{ref}}\right] p_{(CHN)}$$

or

$$u = \left[k_5 \cdot \frac{(E_O - e_O)}{E_H}\right] p_{(CHN)}$$

where $p_{(CHN)}$ is the value of empirical coefficient p determined in the C, H, N determination.

In the simultaneous determination of C, N, S[17,18], the calculation can be carried out as in the determination of O. When related to C:

$$r = 1$$

$$v = \left[0.375 \cdot \frac{(E_S)\ \%S_{ref}\ (E_C - e_C)_{ref}}{(E_C - e_C)\ \%C_{ref}\ (E_S)_{ref}}\right] r_{(CHN)}$$

where $r_{(CHN)}$ is the value of the empirical coefficient r determined in the determination of C, H, N, or

$$v = \left[k_6 \cdot \frac{E_S}{(E_C - e_C)}\right] r_{(CHN)}$$

The calculation of the percentage contents of various elements starts from the ratio of the empirical coefficients obtained by the described procedure and the corresponding molecular mass. If the compounds under analysis also contain other elements (*e.g.*, halogens and P) the contents of these must be known or determined by another procedure. In association with this, the utilization of the response of selective detectors[9] is promising.

2.2. Determination of empirical and molecular formulae

The determination of a molecular formula by multiplying the coefficients of the empirical formula by a small integer is possible in the present instance by comparing

the retention data of the analysed compound with the retention data of a compound of similar composition the molecular mass[19] of which is known.

The requirement of the precision of the determination of the empirical formula is governed by the requirement of the unambiguity of the determination of the number of atoms of individual elements, and it is not identical for all of them. It is the most exacting for H, which has the lowest atomic mass. In questionable cases, the rule that a compound that contains in its molecule an even number of atoms with odd valency cannot contain an odd number of H atoms is valid.

The above procedure provides some advantages that are worth mentioning:

(a) precise weighing and careful manipulation of the sample are eliminated;

(b) as the error due to weighing is avoided, EA can be performed with advantage on the microgram scale;

(c) provided that short-term significant changes in physical parameters of the analyser do not arise, it can be assumed that the results of the determination are not influenced by their changes as the ratio of the detector signals for various pairs of elements remains the same.

2.3. Number of double bond equivalents

The number of double bond equivalents, *i.e.*, the unsaturation number, R, is an aid in the deduction of the structural formula from the empirical formula. It is determined according to the relationship

$$R = 1 - N + \tfrac{1}{2}\Sigma i\, n_i V_i$$

where n_i are the numbers of individual atoms in the empirical formula, V_i is the valency of element i (the concept of valency here denotes the sum of homopolar and heteropolar bonds) and N is the total number of the atoms present, $N = \Sigma i\, n_i$. A double bond corresponds to one ring and a triple bond to two double bonds[6].

2.4. Parameter W

A method for the identification of an unknown compound has recently been described that, combines retention increments compatible with the empirical formula, with the retention of this compound[8]. The parameter W, relating the molecular mass, M, and the Kováts retention index, I, or molecular retention index, Me, is defined as

$$W = Me - M = 0.14\, I - M + 2$$

In this way the limits of the value of W can be determined for certain structural groups on different stationary phases.

3. COMBINATION OF GAS CHROMATOGRAPHY AND ELEMENTAL ANALYSES

GC and EA can be combined by (a) separate use of GC and EA, necessarily involving the isolation of the sample compound (trapping) or (b) on-line connection of GC and EA with or without the trapping of individual peaks.

The former procedure offers universal application without particular requirements for special instrumentation; however, it assumes that a gas chromatograph and an automated elemental analyser, permitting C, H, N, O and also S determinations, are available. It is the type of elemental analyser applied that determines the amount of sample that must be isolated. Usually, this amount is much larger than in an on-line combination, but it represents 100 μg of the sample at the minimum. The problem of the selection of the type of trap is closely associated with the principle of the elemental analyser used[20], systems involving a dilution chamber between the reactor and the separation part can make use of traps of any type, whereas those working under continuous dynamic conditions require the compound to be isolated in the pure state.

However, the weighing, which in general follows the isolation of the compound (with the exception of the procedure without weighing), again restricts the procedure of trapping to isolation in the pure state or to isolation in the packing with a suitable sorbent (differential weighing before and after desorption, which is more suitable with a classical type of microbalance owing to the considerable weight of the trap).

A number of variants of the fundamental arrangement offer in the latter instance an on-line GC–EA combination. They can be classified, with regard to the function of the GC column, into the following systems: (a) with the GC column operating without changing the carrier gas flow; and (b) with the GC column operating as "stop flow" system[21,22].

In the former instance, the GC column outlet must be equipped with a suitable trap if the EA proper is not to be limited to the only peak from the whole chromatogram, whereas in the latter the peaks following the peak under analysis are "preserved" in the GC column after the interruption of the chromatographic process by switching off the carrier gas feed; more attention will be devoted to the realization of individual variants later. Hence the "stop-flow" system does not require any trap and makes it possible to continue chromatographing or subsequent EA after the analysis of the selected peak.

Both chromatographic versions can be connected to different EA systems. As these do not provide results with the same accuracy and may differ in other parameters, it is necessary that these circumstances should be taken into consideration when the type of EA is selected. The following systems can be considered.

4. ELEMENTAL ANALYSIS SYSTEMS FOR ON-LINE CONNECTION WITH THE GAS CHROMATOGRAPHIC COLUMN

The continuously operating GC column is connected to either (a) reactor–elution GC; (b) reactor–detector–selective absorber–detector; (c) reactor–dilution chamber–combination of detectors and selective absorbers; (d) reactor–dilution chamber–frontal GC; (e) reactor–dilution chamber–sampling loop–elution GC.

The following is charachteristic of individual combinations:

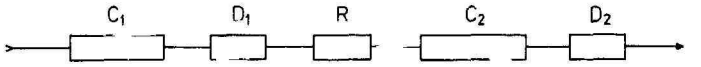

Fig. 2. Flow diagram of a GC column – reactor EA system by elution GC.

4.1. *Reactor elution gas chromatography*

The simplest arrangement[23] is obvious from Fig. 2.

The carrier gas passes through a GC column, C_1, a non-destructive detector, D_1, a reactor, R, a second chromatographic column, C_2, and a detector, D_2. The analysed peak is thus recorded in the detector D_1 first, allowed to react (*e.g.*, oxidation), in reactor R, and the reaction products are separated in column C_2 and detected by detector D_2. Disadvantages of this arrangement are obvious. The direct dependence of individual parts of the arrangement on the flow-rate of the carrier gas restricts this procedure to well separated peaks[24,25] leaving the GC column within relatively short elution times. Sorption phenomena and diffusion then occur in the reactor and, as a result, the zones of reaction products are broadened with adverse effects on the subsequent chromatographic separation.

The system with independent control of the flow-rate of the carrier gas in both the chromatographs and reactor[26], suggested initially for pyrolysis GC, is of greater practical interest. However, even here peaks that follow each other closely cannot be analysed without using a trap or "stop flow" conditions. The function of this device is shown in Fig. 3.

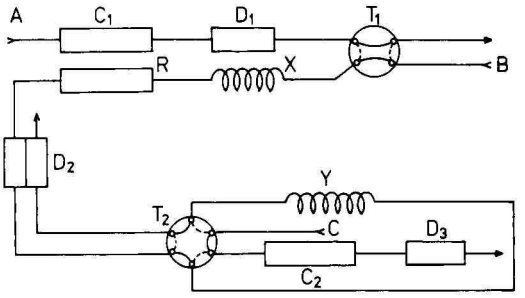

Fig. 3. Arrangement of GC column – reactor – elution GC system with independent control of the flow-rates of gases.

The separation proper proceeds in column C_1, and individual peaks are registered by means of a non-destructive detector, D_1 (this can be replaced with a splitter and a destructive detector) and pass through a valve, T_1, to ambient air. At the same time, the carrier gas is introduced from a source B, via valve T_1, free volume of a delay coil, X, a reactor, R, a non-destructive detector, D_2, a multi-port valve, T_2, and a transfer coil Y, a valve, T_2, again to detector D_2 and to ambient air. In addition, the carrier gas streams from a source, C, via T_2, a column, C_2, and a detector, D_3. If the peak that has just been registered by means of detector D_1 is to be analysed, valve T_1 is turned and the component of the peak together with the carrier gas is introduced into the coil X, reactor R, detector D_2, valve T_2, transfer coil Y, valve T_2 and through the reference section of detector D_2 and out. After completion of the peak registration by detector D_1, valve T_1 is again turned, and further transport of the peak into the reactor is executed by the carrier gas from source B. Detector D_2 plays an auxiliary role, serving to indicate reaction products leaving reactor R or to prevent their elution from transfer coil Y. It can therefore be omitted as it is sufficient if the carrier gas flow-rate from B and the free volume of circuit X, R, Y are known. The analysis

proper of the reaction products is performed by turning valve T_2 and sampling the contents of the coil Y into the column C_2.

4.2. Reactor–detector–selective absorber–detector

A flow diagram of a typical arrangement applied to the determination of C/H ratio[27] is shown in Fig. 4.

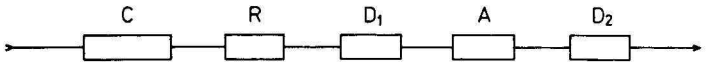

Fig. 4. GC column reactor detector – selective absorber – detector system.

The peaks leaving the GC column, C, pass through a reactor, R, where they are oxidized to carbon dioxide and water and the latter is converted to hydrogen. The mixture passes through a detector, D_1, and an absorber, A, where carbon dioxide is trapped, and then through a detector, D_2, where hydrogen is determined. The amount of carbon dioxide is determined from the difference of the responses of the two detectors.

It is obvious that by selecting a suitable packing and temperature of the reactor and using a selective absorber or by inserting more detector–absorber couples, various elements, *e.g.*, C, H, N, O and S, can be determined. An advantage of this arrangement is that it is not necessary for a trap or a "stop flow" arrangement of the GC column to be used, provided that the peaks leaving the column are well separated.

The same does not apply, however, to the different arrangement shown in Fig. 5, used for the determination of C, H, N[28,29]. It differs from Fig. 4 in that the component represented by the peak is oxidized in the reactor into a mixture nitrogen, carbon dioxide and water and freed from water in the absorber, where it is retained until carbon dioxide or nitrogen is detected (by the same procedure as shown in Fig. 4), and only then thermally desorbed and registered with the aid of a detector, D_1. Carbon dioxide is absorbed in an absorber, AB, and nitrogen is registered by a detector D_2. The amount of carbon dioxide is determined from the difference between the data from detectors D_1 and D_2.

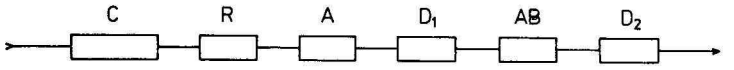

Fig. 5. GC column reactor absorber detector – absorber detector system.

To compare the dynamic systems described so far, based on separation by elution GC and selective absorption, it must be taken into consideration that component S passes the GC column under repeatedly established equilibrium and that its concentration in the gaseous phase varies[30]. If N_S is the number of moles of component S, then its instantaneous concentration at the column outlet can be expressed as

$$c_S = \frac{N_S}{v\,t_R}\sqrt{\frac{L}{2\pi H}}\cdot\exp\left[-\frac{L}{2H}\left(1-\frac{t}{t_R}\right)^2\right]$$

where v is the volumetric flow-rate of the gas, t the elution time (the time period during which concentration c_S is eluted), t_R the retention time, L the column length and H the height equivalent to a theoretical plate.

For the maximal concentration of the eluted component $t = t_R$, so that

$$c_S^{max} = \frac{N_S}{v\, t_R} \sqrt{\frac{L}{2\pi H}}$$

and the integral of the time function of the concentration distribution of the eluted component satisfies the condition of the Gaussian random distribution

$$\int_{-\infty}^{+\infty} c_S \mathrm{d}t = Y$$

and has a maximum c_S^{max} at time $t = t_R$ and two points of inflection at time t_{inf}, situated symmetrically on both the sides of t_R:

$$t_{inf} = t_R \pm \sqrt{\frac{L}{H}}$$

The separation of the eluted component in the column is influenced by simultaneous dissolution and absorption processes, shifts in the equilibrium in the presence of inert components and by the amounts of the eluate and the eluted component.

With a change in the concentration of the eluted component a change also occurs in the concentration of the carrier gas. These changes can be expressed in terms of variations in partial pressures as the total pressure in an open chromatographic system remains constant. As long as the pure carrier gas C streams in the column, its partial pressure at the column outlet is $p_C = p$. After the introduction of a substance S into the column, the partial pressure of the carrier gas is changed. As the total pressure remains unchanged, then

$$p = p_C + p_S$$

With all the dynamic systems described so far, component S, leaving the GC column, passes through the reactor where it is converted into the mixture of reaction products, e.g., nitrogen, carbon dioxide and water if C, H, N are determined and, in the next step, this mixture is separated either chromatographically or by selective sorption.

In GC separation, all of the preceding considerations are valid. Provided that the chromatographic separation is good, in the course of the analysis with a defined time sequence the following relationships are valid for the total pressure at the column outlet and in the measuring section of the sensing element:

$$p = p_C$$

or

$$p = p_C + p_{N_2}$$

or

$$p = p_C + p_{CO_2}$$

or

$$p = p_C + p_{H_2O}$$

With selective sorption the situation is different. Having left the reactor, the whole mixture of reaction products passes through the sensing element so that

$$p = p_C + p_{N_2} + p_{CO_2} + p_{H_2O}$$

After the conversion of water into hydrogen,

$$p = p_C + p_{N_2} + p_{CO_2} + p_{H_2}$$

and having passed the absorption layer, where carbon dioxide is trapped, the mixture enters the second sensing element at the total pressure in the profile of the zone of the reaction products:

$$p = (p_C + p_{N_2} + p_{CO_2} + p_{H_2}) - p_{CO_2}$$

i.e., the initial partial pressure of the carrier gas does not change and, as a consequence, the individual partial pressures of the components present vary.

From the viewpoint of the use of the detector of the concentration type (katharometer), in the profile of the peak being analysed concentration changes occur, which are not negligible, particularly in carbon dioxide absorption (carbon dioxide concentrations in the carrier gas of the order of 10% should be taken into account). These changes are the reason for the non-linearity of the detector response, which can be defined only with difficulty as it is affected by a number of factors: (1) variations in the concentrations of the components present; (2) non-linearity of the dependence of the change in the thermal conductivity on the concentration of the components present; (3) non-linearity of the dependence of bridge unbalance signal on the change in the thermal conductivity; (4) variations in the flow-rate of the gaseous mixture in the peak profile and thus also the non-linearity of the detector response depending on the flow-rate of the analysed mixture; and (5) changes in the viscosity of the gaseous mixture in the profile of the peak being analysed.

The phenomena mentioned above are responsible for the fact that this method is suitable for semi-quantitative applications only. The same factors arise in systems using the dilution chamber. However, in this instance the concentrations of the reaction products being determined are lower by an order of magnitude, and also the resulting errors in the determination caused by non-linearity of the detector response are negligible. A more detailed discussion of these phenomena is given in the sections dealing with individual methods.

Finally, a combined case[28,29] can be considered, which is mentioned, *e.g.*, in Fig. 5, where the considerations concerning GC apply to the determination of water.

4.3. Reactor–dilution chamber–combination of detectors and selective absorbes

In this instance the possibility is considered of connecting a GC column on-line to elemental analysers of the Perkin-Elmer Model 240 and Yanaco types[31–35], which differ in the shape and function of the dilution chamber, *i.e.*, the former uses a glass flask with an auxiliary sampling loop and the latter a cylinder with a mechanically controlled piston.

In the first instance, as follows from the flow diagram in Fig. 6, the components from the GC column pass through valve T_1 into ambient air or, in peak analysis, into reactor R, via the three-way valve S_1 into dilution chamber CH, where the reaction products are diluted and homogenized under steady-state conditions after closing the chamber with the aid of valves S_1 and S_2.

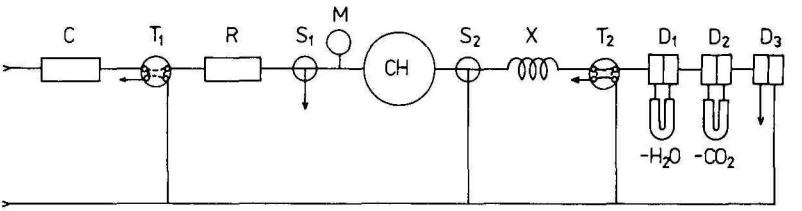

Fig. 6. Arrangement of GC column – reactor dilution system; separation of individual components by selective absorption and differential measurement of thermal conductivities.

After finishing the peak sampling, the system is fed with the pure carrier gas (helium) via T_1, R and S_1 and the pressure is increased to an overpressure, determined in advance, by means of a manometer, M. After finishing the homogenization, the mixture from CH is allowed to expand via S_2, a sampling loop, X, and a valve, T_2, and out. Meanwhile, the carrier gas streams via T_2 into a system of absorbers and thermal conductivity detectors D_1, D_2 and D_3 (the baseline is registered). Subsequently, by switching valves S_2 and T_2 the contents of loop X are purged via T_2 into the system of detectors and absorbers. In C, H, N determination, water is trapped in the first absorber (packed with Anhydrone) and the difference in the corresponding thermal conductivities is registered by a detector, D_1, carbon dioxide is trapped in the second absorber (Ascarite) and D_2 again registers the difference in the thermal conductivities of the mixtures. The difference between the thermal conductivity of the remaining mixture and the pure carrier gas is measured in a third detector, D_3. When the analysis is finished, chamber CH is washed with the carrier gas via S_2 and S_1 and out.

The second case, using the cylindrical dilution chamber and the mechanically controlled piston, is analogous.

The dilution chamber, which apparently makes the whole process complicated and affects adversely the time of analysis (dilution of the reaction products and their homogenization for about 1.5 min), in addition to some smaller drawbacks [adsorption on the walls, particularly in the analysis of small amounts of the sample (below 50 μg), or the dead volume], has a number of important advantages:

(1) It acts as an integrator and thus simplifies the electronic components of the analyser.

(2) It virtually eliminates difficulties associated with sorption of the reaction products in the reactor, or those complicating the subsequent separation.

(3) It makes it possible to vary the flow-rate through the reactor almost at will.

(4) It permits the use of larger amounts of sample compound for the analysis as it makes it possible, to a considerable extent, to avoid non-linearity of the katharometer response caused by its concentration dependence.

(5) It makes it possible to use large-volume traps of any type.

(6) The frontal GC technique can be used in connection with the dilution chamber, giving higher column efficiency, and enables shorter columns to be used, yielding shorter analysis times, thus compensating for the time necessary for homogenization of the gas mixture in the dilution chamber.

It is the following three factors that contribute to the errors caused by non-linearity of the detector with the systems described above:

(a) The relationship between the component concentration and the change in thermal conductivity is linear at low concentrations or in a narrow range of the concentrations. The effect of the non-linearity can be neglected provided that the molar fraction is less than 0.01; hence follows one of the advantages of the dilution of the components.

(b) The other source of error is associated with the presumption of direct proportionality between the change in the thermal conductivity and the signal of the bridge unbalance. It was proved[28] that the relationship between the molar fraction, x, of the component and the signal of the bridge unbalance, ΔE, has the following form

$$\Delta E \sim x(1 - x)$$

and, in contrast to the case of elution GC, it is therefore very small with dilution and gives rise to errors that do not exceed 0.05% if it is diluted to $x \leqslant 0.01$ (ref. 31).

On the other hand, with systems using elution GC a function-generating device must be used to obtain a linear approximation and thus also the possibility of performing the analysis with a wider range of the weighed amounts.

The application of a later version of the katharometer, operating with a constant temperature of the filaments, provides a wider range of linearity of response[30].

(c) With the systems described above it is necessary to take into account the third deviation from the linear relationship between the amount of the component of interest in the sample, and hence the concentration in the dilution chamber of the products of its specific reaction and the change in its thermal conductivity in a separation section after absorption of individual components. The deviation takes the form of an increase in the initial concentrations of the reaction products after homogenization in the dilution chamber as soon as one of the components of the mixture is absorbed.

If the most frequent application, *i.e.*, C, H, N determination, is taken as an example, then, provided that the Pregl–Dumas reaction system is used, after reaction of the sample and equilibration of the mixture of the reaction products the mixture of helium, nitrogen, carbon dioxide and water remains in the dilution chamber, so that

$$x_{He} + x_{N_2} + x_{CO_2} + x_{H_2O} = 1$$

where x_i is the molar fraction of an individual component and is calculated according to the relationship

$$x_i = \frac{G_i T \cdot 22.4 \cdot 760}{M_i V P \cdot 273} \cdot 10^{-3}$$

where M_i (μg) and G_i (μg) are the molecular mass and the mass of component i, respectively, and V (ml), T (^{0}K) and P (Pa) are the volume, temperature and pressure, respectively, in the dilution chamber.

Then, with a simplifying assumption[31,32] of linear additivity of the katharometer response to the concentrations of individual components in the case of multicomponent mixtures and of the estimate or the calculation of the corresponding thermal conductivity, λ_i, it is possible to start from the presumption that after the absorption of the first component, *i.e.*, water,

$$x_{H_2O} = 0$$

and

$$x_{He} + x_{N_2} + x_{CO_2} = 1$$

so that in the measuring section of the katharometer (*viz.*, Fig. 6)

$$\lambda^M = x_{He}\lambda_{He} + x_{N_2}\lambda_{N_2} + x_{CO_2}\lambda_{CO_2} + x_{H_2O}\lambda_{H_2O}$$

and in the reference section

$$\lambda^R = \frac{x_{He}}{1 - x_{H_2O}} \cdot \lambda_{He} + \frac{x_{N_2}}{1 - x_{H_2O}} \cdot \lambda_{N_2} + \frac{x_{CO_2}}{1 - x_{H_2O}} \cdot \lambda_{CO_2}$$

and from the difference between the two thermal conductivities, before and after the absorption of water,

$$\Delta\lambda_{H_2O} = x_{H_2O}\lambda_{H_2O} - \frac{x_{H_2O}}{1 - x_{H_2O}} \cdot (x_{He}\lambda_{He} + x_{N_2}\lambda_{N_2} + x_{CO_2}\lambda_{CO_2})$$

and analogously after the absorption of carbon dioxide,

$$\Delta\lambda_{CO_2} = \frac{x_{CO_2}}{1 - x_{H_2O}} \cdot \lambda_{CO_2} - \frac{x_{CO_2}}{(1 - x_{H_2O})\ [1 - (x_{H_2O} + x_{CO_2})]} \cdot (x_{N_2}\lambda_{N_2} + x_{He}\lambda_{He})$$

and finally, by comparing with pure helium,

$$\Delta\lambda_{N_2} = \frac{x_{N_2}}{1 - (x_{H_2O} + x_{CO_2})} \cdot (\lambda_{N_2} - \lambda_{He})$$

Strictly, generally linear additivity of the thermal conductivity cannot be assumed for multi-component mixtures of gases and therefore for the calculation of the thermal conductivity in the individual katharometer cells after the absorption of the components of the mixture it is more exact to use more complicated relationships, the best being that according to Lindsay and Bromley[36]:

$$\lambda_m = \sum_{i=1}^{n} \frac{x_i}{1 + \frac{1}{x_i} \sum_{\substack{i=1 \\ j \neq 1}}^{n} A_{ij} x_j}$$

where

$$A_{ij} = \frac{1}{4}\left\{1 + \left[\frac{\mu_i}{\mu_j} \cdot \left(\frac{M_j}{M_i}\right)^{3/4} \cdot \frac{1 + \frac{S_i}{T}}{1 + \frac{S_j}{T}}\right]^{1/2}\right\}^2 \cdot \frac{1 + \frac{S_{ij}}{T}}{1 + \frac{S_i}{T}}$$

where μ_i and μ_j are viscosities of gases i and j, respectively, M_i and M_j are the molecular masses of i and j, respectively, S_i and S_j are the Sutherland's constants of i and j, respectively, x_i and x_j are the molar fractions of i and j, respectively, and T is absolute temperature (°K).

For practical reasons, it is advantageous that correction factors, f_i, can be calculated for the conditions given for the analyser and that the differences in the thermal conductivities measured for individual components, $\Delta\lambda_i$, can be corrected with the respective factors. The values obtained in this way vary linearly with the change in concentration i (ref. 32):

$$\frac{\Delta\lambda_i}{f_i} = \Delta\lambda_{i,\text{corr.}} = \Delta\lambda_{i,L}$$

For a binary system with thermal conductivities λ_i and λ_{He} (carrier gas) it is possible to write, as a particular case,

$$\Delta\lambda_{i,L} = x_i (\lambda_i - \lambda_{He})$$

The dependence between $\Delta\lambda_{i,L}$ and x_i is linear, so that from the above relationships we have

$$f_i = \frac{\Delta\lambda_i}{\Delta\lambda_{i,L}} = \frac{\Delta\lambda_i}{x_i (\lambda_i - \lambda_{He})}$$

Factors f_i can be calculated from the corresponding relationships, which is impractical. It is more advantageous to tabulate them in relation to the amounts of other components present, which is valid to a first approximation. These corrections need not be performed in practice because, as a rule, the errors that arise do not exceed $\pm$ 0.2% absolute for each element to be determined.

4.4. Reactor–dilution chamber–frontal gas chromatography

Fig. 7 shows a flow diagram of a device suitable for the realization of reaction frontal GC[15,37], and also reaction elution GC, or a combination of both[18].

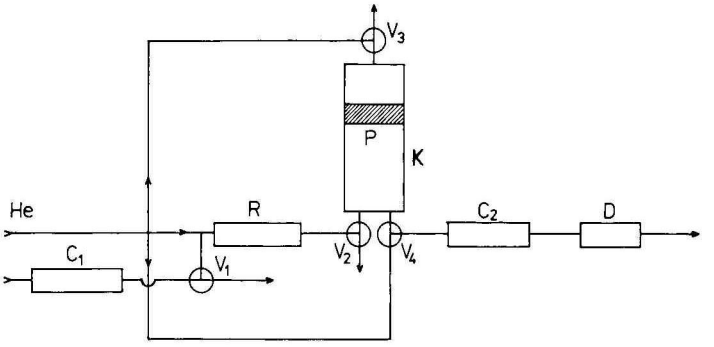

Fig. 7. GC column reaction frontal GC system.

The substance under analysis, leaving the GC column, C_1, or having been captured in a trap first, is introduced into a reactor, R, via a valve, V_1, reacts with the formation of the defined products, which are carried by the carrier gas via a valve, V_2, into a cylindric dilution chamber, K, equipped with a loosely moving piston, P. At the same time, the piston slides towards the opposite face with a valve, V_3, opened to the atmosphere. The piston having reached the face, the pressure in the chamber increases to a value adjusted in advance, then the chamber is closed for about 1.5 min in order to allow for homogenization of the gaseous mixture by diffusion. Simultaneously valve V_3 is switched and the carrier gas is introduced behind the piston under an overpressure which is at the inlet of the chromatographic column. Valve V_4 is switched, the entry of the carrier gas into a GC column, C_2, is interrupted and, at the same time, the inlet into the dilution chamber is opened. The piston of the chamber purges the equilibrated mixture from the dilution chamber into the GC column, filled with a suitable sorbent (*e.g.*, Porapak Q or QS). Frontal chromatographic development occurs provided that the sampling is performed for a sufficiently long period. By selection of the sampling time, combined frontal elution separation[18], having some advantages over a purely frontal process, can be achieved. The residue from the dilution chamber is blown off into the ambient atmosphere and the next analysis can be started.

This system combines some properties of all of the above arrangements. A bypass arrangement of the reactor–dilution chamber system provides all the advantages of the dilution chamber, as mentioned earlier.

On the other hand, some disadvantages of the application of the dilution chamber, particularly in the analysis of amounts of sample less than 50 μg, cannot be disregarded: (1) existence of dead volumes and large surface areas (surfaces of the piston, inside walls of the dilution chamber), the dimensions and the sorption properties of which must be suppressed (*e.g.*, by silane treatment); and (2) demands on smooth piston sliding.

Strictly, no direct linear proportion exists in frontal GC between the concentration of the component in the dilution chamber after the equilibration and the corresponding katharometer response in the course of the adsorption chromatographic process. The concentration established in the dilution chamber initially varies during the adsorption development.

If C, H, N determination is considered, the concentrations of carbon dioxide, nitrogen and water in helium (carrier gas) reach $x = 0.001$–0.01 when competitive sorption in the chromatographic process can be neglected. This mixture is led from the dilution chamber into the chromatographic column packed with Porapak Q or QS and individual components are sorbed until equilibrium is established. The least sorbed component, nitrogen, appears at the column outlet first as a concentration step, the height of which should correspond to the concentration introduced from the dilution chamber. In fact, it is higher as the remaining components, carbon dioxide and water, were sorbed from the initial mixture. The molar fractions of nitrogen and helium are therefore changed. The situation is similar after the elution of carbon dioxide. Only after elution of water do the initial concentrations established in the dilution chamber leave the column.

These concentration changes are equivalent to those described in further detail in the preceding section, on separation by selective absorption.

Moreover, the concentration changes in this instance result from desorption of the less sorbed component by the components proceeding more slowly (in the present instance by carbon dioxide and water). It is a new establishment of the sorption equilibrium, based on the concentration changes over the sorbent, that is relevant here. With respect to low concentrations of substances, this phenomenon can be neglected.

The situation is different in the desorption part of the frontal chromatographic process. Here no concentration changes occur and the component eluted by the sorbent is immediately replaced with the same molar fraction of helium (carrier gas).

A combined frontal elution separation is obtained if the contents of the dilution chamber are sampled into the GC column within a shorter period of time. It is advantageous as any eventual concentration corrections, discussed above, will be omitted for the last component (in the present instance this is water) and the total analysis time will be shortened by about 2 min[18].

The resulting precision of the determination by the above method, as with all other instrumental methods, is complex. In addition to the separation process proper, the parameters of various elements of the instrument, such as the precision of the temperature control in the thermostat, the precision of the stabilization of the katharometer filament voltage and of the carrier gas pressure, the sensitivity and the precision of the pressure establishment in the dilution chamber, the perfectness of the reaction process, the magnitudes of the dead volumes of the valve system, the sorption properties of the inner surface on the connections and the walls of the dilution chamber affect this precision.

Practical experience suggests that the main sources of errors are sorption of reaction products on the inner surface of the dilution chamber and in the inlets (bore holes towards membrane valves in the face of the dilution chamber) and the concentration changes that occur in the course of adsorption frontal GC development.

The precision of the results also depends, to a considerable extent, on the initial

amount of the sample. A precision of $\pm$ 0.2% absolute can be obtained for individual elements with samples of 100–1000 μg. With decreasing amounts of sample, the effects associated with concentration changes and non-linearity of the katharometer response lose their significance and sorption phenomena predominate. The smallest amount of sample that can be analysed is about 10 μg for systems with a dilution chamber, and about 1 μg for based on elution GC.

More precise results are obtained with larger amounts of sample (100 μg) when the conditions are optimal for easy and rapid selective reactions and the ratio of the sample signal to the blank value for a given element remains sufficiently great.

4.5. Reactor–dilution chamber–sampling loop–elution gas chromatography

A suitable instrumental arrangement for this case can be based, *e.g.*, on Fig. 6, the only difference being that the sampling tube X is replaced with a loop and the system of absorbents and detectors with a chromatographic column and one detector. The advantage of this arrangement over that described in Section 4.1 is the possibility of performing an independent reaction and eliminating sorption phenomena in the reactor. On the other hand, the sampling itself with the aid of the loop suffers from an error that is not negligible and restricts the practical applicability of this arrangement.

Use of a loop with a sufficiently large volume is a particular case when the sampling into the chromatographic column is prolonged; during the chromatographic process, the initial concentration in the centre of the zone of the component being separated will be maintained until leaving the chromatographic column, and the resulting chromatogram will consist of stepwise peaks, the height of which will correspond to the concentrations established originally in the dilution chamber[20]. However, this presumes that a simple mixture of gases is produced by the reaction and that the individual components are separated well with a sufficient reserve with respect to elution time. This case does not suffer from the error caused by the sampling loop and is advantageous in practice. It is substantially identical with the case described in Section 4.4.

5. ELEMENTAL ANALYSIS SYSTEMS FOR ON-LINE CONNECTION WITH THE GAS CHROMATOGRAPHIC COLUMN, USING A "STOP FLOW" SYSTEM

EA systems for connection to the GC column operating as a "stop flow" system differ only in that they do not require trapping. The interruption of the carrier gas flow through the GC column for 8 min, which is necessary to perform, *e.g.*, C, H, N analysis, is said[4] not to have any negative effects on the subsequent separation.

6. REACTION CONDITIONS FOR INDIVIDUAL DETERMINATIONS

6.1. Determination of C, H, N

Most instrumental methods determine C, H, N simultaneously. The reaction principle is very simple and can be expressed schematically as follows:

$$\text{Organic N compound} \xrightarrow{(O)} CO_2 + H_2O + N_2 + \text{N oxides} \xrightarrow{(Cu)} CO_2 + H_2O + N_2$$

In general, however, the mechanism of the oxidation process is complicated and it is not always easy to satisfy the requirements of the reaction scheme. In the reaction of chromatographic effluents, however, the situation is usually much simpler as compounds are involved that are relatively volatile or convertible into the gaseous state and, moreover, have a relatively simple qualitative composition.

Sample compounds are mostly combusted with addition of oxygen and the gaseous reaction products pass additionally through an oxidative packing (mostly oxygen donors) in order to be completely oxidized. Copper (II) oxide still has a wide range of application, although a number of others, such as the decomposition product of $AgMnO_4$[35], Co_3O_4[39] and others, described in detail in textbooks on organic elemental analysis[40-44], have been proposed.

In separate determination by GC–EA, the reaction conditions of EA are determined by the instrumentation that is applied.

Various commercial analysers operate on the basis of different reaction principles, which are usually optimal for the given system[40,41]. Retention of unreacted oxygen and reduction of nitrogen oxides are generally performed over copper at 500–650°C, whereas the oxidation packings and pyrolysis section are mostly at 950–1050°C.

Various forms of metallic silver (silver-wool, silver deposited on an inert support, mixture of silver with Co_3O_4, SnO_2, etc.) at 500–800°C[40-42] are used to absorb interfering elements, such as halogens and sulphur.

All of these methods employ relatively high temperatures (1000 $\pm$ 50°C) in the pyrolysis section and the combustion packing in order to effect virtually instantaneous destruction of all types of substances; however, significant corrosion of the quartz tube should not occur. This also results from the fact that, in contrast to classical elemental analysis, reactions are mostly carried out in an inert medium of the carrier gas (helium) with the addition of only a small amount of oxygen in order to prevent rapid exhaustion of the copper packing. Classical donors of oxygen, such as CuO, Co_3O_4 and decomposition product of $AgMnO_4$, are no longer used because at these temperatures they have a high oxygen tension and lose their efficiency (the maximum usable temperatures are 850–900, 750–800 and 500–550°C for CuO, Co_3O_4 and the decomposition product of $AgMnO_4$, respectively). With on-line connection of the GC column to EA, however, it is advantageous to use classical oxygen donors for the oxidative conversion of the sample compound, CuO being the best, or no addition of oxygen, particularly if smaller amounts of compounds (100 μg and less) are analysed.

6.2. Determination of O; C, O; H, O

Oxygen determination is theoretically possible by two reaction procedures:

(a) By pyrolysis of the sample in an inert gas and reductive conversion of the pyrolysis products by passing them through the layer of carbon packing, or nickelized or platinized carbon packing, at a sufficiently high temperature (1120, 950–1050 and 900°C with non-catalysed, nickel-containing and 50% of platinum-containing packings, respectively). Oxygen from the sample is converted quantitatively into carbon monoxide, which is determined as such or converted into carbon dioxide prior to the determination proper. Hydrogen, nitrogen and also methane (if the conversion is performed at temperatures below 1050°C[16,40,41]) are by-products of the reaction.

(b)By pyrolysis of the sample in the stream of hydrogen or hydrogen in a mixture with helium and by conversion of the pyrolysis products on a nickel catalyst at a relatively low temperature 450°C[15]. Oxygen is converted into water and carbon into methane. As the interfering elements, such as halogens and sulphur, poison the catalyst, the application of this reaction procedure, in contrast to the above, is restricted to compounds of qualitatively simple composition.

It follows from the above that in classical elemental analysis, reductive conversion on a carbon packing is used explicitly to determine oxygen. In the present instance it will be used analogously for separate applications of GC and EA, as all the instrumental methods are also based on this principle[40,41].

With on-line systems, hydrogenation cleavage on a nickel catalyst is also possible, as substances that are simple from the viewpoint of qualitative composition are mostly involved[15]. If application to the determination of the empirical formula without weighing is taken into consideration, it is necessary for simultaneous determination of oxygen and carbon, or oxygen and hydrogen, to be obtained[15,16].

In the first instance hydrogenation cleavage of the sample into methane and water can be used successfully, so that methane corresponds to carbon and water to oxygen. This method is particularly suitable for on-line connection[15]. Unreacted hydrogen can be removed by diffusion through a heated capillary made of paladium and silver.

Reductive conversion of the sample on a carbon packing permits the simultaneous determination of oxygen and hydrogen, preferably in the form of carbon dioxide and water after oxidation of the reaction products[16]. With respect to retention of hydrogen on the carbon packing, this procedure is suitable for EA systems with a dilution chamber.

6.3. Determination of S; C, H, N, S; C, N, S

All contemporary instrumental methods can be used for the determination of S or the simultaneous determination of S, C, N or together with H upon total oxidation of the sample[17,45–48]. In contrast to the reaction conditions in C, H, N determinations, it is necessary for some other problems to be solved. Granular WO_3[45,48] and SnO_2[17] at 1000°C, which do not show substantial retention of sulphur oxides, are suitable as combustion packings. Sulphur oxides must be converted into a uniform product, sulphur dioxide. A short layer of copper or copper(I) oxide at 850 ± 20°C was suggested to this purpose, where sulphur trioxide reacts to give copper(II) sulphate in order that the latter may be decomposed into copper(II) oxide and sulphur dioxide. This layer serves simultaneously for the absorption of unreacted oxygen and for reduction of nitrogen oxides, both reactions being accelerated by the presence of sulphur dioxide[49]. It is probable that in this layer [in fact in a mixture of copper(I) and -(II) oxides and copper] partial sorption of sulphur dioxide occurs even at the optimal temperature; however, it can be suppressed to a considerable extent by practical measures[50]. As the method is relative, sufficiently precise the results are obtained in this determination.

With on-line connection of GC and EA, reductive conversion of the sample in a stream of hydrogen over a platinum catalyst to give hydrogen sulphide, methane and water[51] can be considered, as with the determination of oxygen. This reaction is,

moreover, promising for the determination of phosphorus and arsenic as phosphine and arsine, respectively.

7. CONCLUSION

In the identification of unknown compounds leaving the chromatographic column, the determination of their empirical or molecular formulae and hence their molecular masses provides valuable information, which in most instances is sufficient if other chemical properties of the mixture under separation or relative elution data are also known. In practice, combined GC–MS, which moreover provides further information on molecular fragments and thus also on the structure of the compound under study, is mainly used for this purpose. This instrumentation is, however, still expensive and not available in many laboratories. The aim of this present review has been to indicate different ways of acquiring the information mentioned above with the aid of much simpler and less expensive means.

The separate application of GC and EA to the collection of the peak under analysis in a suitable trap can be recommended. The procedure without sample weighing is applicable in most instances, especially in GC. The procedure involving sample weighing is limited to larger amounts of sample (minimum 100 μg), as it is restricted by the sensitivity of currently available ultramicrobalances.

On-line GC–EA connection can be achieved most easily in the form of an adapter for the gas chromatograph (such as a replaceable thermostat head, likewise in the case of exchangeable detectors), providing the possibility of using an electronic modulus of the GC katharometer. The concept using a dilution chamber is advantageous, particularly for the determination of samples with a minimal mass of 50 μg; the possibility of using a trap of any type is an additional advantage. The combination of a GC column operating as a "stop flow" system with elution GC is suitable for samples with masses less than 50 μg.

The application of computers will make data processing and calculation of the empirical or molecular formulae substantially easier[52–54].

8. SUMMARY

General conditions and possibilities are discussed for the identification of GC effluents by determining their elemental composition, thus making it possible to calculate empirical and molecular formulae. Attention is paid to the trapping of GC peaks, to various methods for the direct combination of GC and EA, to elemental analysis without weighing and to the reaction conditions for the EA of GC column effluents, especially for the determination of C,H,N; O; C,O; H,O; S; C,N,S; and C,H,N,S. Individual instrumental methods of EA are discussed in detail, and sources of errors are pointed out.

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Journal of Chromatography, 251 (1982) 61–88
Chromatographic Reviews
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

CHREV. 156

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY: APPLICATIONS TO ORGANOMETALLIC AND METAL COORDINATION COMPOUNDS

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CONTENTS

0378-4355/82/0000–0000/$7.00

1. INTRODUCTION

High-performance liquid chromatography (HPLC) has been applied extensively to the separation of numerous organic and biochemically active compounds during the last 10 years. The technique has also been used for the separation of metal complexes and organometallic systems, although this application has not been as thoroughly studied. It is encouraging to note, however, that the amount of interest in utilizing HPLC for inorganic systems appears to be increasing during recent years. Other chromatographic methods such as gas chromatography (GC), thin-layer chromatography (TLC) and "classical" (gravity feed) column chromatography (CCC) have also been used for the separation and determination of inorganic compounds. GC, however, is severely limited for separating metal-organic compounds because the method usually requires column operating temperatures at which many of these species either lack volatility or undergo decomposition. TLC and CCC suffer from difficulties such as long analysis times and lack of quantitative capability. In HPLC, the columns are usually constructed of stainless steel; the compounds of interest are thus isolated from the atmosphere and light and they can be separated with degassed, inert mobile phases at ambient temperatures. The use of HPLC for determining inorganic metal complexes which are often unstable, therefore, offers obvious advantages over previously employed TLC, CCC and GC techniques. It should be noted, however, that stainless steel can sometimes reduce metal complexes, and one must be alert to this possibility.

This paper will update our earlier review[1] on the same subject; some of the work which was covered in that paper will also be included here. The general subject of HPLC in inorganic analysis has also been reviewed recently by Schwedt[2]. We will review the application of HPLC for two classes of metal–organic systems: (1) organometallic compounds and (2) metal coordination complexes. Any complex containing a metal–carbon bond will be considered under the organometallic classification. Separations of organometallic complexes from coordination compounds and other substances will also be considered here. Within each of these two categories the subject will be subdivided into two classifications: (a) separations involving a single metal, (b) multi-element separations employing one or more ligands. The individual discussions have been organized according to ligand type.

This review has been limited to those applications where metals are complexed *prior* to HPLC elution; work involving post-column derivatization of metal ions with a ligand is not included. Also, ion-exchange separations of metal ions and metal-organic compounds primarily of biological interest are not covered, though some of the latter are included for other reasons. This review includes applications which utilize most of the known HPLC modes of operation, *i.e.* liquid–liquid partition, liquid–solid adsorption, reversed-phase, size exclusion and ion-exchange chromatographic techniques. We have attempted to cover the literature through 1980 along with later articles which happen to be known to us.

2. ORGANOMETALLIC COMPOUNDS

2.1. Single-metal complexes

2.1.1. Arene metal carbonyls

The first use of HPLC for the separation of organometallic species was reported in 1969[3]. A column containing Carbowax 400/Porasil C was used to separate four arene tricarbonylchromium complexes with isooctane as the mobile phase. The order of elution of these compounds (Fig. 1) is the inverse of that found with GC[4] (see also ref. 43). The same experimental conditions were later used to separate the two geometric isomers of 2,3-dimethylnaphthalenetricarbonylchromium[5]. The two geometric isomers of tricarbonyl[4b,5,6,7,8,8a,η-(9,10-dihydro-1,4-dimethoxy-9,10-*o*-benzenoanthracene)]chromium have also been separated, both analytically and on a small preparative scale, on Carbowax 400/Corasil I as well as on uncoated Corasil I[6].

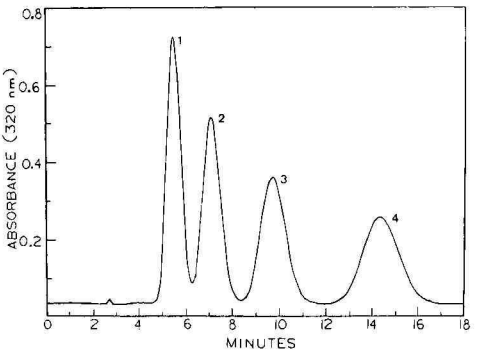
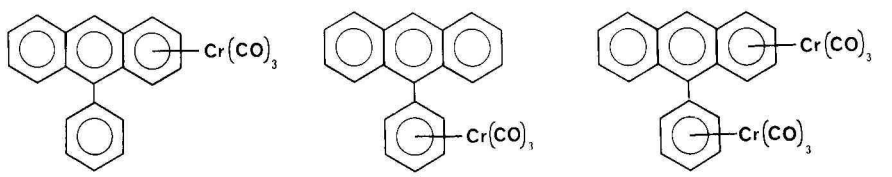


Fig. 1. HPLC separation of tricarbonylchromium complexes of (1) mesitylene, (2) *m*-xylene, (3) toluene and (4) benzene. Reprinted with permission from ref. 3 (Chemical Society, London).

Eberhardt *et al.*[7] have reported an extensive investigation of the HPLC behavior of 23 derivatives of benzenetricarbonylchromium on a Carbowax 400/Corasil column. Three tricarbonylchromium complexes of 9-phenylanthracene (I, II and III) have been separated on a preparative scale on silica with methylene chloride as eluent[8].



(I) (II) (III)

2.1.2. Cyclopentadienyl metal carbonyls

HPLC studies of tricarbonylcyclopentadienylmanganese complexes were first reported in 1973[7]. More recently, Vollhardt *et al.*[9] have separated a mixture of six

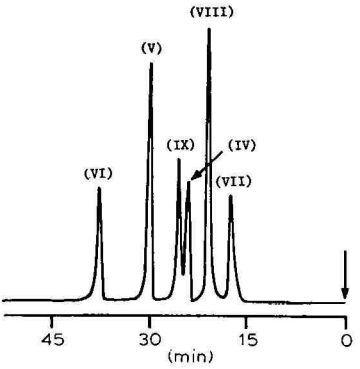
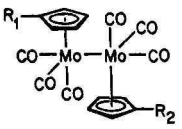
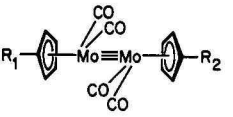


Fig. 2. Chromatogram of a mixture of IV–IX. Reprinted with permission[9].

dinuclear molybdenum complexes (IV–IX), using an Ultrasphere-ODS reversed-phase column with acetonitrile–water (5:1) (see Fig. 2).





(IV)	$R_1 = R_2 = H$	(VII)	$R_1 = R_2 = H$
(V)	$R_1 = CH_3, R_2 = H$	(VIII)	$R_1 = CH_3, R_2 = H$
(VI)	$R_1 = R_2 = CH_3$	(IX)	$R_1 = R_2 = CH_3$

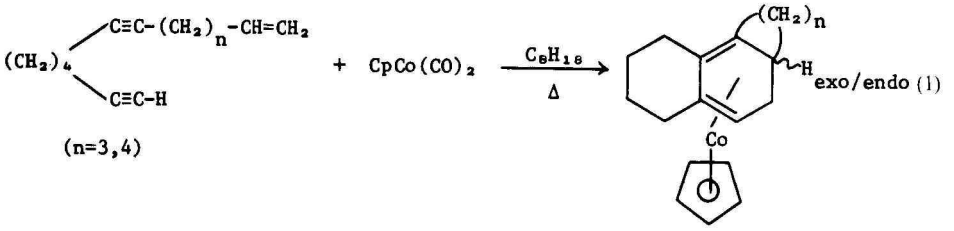
Within each series of single or triply bonded molybdenum complexes, introduction of methyl groups on the cyclopentadienyl rings increases the hydrophobicity and thus also increases the retention time.

2.1.3. Dicyclopentadienyl complexes

A systematic study of the HPLC behavior of 26 dicyclopentadienyl complexes of iron was reported in 1973[7]. Surprisingly, little HPLC work with this type of complex has appeared recently. One important recent advance in HPLC techniques has been the development of element specific detectors. In particular, the inductively coupled plasma (ICP) detector enables one to detect and quantitatively to determine complexes of a single element in the presence of complexes of other elements, even though these different complexes may not be separable under the chromatographic conditions used. Gast *et al.*[10] demonstrated the usefulness of this method of detection in the separation of ferrocene, acetylferrocene and diacetylferrocene on a silica gel column with toluene as the mobile phase. Hausler and Taylor[11] have used size exclusion HPLC (μStyragel 100 Å) to separate ferrocene and 1,1′-diacetylferrocene from iron and copper coordination compounds with toluene as eluent and with ICP detection. They have also separated ferrocene, acetylferrocene, 1,1′-diacetylferrocene and bis(tetrapyrazolylborate)iron(II) with pyridine elution[12]. McKone[13] has developed an undergraduate laboratory experiment involving the separation of ferrocene, acetylferrocene and 1,1′-diacetylferrocene on a C_{18} reversed-phase column with a methanol–water (3:1) mobile phase.

2.1.4. Cyclopentadienyl–cycloolefin complexes

Sternberg and Vollhardt[14] have described the reactions of linear diynenes with dicarbonylcyclopentadienylcobalt to form cyclopentadienyl–cycloolefin cobalt complexes (eqn. 1).



(1)

The *exo*- and *endo*-isomers were formed in approximately equal amounts in 65–75% yields and were separated on a preparative scale on a C_{18} reversed-phase column with dioxane–acetonitrile (5:95) eluent. For the $n=4$ case, a third isomer, presumably arising from hydrogen shifts, was also found. Trimethylsilyl derivatives of the above were also studied (see below).

2.1.5. Metal cluster complexes

Several transition metal cluster complexes, including $H_4Ru_4(CO)_{12}$ and $Ru_3(CO)_{12}$, have been separated on a C_{18} reversed-phase column with methanol–water (3:1) as the mobile phase[15]. The reactions of a number of tetranuclear clusters, including the hydride clusters $H_4Ru_4(CO)_{12}$ and $H_2Ru_4(CO)_{13}$, with carbon monoxide have been studied by Fox *et al.*[16]; the course of the reactions was monitored by infrared spectroscopy and analytical HPLC and the products of the reactions were separated on a μPorasil column. $H_2Ru_4(CO)_{13}$ reacts with carbon monoxide in hexane at 25°C to produce $Ru_3(CO)_{12}$, $Ru(CO)_5$ and H_2. $H_4Ru_4(CO)_{12}$ first reacts with carbon monoxide to produce $H_2Ru_4(CO)_{13}$ and H_2; the $H_2Ru_4(CO)_{13}$ produced then reacts further as described above. The reaction of $H_2Ru_4(CO)_{13}$ is essentially first order in the cluster and first order in carbon monoxide ($\Delta H^{0*} = 12.5$ kcal/mol; $\Delta S^{0*} = -36.6$ cal/mol·°K).

2.1.6. Metal carbonyl complexes containing olefin-type ligands

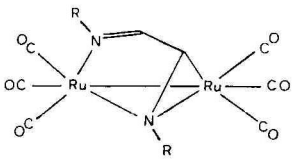
The first application of reversed-phase HPLC to organometallic compounds is apparently that of Graf and Lillya[17] who separated *cis*- and *trans*-tricarbonyl-(3,5-hexadienone)iron; separations of similar *cis–trans* isomeric pairs for five other substituted hexadienones and one substituted pentadienone are also reported. The column contained ODS Permaphase, and 20% aqueous methanol was used as the mobile phase. This reversed-phase mode was chosen so that the synthesized tricarbonylirondienone complexes could be eluted in narrow peaks unobscured by large amounts of less polar starting materials which are strongly retained on the column. The same authors[18] also used HPLC to study the stereospecific synthesis of dienone iron tricarbonyl complexes by Friedel–Crafts acylation. Pryde[19] has reported the use of HPLC to study the thermal rearrangements of four isomeric tricarbonyl(phenylcycloheptatriene)iron complexes. A 5-μm Hypersil silica column was packed under acetone and conditioned with diethyl ether followed by *n*-hexane.

Baseline separations of isomers, which differ from each other only in the position of a hydrogen atom in the 7-membered ring, were achieved in less than 5 min. Peak identifications were confirmed by mass-spectral analysis.

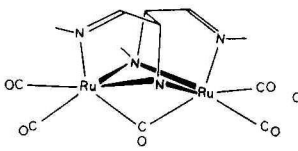
Reversed-phase HPLC has been used to monitor the reactions of $Ru_3(CO)_{12}$ with 1,4-diazabutadienes (DAB) and of $Ru_2(CO)_6$(DAB) with DAB[20]. The first of these reactions proceeds according to eqn. 2 and leads to a product of the structure X.

$$2\ Ru_3(CO)_{12} + 3\ DAB \rightarrow 3\ Ru_2(CO)_6(DAB) + 6\ CO \qquad (2)$$

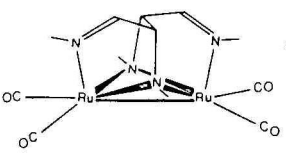
For R = isopropyl, the reaction was found to be second order [first order in $Ru_3(CO)_{12}$ and in DAB]. For R = *tert.*-butyl (*t*-Bu) the reaction again seems to be second order. The rate of reaction of *tert.*-butyl-DAB is slower than that of isopropyl (iso-Pr)DAB by a factor of 40. The reaction of $Ru_2(CO)_6$(DAB) with DAB involves a complicated series of intermediates including those shown (XI-XIII). Notice that formation of these intermediates involves the making and breaking of carbon–carbon bonds. These and other intermediates were identified chromatographically. There is evidence for exchange of coordinated and free DAB when $Ru_2(CO)_6$(iso-PrDAB) reacts with *tert.*-BuDAB. The HPLC characteristics of $M_2(CO)_6$(DAB) (M = Ru, Fe) on silica, CN-bonded, diol-bonded and ODS-bonded stationary phases have also been studied[21].



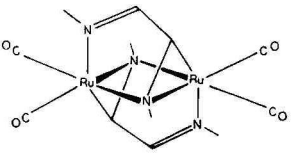
(X)



(XI)



(XII)



(XIII)

2.1.7. Metal carbonyl coordination complexes

The $M_2(CO)_6$(DAB) complexes discussed above, including the intermediates and final products of the reactions, also involve metal–nitrogen coordination and thus could be considered under this heading. The intermediates XI and XII are bis-(alkylimino-alkylaminoethane) complexes (abbreviated as IAE). The capacity factors for silica, CN-bonded, diol-bonded and ODS-bonded stationary phases with a variety of eluents for $Ru_2(CO)_5$(*t*-BuIAE), $Ru_2(CO)_5$(cyclohexyl-IAE), $Ru_2(CO)_4$(cyclohexyl IAE) and $Mo_2(CO)_6$(*t*-BuIAE) have been determined[21]. ICP detection has been used to follow the separation of a mixture of five molybdenum complexes, including tetracarbonylbipyridylmolybdenum, using gradient elution on a C_8 reversed-phase column[10]. Reversed-phase HPLC on a C_8 bonded-silica support has been used to study the reaction of $Fe_2(CO)_9$ with di-*tert.*-butylsulfurdiimine

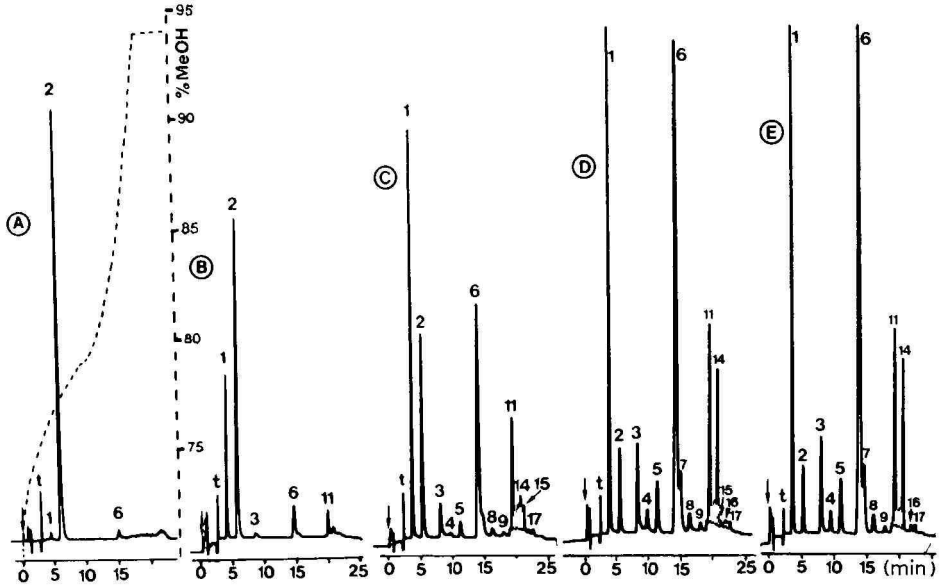
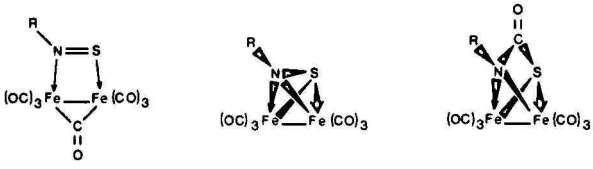
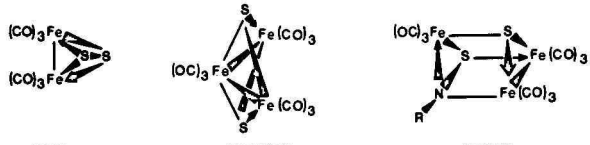


Fig. 3. Example of monitoring the reaction of $Fe_2(CO)_9$ with DBSD using gradient elution. Chromatograms of samples taken from the mixture at various times: A, after 5 min; B, after 1 h; C, after 2 h; D, after $3\frac{1}{2}$ h; E, after $4\frac{1}{2}$ h. Stationary phase: Zorbax C-8. Mobile phase: gradient from 70 to 94% (v/v) methanol–water, gradient shape indicated in A. t = Toluene. Reprinted with permission[22].

(DBSD)[22]. Gradient elution with 70–94% methanol in water resulted in the separation of seventeen products within 30 minutes (see Fig. 3). Structures of some of the products identified are given below (XIV–XIX). ICP detection has also been employed with this system[10].

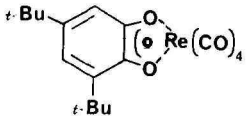


(XIV) (XV) (XVI)



(XVII) (XVIII) (XIX)

Creber and Wan[23] have recently used electron spin resonance as a detector in an HPLC system to demonstrate the formation of the 3,5-di-*tert.*-butyl-*o*-quinonetetracarbonylrhenium complex (XX). The stationary phase was Partisil-10



(XX)

and the eluent was benzene. This first successful use of this detection mode holds promise for future separations and studies of other organometallic radicals.

2.1.8. Metallocarboranes

The original use of HPLC for the separation of several pairs of cyclopentadienylcobaltacarboranes by Evans and Hawthorne[24] has been extended by Plzák *et al.*[25]. Capacity factors on silica-gel columns with four different eluents were measured for eleven cyclopentadienylcobalt complexes of boranes (mostly carboranes), including two bis-cyclopentadienyl complexes. One such complex of iron was also included. Though no actual separations of these complexes were reported, separations were shown to be feasible in many cases. Capacity factors for ten bis-carborane cobalt complexes with bridging groups between the carborane ligands are also reported, and a separation of the methoxy-bridged complex from the ethoxy-bridged complex is demonstrated.

2.1.9. Alkyl and aryl complexes of main group metals

The importance of alkyl and aryl complexes of heavy metals, particularly mercury and lead, in environmental chemistry has brought about investigations of the use of HPLC for their separation and detection. Early work by Fanasaka *et al.*[26], Brinckman *et al.*[27] and Botre *et al.*[28] has been discussed in our earlier review[1]. More recently, electrochemical detection has been used to monitor the separation of Hg^{2+}, $MeHg^+$, $EtHg^+$ and $PhHg^+$ on an ODS column with 40% aqueous methanol containing 0.06 mol/l ammonium acetate and $5 \cdot 10^{-4}$ *M* 2-mercaptoethanol at pH 5.5 as mobile phase[29]. The separation of $MePh_3Sb^+$, Et_3Pb^+, Me_3Pb^+ and $MeHg^+$ on LiChrosorb NH_2 with the same eluent minus the 2-mercaptoethanol is also reported. MacCrehan and Durst[30] have used differential-pulse electrochemical detection with an ODS column and the same eluent to separate $MeHg^+$, $EtHg^+$ and $PhHg^+$. Good separations were obtained even in the presence of interfering reducible cations such as Cd^{2+}, Pb^{2+} and Cu^{2+}. The method has been used for quantitative determination of methyl mercury in tuna fish and shark meat. MacCrehan[31] has recently discussed the advantages of differential-pulse detection (DPD) of organometallic cations; Me_3Sn^+, Et_3Sn^+ and n-Bu_3Sn^+ are simultaneously detected after separation on a cation-exchange column. Alkyltin cations can be more reliably measured with DPD than with amperometric detection.

Gast and Kraak[32] have reported the separation of organomercury compounds on a silica-gel column with 10% butanol in *n*-hexane saturated (*ca.* 0.01%) with tetramethylammonium chloride as eluent. Clean separations of mixtures of diphenylmercury, propylmercury bromide, ethylmercury chloride, methylmercury chloride and phenylmercury chloride were achieved with an elution time of about 20 min. Reversed-phase chromatography on a C_8 column with 40% acetonitrile in 0.1 *M* aqueous sodium bromide (pH 3.5) was also shown effectively to separate a mixture of

mercury(II) chloride, methylmercury chloride, ethylmercury chloride, propylmercury bromide and 3-chlorophenylmercury acetate. Both UV detection at 205 nm and post-column derivatization (dithizone) were used. For alkylmercury compounds, the lowest limit of detection (80 ppb, 10^9) was obtained with dithizone reaction detection while for the phenylmercury compounds, UV detection was most sensitive (60 ppb). ICP has been used for the detection of alkylmercury compounds and of lead compounds in gasoline[10]. This detector shows enhanced sensitivity for alkylmercury compounds compared to UV detection; ICP detection shows clearly the presence of four lead compounds in the gasoline sample while UV detection reveals only a very broad band of unseparated components.

Burns *et al.*[33] have compared gas–liquid chromatography (GLC) and HPLC for the separation of tin tetraalkyls and alkyltin halides. For ethyltin compounds, they found that both GLC and HPLC are satisfactory for identification and quantitative applications. The detection limits for GLC are smaller than those of HPLC (differential refractometric) by factors of 4–5. However, for methyltin compounds, a redistribution reaction (eqn. 3) occurs on the GLC column so that it was not possible to examine all four methyltin compounds simultaneously. Reversed-phase HPLC with acetone–*n*-pentane (60:40) elution produced a satisfactory separation (see Fig. 4). Vickrey *et al.*[34] have described an off-line HPLC–graphite-furnace atomic-absorption sampling procedure which offers certain advantages; this is illustrated by the reversed-phase C_{18} chromatography of tetraphenyllead. Parks *et al.*[35] have described the use of graphite-furnace atomic-absorption detection with size-exclusion HPLC and reversed-phase HPLC for characterizing controlled-release biocidal organometal macromolecules such as organotin silicates and poly(tri-*n*-butyltin methacrylate–methyl methacrylate). Among the compounds studied by Hausler and Taylor[11] using size-exclusion HPLC with ICP detection are $Ph_3Si{-}C{\equiv}C{-}SiPh_3$, $Ph_3Si{-}CH{=}CHCH_3$, Ph_4Pb and $(CH_3CH{=}CH)_4Pb$.

$$(CH_3)_4Sn + CH_3SnCl_3 \rightarrow (CH_3)_3SnCl + (CH_3)_2SnCl_2 \qquad (3)$$

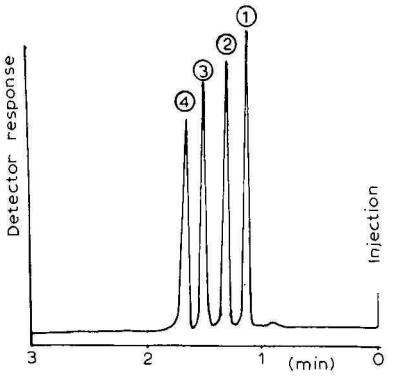
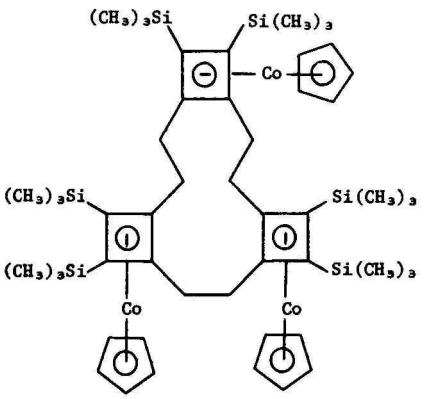


Fig. 4. Chromatogram of admixture of (1) $(CH_3)_4Sn$, (2) $(CH_3)_3SnCl$, (3) $(CH_3)_2SnCl_2$ and (4) CH_3SnCl_3. Reprinted with permission[33].

2.2. *Complexes containing two or more different metals*

2.2.1. *Cyclopentadienyl–cycloolefin complexes*

The work of Sternberg and Vollhardt[14] on the reactions of diynenes with dicarbonylcyclopentadienylcobalt has already been discussed in section 2.1.4. Similar reactions with trimethylsilyl-substituted diynenes have also been carried out leading to trimethylsilyl-substituted cycloolefin complexes. When *exo*- and *endo*-isomers were formed (eqn. 1), these were found also to be separable on a reversed-phase C_{18} column with dioxane–acetonitrile (5:95) as eluent. Gesing and Vollhardt[36] have studied the reaction of 1,6-bis(trimethylsilyl)-1,5-hexadiyne with dicarbonylcyclopentadienylcobalt. Eighteen characterizable complexes were isolated from these reactions, including cyclobutadienecobalt–, cyclopentadienonecobalt– and cobalt cluster–η^5-C_5H_5 complexes. Nine of these complexes, all trimethylsilyl-substituted cyclobutadienes, eluted as a single band from alumina with diethyl ether, but were separable on an ODS reversed-phase HPLC column. The structure of one of these complexes is shown (XXI); this is typical of the compounds synthesized and separated. An extensive discussion of the use of reversed-phase HPLC as an analytical and preparative tool for the characterization and isolation of organometallic compounds, especially CpCo complexes of cyclobutadienes and cyclohexadienes, has recently appeared[9].



(XXI)

2.2.2. *Metal cluster complexes*

$H_2FeRu_3(CO)_{13}$ has been separated from $H_4Ru_4(CO)_{12}$ and $Ru_3(CO)_{12}$ on a C_{18} reversed-phase column using methanol–water (3:1) as eluent[15]. UV irradiation of benzene solutions of $HFeCo(CO)_{10}(PPh_3)_2$ gives at least three products which are separated by elution with methanol–water (4:1)[15,37]. Reactions of the mixed-metal tetranuclear clusters $H_2FeRu_3(CO)_{13}$, $H_2FeRu_2Os(CO)_{13}$, $H_2FeRuOs_2(CO)_{13}$, $H_4FeRu_3(CO)_{12}$, $HCoRu_3(CO)_{13}$, $H_2FeRu_3(CO)_{12}L$ (L = PMe_2Ph, PPh_3), $[HFeRu_3(CO)_{13}]^-$ and $[CoRu_3(CO)_{13}]$ with CO under relatively mild conditions have been monitored by infrared spectroscopy and by analytical liquid chromatography and the products separated by HPLC[16]. These reactions result in fragmentation

to give trimeric and monomeric products which are greatly dependent on metal composition and/or structure. The tetrahydride cluster $H_4FeRu_3(CO)_{12}$ first reacts with carbon monoxide to produce H_2 and $H_2FeRu_3(CO)_{13}$, the latter then fragmenting to form $Ru_3(CO)_{12}$, $Fe(CO)_5$ and H_2. The last reaction proceeds largely by a second-order, [CO]-dependent path for which $\Delta H^{0*} = 20.0 \pm 2.0$ kcal/mol and $\Delta S^{0*} = -25.4 \pm 5.8$ cal/mol·°K) [*cf.* values for $H_2Ru_4(CO)_{13}$ given in section 2.1.5.].

2.2.3. Metal carbonyl complexes containing olefin-type ligands

The work of Gast and Kraak[21] on diazabutadiene complexes of the general formula $M_2(CO)_6(DAB)$ discussed previously (section 2.1.6.) includes a study of two mixed metal species, $CoMn(CO)_6$(iso-PrDAB) and $CoMn(CO)_6$(*t*-BuDAB). Capacity factors for these complexes on silica, CN-bonded, diol-bonded and ODS-bonded stationary phases with a variety of eluents were measured.

2.2.4. Alkyl and aryl complexes of main group metals

Among the compounds studied by Hausler and Taylor[11] were three mixed metal species, $Ph_3SnC{\equiv}CSi(Ph)_2C{\equiv}CSnPh_3$, $Ph_3SnC{\equiv}CSiPh_3$ and $Ph_3PbC{\equiv}CGePh_3$. After size-exclusion HPLC, a refractive-index detector showed separation of the first of these from the other two. Detection of Sn species by ICP shows separation of the first two compounds while Pb monitoring shows a single peak for the last compound.

2.2.5. Organopolymetallic compounds

The recently discovered ability of zerovalent triphenylphosphine complexes of platinum and palladium to insert into Hg–C and M–M′ bonds (M = Hg, Cd, Zn; M′ = Ge, Sn) has resulted in the synthesis of a number of organometallic compounds containing polymetallic chains. Germylpalladium and germylplatinum complexes were also synthesized by oxidative insertion of palladium and platinum triphenylphosphines into Ge–H bonds. Bochkarev *et al.*[38] have used silica columns with UV detection (254 nm) and *n*-hexane–diethyl ether mixtures as eluents to separate pentafluorophenyl complexes containing Ge–Hg–Pt–Ge, Sn–Hg–Pt–Ge and Ge–Cd–Pt–Ge groups. These complexes cannot be separated by GLC because of low volatility and limited thermal stability. HPLC makes it possible to monitor the reactions involved and to obtain information on relative reaction rates, mechanisms and stabilities of reaction products.

3. METAL COORDINATION COMPLEXES

3.1. Separations of single-element multi-ligand coordination complexes

A number of reports have appeared in which coordination complexes of the same metal with a variety of ligands are separated by HPLC. Many of these studies have led to interesting observations concerning stereochemical, kinetic, thermodynamic and structural properties of metal coordination compounds.

3.1.1. β-Diketonates

The elution behaviour of various β-diketone ligands and β-diketonates of

Cr(III) on size-exclusion columns has been studied by Saitoh *et al.*[39] and by Suzuki *et al.*[40]. In the initial paper, acetylacetone and its Cr(III) chelate were selected as model compounds for studying their gel chromatographic behavior on Merckogel OR-2000[39]. Two poly(vinyl acetate) gels and two polystyrene gels were investigated in the later work[40]. The ligands included acetylacetone, trifluoroacetylacetone, benzoylacetone, furoyltrifluoroacetone, benzoyltrifluoroacetone, theonyltrifluoroacetone and dibenzoylmethane, as well as their corresponding Cr(III) chelates. It was found that the elution order of neither the β-diketone ligands nor their Cr(III) chelates on any gel followed the expected order of their molecular weights; the distribution coefficients of the ligands and chelates were found to depend strongly on the gel and could not be correlated with the molecular weights of the compounds.

A recent communication by Noda *et al.*[41] demonstrated the feasibility of gel permeation chromatography for the separation of mixed-ligand complexes of several beryllium(II) β-diketonates. The chelates studied included the bis(acetylacetonato), the bis(theonyltrifluoroacetonato) and the mixed-ligand complexes.

Uden *et al.*[42] employed normal-phase HPLC on silica for the separation of pairs of geometrical isomers of octahedral cobalt(III) and chromium(III) chelates of trifluoroacetylacetone [H(TFA)], benzoylacetone [H(BAA)] and 2,2-dimethylhexane-3,5-dione [H(PAM)]. Both isocratic and gradient elution modes were used with UV detection at 254 nm. It was found that adsorption HPLC with a mobile phase of medium polarity (6% acetonitrile in methylene chloride) permits ready resolution of isomer pairs of non-volatile complexes such as $Co(BAA)_3$ and $Co(PAM)_3$. In all cases, it was found that the *mer* isomer elutes before the *fac* isomer, and for a given ligand, chromium complex isomers elute before the analogous cobalt species. The authors also found that it was possible to achieve good resolution of mixed ligand complexes of chromium with hexafluoroacetylacetone [H(HFA)] and HTFA using either isocratic or gradient elution.

Acetylacetonato-cobalt(II) and -cobalt(III) complexes as well as those of copper(II) have recently been separated using size-exclusion HPLC and detected with an ICP atomic-emission spectrometer by Hausler and Taylor[12].

3.1.2. β-Ketoamines

Uden *et al.*[43] applied reversed-phase HPLC successfully to the separation of neutral tetradentate chelates of copper(II), nickel(II) and palladium(II) with a range of fluorinated and non-fluorinated β-ketoamine and salicylaldimine ligands. A bonded octadecyl substrate was used as the reversed-phase functionality, and elution was carried out isocratically using methanol–water–acetonitrile. UV detection at 254 nm was employed. The separation of the N,N′-ethylenebis(acetylacetoneimine) [$H_2(enAA_2)$], N,N′-propylenebis(acetylacetoneimine) [$H_2(pnAA_2)$] and N,N′-butylenebis(acetylacetoneimine) [$H_2(bnAA_2)$] chelates of nickel(II) showed that substitution of methyl groups into the ethylene bridge leads to greater partition on the C_{18} substrate and longer retention times. It is interesting to note that the order of elution is the reverse of that noted in gas chromatography for the same compounds (see also ref. 3). Other interesting variations of retention and resolution as a function of ligand structure and the metal used are reported in this paper.

3.1.3. Hydrazones

Gasparrini *et al.*[44] have separated several palladium(II) complexes of substituted hydrazones on a LiChrosorb DIOL column with *n*-hexane–dichloromethane mixtures. Complexes which were separated included those of the type *trans*-$[PdL_2Cl_2]$ where L is the substituted hydrazone ligand. Ligands which were studied included the N-methyl-N-phenylhydrazones of methyl isopropyl ketone, of diethyl ketone, of methyl-*n*-propyl ketone, of acetone and of acetaldehyde as well as the N,N-dimethylhydrazone of acetone. Mixed ligand complexes of the type *trans*-$[PdLL'Cl_2]$ were also studied. They found that unmodified silica columns were not effective, as the complexes were generally unstable and strongly adsorbed. The LiChrosorb DIOL column was found to be very effective and permitted the use of non-polar mobile phases in which the complexes were readily soluble. A number of six-component separations were reported using either isocratic or gradient elution operating conditions. It was also found that the capacity factor (k') decreases as the chain-length of the ketone increases, a result which is consistent with normal-phase HPLC separations. The high sensitivity of the technique permits trace amounts of these complexes to be determined. The method can also be employed to monitor the progress of the synthesis of these complexes and can provide information about their purity and rate of ligand exchange.

3.1.4. Dithiocarbamates

A distinct advantage of the use of the dialkyldithiocarbamate (DTC) anion over many other ligands is its insolubility in organic solvents and its ability to form complexes with many metal ions. Extraction into organic solvents transfers only the neutral chelate, leaving behind excess ligand. Hence, DTC complexes lend themselves uniquely to HPLC studies.

Liška *et al.*[45] have studied the separation and identification of a series of nickel(II) bisdialkyldithiocarbamate complexes by HPLC. Silica gel was used as the stationary phase and chloroform–cyclohexane mixtures as the mobile phase with UV detection at 325 nm. The capacity factors (k') of a series of DTC complexes from the dimethyl- to dioctyl- were measured as a function of mobile phase composition. It was found that there was a substantial influence of the N-alkyl substituent and the mobile phase composition on the capacity factors; the limit of detection was in the range of 10^{-9}–10^{-10} *M* Ni(II). The separation and quantification of Ni(II)–DTC complexes was found to be severely complicated by the formation of new complexes via ligand exchange reactions. These side reactions were investigated further in a later paper by Liška *et al.*[46]. In this study it was observed that a third compound was formed for every pair of Ni(II) complexes with different N-alkyl substituents. For example, a mixture of nickel(II) bisdiethyl- and bisdihexyl-DTC separated by either HPLC or TLC produced a mixture of three substances of which two were identical with the original symmetrical complexes. It was demonstrated that mixed-ligand complexes were formed by ligand-exchange reactions in solution. The results were verified by TLC analysis. Some of these compounds could be isolated and were characterized by molecular-weight determinations and elemental analysis. The HPLC separation of multi-ligand DTC complexes of Cu(II), Co(II), Zn(II) and Pb(II) was reported in a subsequent paper by Lehotay *et al.*[47]. It was demonstrated again that symmetrical Zn(II) bisdialkyl–DTC complexes as well as symmetrical Pb(II) bisdial-

kyl–DTC complexes used to prepare the sample mixture produced mixed ligand complexes as described by the equilibrium shown in eqn. 4.

$$MA_2 + MB_2 \rightleftharpoons 2\ MAB \tag{4}$$

This reaction was found not to occur between bisdialkyl–DTC complexes of Cu(II) or Co(II). A typical chromatogram showing the separation of Zn(II) bisdialkyl–DTC complexes is shown in Fig. 5. The authors have concluded from this work that HPLC is an effective method to separate mixtures of metal bisdialkyl–DTC complexes, although the determination of these compounds in the environment or in fungicides is hindered by the ease with which ligand exchange between them occurs.

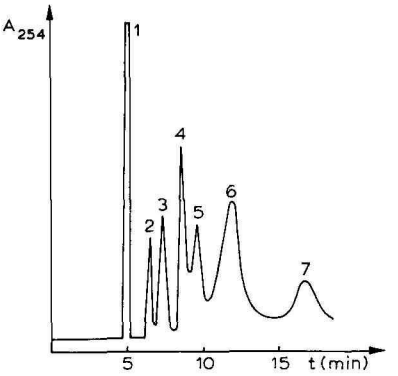


Fig. 5. Separation of Zn(II) bisdialkyl–DTC complexes. Mobile phase: chloroform cyclohexane (10:90). Column: 25 × 0.46 cm, LiChrosorb SI 60, particle size 10 μm. Peaks: 1 = inert substance; 2 = Zn(II) bisdibutyl–DTC; 3 = mixed-ligand complex, Zn(II) dipropyl–DTC dibutyl–DTC; 4 = Zn(II) bisdipropyl–DTC; 5 = Zn(II) diethyl–DTC dibutyl–DTC; 6 = Zn(II) diethyl–DTC dipropyl–DTC; 7 = Zn(II) bisdiethyl–DTC. Reprinted with permission[47].

Moriyasu and Hashimoto[48] have carried out additional studies on the lability of ternary Ni(II) chelates of DTC by HPLC. They describe an HPLC method to determine the kinetic rate constants for the formation and disproportionation of these complexes. They verified that thoroughly deactivated silica columns produced satisfactory chromatograms for Hg(II), Cu(II), Ni(II) and Co(III) without dissociation of the chelates during chromatography. Contrary to the findings of Lehotay *et al.*[47], Moriyasu and Hashimoto[48] found that the Cu(II) chelates are more labile than those of Ni(II) and that during chromatography, disproportionation did in fact occur. Moriyasu and Hashimoto[48] also state that Lehotay *et al.*[47] claim non-lability for Co(III) complexes. This is incorrect; Lehotay *et al.* claimed non-lability for Co(II) complexes. Moriyasu and Hashimoto[49] have also done a more extensive kinetic and equilibrium HPLC study of dialkyl–DTC chelates of Ni(II) and Cu(II). The equilibrium of ternary complex formation from two symmetrical complexes is the same as that shown in eqn. 4. They found that the HPLC separation process was sufficiently rapid, and that kinetically unstable ternary complexes can be eluted without disproportionation during chromatography. It was found possible to halt the progress of fast exchange reactions at the moment of injection. The chromatograms, therefore,

indicated the concentration of the species before chromatography, facilitating the determination of equilibrium constants for the Ni(II) and Cu(II) chelates. The equilibrium equation for the process shown in eqn. 4 is given in eqn. 5.

$$K = \frac{[MAB]^2}{[MA_2]\,[MB_2]} \tag{5}$$

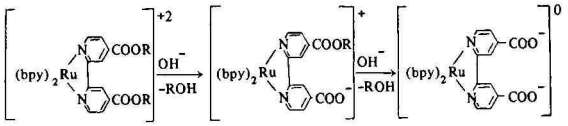
Experimentally determined K values were always found to be 4.0. The authors suggest that disproportionation takes place by collision of two ternary metal chelates, the rate constants being on the order of 10^1–10^2 l/mol · sec for Ni(II) chelates and 10^3 l/mol · sec for Cu(II) chelates.

The interesting possibility of simultaneously determining Cr(III) and Cr(VI) in waste water by separating the complexes of these chromium species with ammonium pyrrolidinedithiocarbamate using reversed-phase HPLC has been examined by Schwedt[50]. Reaction conditions and products were examined and the procedure was found to be applicable to the simultaneous determination of both chromium species. Tande *et al.*[51] have used DTC for the simultaneous determination of Cr(III) and Cr(VI) in water by means of reversed-phase HPLC with methanol–water (65:35) as the mobile phase and UV detection at 254 nm. They found that at room temperature and pH 5.8, DTC reacts with both Cr(III) and Cr(VI). Detailed examination of this reaction by HPLC revealed that three products were formed, an unidentified disulfide and two chromium–DTC chelates of different composition.

3.1.5. Bipyridines

Paired-ion HPLC is rapidly becoming very useful and popular for the separation of ionic compounds on reversed-phase columns. This can be accomplished by the addition of a counter-ion (usually an ionic alkyl compound) to the mobile phase causing the ionic species of interest to form ion pairs. The ion pair behaves as though it were a non-ionic, neutral species, thus favoring sorption on hydrophobic stationary phases. The mechanism and application of reversed-phase paired-ion HPLC has been described extensively[52,53]. It can be seen, therefore, that this chromatographic technique is especially useful for reversed-phase HPLC studies of cationic and anionic complexes, an area which has received very little attention thus far.

Valenty and Behnken[54] have reported the first use of reversed-phase *paired-ion* HPLC to separate ionic metal complexes. They investigated the elution of the diester, monoester-monocarboxylate and dicarboxylate derivatives of tris(2,2′-bipyridyl)ruthenium(II). These compounds are of interest because they can be employed as photopromoted electron transfer reagents and they can be distinguished on the basis of water solubility and molecular charge as shown in the hydrolytic reactions of I and II (eqn. 6).



(6)

I, R = R = $C_{18}H_{37}$ IV, R = $C_{18}H_{37}$ III
II, R = R = C_2H_5 V, R = C_2H_5

Linear gradient elution with aqueous tetrahydrofuran (THF) containing ion-pairing reagents was used on a reversed-phase C_{18} column. Eluted components were monitored with UV detection at 254 and 280 nm. The ion-pairing reagents which were added to the mobile phase were 0.015 *M* methanesulfonic acid (for the separation of I, III and IV) and 0.005 *M* heptanesulfonic acid (for the separation of II, III and V). The chromatogram in Fig. 6 shows a separation of II, III and V accomplished in 11 min with a linear gradient programmed from 10 to 40% THF in water. The order of elution is exactly what one would predict; compound III is the most polar, although it has a net charge of zero; the two carboxylate groups cannot form an ion pair with the counter ion since they are anionic. Compound II is the least polar, even though it has a net cationic charge of +2, and can thus form a relatively non-polar ion pair with the sulfonate counter ion. This paper is a particularly interesting example which illustrates the versatility of paired-ion reversed-phase HPLC for the separation of ionic metal complexes.

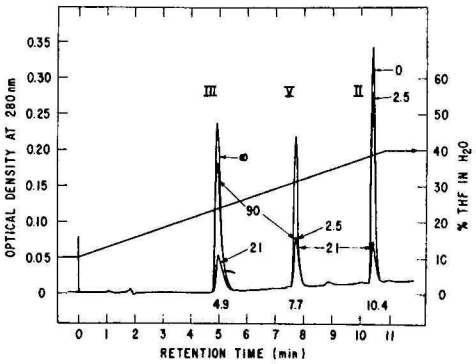


Fig. 6. Reversed-phase paired-ion HPLC separation of tris(2,2′-bipyridyl)ruthenium(II) derivatives. Chromatograms of the hydrolysis reaction of II in aqueous $1.0 \cdot 10^{-2}$ *M* pH 10 borate buffer. Traces shown at reaction times of 0, 2.5, 21, 90 and ∞ min. The diagonal line across the figure shows the composition of mobile phase with 0.005 *M* *n*-heptanesulfonic acid buffered to pH *ca.* 3.5. Reprinted with permission from ref. 54 (American Chemical Society).

3.1.6. EDTA, ethylenediamines and amino acids

Jones and Manahan[55] have demonstrated that copper(II) chelates of EDTA, NTA (nitrilotriacetic acid), EGTA [ethylenebis(oxyethylenenitrilo)tetraacetic acid] and CDTA [(1,2-cyclohexylenedinitrilo)tetraacetic acid] can be separated by HPLC using a 5-cm weak anion-exchange resin column and an aqueous 0.05 *M* ammonium sulfate mobile phase. The eluted copper chelates were detected by atomic absorption. The chelates were observed to elute from the column as a function of charge and size in the order: $Cu_2(EGTA)$, $Cu(NTA)^-$, $Cu(EDTA)^{2-}$, $Cu(CDTA)^{2-}$. This analysis was developed principally to determine chelating agents as pollutant components of natural and waste waters. The chelated copper(II) ion, added to the ligand mixture, served as a convenient "indicator metal" to provide sensitive and selective detection of the ligands by atomic absorption. This work illustrates the utility of atomic absorption as a species-specific detector. Yoshikawa *et al.*[56] have also reported an ion-exchange HPLC separation of cationic and anionic mixed ligand complexes of

Co(III) with NH_3, NO_2^-, en, dien, trien, carbonate, oxalate, EDTA and iminodiacetic acid. Separations were achieved on strongly acidic and strongly basic resins using aqueous sodium chloride, and UV detection at 254 nm. The method was applied to kinetic studies and isomer separations.

It has been shown recently by Warner and Legg[57] that the geometric diastereoisomeric forms of en–amino acid- Co(III) complexes can be separated by analytical and preparative HPLC on Whatman LP-1 (10–20 μm) silica particles with an isopropanol–triethylammonium bicarbonate (70:30) buffer system (pH 9). The absorbance at 510 nm was monitored. The complexes studied included $[Co(en)_2tyr]^{2+}$, $[Co(en)_2asp]^{2+}$ and $[Co(EDDA)(en)]^+$, where tyr = tyrosine, asp = asparagine and EDDA = ethylenediaminediacetic acid. Even though complete resolution was not achieved for the diastereoisomeric $[Co(en)_2aa]^{2+}$ complexes (aa = amino acid), the compounds were rapidly separated from several other mixture components and mirror-image circular dichroism spectra were generated. Typical analytical and preparative chromatograms for $[Co(en)_2(tyr)]^{2+}$ are shown in Fig. 7. The *sym-cis* and *unsym-cis* isomers of $[Co(EDDA)(en)]^+$ were also rapidly and completely resolved by this method. The results were consistent with those previously obtained by TLC.

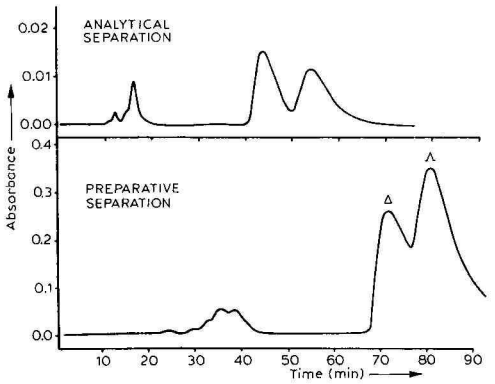


Fig. 7. Analytical and preparative separations of $[Co(en)_2(tyr)]^{2+}$ diastereomers. Analytical separation conditions: flow-rate, 6 ml/min; approximately 2 mg of mixture were applied in a 20-μl sample volume; elution monitored at 510 nm. Preparative separation conditions: flow-rate, 3 ml/min, approximately 100 mg of mixture were applied in a 500-μl sample volume with a 20-min flow program; elution monitored at 510 nm. Reprinted with permission from ref. 57 (American Chemical Society).

Paired-ion reversed-phase HPLC was recently employed by Buckingham *et al.* for the rapid separation of similar cobalt(III) bis(ethylenediamine) amino acid complexes[58]. The complexes studied were of the diastereomeric type $\Delta[Co(en)_2aa]X_2$ where aa represents an amino acid (gly, pro, val, leu or phe). It was possible to separate these five complexes on a reversed-phase packing in less than 15 min with *p*-toluenesulfonate or hexanesulfonate as the pairing-ion. The complexes were detected at 480 nm. It was shown that these complexes elute in the order of the relative hydrophobicities of the parent amino acid. At high sample loadings, concentration-dependent peak-splitting effects were noted.

A reversed-phase paired-ion separation of L-methionine dipeptide complexes of palladium(II) was recently reported by Lam-Thanh *et al.*[59]. They used quaternary ammonium salts as the pairing ions with UV detection at 380 nm.

3.1.7. Carbonates, benzoates and camphorates

Strazza and Polcaro have used HPLC for rapid separations of relatively labile carbonato- and benzoatocobalt(III) complexes formed in solid-state reactions[60]. Included in the study were the *cis* and *trans* forms of $[Co(en)_2Cl_2]^+$ and $[Co(en)_2(OCOC_6H_5)_2]^+$. HPLC was performed on a silica column (10 μm particles) using a gradient which was programmed from an eluent which was a mixture of 95% ethanol, 2-propanol and 25% aqueous ammonium nitrate to an eluent consisting of 1 *M* acetic acid in methanol. The column effluent was monitored at 340 and 580 nm respectively for each of the two separations.

An interesting HPLC separation of the four diastereomers of tris[(+)-3-acetylcamphorato]chromium(III) on Corasil II with 15% THF in *n*-hexane has been reported by Minor and Evcrett[61]. Retention times were 12 min for *Δ-trans*, 14 min for *Λ-trans*, 20 min for *Λ-cis*, and *ca.* 50 min for *Δ-cis*.

3.1.8. Diphosphonates

Diphosphonate complexes of technetium-99m are widely used as skeletal imaging agents in nuclear medicine. A typical skeletal imaging agent is prepared on the day of use by mixing $^{99m}TcO_4^-$ and its daughter $^{99}TcO_4^-$ with a reducing agent (Sn^{2+}, $NaBH_4$ or NH_2OH), a diphosphonate ligand [hydroxyethylidene diphosphonate, $CH_3C(PO_3H_2)_2(OH)$, HEDP] and an antioxidant stabilizer (ascorbic acid). The resultant radio-pharmaceutical solution contains Tc–diphosphonate complexes, reductant diphosphonate complexes, excess diphosphonate and stabilizer. Pinkerton *et al.*[62] have developed the first ion-exchange HPLC procedure to separate and characterize the component complexes in radio-pharmaceutical Tc–HEDP solutions. They used a 25-cm column containing Aminex A-27 anion-exchange resin and aqueous sodium acetate (pH 8.4) as the eluent. Three modes of detection were used: (1) single wavelength absorbance at 405 nm, (2) UV–visible rapid scanning (250–660 nm) and (3) radiometric scintillation. The results indicated that the radio-pharmaceutical solutions consist of mixtures of numerous Tc-containing species of unknown oxidation state, configuration and coordination environment. A typical chromatogram, shown in Fig. 8, illustrates that there are at least seven components containing ^{99m}Tc activity.

Wong[63] has reviewed the application of HPLC for the analysis of mixtures containing ^{99m}Tc labeled radiopharmaceuticals. The review includes work which has been reported on ^{99m}Tc adducts with pyrophosphate, methylene diphosphonate, ethylene diphosphonate, diethylenetriamine pentaacetic acid and human serum albumin.

3.1.9. Crown ethers

Reversed-phase HPLC was employed by Mangia *et al.*[64] to study the chromatographic behavior of Hg(II) halide complexes with the macrocyclic polyether, dibenzo-18-crown-6(2,3,11,12-dibenzo-1,4,7,10,13,16-hexaoxacyclooctadeca-2,11-diene) (DBC). This compound acts as a hexadentate ligand with Hg(II) halides. Micropak CH (octadecylsilane on silica) columns were used with mobile phases consisting of methanol–phosphate or borate buffer mixtures (pH 7–10) to separate the complexes which were detected by monitoring the UV absorbance at 254 nm. It was shown that several of the mercury halides and mixed halides could be separated very rapidly as their crown ether complexes by this method.

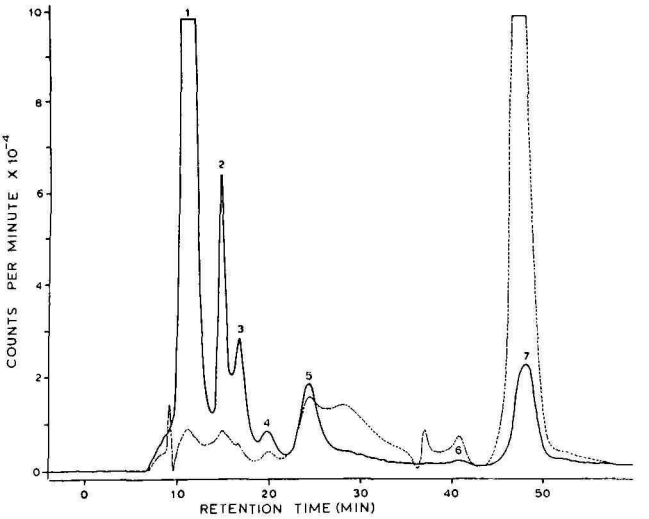


Fig. 8. HPLC of 3.3 m*M* Tc($NaBH_4$)-HEDP spiked with ^{99m}Tc and prepared in air (flow-rate, 0.156 ml/min; temperature, ambient). Solid line, γ detection at 140 keV (50-keV window); broken line, visible detection at 405 nm (0.1 a.u.f.s.). Reprinted with permission from ref. 62 (American Chemical Society).

3.1.10. Other ligands

Hausler and Taylor have shown[12] that complexes such as bis(tetrapyrazolylborate)iron(II) and bis(*o*-aminobenzaldehyde)ethylenediimine copper(II) can be separated from a series of metal–organic complexes by size-exclusion HPLC. The main objective of this work was to demonstrate the application of HPLC to the determination of organically bound metals in coal-derived materials. Metal-specific detection was employed.

The capacity factors of various bis(tetraazadiene)nickel complexes were measured by Gast and Kraak[21] on silica, CN-bonded and ODS-bonded stationary phases with THF–*n*-heptane mixtures. In the reversed-phase mode, the order of elution was exactly reversed from that observed in the normal-phase mode.

3.2. Multi-element separations of coordination complexes

3.2.1. β-Diketonates

The first HPLC separation and determination of metal coordination complexes was reported in 1972 by Huber *et al.*[65]. They showed that liquid–liquid partition HPLC with a ternary two-phase system consisting of water–2,2,4-trimethylpentane–ethanol could be used for the isocratic separation of several metal acetylacetonates. The water-rich (polar) phase served as the stationary liquid and was supported on silica particles (5–10 and 10–20 μm). The hydrocarbon-rich (non-polar) phase served as the eluent. The column effluent was monitored at 310 nm. It was shown that six metal acetylacetonates, Be(II), Cu(II), Al(III), Cr(III), Ru(III) and Co(III), could be separated and determined in 25 min. Three of the complexes, Ni(II), Al(III) and Fe(III), produced asymmetric peaks due to hydrolytic reactions of the complexes with the water-rich stationary phase. The Al(III) acetylacetonate was

found to undergo hydrolysis in the stationary phase producing several hydroxoacetylacetonatoaluminum(III) species which could be separated on the column. This initial paper on HPLC of metal coordination complexes demonstrated the utility of the technique for separating such compounds and for studying their dissociation equilibria.

Size-exclusion HPLC has found very limited application for the study of metal complexes; however, several reports dealing with this mode of separating metal acetylacetonates are of interest and are included in this review. Yamamoto *et al.*[66] studied the elution of metal acetylacetonates by use of size-exclusion chromatography. They used a 10 Å pore size styrene–divinylbenzene copolymer gel with a variety of organic solvents (carbon tetrachloride, chloroform, benzene, toluene, and *p*-xylene) as the isocratic mobile phase. Differential refractometry was employed for detection. They found (surprisingly) that, by a suitable choice of solvent, polystyrene gel columns can be used for the separation of chelates with very similar sizes such as cobalt(III) and chromium(III) acetylacetonates. They speculated that in certain solvents such as chloroform (known to form solvated complexes with metal acetylacetonates), it is the solvated species which are the effective entities being separated according to molecular size of the polystyrene gel. The chromatographic behavior of the Co(III), Fe(III), Cr(III), Al(III), Cu(II), Ni(II) and Be(II) acetylacetonates on size-exclusion Merckogel OR-2000 columns was studied by Saitoh and Suzuki[67]. They used THF as the mobile phase and refractometric detection. They reported the elution characteristics and distribution coefficients for the chelates comparing the data with several alkanes. In a later paper by Suzuki and Saitoh[68], the elution characteristics of Al(III), Cr(III), Fe(III), Co(III) and Be(II) acetylacetonates on the same gel with a variety of organic solvents (chloroform, benzene, toluene, 1,4-dioxane, ethyl acetate, butyl acetate, acetone, ethyl methyl ketone and methanol) were reported.

An extensive study of the elution behavior of a series of metal β-diketonates on alumina, silica gel, bonded-phases, and open-pore polyurethane HPLC columns has been carried out by Tollinche and Risby[69]. Metal β-diketonates which were studied included: (1) acetylacetonates (acac), $Cu(acac)_2$, $Co(acac)_2$, $Cr(acac)_3$, $Al(acac)_3$, $Be(acac)_2$, $Ru(acac)_3$, $Rh(acac)_3$, $Co(acac)_3$; (2) 2,2′,7,7′-tetramethyl 3,5-heptanedionates (thd), $Co(thd)_3$, Cr)thd)$_3$, $Fe(thd)_3$, $Cu(thd)_2$, NNi(thd)$_2$; (3) 1,1,1-trifluoro-2,4-pentanedionates (tfa), $Al(tfa)_3$, $Cr(tfa)_3$, $Co(tfa)_3$, $Ru(tfa)_3$, $Rh(tfa)_3$; and (4) 1,1′,1′,2,2′,3,3′-heptafluoro-4,6-octanedionates (fod), $Co(fod)_3$, $Cr(fod)_3$. It was found that the best separations were obtained on normal-phase silica gel columns with non-polar eluents and UV detection at 280 nm. A typical six-component separation of several metal acetylacetonates is shown in Fig. 9. The authors also studied a number of different mobile phases and found that several *cis trans* isomers of the metal β-diketonates could be separated in the normal-phase mode.

3.2.2. *β-Ketoamines*

Normal-phase HPLC (liquid–solid) was employed by Uden and Walters[70] for the isocratic separation of neutral copper and nickel chelates of N,N′-ethylenebis(acetylacetoneimine) [$H_2(enAA_2)$] and N,N′-ethylenebis(salicylaldimine) [$H_2(enSal_2)$] on 10-μm diameter silica with UV detection at 254 nm.

Reversed-phase and normal-phase HPLC separations of several β-ketoamine metal chelates were reported by Gaetani *et al.*[71]. Included in the study were several

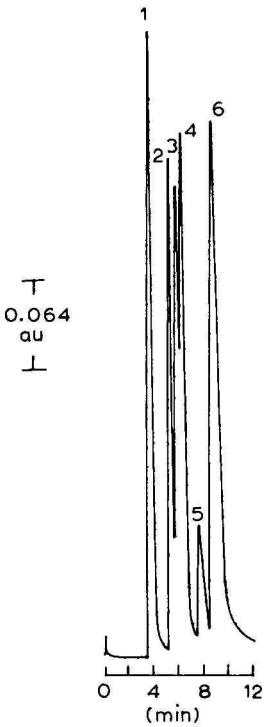


Fig. 9. HPLC separation of metal acetylacetonates on a 5-μm Partisil column (25 cm). Conditions: eluent, 1,2-dichloroethane–methanol (98.6:1.4); detector, UV at 280 nm. Components: (1) $Be(acac)_2$; (2) $Ru(acac)_3$; (3) $Rh(acac)_3$; (4) $Cr(acac)_3$; (5) $Al(acac)_3$; (6) $Co(acac)_3$. Reprinted with permission from ref. 69 (Preston Publications).

chelates of $H_2(enAA_2)$, N,N′-trimethylenebis(acetylacetoneimine) $[H_2(tmAA_2)]$ and N,N′-ethylenebis(benzoylacetoneimine) $[H_2(enBA_2)]$ with Co(II), Ni(II), Cu(II) and Pd(II). Rapid separations of $CoenAA_2$, $NienAA_2$ and $CuenAA_2$ (< 5 min) were obtained on a reversed-phase C_{18} column with methanol–phosphate buffer (pH 7.8). Also, $Ni(en)BA_2$ and $Cu(en)BA_2$ were successfully separated on a polar (normal-phase)–NH_2 bonded-phase column using a methanol–phosphate buffer. Detection limits were in the sub-nanogram range for nickel and copper.

3.2.3. Hydrazones and semicarbazones

In 1973 Heizmann and Ballschmiter[72] reported the first successful liquid–solid HPLC separations of mercury(II), copper(II), lead(II) and zinc(II) chelates of bis-acetylbisthiobenzoylhydrazone. They used Merckosorb SI 60, Perisorb A and alumina as the column packing and reported satisfactory correlation with TLC data and limits of detection in the nanogram range for Hg(II) and Cu(II). This paper represents one of the important early breakthroughs in the field of HPLC of metal chelate systems.

In a later paper, Heizmann and Ballschmiter[73] demonstrated the possibilities of separating the 1,2-diketobisthiosemicarbazone and 1,2-diketobisthiobenzhydrazone chelates of Cd(II), Hg(II), Cu(II), Ni(II) and Co(III) isocratically on silica as

the adsorbent. Mobile phases which were studied consisted mostly of binary mixtures containing benzene, *n*-hexane, chloroform, acetonitrile, cyclohexane and *n*-heptane. Detection was performed by monitoring the UV absorbance at 360 nm. It was shown that the elution characteristics of the chelates were influenced by varying the substituents on the ligand molecules. The metal chelates could be detected at the nanogram level.

3.2.4. Dithiocarbamates

Uden and Bigley[74] employed the liquid–solid adsorption mode for the isocratic separation of Cu(II), Ni(II) and Co(III) diethyldithiocarbamates on 8-μm spherical silica particles with an eluent consisting of 5% acetonitrile, 15% diethyl ether and 80% Skelly B (light petroleum hydrocarbon). Detection of the chelates was accomplished by means of UV absorption at 254 nm; this method was supplemented by using a metal-specific argon-plasma detector in order to demonstrate that the observed peaks corresponded to the metal chelates and not decomposition products. A detailed description of the construction and evaluation of this metal-specific HPLC detection system has been published by Uden *et al.*[75]. This paper was the first report in which a d.c. argon plasma emission source (DCP) was used for the detection of metallic species after HPLC separation. The authors pointed out that the advantages of plasma sources are that they generally exhibit superior sensitivities (sub-nanogram levels) and detection limits compared to flame techniques and that they are also suitable for multi-element determinations. Model compounds which were used to evaluate the DCP included β-diketonate, β-ketoamine and diethyldithiocarbamate complexes of Cu(II), Ni(II), Cr(III), Co(III) and Hg(II).

Heizmann and Ballschmiter, in their paper[73] dealing with the separation of semicarbazone and hydrazone metal complexes, also included a study of the separation of the dithiocarbamates of Cd(II), Hg(II), Cu(II), Ni(II) and Co(II) using normal-phase HPLC and UV detection at 360 nm.

In 1979, Liška *et al.*[76] described the normal-phase separation of mixtures of Zn(II)–, Cu(II)–, Mn(II)–, Ni(II)–, Pb(II)–, Co(II)–, Cd(II)– and Fe(II)–DTC complexes on LiChrosorb SI 60 (10 μm) with chloroform–hexane as the mobile phase and UV detection. They also studied the influence of the nature of the ligand N-substituents and mobile phase composition on the chromatographic properties of the Cu(II)–DTC complexes.

Schwedt[77] has reported a 12-min isocratic separation of Se–, Cu(II)–, Ni(II)– and Pb(II)–DTC complexes by reversed-phase HPLC using acetonitrile–water mixtures as the mobile phase and UV detection at 254 nm. He has also reported the separation of Se, Cr(III), Ni(II) and Co(II) chelates by reversed-phase HPLC[78]. Additionally, the possibilities of reversed-phase HPLC separation of the tetramethylenedithiocarbamates of Cd(II), Pb(II), Ni(II), Co(II), Zn(II), Cu(II) and Hg(II) on LiChrosorb RP-8 using methanol–water (70:30) and UV detection was studied[79]. This separation is shown in Fig. 10.

Edward-Inatimi and Dalziel[80] have reported a multi-element analysis scheme using solvent extraction and normal-phase HPLC on Hypersil with spectrograde benzene and UV detection at 280 nm. They separated mixtures of DTC complexes of Cu(II), Ni(II), Hg(II), Pb(II), Co(II), Mn(II) and Bi(III) and were able to determine these in the ppb range. They tested their procedure successfully using standard steels.

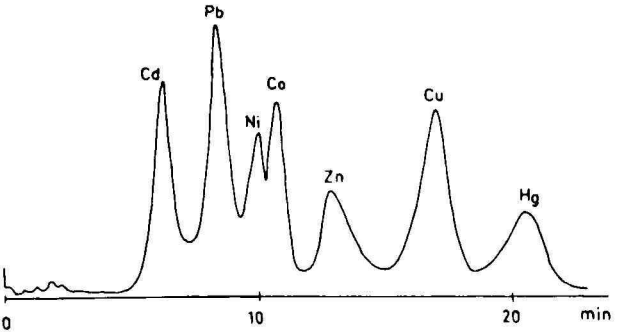


Fig. 10. Reversed-phase HPLC of metal tetramethylenedithiocarbamates. Column, 250 × 4.6 mm I.D., LiChrosorb RP-8 (10 μm); mobile phase, methanol–water (70:30); flow-rate, 1.2 ml/min; detector, 254 nm. Reprinted with permission from ref. 79 (Vieweg).

Reversed-phase HPLC with electrochemical detection has been used by Bond and Wallace[81] for a specific and very sensitive determination of copper as its diethyldithiocarbamate or its pyrrolidinedithiocarbamate complex [$Cu(DTC)_2$]. Two procedures are reported. In one of these, the copper is complexed prior to injection; the second method involves adding a dithiocarbamate salt to the moving phase causing complex formation *in situ* when the metal ion solution is injected. Cyclic voltammograms showed that these $Cu(DTC)_2$ chelates undergo reversible one-electron reduction and oxidation steps at platinum, gold and glassy carbon electrodes in an acetonitrile–water medium. It was found that electrochemical detection is extremely sensitive with a limit of detection of 1 ng of copper. A large number of anions and cations (in 20-fold excess) were tested as potential interferents. Several of the metal ions such as Cd(II), Pb(II), Co(III) and Fe(III) were found to produce HPLC peaks, but did not interfere with the determination. Applications to a wide range of industrial effluents were successful. The detection limit of the HPLC method is comparable to that of atomic absorption, but the HPLC procedure would be the preferred technique when only small volumes of samples are available.

3.2.5. *Dithizonates*

Dithizone (DZ) has been used extensively as an effective analytical solvent-extraction reagent in the past. Its advantages as a potential ligand for multi-element HPLC analysis include its ability to form neutral chelates with a large number of divalent metal ions as well as high chelate molar absorptivities (30,000–100,000 l/mol · cm) in the visible region of the spectrum (500–530 nm). This property permits the elution of DZ complexes from HPLC columns with UV-absorbing solvents.

Lohmüller *et al.*[82] reported the first successful separation of several groups of DZ chelates including those of Pb(II), Zn(II), Cd(II), Hg(II), Cu(II) and Co(II) using normal-phase HPLC on 30-μm silica particles with a series of organic moving phases (benzene, carbon tetrachloride, tetrahydrofuran, acetonitrile, toluene and chloroform). The absorbance at 525 nm was monitored. Several three- and four-component mixtures were successfully separated. A typical three-component separation of the mercury, nickel and cobalt chelates is shown in Fig. 11. An HPLC separation of several metal dithizonates, $M(DZ)_2$, was developed concurrently with the work of

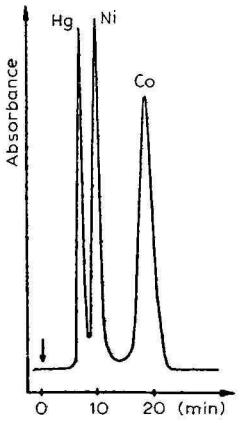


Fig. 11. HPLC separation of metal dithizonates. Packing, LiChrosorb SI 60 (30 μm); glass column, 300 × 2 mm; solvent, benzene; flow-rate, 10 ml/h. Reprinted with permission[82].

Lohmüller *et al.* and was reported in 1978 by O'Laughlin and O'Brien[83]. This study included an investigation of the behavior of Ni(II), Co(II), Cu(II), Zn(II), Hg(II) and Pb(II) dithizonates on 37–50-μm μ-Corasil and 10-μm μ-Porasil with a variety of mobile phases ranging in polarity from heptane to isopropanol. The best separations, however, were obtained on μ-Porasil with toluene as eluent and a variable-wavelength UV–visible spectrophotometer operated at 275 nm. Detection limits for the metals ranged from 10 to 100 ng.

Henderson *et al.*[84] have recently published a normal-phase HPLC separation of a number of divalent dithizonates using non-aromatic solvents and glass-lined columns with a variety of binary and ternary mobile phases containing acidic or basic modifiers such as acetic acid and various amines. Detection was accomplished by visible absorption at 475–525 nm. They also emphasize the advantages of DZ as a multi-element ligand for HPLC analysis, *i.e.* many divalent complexes have very high formation constants and very high molar absorptivities. HPLC separations of DZ complexes of Co(II), Cu(II), Pb(II), Zn(II) and Cd(II) are reported.

3.2.6. 8-Hydroxyquinolates

Several 8-hydroxyquinolates including those of Cu(II), Co(II), Ni(II), Hg(II) and Fe(II) were separated by Berthod *et al.*[85]. They used reversed-phase HPLC and three modes of detection: UV absorption, electrochemical and atomic absorption. This was the first reported HPLC separation of metal 8-hydroxyquinolates.

The 8-hydroxyquinoline chelates of Al(III) and Co(III) were recently separated by Hambali and Haddad[86]. They used a silica column and 5% methanol chloroform as the mobile phase with UV detection at 254 nm. A rapid separation was obtained for the two compounds in less than 5 min and detection limits were in the nanogram range for both metals.

It appears that 8-hydroxyquinoline would be an ideal ligand to use for the separations of multi-element mixtures by HPLC because of its ability to complex with many metal ions to produce neutral chelates. Furthermore, since several of these chelates possess native fluorescence, it is likely that the sensitivity could be improved considerably using this mode of detection.

3.2.7. 1,10-Phenanthrolines and ethylenediamines

Paired-ion HPLC was used successfully for the separation of the 1,10-phenanthroline (phen) chelates of Fe(II), Ni(II) and Ru(II) by O'Laughlin and Hanson[87]. They found that an excellent separation of $Ni(phen)_3^{2+}$ or $Ru(phen)_3^{2+}$ from $Fe(phen)_3^{2+}$ could be achieved on a μBondapak-CN column with methanol–(0.5%) aqueous acetic acid (20:80) containing 0.015 *M* methanesulfonate as the counter-ion. Detection was achieved by monitoring the UV absorption signal at 265 nm. Nickel was determined in the 0–30 ng range by monitoring the elution of $Ni(phen)_3^{2+}$ at 265 nm. The $Ru(phen)_3^{2+}$ and $Ni(phen)_3^{2+}$ species eluted with the same retention volume. Iron and nickel or iron and ruthenium can thus be determined simultaneously. The elution of $Ru(phen)_3^{2+}$ was confirmed with a fluorescence detector and that of $Ni(phen)_3^{2+}$ with atomic absorption. The effect of various ion-pairing reagents, pH and mobile phase composition on the retention of these complexes was studied. No elution peaks were observed for the labile phen complexes of Co(II), Zn(II) or Cd(II). In a later report by O'Laughlin[88], the three complexes $Ni(phen)_3^{2+}$, $Ru(phen)_3^{2+}$, and $Fe(phen)_3^{2+}$ were separated from one another as well as from the ligand and from a bis-$Ru(phen)_2X^{2+}$ chelate. This separation is shown in Fig. 12 and was accomplished by paired-ion HPLC on Partisil SCX with acetonitrile–water–perchloric acid mixtures as the mobile phase, and detection at 265 nm.

Cation-exchange chromatography was employed by Yoneda *et al.*[89] who separated two pairs of tri- and divalent ethylenediamine (en) and phen chelates:

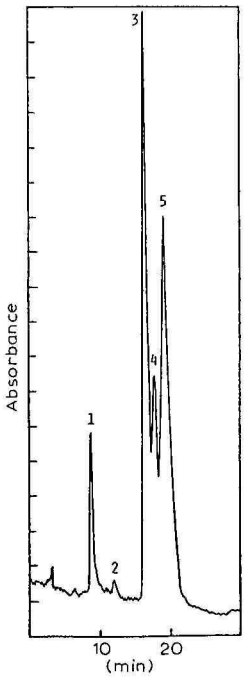


Fig. 12. HPLC separation of $Ni(phen)_3^{2+}$, $Ru(phen)_3^{2+}$ and $Fe(phen)_3^{2+}$. Column: μPartisil-SCX; mobile phase: acetonitrile–water (80:20)–0.06 *M* $HClO_4$; detector: UV at 265 nm. Components: (1) phen; (2) $Ru(phen)_2X^{2+}$; (3) $Ni(phen)_3^{2+}$; (4) $Ru(phen)_3^{2+}$; (5) $Fe(phen)_3^{2+}$. Reprinted with permission[88].

$[Co(en)_3]^{3+}$–$[Ni(en)_3]^{2+}$ and $[Co(phen)_3]^{3+}$ $[Fe(phen)_3]^{2+}$. The separation was achieved on an SP-Sephadex cation-exchange column using various concentrations of aqueous potassium bromide and potassium sulfate solution as eluents. The absorbance of the various complexes was monitored at appropriate visible wavelengths. The chromatographic behavior and order of elution of these complexes as a function of ionic strength was also studied. Yoneda[90] has discussed the stereochemical aspects of optical resolution of several octahedral ethylenediamine, diethylenetriamine, triethylenetetramine and amino acid metal chelates of Co(III) and Cr(III) on ion-exchange resins.

3.2.8. Porphyrins

A report published recently by Richter and Rienits[91] deals with the HPLC separation of zinc and magnesium chelates of protoporphyrin IX as the dimethyl ester derivatives. The complexes were eluted on 10 μm LiChrosorb with acetone–hexane (15:85) with detection at 405 nm. This determination was successfully applied to the examination of the protoporphyrin IX chelates arising from the incubation of cucumber etiochloroplast and wheat etioplast.

4. CONCLUSION

HPLC was first applied to metal–organic systems in 1969; approximately 50 papers on the subject appeared in the 9 years since then. This number has approximately doubled in the past 3 years, a fact which indicates that applications of HPLC in organometallic and coordination chemistry are being developed rapidly. This trend may be expected to continue.

HPLC has been shown to be an effective means for the separation and determination of many different types of coordination complexes and organometallic compounds. One area which holds a great deal of potential is the use of HPLC for multi-element determinations by employing a single ligand to complex mixtures of dissolved metal ions prior to HPLC separation. The advantage of such an approach is the possibility of achieving multi-element determinations in a single run. It should be pointed out, however, that chromatographic adjustment of selectivity for the elution of metal chelates is characterized by narrower limits in reversed- or normal-phase HPLC than would be possible in ion-exchange separations of the free metal ions or their halo-complexes. Also, the development of HPLC element specific detectors such as ICP and others based on atomic absorption and electrochemical properties has made available sensitive and selective methods for the detection and determination of trace amounts of metals.

Among the exciting developments in organometallic chemistry in recent years has been the use of HPLC to monitor the course of reactions, identify intermediates and determine reaction rates and the composition of equilibrium mixtures. The separation of isomeric complexes is often possible by HPLC when all other methods fail. The most significant advantages of HPLC for the study of inorganic systems are that the method is capable of excellent resolution and low level detection of highly non-volatile and thermally unstable systems. It is principally because of these reasons that we expect to see much greater use being made of this technique in inorganic chemistry as more chemists become aware of its possibilities.

5. SUMMARY

A review of the applications of high-performance liquid chromatography to organometallic compounds and metal coordination complexes since 1969 is presented. The review covers high-performance liquid chromatographic separations which have been reported for arene metal carbonyls, cyclopentadienyl metal carbonyls, dicyclopentadienyl complexes, metal cluster complexes, metallocarboranes, cyclopentadienyl–cycloolefin complexes, alkyl and aryl complexes and organopolymetallic compounds. The second part of the review summarizes separations of metal coordination compounds, including complexes of the following ligand types: β-diketonates, β-ketoamines, hydrazones, semicarbazones, dithiocarbamates, dithizonates, bipyridines, phenanthrolines, 8-hydroxyquinolates, EDTA, ethylenediamines, amino acids, carbonates, camphorates, diphosphonates, crown ethers and porphyrins.

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can only be fully exploited with a complete system of instruments for sample application, chromatographic development and evaluation

Here is a complete system for high performance TLC with linear chromatogram development

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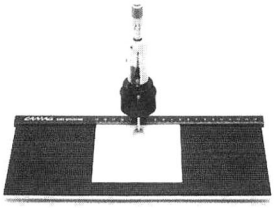
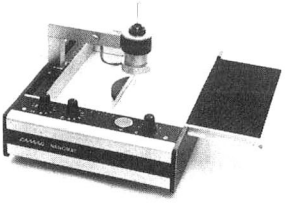


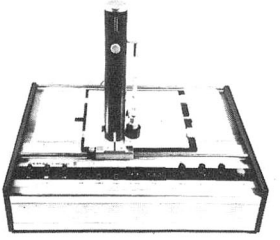
CAMAG Nano-Applicator
variable sample volumes 50–230 nl (spotwise), reproducibility 1%

or



CAMAG Nanomat
fixed sample volumes 100 or 200 nl with nanopipette, or larger volumes using the automatic repetition device

or



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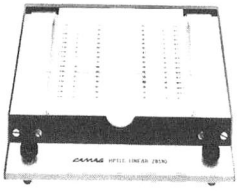
applies dilute sample solutions (2–100 µl) in form of a narrow band; reproducibility 1%

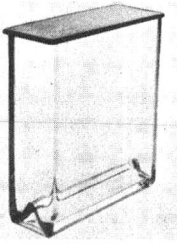
2. Chromatogram Development



CAMAG Twin-Trough Chamber ▶
versatile, economical in solvent consumption

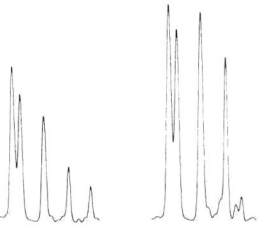
or

◀ **CAMAG HPTLC Linear Developing Chamber**
up to 32 samples can be separated on one 10 x 10 cm HPTLC-plate or 72 samples on a 20 x 10 cm plate



3. Quantitative Evaluation

CAMAG TLC/HPTLC Scanner
Available in two options:
economy-priced filter version or monochromator equipped.
Scanning modes: absorbance, fluorescence, fluorescence quenching.
Automatic scanning of all tracks of a chromatogram, or of the selected chromatogram segment.



If you are planning to set up a new TLC laboratory, or to complement your existing TLC equipment, ask the CAMAG specialist. He will help.

Ask for full information on COMPLETE CAMAG SYSTEMS for circular HPTLC and for conventional TLC also.

CAMAG

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CAMAG has agents in almost all countries