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Supercritical Fluid Chromatography

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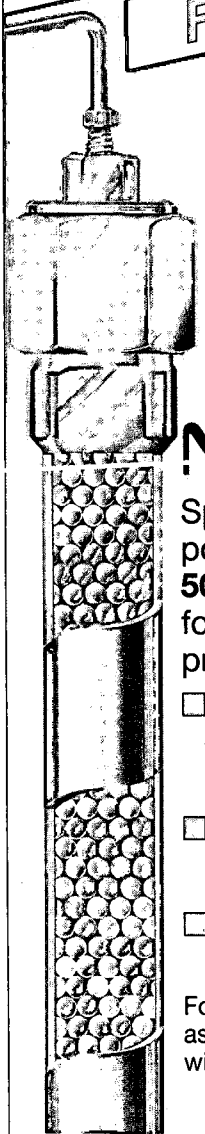

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An authoritative review... highly recommended...

Optimization of Chromatographic Selectivity

A Guide to Method Development

by **P. Schoenmakers,** *Philips Research Laboratories, Eindhoven, The Netherlands*

(Journal of Chromatography Library, 35)

"The contents of this book have been put together with great expertise and care, and represent an authoritative review of this very timely topic... highly recommended to practising analytical chemists and to advanced students." (Jnl. of Chromatography)

"...an important contribution by a worker who has been in the field almost from its inception and who understands that field as well as anyone. If one is serious about method development, particularly for HPLC, this book will well reward a careful reading and will continue to be useful for reference purposes." (Mag. of Liquid & Gas Chromatography)

This is the first detailed description of method development in chromatography - the overall process of which may be summarized as: method selection, phase selection, selectivity optimization, and system optimization. All four aspects receive attention in this eminently readable book.

The first chapter describes chromatographic theory and nomenclature and outlines the method development process. Guidelines are then given for method selection and quantitative concepts for characterizing and classifying chromatographic phases. Selective separation methods (from both GC and LC) are given - the main parameters of each method are identified and simple, quantitative relations are sought to describe their effects. Criteria by which to judge the quality of separation are discussed with clear recommendations for different situations. The specific problems involved in the optimization of chromatographic selectivity are explained. Optimization procedures, illustrated by examples, are described and compared on the basis of a number of criteria. Suggestions are made both for the application of different procedures and for further research. The optimization of programmed analysis receives special attention, and the last chapter summarizes the optimization of the chromatographic system, including the optimization of the efficiency, sensitivity and instrumentation.

Those developing chromatographic methods or wishing to improve existing methods will value the detailed, structured way in which the subject is presented. Because optimization procedures and criteria are described as elements of a complete optimization package, the book will help the reader to understand, evaluate and select current and future commercial systems.

Contents: 1. Introduction. 2. Selection of Methods. 3. Parameters Affecting Selectivity. 4. Optimization Criteria. 5. Optimization Procedures. 6. Programmed Analysis. 7. System Optimization. Indexes.

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Proceedings of the Sixth International Conference, Torino, Italy, 15-18 September 1987

edited by **L. Pawlowski,** *Technical University of Lublin, Poland,* **E. Mentasti,** *University of Torino, Italy,* **W.J. Lacy,** *Alexandria, VA, USA,* **C. Sarzanini,** *University of Torino, Italy*

(Studies in Environmental Science, 34)

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AQUEOUS SIZE-EXCLUSION CHROMATOGRAPHY

edited by **P.L. DUBIN,** *Indiana-Purdue University*

(Journal of Chromatography Library, 40)

The rapid development of new packings for aqueous size-exclusion chromatography has revolutionized this field. High resolution non-adsorptive columns now make possible the efficient separation of proteins and the rapid and precise determination of the molecular weight distribution of synthetic polymers. This technology is also being applied to the separation of small ions, the characterization of associating systems, and the measurement of branching. At the same time, fundamental studies are elucidating the mechanisms of the various chromatographic processes.

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- High-performance packings based on derivatized silica are reviewed in detail.
- Special techniques are thoroughly described, including SEC/LALLS, inverse exclusion chromatography, and frontal zone chromatography.
- Attention is focussed on special applications of size-exclusion methods, such as the characterization of micelles, separations of inorganic ions, and Hummel-Dreyer and related methods for equilibrium systems.
- Protein chromatography is dealt with in both dedicated sections and throughout the book as a whole.

This is a particularly comprehensive and authoritative work - all the contributions review broad topics of general significance and the authors are of high repute.

The material will be of special value for the characterization of synthetic water-soluble polymers, especially polyelectrolytes. Biochemists will find fundamental and practical guidance on protein separations. Researchers confronted with solutes that exhibit complex chromatographic behavior, such as humic acids, aggregating proteins, and micelles should find the contents of this volume illuminating.

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Y. Michotte *and* **L. Kaufman,** *Vrije Universiteit Brussel, Belgium*

(Data Handling in Science and Technology, 2)

Most chemists, whether they are biochemists, organic, analytical, pharmaceutical or clinical chemists and many pharmacists and biologists need to perform chemical analyses. Consequently, they are not only confronted with carrying out the actual analysis, but also with problems such as method selection, experimental design, optimization, calibration, data acquisition and handling, and statistics in order to obtain maximum relevant chemical information. In other words: they are confronted with chemometrics.

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SPECIAL ISSUE

SUPERCRITICAL FLUID CHROMATOGRAPHY

Guest Editor

PETER J. SCHOENMAKERS
(Eindhoven, The Netherlands)

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SUPERCRITICAL FLUID CHROMATOGRAPHY

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Journal of Chromatography, 505 (1990) 1
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

PREFACE

This special issue of the *Journal of Chromatography* contains a number of reviews and original papers in the field of supercritical fluid chromatography (SFC). Special volumes covering a specific field within chromatography are a relatively new departure for the journal. This formula of combining reviews on key aspects of a technique with some of the latest developments will provide the opportunity for both experienced SFC users and other chromatographers to augment their knowledge of the technique.

SFC is not a new technique. It was invented in the early 1960s and in that decade a number of studies were conducted. Much of this work is still highly relevant today. In the following decade, the development of SFC virtually came to a standstill. A few researchers barely managed to keep the flow going. The 1980s saw a revival of SFC, sparked by a number of significant breaktroughs:

(i) the introduction of open-tubular columns;

(ii) the use of columns packed with very small particles ($d_p \leqslant 10$ μm);

(iii) modification of the flame ionization detector for routine use in SFC; and

(iv) the coupling of SFC to solute identification methods, such as mass spectrometry (MS) and infrared spectrometry.

In the last 10 years the *technology* required for SFC has been developed, but further progress remains to be made. With reliable commercial instrumentation now available from a number of manufacturers, it may be expected that the next decade will give rise to a significant increase in the number of *applications* of SFC. The contributions to this volume illustrate that both the technology and applications of SFC are being actively researched at the moment.

The general advantages of SFC over gas chromatography (*i.e.*, lower operating temperatures) and liquid chromatography (*i.e.*, potentially faster and/or more efficient separations and better compatibility with certain detectors) have been amply demonstrated. In the next decade, I would expect more specific advantages of SFC to be more thoroughly explored. These include the safety aspects of solvents (carbon dioxide being highly attractive in this respect), the potential high purity of supercritical fluids (including the virtual absence of particulate contamination) and the relative ease of removal of supercritical solvents. The last two advantages are relevant for SFC–MS, for supercritical fluid extractions (possibly followed by chromatography), for multi-dimensional chromatography and for preparative chromatography. Most of these areas are still largely unexplored, but they receive considerable attention in this volume.

This has been my first experience as a Guest Editor of the *Journal of Chromatography*. It has been a pleasurable experience to work with the staff of the journal, but most of all it has been a learning exercise. What I have learned most of all is to appreciate the efforts of manuscript reviewers. Their task is difficult, anonymous, unrewarding and yet invaluable. I am truly grateful to the many expert chromatographers who assisted in the reviewing process for this volume.

Eindhoven (The Netherlands) PETER SCHOENMAKERS

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Review

Packed columns in supercritical fluid chromatography

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CONTENTS

1. INTRODUCTION

In recent years, the developments in supercritical fluid chromatography (SFC) have tended to be in capillary SFC, especially with respect to the equipment. The aim has been to combine the high efficiency of gas chromatography (GC) with the solvation power of liquid chromatography. However, the high diffusion coefficients of gases can only be approached when the temperature is relatively high and the density is low. The solvation power, on the other hand, increases with increasing density, which is achievable at low temperatures.

A comparison of the efficiencies obtainable with packed and open-tubular columns shows that the number of theoretical plates per unit length is approximately identical when the inner diameter of the open tubes is as large as the particle size in the packed columns[1]. However, as the inner diameter of capillaries is usually at least 50 μm and the particle size in packed columns is usually in the range 3–10 μm, the number of theoretical plates per unit length is higher in packed columns.

Large differences exist in the permeability. The permeability of a column influences the pressure drop over the column. With $p \approx 32/d^2$ for capillaries and $p \approx 1000/d_p^2$ for packed columns, where d is the inner diameter of the capillary and d_p is the particle size in packed columns, the pressure drop for packed columns is 30 times higher than for capillaries. Consequently, for a given pressure drop, a capillary can be longer, resulting in an increase in the number of theoretical plates achievable.

The use of capillaries is favoured when many theoretical plates are necessary for separating a complex mixture. Also, it is convenient to use capillary columns for the separation of polar compounds. The phase ratio, V_s/V_m, is larger for packed columns

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owing to the higher specific surface area. A small V_s results in a low sample capacity and in a short analysis time. Applying the technology of GC, very good deactivated capillary columns can be produced. Consequently, disturbing silanol–sample interactions can be ignored.

However, working with packed columns also has advantages. In many instances short columns (10–30 cm) have sufficient numbers of theoretical plates. A separation can be achieved in a short analysis time. Compared with high-performance liquid chromatography (HPLC), the minimum in the *H vs. u* plot is shifted towards higher linear velocities, as can be seen in Fig. 1. Owing to the higher phase ratio, a higher sample capacity is achievable, which makes a transfer to semi-preparative sample amounts relatively easy.

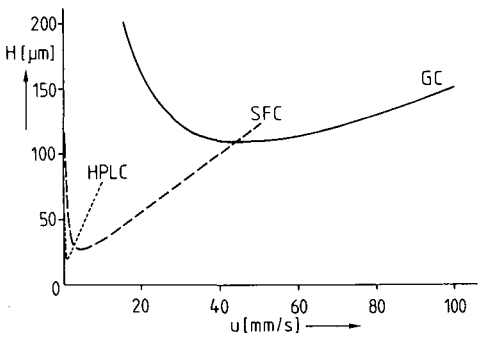

Fig. 1. *H vs. u* curves for packed columns used in HPLC, SFC and GC. $d_p = 10$ µm. Mobile phase: HPLC, methanol; SFC, CO_2; GC, N_2. Sample: HPLC, benzene; SFC and GC, methane.

2. INSTRUMENTATION

Using the same column dimensions in SFC as in HPLC, the flow-rates of the supercritical fluid are relatively high. Consequently, most of the components in packed-column SFC equipment are derived from HPLC.

The eluent is cooled and pumped as a liquid by a common HPLC reciprocating pump. For sample introduction, HPLC injection valves are usually used. The sample is dissolved in a solvent prior to injection. Because the sample has to be resolved again by the supercritical eluent, there might be a discrimination of samples with high molecular weight or high polarity during the solvating process. Owing to the large amount of solvent introduced, the phase equilibrium is disturbed, resulting in system peaks.

A sample introduction method has been described in which the sample is supercritically extracted and subsequently, while still in the supercritical state, injected onto the column[2]. In this instance the above problems can be neglected, but other problems arise concerning the handling of the extraction system. For each extraction, *i.e.*, for each injection, the whole system has to be cleaned properly.

An important unit for in packed-column SFC is the controlling system for pressure and flow. The supercritical pressure can be attained only when a restrictor is

at the end of the system, and this restrictor may have different functions and forms. One kind, a straight piece of small-bore fused-silica tubing, is taken from capillary SFC. Depending on the inner diameter and the length of this capillary restrictor, a different amount of mobile phase passes through. A capillary restrictor is advantageous when only small flow-rates are to be handled, *i.e.*, when packed microcolumns are used[3,4]. Using a capillary restrictor, the flow-rate cannot be kept constant during a pressure or density programme. Usually a flow programme is superimposed on the density programme.

When working with columns with greater dimensions, the volume flow-rate is much higher, which allows the use of manually or electrically adjustable valves. Such a valve is able to control the pressure independently of the flow-rate[5,6].

For density programming, regulation of the valve is required. Several methods for achieving reproducible density or pressure programmes have been described[2,7,8]. Owing to the high flow-rates, pressure- and flow-regulation circuits can be separated, making it possible to keep the linear velocity constant during a pressure programme.

3. DETECTION

In SFC, two groups of detectors can be distinguished. With GC-like detectors the detection takes place under ambient pressure conditions, *i.e.*, subcritical. Prior to detection the system has to be expanded. The other group consists of HPLC-like detectors. Here detection occurs under pressure, *i.e.*, under supercritical conditions. Owing to the high flow-rates in packed-column SFC, UV detectors equipped with a high-pressure flow cell are generally used. The detection occurs under supercritical conditions. The UV detector is a selective detector, responding only to UV-active components.

The most often used GC detector is the flame ionization detector, because of its sensitivity and universality. As it is a flame-based detector, decompression of the supercritical eluent to ambient pressure has to occur before detection. If the expansion is carried out along a capillary (linear restrictor), condensation can occur, because the fluid loses its solvation power during decompression. Therefore, preferred restrictors are either a frit restrictor or an orifice, allowing the expansion to take place along a very short path length.

Using a flame-based detector, the variety of possible mobile phases is limited to non-flammable fluids, such as carbon dioxide or sulphur hexafluoride. Employing commercially available detectors, the amount of column effluent has to be reduced in order to prevent the flame from being extinguished. Separations have to be carried out on microcolumns in this way.

Detectors specific for packed-column SFC have also been used, *e.g.*, light-scattering detection[9], photo-ionization detection[10,11] and combined methods involving mass spectrometry (MS)[12–14] and IR spectrometry[15] but these will not be described in this review.

4. MOBILE PHASE

Supercritical fluids are able to dissolve compounds and in addition to their structural properties, physical parameters such as pressure and temperature are

responsible for differences in solvation behaviour. Consequently, the chromatographic properties can easily be changed.

The main parameter affecting the solvation power of the mobile phase is the density. Increasing the density leads to an increase in solvation power and thus a decrease in retention. At constant temperature, the solvation power increases with increasing pressure. This effect has been taken advantage of in pressure programming ever since the beginning of SFC[77–79].

Temperature variation can also be used to adjust chromatographic properties. At a constant pressure, the volatility of compounds increases with increasing temperature, but the mobile-phase density decreases. The solubility reaches a maximum which is specific for each compound, depending mainly on its vapour pressure[24,80]. Consequently, the temperature dependence can be used in two ways: negative temperature gradients increase the density of the mobile phase, and together with pressure programming the solvation power of the eluent is increased[81].

For relatively volatile solutes or at relatively high temperatures, the vapour pressure has a strong effect on chromatographic properties. Increasing the temperature during an analysis then results in a faster and more efficient separation[67,82].

To find a suitable mobile phase for a given separation problem, structural and transport properties of the eluent should be considered. So far, no theoretical study considering possible mobile phases has been carried out, but the number of experimentally examined supercritical fluids has grown. Thoroughly examined are the lower alkanes, diethyl ether, carbon dioxide, xenon, sulphur hexafluoride, fluorchloro hydrocarbons and methanol. Methanol is of special interest, because it is the only polar mobile phase. The investigations by Takeuchi *et al.*[22] are based on samples which are not very polar. Consequently, no clear statement about the applicability of methanol for polar solutes could be made.

Leyendecker *et al.*[18] made a systematic study of the differences in the solvation behaviour of various mobile phases. As a basis for an empirical correlation, the retention and resolution of different polyaromatic hydrocarbons were determined. Comparisons were carried out at equal pressures, equal reduced pressures and equal capacity ratios. At equal pressures, the solvation power increased in the order $C_2H_6 < CO_2 < N_2O < CHF_3$. Under the same conditions the resolution increased in the order $CO_2 < N_2O < CHF_3 \approx C_2H_6$. As shown in Fig. 2, trifluoromethane combines high resolution with low capacity factors.

In addition to theoretical considerations, practical aspects affect the choice of a particular mobile phase. Easy handling, little or no toxicity, no spontaneous decomposition and high purity, combined with cheapness, favour the use of lower alkanes and carbon dioxide.

Another aspect of selecting a mobile phase is the detection method employed. Using a UV detector, any of the fluids mentioned above can be applied. A restriction to non-flammable fluids is implied by using a flame ionization detector, *i.e.*, only CO_2, SF_6, N_2O and Xe can be applied. SF_6 has been reconsidered recently. Schwartz and Brownlee[63] described a method for group separations in a gasoline sample. Hellegeth *et al.*[21] compared SF_6 and CO_2, and found that SF_6 is a weaker eluent than CO_2 and limited towards polar compounds, eluting only monofunctional species. Xe seems to be the mobile phase of choice when using IR detection. The noble gas has no absorption within the common range of spectroscopy, making the use of a flow cell

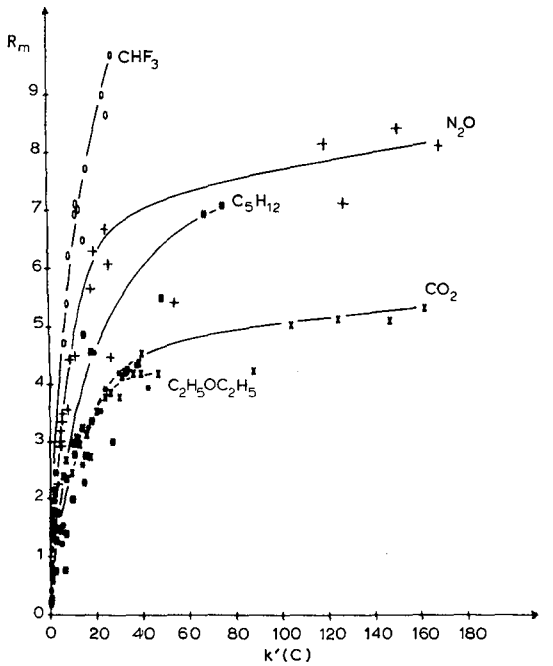

Fig. 2. Dependence of the resolution, R_m, between naphthalene, anthracene, pyrene and chrysene on k' (C) in different mobile phases at different temperatures and pressures. Column: 250 × 4.6 mm I.D., unmodified silica, 10 μm. (Redrawn with permission from ref. 18.)

relatively easy[23]. Because of its high price, Xe can only be used for miniaturized systems. For SFC–MS preferably those fluids are chosen which are gaseous under ambient conditions. In this instance a vacuum can be employed without the simultaneous vaporization of the analyte[12–15].

As can be deduced from the application examples in Tables 1, 3, 4 and 5, CO_2 is the most commonly employed fluid phase. In the following, the use of CO_2 as eluent will be described.

Early extraction studies showed that CO_2 is a non-polar solvent[24]. Non-polar components of low molecular weight (up to 400 g/mol) dissolve well. The solubility decreases as the polarity or molecular weight increases. In Table 2 the solubilities of various substances in liquid CO_2 are listed. The low solvation power of CO_2 for polar substances causes problems, because most real samples contain compounds with polar functional groups.

To increase the polarity of the mobile phase, polar modifiers can be added to the supercritical fluid. As shown in Table 3, several investigations concerning the influence of different modifiers have been carried out. As an example, the effects of different modifiers on the capacity factors of hexachlorobenzene and 4-nitroaniline are illustrated in Fig. 3. The effect of modifiers depends strongly on the structure of the compound under examination. Generally, a modifier affects the solubility of polar compounds in the fluid[25–27] and also the activity of the stationary phase by blocking the strongest adsorption sites[26–29]. Apart from modification with a polar solvent, very

TABLE 1

PUBLICATIONS DEALING WITH STATIONARY PHASES

Column	*Mobile phase*	*Application*	*Detection*[a]	*Ref.*
Zorbax-ODS LiChrosorb Si 60 silica Aminopropyl-LiChrosorb Pirkle-type: DNBPG[b]	CO_2 CO_2 with different modifiers	Phosphine oxides	UV	48
Porous glass Silica gel	Methanol Diethyl ether	Oligomers of styrene and methylphenylsiloxanes	UV	42
Cyanopropyl Cyanopropyl-polysiloxane	CO_2	Basic N-containing compounds	FID	39
ODS with different pore diameters and C content	CO_2	Polystyrene oligomers	UV	49
YMC-Gel PVA-Sil YMC-Gel Phenyl YMC-Gel Silica Nucleosil Cyano Deltabond Methyl Deltabond Octyl Deltabond Cyano	CO_2 CO_2 and water	Separation of free fatty acids	FID	28

[a] FID = Flame ionization detection.
[b] DNBPG = (*R*)-N-(3,5-Dinitrobenzoyl)-phenylglycine.

specific substances have been added to the eluent. Steuer *et al.*[30] modified CO_2 with ion pairing agents and applied this versatile system to the separation of enantiomeric 1,2-amino alcohols as diastereomeric ion pairs. Berger *et al.*[31] added tetramethylammonium hydroxide (TMAOH) in methanol to CO_2 for the separation of PTH-amino acids. Because their gradient ended with 33% of methanol–TMAOH in the eluent, it can be concluded that they probably performed liquid chromatography towards the end of the separation.

TABLE 2

SOLUBILITIES OF VARIOUS COMPOUNDS IN LIQUID CARBON DIOXIDE[24]

Soluble	*Partly soluble*	*Solubility (wt.-%)*	*Insoluble*
Tin(II) chloride	Water	0.1	Urea
Benzene	Iodine	0.2	Glycine
Pyridine	Naphthalene	2.0	Phenylacetic acid
Acetic acid	Aniline	3.0	Succinic acid
Glycerol diacetate	*o*-Nitroanisole	2.0	Hydroxysuccinic acid
Ethanol	Lactic acid	0.5	Tartaric acid
Hexanol	Glycerol monoacetate	1.0	Dextrose
Benzaldehyde	Glycerol	0.05	Saccharose
Camphor	*n*-Decanol	1.0	
Limonene			

TABLE 3
PUBLICATIONS DEALING WITH MOBILE PHASES

Mobile phase[a]	*Column*	*Application*	*Detection*	*Ref.*
Dimethyl ether	LiChrosorb Si 60	Influence of T, P and flow-rate on the behaviour of dimethyl and diethyl ether	UV	19
CO_2 modified with $(C_1–C_{10})OH$, CH_2Cl_2, THF, n-C_6, dioxane, acetonitrile, ethers	CP-Spher C_{18} Nova-Pak C_{18}	Modifier effects	UV	26
CO_2 modified with C_1OH, C_3OH, THF, DMSO, SF_6, acetonitrile, methoxyethanol, Freon 11	Diol Octyl Octyl, end-capped	Modifier effects	UV	27
CO_2 modified with C_1OH, C_3OH, C_6OH, DMSO, THF, dimethylacetamide, methoxyethanol, dimethylacetamide, CH_2Cl_2, propylene carbonate, acetonitrile, dioxane	Diol, 5 μm Cyano Silica Diol, 10 μm ODS	Use of modifiers	UV	50
n-C_3, n-C_4, n-C_5 n-C_5 modified with 1,4-dioxane	Silica	Influence of T, P, density, type and composition of the mobile phase on k' and resolution	UV	17
n-C_5 CO_2	LiChrosorb Si 60	Comparison of n-C_5 and CO_2 as eluents based on the free volume	UV	51
n-C_5 modified with diethylene glycol dimethyl ether	LiChrosorb Si 100	Studies of binary eluents	UV	52
CO_2 modified with C_1OH, C_3OH, C_6OH, propylene carbonate, dimethylacetamide, THF, acetonitrile, DMSO, CH_2Cl_2	Diol	Solvatochromic polarity studies of modifiers	UV	29
CO_2 modified with 1,4-dioxane C_2OH modified with 1,4-dioxane	LiChrosorb Si 100	Eluent mixtures containing 1,4-dioxane	UV	53
CO_2, N_2O, $CHCl_3$, $CClF_3$ n-C_2, n-C_3, n-C_4, i-C_4, n-C_5, dimethyl ether, diethyl ether		Comparison of different eluents	UV	18
C_1OH Diethyl ether	Develosil-100-5 Develosil-ODS-5	Styrene oligomers, polysiloxane, non-ionic detergents	UV	22
CO_2 modified with acetonitrile solution containing a counter- and a competing ion	CS-GU cyano-bonded phase	Separation of enantiomeric 1,2-amino alcohols as diastereomeric ion pairs	UV	30
SF_6 CO_2	Cyano Phenyl Silica	Comparison of SF_6 and CO_2 Separation of aromatic hydrocarbons	UV	21
CO_2 modified with C_1OH, C_2OH, i-C_3OH, C_6OH, THF	Silica	Agricultural products	UV	54

[a] THF = Tetrahydrofuran; DMSO = dimethyl sulphoxide; $C_1OH–C_{10}OH$ = methanol to decanol; n-C_2–n-C_6 = ethane to hexane.

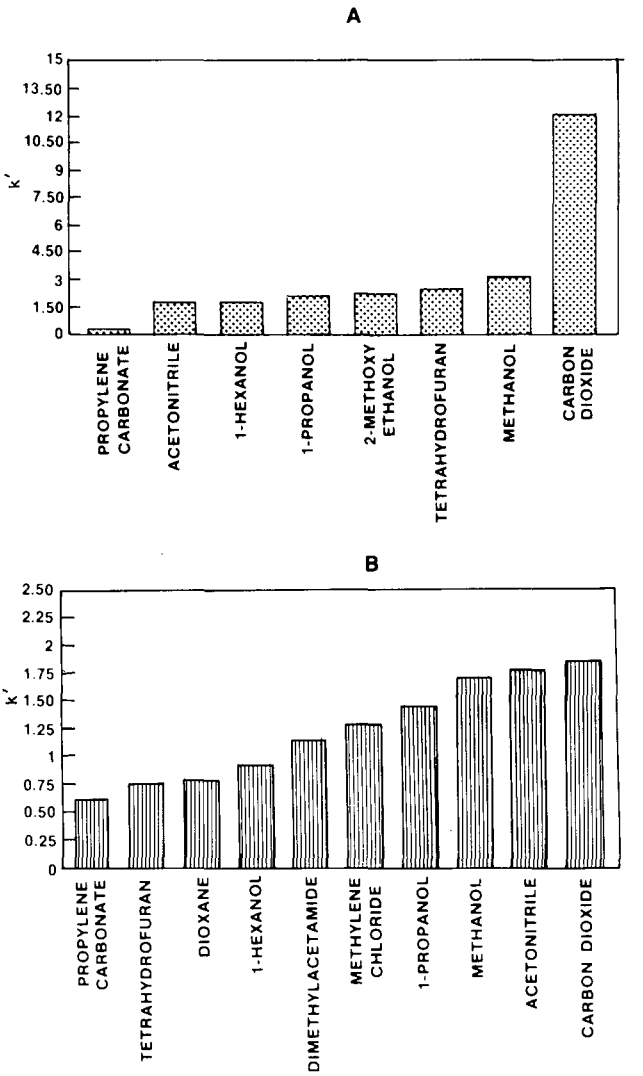

Fig. 3. Effects of 6 mol-% modifier in CO_2 on the capacity factor of (A) 4-nitroaniline and (B) hexachlorobenzene. Conditions: (A) column, 250 × 4.6 mm I.D., cyano, 5 μm, 4500 p.s.i., 40°C; (B) column, 200 × 4.6 mm I.D., ODS, 5 μm, 2800 p.s.i., 40°C. (Redrawn with permission of Preston Publications from ref. 50.)

5. STATIONARY PHASES

In SFC the density of the mobile phase is the parameter which most strongly affects the chromatographic properties. During chromatography the retention is commonly adjusted by a pressure or density programme.

The strong dependence of the capacity factor on density leads to an argument often cited against the use of packed columns in SFC. Packed columns have a lower

permeability than capillaries, resulting in a higher pressure drop over the column. Owing to the pressure drop and because supercritical fluids are compressible, a density gradient is created along the column. In chromatographic terms, this is a negative gradient, starting with a strong elution power at the column head and ending with a low solvation power at the column outlet. This problem has been discussed in various publications[33–36].

The density changes of supercritical fluids are a function of the pressure employed. Close to the critical point, the variation in density is much larger than when working at relatively high pressures. Hence it has been found that as soon as the pressure applied is at least 20% above the critical pressure, the density changes caused by the pressure drop do not affect the chromatographic results and packed columns can be used for SFC.

Owing to the high phase ratio in packed-column SFC, the interactions between the stationary phase and the sample are significant. Consequently, a way to change the properties of the system is to vary the stationary phase. The stationary phase can be selected from a wide variety of types with different selectivities that have been developed for HPLC. As can be seen in Fig. 4, the influence of the stationary phase is strong. Fig. 4 shows a separation that has been carried out on a silica and a reversed-phase column. The retention is much higher in the latter instance, but selectivity studies showed that the effect of the density on the retention is similar for both phases[36].

As soon as polar or basic samples have to be separated, one of the main problems in packed-column SFC arises, namely that basic and polar compounds elute as asymmetric peaks and often incompletely owing to strong interactions with the stationary phase. It is commonly accepted that these interactions derive from residual silanol groups on the silica surface.

Various efforts have been made to reduce the influence of unshielded silanols. Blilie and Greibrokk[26] found that polycyclic aromatic hydrocarbons elute better when an "end-capped" phase is employed, instead of a traditional reversed-phase column.

Owing to steric hindrance, in a usual silanization reaction only a fraction of the

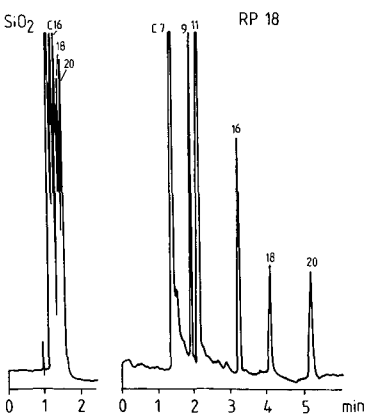

Fig. 4. Separation of *n*-alkanes on a silica and a reversed phase. Conditions: columns, 250 × 4.1 mm I.D., silica, 10 μm and 250 × 4.1 mm, RP-18, 10 μm; CO_2, 115 bar, 40°C. (Redrawn with permission from ref. 2.)

silanols are able to react, which led to a new way to modify the silica gel. Schomburg *et al.*[37], Engelhardt and Löw[38] and Ashraf-Khorassani *et al.*[39] tried to achieve complete coverage of the silanols by treating the silica with various oligomers and monomers. In a second step, these oligomers are immobilized on the surface by a polymerization. Owing to the coverage with polymer, the silanols should be inaccessible. Fig. 5 shows that the peak shapes of polar components are definitely improved in comparison with a conventional stationary phase.

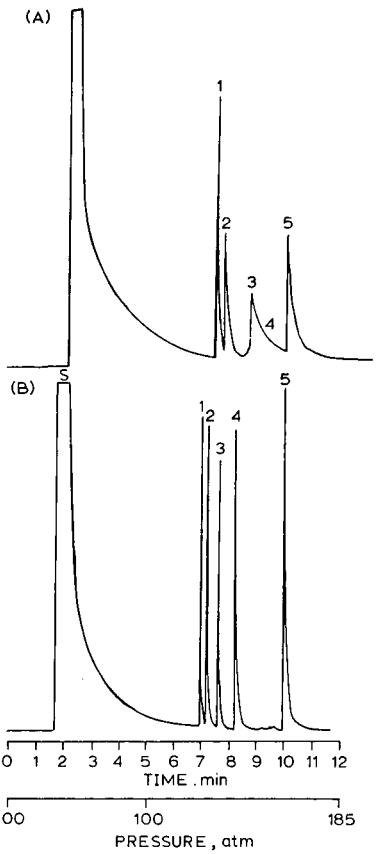

Fig. 5. Separation of a polarity test mixture on (A) a conventional cyanopropyl and (B) cyanopropyl Deltabond column. Conditions: CO_2, 60°C. 1 = *n*-Pentadecane; 2 = phenyl acetate; 3 = acetophenone; 4 = 2,6-dimethylaniline; 5 = phenol. (Redrawn with permission from ref. 39.)

In addition to attempts to cover silica surfaces completely, studies have been carried out employing different matrices. Investigations concerning the use of a copolymer of styrene and divinylbenzene showed that specific π–π interactions between the aromatic rings of the sample and stationary phase occur[40,57,68]. Solutes containing aromatic rings eluted with tailing. A high retention of all compounds is observed. Takeuchi *et al.*[42] compared porous glass beads with silica as a stationary phase. The low retention times achieved on the glass bead column were caused by their

lower surface area compared with the silica column. It was found that silanols determine the separation mechanism also when a glass bead column is employed. The retention behaviour of various compounds was comparable in both systems.

A different way to reduce the disturbing effects of silanols is to use modifiers. As mentioned before, polar modifiers increase the solvation power of the mobile phase. They also modify the stationary phase, the most active adsorption sites being blocked with the modifier molecules. In this way, the interactions between the stationary phase and the sample are reduced. As shown in Fig. 6, the peak shape improves and the retention time decreases with the use of modifiers.

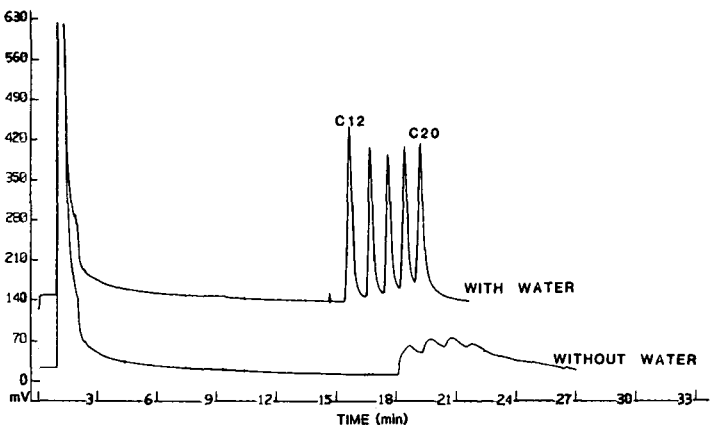

Fig. 6. Influence of water as a modifier on an SFC separation of C_{12}, C_{14}, C_{16}, C_{18} saturated free fatty acids. Conditions: column, 100 × 1 mm I.D., Nucleosil cyano, 5 μm; 1800 p.s.i. for 2 min, 1800–6000 p.s.i. at 100 p.s.i./min; 70°C. (Redrawn with permission from ref. 28.)

6. APPLICATIONS

In Table 4, some applications described in the literature are listed. It is striking that predominantly synthetic mixtures consisting of non-polar hydrocarbons are separated. CO_2 is usually applied as the mobile phase in combination with UV detection. In this way, modifiers can be added, but the same detection problems occur as with HPLC.

There are also some applications specific to packed-column SFC. The use of chiral stationary phases for the separation of enantiomers should be mentioned. Pirkle-type stationary phases, cylodextrins and bonded chiral diamides have been employed for the separation of phosphine oxides and amino acid derivatives. These applications are summarized in Table 5.

The separation of enantiomers reveals another advantage of the use of packed columns in SFC. For these applications special interactions between the sample and the stationary phase are necessary. The slow adsorption and desorption velocities needed for building up the specific interactions necessary for enantiomeric separations can often be achieved only at relatively low temperatures. Owing to the high phase ratio, it is possible to perform packed-column SFC at low temperatures. Consequent-

TABLE 4

APPLICATIONS OF PACKED-COLUMN SFC

Application	*Column*	*Mobile phase*[a]	*Detection*	*Ref.*
Polycyclic aromatic hydrocarbons	ODS, 3 μm Silica, 3 μm Silica, 5 μm	CO_2 CO_2 modified with C_1OH	UV	55
Ubiquinones	ODS, 3 μm ODS, 5 μm	CO_2 CO_2 modified with C_1OH	UV	56
Polystyrene oligomers	ODS, 3 μm ODS, 5 μm Silica, 3 μm PRP-1, 10 μm	CO_2 CO_2 modified with C_1OH	UV	57
Aromatic peroxides and their reaction products with methylvinylsilicones	MOS, 5 μm ODS, 10 μm RP-8, 10 μm	CO_2 CO_2 modified with C_1OH	UV	58
Carotenoids in paprika oleoresin	ODS, 3 μm ODS, 5 μm	CO_2 CO_2 modified with C_1OH, C_2OH	UV	59
Caffeine in beverages	ODS, 3, 5, 10 μm RP-8, 10 μm	CO_2 CO_2 modified with C_1OH	UV	60
Group separation of oil residues	CP-Spher silica Spheri-5-cyano Silica + $AgNO_3$	CO_2	UV, FID	61
Fractionation of petroleum- and coal-derived mixtures	Silica with intermediate particle size (30–70 μm)	CO_2	UV	62
Group analysis of gasolines	Spheri-5, 5 μm	SF_6	FID	63
N-Vinylcarbazole oligomers	LiChrosorb Si 100, 10 μm LiChrosorb Si 60, 10 μm	C_5 modified with dioxane	UV	64
Liquid crystal mixtures	CP-Spher C_{18}, 8 μm	CO_2	UV	65
Styrene oligomers	LiChrosorb Si 100, 10 μm	C_5	UV	66
Optimization strategy for oligomer separations	LiChrosorb Si 60, 10 μm	Various gradients	UV	67
Substituted ferrocenes Metal β-diketonates	Silica, 7 μm Cyano, 10 μm Methyl, 10 μm Phenyl, 5 μm Octyl, 10 μm ODS, 5 μm PRP-1, 5 μm	CO_2 modified with C_1OH	UV	68
Hydrocarbon groups in gasoline and middle distillate fuels	Silica Ag-loaded strong cation exchanger	SF_6 modified with 10% CO_2	FID	69
Ecdysteroids	Spherisorb cyanopropyl Spherisorb ODS-2, 5 μm	CO_2	UV	4
Opium alkaloids from poppy-straw extract	LiChrosorb Si 60, 5 μm Aminopropyl, 10 μm	CO_2 modified with C_1OH, methyl-, ethyl-triethylamine, H_2O	UV	70
Propellant stabilizer (synthetic mixture)	Deltabond, cyano Cyanopropyl, 5 μm Propylamino, 5 μm	CO_2 CO_2 modified with C_1OH	FTIR UV FID	3
Amino acids after a pre-derivatization step	Nucleosil-100-5, 5 μm	CO_2 modified with C_1OH, H_2O, methylamine	UV	71

[a] For abbreviations see footnote to Table 3.

TABLE 5

CHIRAL SEPARATIONS

Application	*Column*	*Mobile phase*[a]	*Detection*	*Ref.*
Enantiomeric pairs of phosphine oxides	Pirkle-type, DNBPG	CO_2 modified with C_1OH, C_2OH C_3OH modified with 5% H_2O	UV	72
Racemic N-acetylamino acid *tert.*-butyl esters	(N-Formyl-L-valylamino)-propylsilica	CO_2 modified with C_1OH	UV	73
α-Amino acid derivatives	(N-Formyl-L-valylamino)-propylsilica	CO_2 modified with C_1OH	UV	74
Homologous series of enantiomeric amides. Comparison of subcritical fluid chromatography and LC	Pirkle-type, DNBPG	CO_2 modified with C_1OH, *i*-C_3OH, 2-, *n*-, *tert.*-C_4OH	UV	43
Racemic amides and phosphine oxides in subcritical fluid chromatography	β-Cyclodextrin-bonded	CO_2 modified with C_1OH	UV	75
Racemic N-4-nitrobenzoylamino acid isopropyl esters	Chiral valine–diamide phase	CO_2 modified with C_1OH	UV	76

[a] For abbreviations see footnote to Table 3.

ly, chiral separations are often carried out under subcritical, *i.e.*, liquid, conditions. Although the diffusion coefficient in liquid CO_2 approximate those in other liquids, subcritical fluid chromatography has been found to be superior to LC in terms of efficiency, stereoselectivity and analysis time[41].

The use of packed columns in SFC is worthwhile if a transfer to preparative chromatography is planned. Owing to the small diffusion paths in the column the system is more efficient, and because of the short columns a separation can be achieved faster than with capillary SFC.

Recent developments in packed-column SFC include a so-called "unified chromatography"[42,43]. In this instance, the eluent passes through the liquid, supercritical and gaseous states during a chromatographic run. Starting at a low pressure, a temperature programme is carried out (GC), followed by pressure programme at constant but supercritical temperature. The increasing pressure transfers the mobile phase into the supercritical state. Subsequently, a negative temperature pogramme brings the supercritical fluid into the liquid state.

Many other gradient systems are being actively developed. Especially modifier gradients seem to be of future interest. With these gradients the possibility is given of varying substantially the properties of the eluent and the stationary phase[44,45]. As Berger *et al.*[31] have shown by the separation of PTH-amino acids, modifier gradients enable packed-column SFC to be applied to polar and analytically interesting compounds. For the separation of biologically active substances, such as ouabain, capillary SFC has to be employed[83].

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8. SUMMARY

The application of packed columns in supercritical fluid chromatography is reviewed. Instrumental aspects are discussed briefly. The main emphasis is put on chromatographic selectivity, and the selection of mobile and stationary phases. The influence of physical parameters, such as pressure and temperature, on a separation and the use of gradient techniques are discussed. Applications of the use of packed columns in supercritical fluid chromatography are discussed and summarized.

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Review

Some aspects of capillary supercritical fluid chromatography

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CONTENTS

1. COMPARISON OF CHROMATOGRAPHIC PERFORMANCE OF COLUMNS IN SUPERCRITICAL FLUID CHROMATOGRAPHY

In order to compare chromatographic conditions in different types of columns, it is very convenient to use dimensionless parameters, and this is considered in the following sub-sections.

1.1. Capillary columns

1.1.1. Reduced plate-height equation. For open-tubular columns, the relationship between the plate height, H (cm), and the linear velocity of the mobile phase, u (cm s^{-1}), is given by the Golay equation[1]:

$$H = \frac{2D_m}{u} + f(k) \cdot \frac{d_c^2 u}{D_m} + g(k) \cdot \frac{d_f^2 u}{D_s} \quad (1)$$

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TABLE 1

DEFINITIONS OF REDUCED (DIMENSIONLESS) PARAMETERS

Parameter	*Capillary columns*[a]	*Packed columns*[a]
Reduced plate height	$h = \frac{H}{d_c}$	$h = \frac{H}{d_p}$
Reduced velocity	$v = \frac{u\ d_c}{D_m}$	$v = \frac{u\ d_p}{D_m}$
Dimensionless film thickness	$\delta_f = \frac{d_f}{d_c} \cdot \frac{D_m}{D_s}$	–
Capacity factor	$k = K \cdot \frac{V_s}{V_m}$	$k = K \cdot \frac{V_s}{V_m}$

[a] d_c, column diameter (cm); d_p, particle size (cm); V_s, V_m, volumes of stationary and mobile phase, respectively.

where D_m and D_s are the diffusion coefficient of the solute in the mobile and the stationary phase, respectively ($cm^2\ s^{-1}$), d_f is the thickness of the stationary-phase film (cm) and k is the capacity factor (dimensionless retention time):

$$f(k) = \frac{1 + 6k + 11k^2}{96(1+k)^2} \tag{2}$$

$$g(k) = \frac{2k}{3(1+k)^2} \tag{3}$$

By using the dimensionless parameters as defined in Table 1, the Golay equation can be rewritten in a simple reduced form[2]. The dimensionless plate-height equation is

$$h = \frac{2}{v} + f(k)v + g(k)\ \delta_f^2\ v \tag{4}$$

where

$$\delta_f^2 = \frac{d_f^2}{d_c^2} \cdot \frac{D_m}{D_s} = \frac{\tau_s}{\tau_m}$$

is the ratio of the diffusion times in the stationary and the mobile phase.

1.1.2. Speed of analysis. Based on reduced parameters (Table 1), a very interesting equation for the speed of analysis in all forms of column chromatography can be derived[2]. Starting from the retention time equation:

$$t_R = t_0(1+k) = \frac{L}{u}(1+k) \tag{5}$$

where t_R, t_0 are the retention time of a solute and an unretained component, respectively. Substituting $L = NH$ and the reduced parameters as defined in Table 1 it follows that

$$t_R = N \cdot \frac{h}{v} \cdot \frac{d_c^2}{D_m} (1+k) \tag{6}$$

Under identical conditions (N, h, v and k), the analysis time is proportional to d_c^2/D_m (or d_p^2/D_m for packed columns). The order of magnitude of D_m in supercritical fluid chromatography (SFC) is about 10^{-4} cm^2 s^{-1} compared with 10^{-5} and 10^{-1} cm^2 s^{-1} for liquid and gaseous mobile phases, respectively. From eqn. 6 and the respective diffusion coefficients, it can be concluded that in order to obtain equal speed of analysis in gas chromatography (GC), SFC and liquid chromatography (LC), the diameters of the columns should be chosen in the ratio of 1.0:0.03:0.01.

Table 2 gives a comparison of d_c^2/D_m and thus the relationship between the analysis times for the three forms of capillary fluid chromatography. A dimensionless film thickness of 0.3 is used for all columns. The very narrow capillary columns essential in open-tubular LC possess a very limited sample capacity. This, together with the requirement for extremely small detector volumes poses enormous technological problems. Therefore, it can be concluded that capillary SFC, because of the larger column diameters involved, is more within the scope of current technology than is capillary LC. An additional advantage of SFC is the fact that if the correct mobile phase is selected, sensitive GC detectors with low effective dead volumes may be used (*e.g.*, flame ionization, mass spectrometric or nitrogen–phosphorous-specific detectors).

TABLE 2

COMPARISON OF RETENTION TIMES IN OPEN-TUBULAR CHROMATOGRAPHIC METHODS (δ_f = 0.3)

Method	d_c (μm)	d_f (μm)	D_m (cm^2 s^{-1})	d_c^2/D_m (s)	*Relative time*
GC	250	0.25	10^{-1}	0.006	1
SFC	50	0.79	10^{-4}	0.250	40
	8	0.13	–	0.006	1
LC	10	0.71	10^{-5}	0.100	16
	5	0.35	–	0.025	4

For the exact calculation of retention times by means of eqn. 6 (at a given value of N), values for h and v have to be included (see Table 3). For fairly high values of k, $v = 45$ and $h = 4.5$ are reasonable values for columns with thin films ($\delta_f \approx 0.3$) as used in SFC. A further increase of v in order to increase the speed of analysis does not make sense, because at high velocities h is proportional to v and hence h/v remains constant.

For an excellent discussion on the concept of reduced film thickness and its implications for values of h and v the reader is referred to ref. 2.

TABLE 3

COMPARISON OF OPEN-TUBULAR AND PACKED COLUMNS (DIMENSIONLESS PARAMETERS) UNDER OPTIMUM AND DAILY PRACTICAL CONDITIONS

Type of column	φ_0	h	h_{min}	E	E_{min}	v	v_{opt}
Capillary	32	4.5	0.8	650	20	45	5
Drawn (packed) capillary	150	3	2	1350	600	10	5
Packed	1000	3	2	9000	4000	10	2.5

1.1.3. Speed and pressure drop. For non-compressible fluids (liquids) and for conditions of relatively low pressure drops in GC and SFC, the pressure drop is described by the Darcy equation:

$$\Delta P = B_0 \eta L u \tag{7}$$

The permeability B_0 for open-tubular columns equals $32/d_c^2$, or expressed in general terms φ_0/d_c^2, where φ_0 is the column resistance factor. Rewriting with the dimensionless parameters h and v:

$$\Delta P = \frac{32}{d_c^2} \cdot \eta N h v D_m \tag{8}$$

or with

$$v = \frac{u d}{D_m} = \frac{L}{t_0} \cdot \frac{d}{D_m} = \frac{N h d^2}{t_0 D_m}$$

$$\Delta P = \frac{\varphi_0 N^2 h^2 \eta}{t_0} = \frac{E N^2 \eta}{t_0} \tag{9}$$

$h^2\varphi_0 = E$ is defined as the separation impedance. From eqn. 9, it follows that

$$\Delta P t_0 = \text{constant} \tag{10}$$

For a given fluid state, the analysis time (proportional to t_0) can be reduced proportionally to d_c^2 (eqn. 6) at the price of a proportionally increasing pressure drop. For a given plate number N, the constant depends on the fluid state (η). For high pressure drops (packed columns) with high plate numbers in SFC, η decreases along the column length. In open-tubular columns this effect can generally be neglected and eqn. 10 can be applied.

Table 4 gives values for $N/\Delta P$ (plates per bar pressure drop) for two column diameters in open-tubular SFC (after eqn. 8). For the calculation of the data in this table, values of $h = 4.5$ and $v = 45$ were used.

In SFC, in contrast to GC and LC, retention is very dependent on the pressure

(density) of the mobile phase. A large pressure drop will result in a density gradient along the column. The effect is similar to a negative temperature gradient along the axis of a GC column. The migration of the components will slow down along the column and (in extreme cases) will eventually stop. This problem can be solved partly by pressure (density) programming. A disadvantage of pressure programming, however, is the increasing linear velocity of the mobile phase and thus a reduction in the number of plates attainable.

From Table 4 it can be concluded that the pressure drop is relatively small in open-tubular SFC; even with 8-μm columns about 10 000 plates can be achieved with a pressure drop of 10 bar (column inlet 210 bar, outlet 200 bar).

1.2. Packed columns

1.2.1. Reduced plate-height equation. For packed columns, no exact analytical H–u or h–v equation analogous to the Golay equation for capillary columns exists. The form of the equation for packed columns is adequately described by the reduced plate-height equation given by Knox:

$$h = Av^{1/3} + \frac{B}{v} + Cv \tag{11}$$

The dimensionless quantities h or v for packed columns are defined in Table 1.

Typical values[3] for the constants in eqn. 11 are A = 1–2, B = 2 and C = 0.05–0.5. Typical values for h and v in practice are 3 and 10, respectively.

1.2.2. Speed of analysis. Eqn. 6 describes the speed of analysis for packed columns, if d_c is replaced by the particle size d_p and if the appropriate values for h and v as given above are used. Contemporary packed columns contain particles of size 10, 5 or even 3 μm. From eqn. 6, it can be concluded that open-tubular fluid chromatography cannot compete with packed columns with respect to speed of analysis unless very small column diameters are used. This, however, is not within the scope of today's technology.

1.2.3. Speed and pressure drop. Eqns. 7–10 are applicable to packed columns by inserting d_p instead of d_c and using the values for h, v and E from Table 3. It should be borne in mind, however, that these equations are strictly valid only for incompressible fluids. For GC and SFC this limits the validity to situations of low pressure drop and hence low plate numbers. In SFC both the linear velocity and the viscosity η are

TABLE 4

PLATES PER BAR PRESSURE DROP IN OPEN-TUBULAR COLUMNS (h = 4.5; v = 45) AND PACKED COLUMNS (h = 3; v = 10) $\eta = 10^{-3}$ g cm^{-1} s^{-1}; $D_m = 10^{-4}$ cm^2 s^{-1}.

Conditions	*Capillary columns*		*Packed columns*	
	d_c (μm)	*Plates per bar pressure drop*	d_p (μm)	*Plates per bar pressure drop*
Supercritical CO_2, 40°C, 200 bar	50	38 600	10	333
	8	1000	5	83

dependent on the pressure drop. In high-pressure GC, pressure-drop correction factors have to be included in the plate-height equations. One of the results is that the speed of analysis is no longer proportional to d_c^2 or d_p^2 and eventually (at high-pressure drops) it becomes proportional to d_c or d_p (refs. 4 and 5).

Table 4 gives values of plates per bar pressure drop for packed columns (φ_0 = 1000) and open-tubular columns (eqn. 8). For drawn (packed) capillary columns, the numbers given have to be multiplied by 1000/150, *i.e.*, the ratio of the column resistance factors of the respective column types (Table 3). For packed capillaries, the ratio is close to 1000/500.

From Table 4, it is clear that for a given allowable pressure drop, ΔP, capillary columns always allow much higher plate numbers to be achieved. This is illustrated in Fig. 1, which shows the analysis of a paraffin wax on a 10 m × 50 μm I.D. column. Apart from the almost Gaussian peak profiles of the main components two fine structures can be observed. The plate numbers that can be generated using packed columns would definitely be insufficient for separating the sample in these three series. At 170 bar and 100°C this column had approximately 60 000 plates for docosane as the test solute (k = 0.75, linear velocity 1.2 cm s^{-1}). The maximum number of plates that can be obtained from any type of column in the three forms of fluid chromatography depends on the maximum allowable pressure drop. In GC and LC this is dependent on instrument design. In SFC retention depends heavily on pressure (or density). The effect of the pressure drop in SFC on retention and plate height is one of the research goals in the near future.

Doubtless in the near future, instrument companies will provide practising chromatographers with the dedicated instrumentation needed to benefit from all the advantages that narrow-bore capillary columns potentially offer. For typical applications, especially high-speed analysis, columns packed with small particles will remain essential in the forthcoming decade.

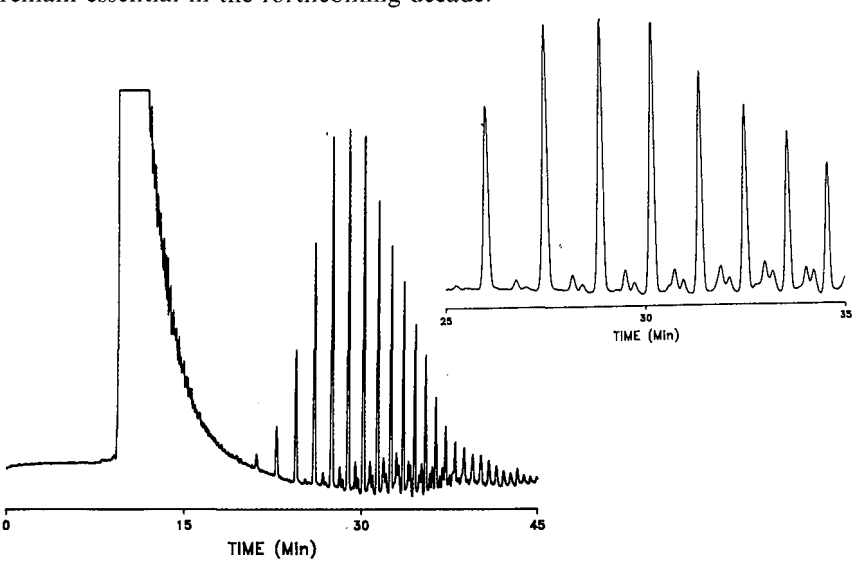

Fig. 1. Analysis of a paraffin wax. Column, 10 m × 50 μm I.D.; stationary-phase, SE-30, 0.25 μm; temperature, 120°C; pressure program, 150 bar (10 min), 5 bar/min to 305 bar.

The choice of the column type for a particular separation is not only governed by the speed of analysis or the maximum plate number. Several other aspects can affect the ultimate choice. One aspect to consider is the volumetric flow-rate through the system. Mass spectrometric detection seems to be better compatible with the lower flow-rates from capillary columns. In contrast, infrared detection, using relatively large-volume detection cells is easier to combine with packed columns. Another aspect influencing the choice of the column is the chemical nature of the solutes. The analysis of polar compounds in packed columns often requires modified mobile phases. If the use of modifier-containing mobile phases is precluded by the detector, the use of capillary columns might be the only alternative. The effect of modifiers added to the mobile phase in packed and capillary SFC will be discussed below. We shall discuss the problems that arise in the on-line combination of capillary SFC with FT-IR.

2. MODIFIERS IN CAPILLARY SFC

2.1. Effects of modifiers on retention

To date, very few studies on the effects of modifiers in capillary SFC have been described. In capillary SFC, relatively polar components can be eluted with pure carbon dioxide. Elution of these components in packed-column SFC often requires the addition of a polar modifier to the mobile phase. Fig. 2 shows the analysis of a polar liquid crystal mixture using both packed and capillary columns. Whereas the polar constituents of the sample show up as broad, tailing peaks in packed columns without modifiers (Fig. 2a), these components can be eluted as sharp, symmetrical peaks from the capillary column (Fig. 2b). The peak shapes on the packed column can be improved by using a mobile phase containing a few percent of methanol, as illustrated in Fig. 2c.

The limited number of reports on mixed mobile phases in capillary SFC have shown that relatively large retention changes, although not as large as in packed columns, can also occur in capillary columns. For example Yonker and Smith[7] observed a 40-fold reduction in the capacity factor of myristophenone on increasing the concentration of the modifier (2-propanol) in CO_2 from 0 to 20 mol-%. Fields *et al.*[8] found an approximately ten-fold reduction in the capacity factor of coronene on adding 9 mol-% of 2-propanol to CO_2. Wright *et al.*[9] used 2.5% (w/w) methanol in CO_2. A direct comparison of their data with the values with pure CO_2 was not possible as these experiments were carried out at different pressures and temperatures. In a recent study[10] using packed columns, we observed a 50-fold reduction in the capacity factor of 2-hydroxyethyl methacrylate on a C_{18} packed column on adding only 0.5% of ethanol to the CO_2. The mechanisms that underlie these observations are not yet fully understood.

We recently identified three different ways in which modifiers may influence retention in packed-column SFC[10]: an increase in the mobile-phase polarity, which can give rise to the occurrence of specific interactions between the solute and the modifier in the mobile phase; an increase in the mobile-phase density; and deactivation of active sites on the surface of the packing material.

Whereas the first two effects represent mobile-phase modifications, the third is related to stationary-phase effects. In our classification of the effects of modifiers, we have neglected stationary-phase swelling and increased solvation of the stationary phase by the organic modifier. Recent work by Yonker and Smith[11] indicates that

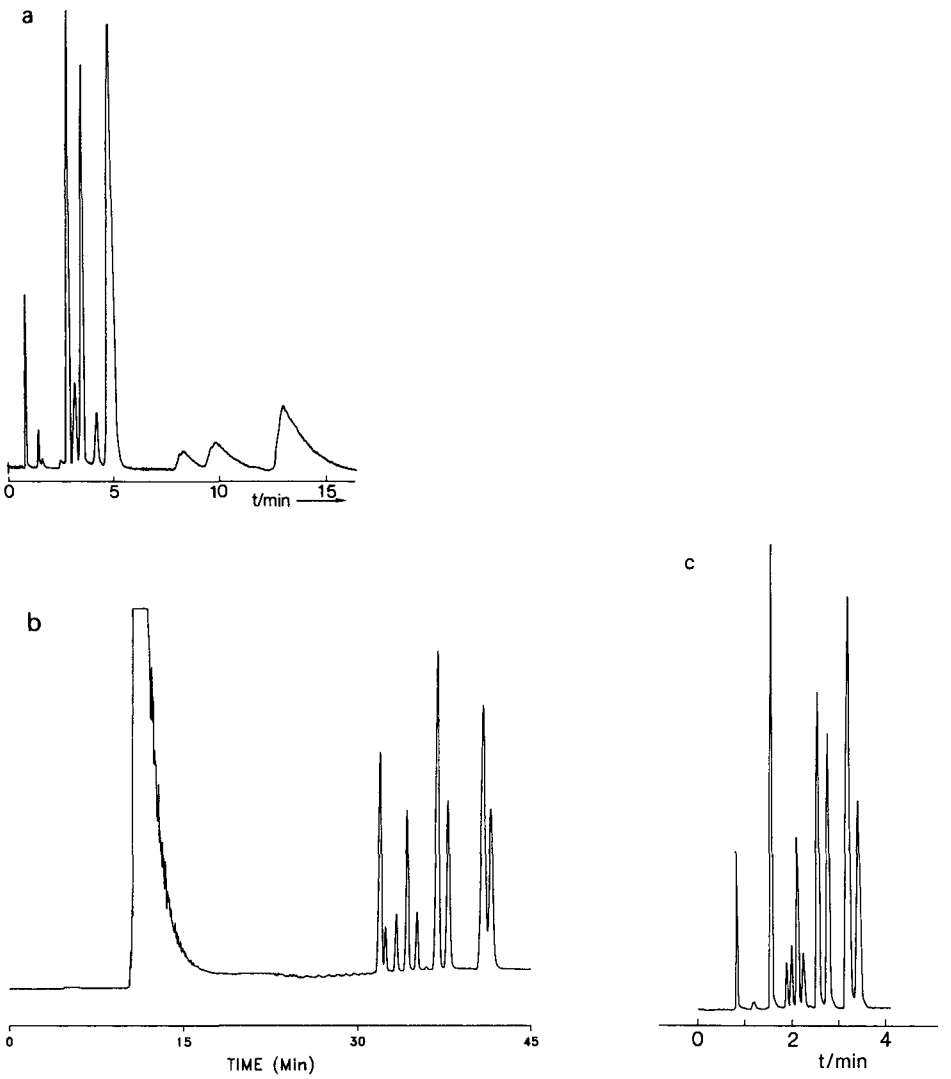

Fig. 2. Analysis of a polar liquid-crystal mixture using packed and capillary columns. (a) C_{18} packed column, 150 × 4.6 mm I.D., particle size 5 μm; P_{in} = 190 bar; ΔP = 16 bar; temperature 45°C; detection, UV at 254 nm. Reprinted from ref. 6 with permission. (b) Capillary column, 10 m × 50 μm I.D.; temperature, 80°C; stationary-phase, SE-30, 0.25 μm; pressure program, 140 bar (10 min), 5 bar/min to 305 bar. (c) C_{18} packed column, 150 × 4.6 mm I.D., particle size 5 μm; P_{in} = 188 bar; ΔP = 20 bar; temperature 50°C; mobile phase, methanol–CO_2 (5:95, v/v); detection, UV at 210 nm.

solvation of the stationary phase by the organic modifier can occur. Data from Strubinger and Selim[12] seem to indicate that a change in the polarity of the stationary phase can occur due to its enrichment with the polar modifier. The effect of this on retention is still unknown. For a true fundamental understanding of the effects of modifiers, it is of considerable importance to know the relative influence of the

processes described above. For capillary columns with low surface areas and a high degree of deactivation we can neglect the third process. The relative magnitude of the two remaining processes can be estimated from plots of the capacity factor of a solute *versus* the density of the CO_2–modifier mixture for various modifier concentrations. In these plots, the course of the capacity factor *versus* the density of the fluid at a certain modifier concentration reflects the influence of the mobile phase density on retention, whereas the change in the capacity factor with the modifier concentration at constant density reveals the influence of the mobile-phase polarity on solute retention. An increased solvating strength of the mixed mobile phase would result in the retention being lower with the mixed phase than with a pure CO_2 phase. This method of comparing retention in mixed fluids and pure CO_2 requires accurate density values for binary fluids. Experimental density data for CO_2-containing mixtures, however, are scarce.

If experimental data are not available, estimation methods must be employed. These methods are mostly based on reduced temperatures and pressures, which in turn also have to be estimated. Several methods for the estimation of the critical properties of binary fluids have been described[13]. Since estimation methods for critical properties of polar fluids can be subject to significant errors, methods for calculating densities will hence inherently be subjected to the same errors. In Fig. 3, a comparison of three methods for estimating critical properties of binary fluids is given. The Lee–Kesler method (data taken from Schoenmakers and Uunk[6]), the Kreglewski–Kay method[14] and the Chueh–Prausnitz method[15] were employed for estimating the critical pressures of methanol and CO_2 mixtures as a function of the mole fraction of methanol. Fig. 3 clearly indicates the differences in the estimated critical pressures for the three methods.

Yonker and Smith[7] compared the critical loci calculated for methanol–CO_2 according to the Chueh–Prausnitz method with experimental data. For this particular

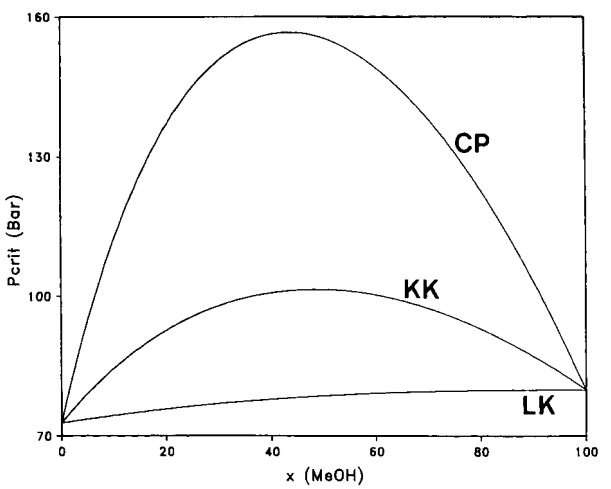

Fig. 3. Calculated critical pressures of CO_2–methanol (MeOH) mixtures as a function of the molar percentage of methanol. LK = values estimated according the Lee–Kesler method; KK = estimated according to the Kreglewski–Kay method; CP = estimated according to the Chueh–Prausnitz method. In the Chueh–Prausnitz method, a binary interaction parameter of 0.15 was used[16].

example, a reasonable fit was found between the experimental data and the estimated values.

The approach of comparing retention in mixed mobile phases with varying modifier concentration as a function of the density was adopted by Yonker *et al.*[17] and by Fields *et al.*[8], and Fig. 4 shows the data from the former, the capacity factor of 9-phenanthrol at 127°C being plotted against the density at three different mole fractions of 2-propanol. The densities of the binary fluids were calculated using the Peng–Robinson equation of state.

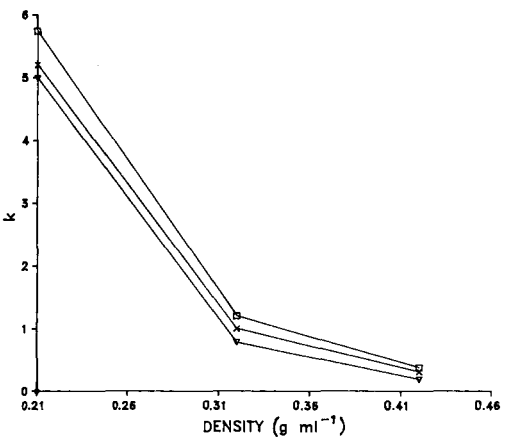

Fig. 4. Capacity factors of 9-phenanthrol *versus* mobile phase density at various modifier concentrations. Modifier mole fractions: □, 0; ×, 0.018; ▽, 0.04. Column, 10 m × 50 μm I.D.; stationary-phase, 5% phenylmethylpolysiloxane; temperature, 127°C. Data taken from ref. 17.

Fig. 4 shows two trends. First, there is a decrease in the capacity factor with increasing density at constant composition. Second, a decrease in k is observed with increasing mole fraction of the polar modifier at constant density. The first observation reflects the general trend in SFC with pure mobile phases, *i.e.*, a decreased retention as the fluid density increases. The decrease in solute retention as the mole fraction of the solvent modifier increases at constant density can be attributed to a qualitative change in the solute–solvent interactions. The polar modifier 2-propanol is believed to interact effectively with the 9-phenanthrol owing to its polar substituent.

Independent evidence of a change in the nature of the solute–solvent interactions can be obtained from spectroscopic measurements. Yonker *et al.*[18] used spectroscopic measurements of solvatochromic shifts to probe the solute–solvent interactions for 2-nitroanisole in CO_2–methanol. Different solvatochromic shifts were observed for different modifier concentrations. From Fig. 4, it appears that the effect of an increase in the mobile-phase density on retention can be much larger than the effect of adding a polar modifier to the fluid. This indicates that comparing the retention in binary fluids with reference values in pure CO_2 at constant pressure instead of constant density can give a distorted picture. On adding a modifier to pure CO_2, the density increases because the critical parameters of the mixed fluid are generally higher than those of pure CO_2. This density increase at constant pressure can have an appreciable

influence on retention. If the density change is taken into account, the effect of adding a modifier will be smaller.

In Fig. 5 the results of Fields *et al.*[8] are shown. The logarithm of the capacity factor of coronene is plotted as a function of the estimated mobile phase density for pure CO_2 and for CO_2–2-propanol mixtures. Fields compared the retention data obtained with CO_2–2-propanol binary fluids with retention data obtained at the same temperature and density with neat CO_2. In contrast to what is expected, *i.e.*, the retention being less with mixed phases than with pure CO_2, the plots of log *k versus* density for CO_2 cross over the lines representing log *k versus* ρ for the mixed phase. This would indicate that retention would be greater for a polar liquid–CO_2 mixture than for a CO_2 mobile phase at the same density and temperature. This observation does not seem to be reasonable and is in contradiction with the results from Yonker *et al.* described earlier. Most probably, it implies that the estimated density values of the CO_2–2-propanol mixture are not correct, either due to an inaccurate estimate of the critical properties of the mixture or to an erratic calculation of the density.

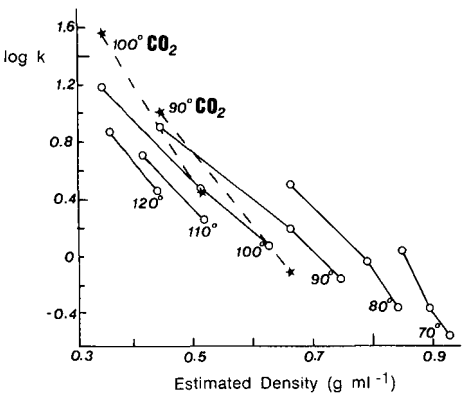

Fig. 5. Log retention of coronene as a function of the estimated density of 8.9 mol-% 2-propanol–CO_2 (solid lines) and density of CO_2 (dashed lines) mobile phases for different temperatures. Capillary column, 10 m × 50 μm I.D.; stationary phase, 100% methylpolysiloxane, 0.25 μm. Redrawn from ref. 8 and reproduced with permission.

2.2. Preparation of binary mobile phases

Owing to the absence of polar single-component solvents with acceptable critical properties and safety requirements for SFC, mixed mobile phases are the only alternative when more polar fluids are needed. The preparation of binary fluids is by no means trivial. Here, we shall discuss some methods for the preparation of mixed fluids. We shall limit ourselves to situations where the main solvent is a gas under ambient conditions (*e.g.*, carbon dioxide).

Three methods for preparing mixed fluids for capillary SFC have been described. The simplest is the use of cylinders with premixed mobile phases. These cylinders can either be obtained directly from commercial gas manufacturers or can be prepared in the laboratory. Two reports have been published which describe techniques for the preparation of known compositions of supercritical mobile-phase fluids[19,20]. Both

methods include subsequent steps of evacuating a small gas cylinder, introducing a small (weighed) amount of the (liquid) modifier in the cylinder, adding the appropiate amount of CO_2 and finally mixing the cylinder by agitation. The mole fraction of the modifier in the resulting solution can be determined from the masses of the modifier and CO_2. A distinct disadvantage associated with the use of such premixed fluids, however, is the continuous change in the composition of the residual liquid in the cylinder during usage. As the vapour pressure of CO_2 is much higher than that of the modifier, the gas phase in the cylinder can be assumed to be pure CO_2. Owing to the selective evaporation of the CO_2, the concentration of the modifier in the liquid phase will increase during usage[6].

The second method for preparing mixed fluids for SFC uses mixing of the eluents in the syringe of the pump prior to compression[9,21,22]. The mixtures are prepared by preloading the syringe pump with the proper volume of modifier and then filling the remaining volume with CO_2. Equilibration of the mixture can be accelerated by rapidly increasing and decreasing the pressure of the pump.

The third method is analogous to high-pressure mixing in LC. Here, the fluid flows of two pumps are combined and mixed prior to entering the chromatographic system. Owing to the extremely low volumetric flow-rates in capillary SFC, this method can only be used in combination with high splitting ratios. In contrast to the methods described above, this method is very flexible with regard to the modifier concentration and even allows the use of composition gradients. The applicability of the dual-pump system for gradient elution in open-tubular SFC was first demonstrated by Yonker and Smith[23].

3. HYPHENATED TECHNIQUES

The ultimate aim of a chromatographic analysis is often not only the separation of the sample into its constituents, but also the elucidation of the structure of the components present in the unknown sample. This necessitates coupling of the chromatographic separation technique with identification techniques. Three identification techniques commonly used in combination with SFC are (Fourier transform) infrared spectroscopy (FT-IR), mass spectrometry (MS) and multi-channel UV detection[24,25]. Here we shall give a short discussion of the problems that arise when capillary SFC is being interfaced with IR spectroscopy.

3.1. SFC–FT-IR

There are two methods for monitoring the IR absorption of the eluent eluting from a chromatographic column[26–28]: on-line flow-through cells[29] or off-line solvent-elimination techniques[30].

In the flow-cell approach, the effluent passes through a high-pressure light pipe. IR spectra of the effluent are collected in real time while the effluent flows through the cell. In the solvent-elimination approach, the chromatographic effluent is deposited on a surface, the mobile phase is evaporated and the residual sample is examined by FT-IR spectroscopy. Here, the position of SFC between GC and LC becomes apparent. Whereas GC–IR is almost exclusively performed with the flow-cell approach and LC–IR with the solvent-elimination technique, both methods are applied in SFC–IR. Here, we shall give only a short discussion of the on-line flow-cell approach.

The success of the flow-cell approach in the coupling of chromatographic techniques with IR detection depends on the ability to cope with the background absorption of the mobile phase. In GC–FT-IR this is not a problem as normal carrier gases are transparent in the mid-IR region. In LC, the applicability of flow-cell techniques is severely hampered by the high background absorption. A detailed study of the IR transparency of CO_2 was published by Morin *et al.*[31]. Gaseous CO_2 was shown to have large transparent IR regions. However, two important groups of bands obscure the IR spectrum in the regions 3500–3800 and 2200–2500 cm^{-1}. In the supercritical state and the liquid state, the two gaseous bands broaden and additional pairs of bands appear at 2070 and 1944 and at 1387 and 1282 cm^{-1}. The intensity of the latter pair of bands depends on the mobile-phase density. Higher mobile-phase densities lead to a lower transparency in this region. According to Wieboldt *et al.*[32], spectral subtraction in the 1475–1225 cm^{-1} region is possible when using cells with an optical path length shorter than 5 mm.

The problem of background IR absorbance can be eliminated by using an IR-transparent supercritical mobile phase. French and Novotny[33] demonstrated the use of the optically transparent xenon for SFC–FT-IR. Although xenon has convenient critical parameters (T_c = 289.8 K, P_c = 58.0 atm), and diffusion coefficients and a solvent strength comparable to those of CO_2[33,34], its widespread use is hindered by its extremely high price. Whereas the background absorption due to CO_2 can be partially corrected for, the situation is aggravated if modifiers have to be used[35]. In this instance the application of on-line SFC–FT-IR for identification is virtually impossible.

Another problem associated with the use of flow cells in capillary SFC–FT-IR is the extremely low cell volume that can be allowed without affecting the quality of the separation. The design of a flow cell is a compromise between chromatographic and spectrometric requirements. From the chromatographic point of view, the cell volume of the detector must be much smaller than the volume of a chromatographic peak eluting from the column. The maximum allowable cell volume is determined by the maximum acceptable loss of chromatographic resolution.

An equation for the maximum allowable cell volume can be derived starting from the width of a chromatographic peak. For the variance of a peak, σ_c, we can write

$$\sigma_c^2 = \frac{V_r^2 H}{L} \tag{12}$$

where σ_c is the standard deviation of the peak (cm^3), H is the plate height (cm), L is the column length (cm) and V_r the retention volume (cm^3). In this equation we can substitute equations for the retention volume of a peak and for the plate height:

$$V_r = V_0 (1+k) \tag{13}$$

with

$$V_0 = \frac{\pi}{4} \cdot d_c^2 L \tag{14}$$

If we neglect the stationary-phase contribution to chromatographic band broadening and if we assume that the operating velocity is far above the optimum value, the plate-height equation becomes

$$H = \frac{f(k)d_c^2 u}{D_m} \tag{15}$$

Substitution of eqns. 13, 14 and 15 into eqn. 12 and rearrangement yield the following expression for the peak width:

$$\sigma_c = \frac{\pi d_c^3(1+k)}{4}\left[\frac{f(k)\quad u\quad L}{D_m}\right]^{\frac{1}{2}} \tag{16}$$

For the band broadening, σ_d, that occurs in the detector cell we can write[36]

$$\sigma_d = \frac{V_d}{\sqrt{12}} \tag{17}$$

where V_d is the cell volume of the detector. Here it is assumed that plug flow occurs in the detector cell. The total band width of the peak, σ_{tot}, can be obtained using the rule of the additivity of variances:

$$\sigma_{tot}^2 = \sigma_c^2 + \sigma_d^2 \tag{18}$$

If we accept a 10% loss of theoretical plates we finally arrive at an expression for the maximum allowable cell volume:

$$V_d = \frac{\pi d_c^3(1+k)}{\sqrt{12}}\left[\frac{f(k)\cdot u\cdot L}{D_m}\right]^{\frac{1}{2}} \tag{19}$$

On substitution of typical values for the various parameters into this equation (*i.e.*, $d_c = 50\,\mu m$, $L = 10\,m$, $k = 2$, $u = 2\,cm\,s^{-1}$, $D_m = 10^{-4}\,cm^2\,s^{-1}$), we obtain a maximum allowable cell volume of *ca.* 400 nl. A larger cell will unavoidably lead to appreciable band broadening. Hence, from the chromatographic point of view, cell volumes in excess of 400 nl are not permissable.

From a spectroscopic viewpoint, three restrictions are placed on the dimensions of the flow cell. For reasons of sensitivity, the path length of the cell should be long. Background absorption of the CO_2, however, limits the path length to a maximum value. Further, the diameter of the light beam is limited to a certain minimum value. For modern instruments, using beam condensing optics, the minimum cross-sectional area of the light beam is *ca.* 1 mm^2 (ref. 31). In designing flow cells for SFC–FT-IR, a conflicting situation arises between chromatographic and spectroscopic requirements. Starting with a beam cross-sectional area of 1 mm^2, the maximum allowable cell volume is already obtained at a path length of only 0.4 mm. Such a short path length is highly unfavourable with regard to FT-IR sensitivity. An increased sensitivity can only be obtained at the expense of chromatographic resolution by increasing the path length of the cell.

The first report on capillary SFC–FT-IR[37] used a capillary column of 60 m × 0.33 mm I.D. with an 8-μl flow cell that had a path lengh of 10 mm. French and Novotny[33] described 1-μl flow cells with path lengths of 1 mm in conjunction with 150-μm fused-silica capillary columns. Recently, Raynor *et al.*[38] described the use of 50-μm capillary columns in combination with a 0.8-μl light pipe. This cell had a path length of 4 mm. To avoid a significant loss of chromatographic resolution, make-up fluid was added just prior to the flow cell. With this experimental set-up, "library-searchable" spectra could be obtained for minimum amounts ranging from 10 ng for compounds with intense IR absorption to 100 ng for poor absorbers. In the off-line solvent-elimination approach for SFC–FT-IR, most of the problems encountered in the flow-cell method are eliminated. As the mobile phase is evaporated, background absorption is absent. Hence, it is possible to use both neat CO_2 and modified CO_2. Further, the unavoidable loss of resolution in the flow-cell coupling is not a source of concern in the off-line combination, For a detailed discussion of the solvent-elimination technique, the reader is referred to recent reviews[27,28].

4. CONCLUSIONS

The use of equations based on dimensionless parameters allows a direct comparison of the speed of analysis and the maximum obtainable plate numbers in packed-column and open-tubular column SFC. The plate number per bar pressure drop is much higher in capillary columns. The number of plates generated per second, however, favours the use of packed columns. A considerable reduction in the inner diameter of the capillary column is needed to obtain a similar analysis speed in packed and capillary columns. The extremely low sample capacity and the stringent requirements placed on the sample introduction and the detection system currently hamper the use of capillary columns with smaller diameters.

A definite advantage of capillary columns is the high degree of surface deactivation. The low activity of capillary columns allows the elution of relatively polar solutes without the use of solvent modifiers. Although the deactivation effect of modifiers is absent in capillary columns, here also the introduction of a modifier can cause a considerable reduction in the capacity factors of polar solutes. The mechanisms that underlie these effects are not yet fully understood.

The coupling of capillary SFC with FT-IR is a compromise between chromatographic and spectroscopic requirements. The minimum cell volume required for good spectroscopic performance exceeds the maximum value that can be accepted without the loss of chromatographic resolution. For packed columns, the necessity to add modifiers often precludes the on-line coupling of SFC with FT-IR. The ability to elute components covering a wide span of polarities with pure carbon dioxide adds to the attractiveness of capillary SFC–FT-IR.

5. SUMMARY

Based on dimensionless parameters, equations are given which compare speed of analysis, pressure drop and plates per bar pressure drop for capillary GC, SFC with capillary columns and SFC with packed columns. With respect to speed of analysis, contemporary capillary SFC cannot compete with packed-column SFC. A further

decrease in capillary column diameter will be needed to reach this goal. Decreasing the column diameter of capillary columns at the same time decreases the sample capacity and also places extremely stringent requirements on the speed of sample introduction and on the time constants of the detection systems. If the allowable pressure drop is a serious factor, as is expected from theory, open-tubular columns are to be preferred in terms of the maximum obtainable plate number.

The effects of polar organic modifiers in capillary SFC are described and compared with those in packed columns. The introduction of a modifier can cause a considerable reduction of the capacity factors in capillary SFC. Generally, the effects of modifiers in capillary SFC are not as large as those observed in packed columns. The interpretation of retention data is severely hindered by the unavailability of accurate density data for binary supercritical fluids. Three methods for the calculation of critical properties of mixed fluids are compared.

The coupling of SFC with FT-IR detection is discussed. For capillary SFC, a conflicting situation arises in which the cell volume that can be allowed without loss of resolution is much lower than the minimum volume required for good spectroscopy. It is shown that the on-line coupling of capillary SFC with IR spectroscopy will always be a compromise between chromatographic and spectroscopic requirements. For packed columns restrictions placed on the detector-cell volume are less stringent. Here, however, the on-line approach is severely hampered by the necessity to add modifiers to the mobile phase for the analysis of (even mildly) polar solutes.

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Review

Preparative supercritical fluid chromatography

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CONTENTS

1. PREPARATIVE CHROMATOGRAPHIC PROCESSES

Elution chromatography was first discovered as a preparative process by Tswett[1] for the fractionation of chlorophyll. It is therefore not surprising that preparative supercritical fluid chromatography (SFC) was proposed at the very beginning of the development of SFC by Klesper *et al.*[2], who stated that "the porphyrins could be recovered at the outlet valve". In fact, the unique physico-chemical properties of supercritical fluids, leading to the easy separation of fractionated compounds from the eluent, have convinced many workers that preparative SFC might be a very useful and relative easy tool in comparison with preparative gas chromatography, which is unsuitable for heavy and thermolabile compounds and preparative liquid chromatography, in which fraction–eluent separation can be problematic.

As shown in Fig. 1, preparative elution chromatographic processes are based on the same concept, whatever the eluent, with the following steps: periodic injection of the feed into a continuous flow of eluent; chromatographic separation due to selective interactions of the feed with both the eluent and the stationary phase; detection at the column outlet and fraction collection; separation of the fractionated compounds from the eluent; and purification and recycling of the eluent when economical (*e.g.*, large-scale production).

Obviously, as long as small amounts of pure products (10^{-3}–1 g) are required, *e.g.*, for structure analysis, bench-scale equipment derived from common analytical apparatus can be used with adoption of non-destructive detection, fraction collection and eluent removal. However, for industrial-scale production (1 g/h–1 kg/h),

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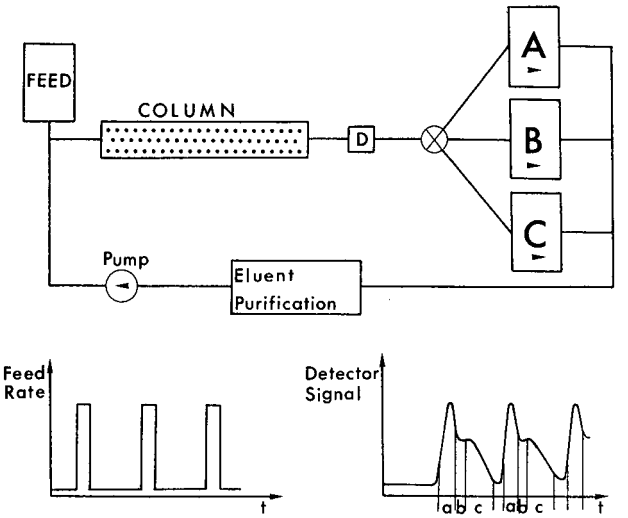

Fig. 1. Schematic diagram of the concept of preparative elution chromatography.

large-scale equipment will have to be designed, posing very different problems, even if the same concept is applied. In preparative SFC, most studies have been devoted to the former aspect, but very promising results are just starting to appear concerning the latter.

2. SMALL-SCALE PREPARATIVE SFC

As the general design of an analytical packed-column SFC instrument (Fig. 2) is always the same whatever the source [commercial or laboratory made from high-performance liquid chromatographic (HPLC) components], the equipment described in the literature is similar and differs only in the way fraction collection is performed: at atmospheric pressure; at high pressure; by adsorption on a solid followed by elution with a liquid solvent; or by dissolution in a liquid solvent.

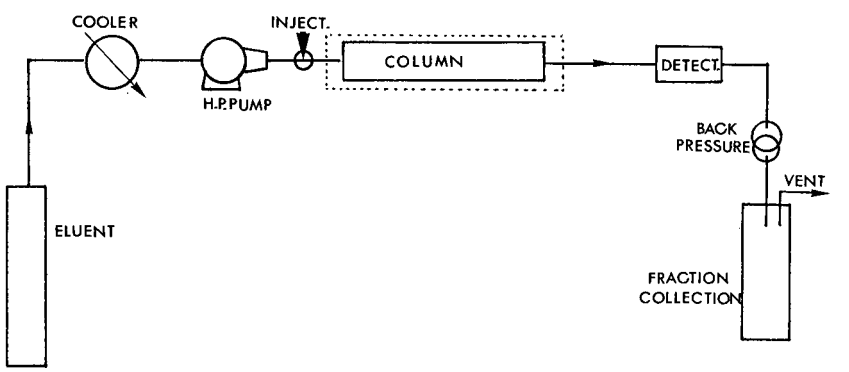

Fig. 2. General scheme of small-scale preparative SFC. H.P. = High-pressure; DETECT. = detection.

2.1. Collection at atmospheric pressure

This was first been used with mobile phases that are liquid at room temperature. As the pressure is reduced to atmospheric pressure by means of a capillary restrictor or a micrometric valve, liquid fractions can be collected at the outlet with a conventional LC fraction collector[3]. However, with mobile phases that are gaseous at room temperature and atmospheric pressure, fractions can also be collected directly at the outlet of a capillary restrictor or a back-pressure regulator, provided that the flow-rate remains relatively low and that the pressure reducer is sufficiently heated[4,5]. In most instances and particularly for volatile compounds, a special design of traps will be required to ensure efficient condensation of the products. An example was given by Flament and Keller[5].

2.2. Collection at high pressure

The first attempts at collection at high pressures were made by Gouw and Jentoft[3] for collecting solutes dissolved in gaseous eluents, such as carbon dioxide or nitrous oxide. They developed an original system, consisting essentially of a rotating LC fraction collector mounted in a high-pressure container pressurized with nitrogen. The pressure in the container was adjusted so that the emerging mobile phase was still a liquid at the detector outlet. This liquid could then be evaporated, whereas the solutes were left as dry residues in test vials placed in the fraction collector. However, this type of device has rapidly been replaced by a more convenient system, consisting of a series of high-pressure vessels, selected by a switching valve. The vessels used by Campbell and Lee[6] were pressurized with nitrogen and cooled to about 3°C, so that solutes could be collected by slowly reducing the pressure using a micrometric valve.

Other workers have used the knowledge acquired with small-scale supercritical fluid extraction for trapping pure components in high-pressure separators after pressure reduction and for recycling the eluent after cooling and recompression[7,8]. This approach will be useful for larger-scale preparative SFC.

Alternative methods described include adsorption of the solutes on a solid followed by elution with a liquid solvent[9,10] and dissolution in a liquid solvent in which the solutes dissolve preferentially[11].

2.3. Applications

Like other preparative chromatographic methods, preparative SFC is useful for the production of pure fractions when verification of the separation, identification (by mass, NMR or IR spectrometry) or thermodynamic studies are required.

Most of the early preparative studies, such as those of Gouw and Jentoft[3], were carried out to demonstrate the feasibility of SFC by proving first that the observed peaks were not artefacts and second that no degradation was occurring in the column. The collected fractions were either recombined and run again or identified by mass spectrometry[11–15].

To check the quality of flavour and essential oil (black pepper and clove extract) fractionations, Flament and Keller[5] developed an original two-dimensional chromatographic method by coupling SFC and thin-layer chromatography (TLC) (Fig. 3).

Preparative SFC can also be considered as a final enrichment step for trace analysis (off-line quantification or identification), as reported in Table 1[3,5,6]. Moreover, coupling with supercritical extraction or solute recycling can be performed to concentrate the solute and to increase the resolution of the separation[4,16].

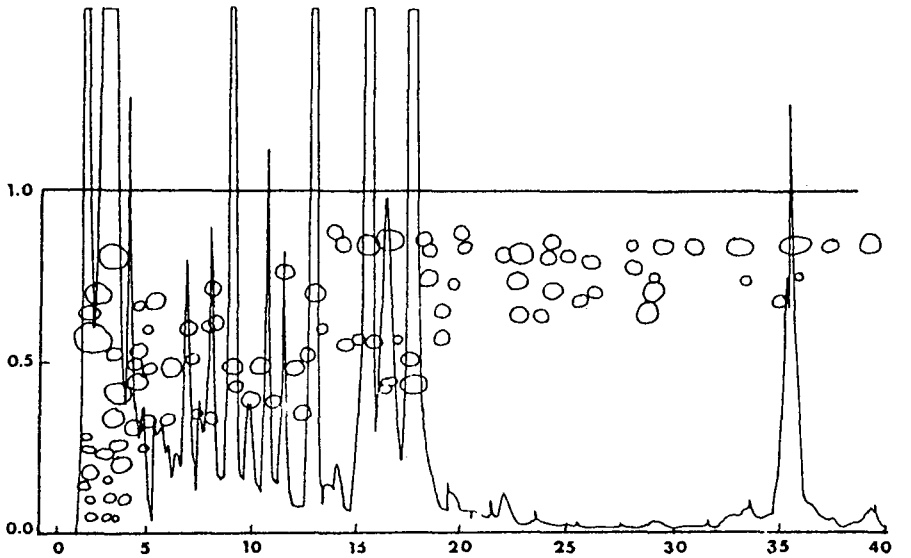

Fig. 3. Fractionation of clove essential oil by combined SFC–TLC. SFC conditions: column, C_{18}, 25 × 1 cm I.D.; CO_2 flow-rate, 4 ml min^{-1} at 32 MPa, 40°C; modifier, methanol (5%); laser detection at 150°C. TLC conditions: silica gel plate; Eluent, hexane–ethyl acetate (3:2). Horizontal axis: time in min; vertical axis: laser detector signal and TLC chromatogram. Reproduced from ref. 5, with permission.

3. LARGE-SCALE PREPARATIVE SFC

Large-scale preparative SFC seems to be a promising method for producing fractions free from solvent and, at first sight, it is surprising that there have been so few reports on semi-industrial preparative SFC separations. In practice, it turns out to be very difficult to cope with many of the problems, such as eluent recycling, eluent–product separation, periodic injection of the feed, fraction collection and column technology, all of which are much more crucial than on a smaller scale. However, we can mention here the work done by Khosah[18] on the laboratory scale on elution with a supercritical fluid of compounds adsorbed on a porous material (natural or not, mineral or polymeric). Khosah discussed the technical and economic feasibility of the process on the pilot scale. Alkio *et al.*[7] more recently published some results obtained with a preparative SFC unit built by conversion of a supercritical fluid extractor. Columns of 0.3–2 l could be mounted in this unit, which were swept at flow-rates of up to 8 kg/h of liquid carbon dioxide. However, both devices lack experience and automation and industrial development in the near future is unlikely.

Promising results have been obtained in our laboratory since 1982. We have built a fully automated pilot unit, which can accommodate columns of I.D. up to 6 cm and lengths up to 1 m. The eluent can be totally recycled and its flow-rate through the column can reach 50 kg/h[19]. A general scheme of the process is shown in Fig. 4. The first studies carried out with a synthetic mixture of naphthalene derivatives showed the feasibility of the process, its good reliability and good stability of the hydrodynamics. Some technological difficulties regarding eluent–product separation and column

TABLE I

EXAMPLES OF SMALL-SCALE PREPARATIVE SFC FRACTIONATIONS

Solute	*Eluent*	*Column*	*Recovery*	*Ref.*
Benzo[*a*]pyrene, benz[*a*]anthracene in automobile exhaust or refinery solid waste	CO_2	Alumina	Concentrate for off-line quantification	3
Polyaromatic hydrocarbons from mineral oil	CO_2	Alumina	Concentrate for chromatographic analysis	3
di-n- and di-isobutyl-phthalate (1 mg)	CO_2	Bare silica (5 μm), 1 cm I.D.		4
Black pepper extract	CO_2–methanol	C_{18}, 1 cm I.D.	TLC coupling	5
Clove extract	CO_2–ethanol	C_{18}, 1 cm I.D.	TLC coupling	5
Coal tar (10–20 g)	CO_2	Bare silica (40–63 μm) or NH_2-bonded silica (30–70 μm), 4.6 mm I.D.		6
Mineral oil distillate (35 g)	CO_2	Molecular sieve 5 Å	1.7 g isoalkanes, 1.65 g *n*-alkanes	8
Styrene oligomers (100 mg)	*n*-Pentane–methanol	Porasil A, 5 mm I.D.	20 mg of 15 pure oligomers	9,15
Styrene oligomers (35 mg)	CO_2	Porasil C, 2.6 mm I.D.		12–14
	CO_2	Reversed-phase, 4.6 mm I.D.	100 mg	17

efficiency were encountered and led to the design of high-performance separators[20] and to the adaptation of axial compression[21] to preparative SFC. We also investigated the possibility of adding a modifier, which would open the field of preparative SFC to more polar molecules[22].

The real potential of the process has been shown to be in the area of industrial separations. The purification of a vitamin intermediate and the fractionation of polyunsaturated fatty acid esters that may be useful in the treatment of cardiovascular and heart diseases [eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) esters], were chosen as illustrative examples. The purification of a vitamin intermediate has been carried out on a 60 × 6 cm I.D. silica column using a carbon dioxide–methanol mixture as the eluent. Very high purities (>99%) could be achieved, but the yields were relatively low[23,24].

Regarding fatty acid ester fractionation, the first attempts at using an axial compression column led to a pure EPA fraction (>90%) with a relatively high yield

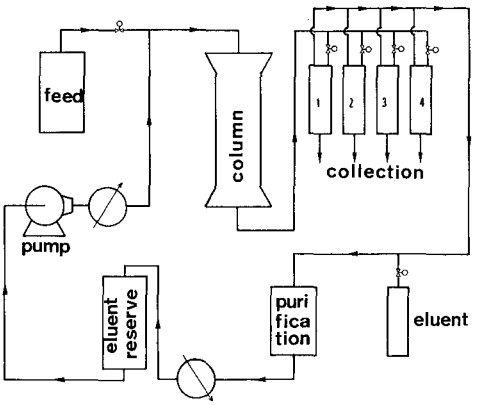

Fig. 4. General scheme of a large-scale preparative SFC unit.

(*ca.* 15 g/h) and to a DHA fraction (80%) with a yield of up to 20 g/h. These results were obtained on a 22 × 6 cm I.D. silica column with pure carbon dioxide as the eluent; a comparison with results obtained by HPLC indicated that preparative SFC will be a competitor to HPLC in the near future for this type of separation[24].

4. FUTURE DEVELOPMENTS

Small-scale preparative SFC can be very useful for purifying small amounts of key products and for the identification of the main impurities. It has a wider range of applicability than gas chromatography (GC) (extended to heavy compounds) and it is much faster than HPLC. However, although several collection device are discussed in this paper preparative, no SFC apparatus has reached the stage of commercial availability, apart from that from JASCO[4,16], which does not seem to be reliable and quantitative. Special attention must be paid to pressure reduction, eluent–product separation and fraction recovery for preparative SFC to become a quantitative method of fractionation.

Sample preparation by preparative SFC seems to be of a great interest for many studies, but it does not seem to be the most convenient method for trace analysis, for reasons given in this survey. Direct coupling of supercritical extraction with capillary SFC and with mass or infrared spectrometry would be preferable and would give a better performances for this kind of study.

Large-scale preparative SFC, on the other hand, is just reaching commercial development and seems to be very promising for the final purification or fractionation of fine chemicals or natural compounds. Unlike preparative GC, heavy, thermally labile compounds can be treated, and unlike preparative HPLC, the products can be recovered free from solvent and thus be directly usable for tests, reactions or final uses. Moreover, the coupling of supercritical fluid extraction and preparative SFC makes it possible to produce highly purified substances without any contact with organic solvents, which is of great interest for the pharmaceurical industry. However, the necessary use of a modifier (co-solvent) for the elution of polar compounds reduces the interest in preparative SFC for application to the fractionation of such compounds although, even in this instance, solvent removal is often easier than it is in HPLC.

5. SUMMARY

Preparative SFC has been investigated since the first development of SFC in order to collect pure components for identification (1962) and new devices for sample collection have been investigated. During the 1980s, SFC has come of age as an analytical tool complementary to GC and HPLC. Similarly, preparative SFC is being developed as a large-scale production process in parallel with recent developments in preparative GC and HPLC.

The various versions of preparative SFC that have been proposed, differing essentially in fraction collection and eluent recycling, have been surveyed. Future developments of both small- and large-scale preparative SFC are discussed in comparison with competitive GC and HPLC processes.

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Review

Coupling of supercritical fluid extraction with chromatographic techniques

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CONTENTS

1. INTRODUCTION

The growing interest in supercritical fluid extraction (SFE) is highlighted by the many monographs and reviews which have appeared in the literature since 1978[1–16]. The ability of a supercritical fluid (SF) to solubilize solids was first reported by Hannay and Hogarth[17] in 1879, when they noted that metal halides became soluble in supercritical ethanol as pressure was increased. Studies of solubilities in SFs continued during the following decades on a sporadic basis. An admirable review by Booth

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and Bidwell[18] covers this research up to 1949. About this time SFE was being scrutinized closely for its potential to reduce energy costs in process engineering, compared with conventional separation processes such as distillation or solvent extraction. Work by Kerr-McGee Refining (USA) led to the Residual Oil Supercritical Extraction (ROSE) process being developed in the 1950s for the removal of lighter products from the residue of the commercial distillation of crude oil[8]. Numerous other process applications were developed[2,10,15]. In the 1970s, foodstuffs became the centre of focus for SFE. Many patents resulted from these early studies, covering the SFE of hops, coffee, tea, tobacco and spices. In 1979, Hag (F.R.G.) built the first large-scale production plant using SFE to remove caffeine from green coffee beans[13].

In the 1980s, increasing attention was given to the use of SFE as a means of sample preparation in analytical chemistry. The main advantages of SFE are (i) an improved efficiency, as extraction times, in comparison with Soxhlet extractions, are reduced from hours to minutes; (ii) the use of a non-toxic and cost-effective extraction solvent (carbon dioxide); (iii) the potential of extracting thermally labile compounds; (iv) the simplicity of controlling the extraction conditions; (v) the ease of separating the analytes from an SF; (vi) the possibility for direct analysis of complex matrices, thus reducing the risk of sample contamination; (vii) the potential of fractionation; (viii) the compatibility of the method with on-line methods; and (ix) the possibility of class-selective extraction by choosing the proper fluid polarity, density and/or entrainer. In general, the transport properties of the SFs, high diffusivity and low viscosity, favour high mass-transfer rates and the low fluid density facilitates phase separations in solid–fluid or liquid–fluid operations. Excellent qualitative and quantitative results have been documented, confirming the potential of SFE when coupled with various methods of analysis.

2. PRINCIPLES OF SFE

SFs possess physico-chemical properties intermediate between those of liquids and gases (Table 1)[19,20]. The density of an SF is typically 100–1000 times higher than that of a gas and comparable to that of a liquid. Consequently, molecular interactions can be strong owing to short intermolecular distances[21]. As a result, the solvation properties are similar to those of liquids, but with significantly lower viscosities and higher diffusion coefficients. The 10–100 times lower viscosities and the 10–100 times higher diffusion coefficients in SFs compared with liquids result in a significantly enhanced mass transfer of solutes in extractions with SFs than in extractions with liquids[1,15,22].

TABLE 1

PHYSICAL PROPERTIES OF SUPERCRITICAL FLUIDS, LIQUIDS AND GASES

Property	*Gas*	*Supercritical fluid*	*Liquid*
Density (g/ml)	10^{-3}	0.2 – 0.9	1.0
Viscosity (g/cm·s)	10^{-4}	10^{-4}–10^{-3}	10^{-2}
Diffusity (cm^2/s)	10^{-1}	10^{-3}–10^{-4}	$<10^{-5}$

The potential advantages of SFE accrue from the physico-chemical properties of the SFs[23], *viz.*, large changes in SF density (and hence solvating power) can be effected by small changes in pressure, because the compressibility of SFs is large just above the critical temperature (T_c)[10]. As the solvent strength of an SF is directly related to its density, the solvating ability of an SF towards a particular species can easily be modified by changing the extraction pressure (and, to a lesser extent, the temperature)[24]. SFs thus have "tunable" solvent strengths which make selective extraction possible. With the greatly enhanced mass-transfer properties in comparison with liquids, the use of SFs provides more rapid extraction rates and improved extraction efficiencies owing to better penetration of the matrix[25]. In addition, SFs do not have the surface-tension or wetting problems associated with liquid extraction[17].

Schneider[1] made the important point that the solvent power of an SF cannot exclusively be explained from its density increase, and Giddings *et al.*[26] elaborated this by stating that the solvent power of an SF has two facets, a "state effect" and a "chemical effect". The principal variable of the "state effect" is the density of the SF, whereas the "chemical effect" is unique to each solute and dependent on its polarity, acid–base properties and hydrogen-bonding properties. Some general rules regarding the observed relationship between the extractability with supercritical carbon dioxide and the chemical structure of model substances from various groups of naturally occurring materials have been formulated by Stahl[27].

In practice, the choice of the SF depends on the polarity of the analyte(s), the solvent strength and selectivity required, the thermal stability of the extracted compound at the necessary operating temperature and the instrumental limitations which are associated with the high critical pressures (P_c) of some of the SFs. Usually the SF is applied at a temperature higher than its critical value and at a pressure significantly higher than the critical pressure of the fluid[12]. A considerable variety of SFs have been used in SFE covering a wide range of critical temperatures and pressures, molecular size and polarity[7,14]. Among these carbon dioxide is the fluid most frequently used. It has a moderate critical pressure (73.8 bar) and, with its low critical temperature (31.1°C), it is ideal for the extraction of many thermally labile compounds. However, carbon dioxide has its limitations, especially for the extraction of polar compounds.

A way to increase the polarity of an SF extraction solvent is to add small amounts of polar liquids (*e.g.*, acetonitrile, methanol or water), which are referred to as entrainers in SFE[11,13,28] and as modifiers in SFC[19]. The effect of adding as little as 1 mol-% of entrainer to an SF can be dramatic and the solubility may be increased by several orders of magnitude. This increase appears to be restricted to solutes with a certain chemical functionality (polarity)[11]. It should be noted that the addition of entrainers will also change the critical properties of the mixture; these can be approximated by the equations used by Reed and Sherwood[29]:

$$T_c = X_a T_a + X_b T_b \quad (1)$$

$$P_c = X_a P_a + X_b P_b \quad (2)$$

Where T_c and P_c are the critical parameters for the mixed extraction solvent, X_a and X_b are the mole fractions of the solvent A and B, T_a and T_b are the critical temper-

ature of the solvents A and B, respectively and P_a and P_b are the corresponding critical pressures. More elaborate treatments are based on the methods of Cheuh and Prausnitz[30] for T_c and Kreglewski and Kay[31] for P_c. The use of different extraction pressures, entrainers and fluids with varying polarity is particularly valuable in allowing "class-selective" extraction methods to be developed[24].

To determine if an extraction process of interest is practically feasible, it is necessary to have an adequate quantitative representation of the phase equilibria for the extracted compound(s) and fluid(s) involved[1]. Without this information, process models cannot be made, and operating conditions, solvent flow-rates and extraction yields cannot be predicted[13]. A great deal of effort has been focused on correlating, and in some instances predicting, the solubility of various solutes in SFs using basic thermodynamics. In addition, extensive work has been carried out to depict the phase diagrams to allow a better understanding of the pressure–temperature domains which may be of interest in SFE[1,5,12,13,32–34].

Because SFE combines the processes that are involved in distillation and in liquid–liquid extraction, SFE has also been termed "destraction"[35]. Randall[4] preferred the term "dense gas" for SFs to emphasize the fact that the most important parameter in SFE is, in fact, neither the absolute pressure nor the absolute temperature, but the density.

3. SFE IN ANALYTICAL APPLICATIONS

3.1. General aspects

Until recently, the use of SFE has generally been confined to relatively large-scale chemical processing applications[7,8,10,11,14,15]. SFE is now also attracting increased attention for analytical purposes. SFs exhibit a large compressibility above their critical temperature, and small changes in pressure result in large changes in density and, therefore, in a variable solvating power of the SF. In addition, various SFs (or entrained SFs) that exhibit different specific chemical interactions can be used for selective extractions with efficiencies comparable to or better than those of conventional techniques. Other positive features of SFE in analytical applications are (i) its potential to reduce the sample preparation time, which results in faster analyses, reduced costs and greatly reduced sample and solvent consumption; (ii) the ability to analyse complex matrices directly and thus minimize contamination in work-up; (iii) the ease of separating an analyte from the SF; (iv) the possibility of fractionating during collection; (v) running multiple concurrent analyses from the same extraction or concentrate during decompression (focusing); and (vi) compatibility with on-line methods of analysis[13,24,25,36–38]. A logical extension of SFE is to combine the process with chromatographic techniques such as thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), gas chromatography (GC) or supercritical fluid chromatography (SFC), so that sample preparation and analysis are instrumentally linked.

Safety must also be an important consideration. The complete SFE system, *i.e.*, the pump, extraction vessel, connecting tubing, inlet and outlet fittings, valves and in-line monitoring detectors, must be able to withstand the high pressures used in SFE. Saito *et al.*[39] recommend testing each item by pressurizing it prior to use with water at 1.5 times the maximum intended working pressure. They also provide useful

examples of calculating the minimum wall thicknesses of high-pressure vessels and tubing. Of course, one must also consider the nature of the SF (caustic, toxic, etc.) and any likely chemical reactions with the solutes. For example, nitrous oxide is a strong oxidant and for extractions involving large amounts of easily oxidized material, particularly at elevated temperatures, an explosion hazard exists[40] and ammonia is extremely toxic and corrosive[41].

3.2. Closed- and open-loop systems

Analytical SFE systems utilize either a closed- or an open-loop system. In the closed-loop system the SFE vessel is raised to the desired extraction pressure and a solubility equilibrium is allowed to be reached. This can be done in a static manner or by recirculating the SF through the closed-loop system. A sample can be diverted at any time to another analytical device by valve switching. The advantage of a closed-loop system is that parts of the extract of one sample can be taken for concurrent or consecutive analysis with virtually no difference in extraction profile. The use of a recirculating pump is likely to decrease the time required for the extraction equilibrium to be reached, although a disadvantage is that the whole system, including the pump, can become contaminated with the extract, necessitating extensive and time-consuming cleaning if another sample needs to be run. Another disadvantage of the closed-loop system is that only a fraction of the total extract is taken for analysis.

In the open-loop system, the SF passing through the sample is fed through a detector, usually an ultraviolet or flame-ionization detector and then led to waste. At periodic intervals the extract-laden SF can be led to other devices by means of valve switching (dynamic sampling). By utilizing an SFE density programme, selective fractions (dynamic fractionation) can be obtained or, alternatively, the entire extract can be analysed by connecting the extraction module with the analytical device right from the start of the SFE process, as is often done in SFE–GC, which will be discussed below. In trace analyses either a closed- or an open-loop system can be applied provided that some form of extract concentration is possible.

3.3. Isolation of extracts

In an SFE process, one or more components have to be removed from the sample matrix. The SF and sample are brought into intimate contact and compounds that are soluble in the SF are (selectively) extracted from the sample. After extraction, the extract can be separated from the SF in different ways[15]. Two methods are based on precipitating the components from the extract by reduction of the fluid density, *i.e.*, via (i) temperature increase (isobaric method) or (ii) pressure reduction (isothermal method). The third technique is based on the adsorption of the solute on an appropriate stationary phase.

In the isobaric method, the SF and the dissolved material move from the extraction vessel to a heat exchanger where the SF is heated. The result is a decrease in density and the dissolved material precipitates and can be collected in a separation vessel.

The most common technique used for isolating the extract after SFE in analytical applications is depressurization. However, depending on the conditions, it is possible for analytes to nucleate and become entrained in the expanding gas to form an aerosol which can be easily lost into the atmosphere[25]. The effects of aerosol

TABLE 2

COMPARISON OF EXTRACTION RECOVERIES FROM XAD-2 RESIN USING DIFFERENT COLLECTION METHODS AND EXTRACTION CONDITIONS

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Analyte	*Recovery (%)*				
	Collection mode[a]		*Extraction conditions*[b]		
	Open	*Closed*	*CO_2 (150°C)*	*CO_2 (50°C)*	*CO_2–CH_3OH (80:20 mol/mol) (150°C)*
Chrysene	2.2	75	2.2	25	60
Benzanthrene	2.5	79	2.5	28	62
1-Nitropyrene	1.8	83	1.8	24	29
Dibenzo[*a,i*]carbazole	6.3	95	6.3	10	65
Coronene	8.2	81	8.2	6.5	62
Rubrene	0	25			

[a] Extraction conditions: CO_2 at 150°C and 410 bar with *ca.* 250 ml of liquid.
[b] Open collection applied. Extraction performed at 410 bar with 250 ml of liquid.

formation on analyte losses during collection are illustrated in Table 2, where a comparison is made between the extraction recoveries from XAD-2 resin using open collection and sealed collection. Open collection was performed in a narrow-necked flask cooled to 0°C and for sealed collection a liquid nitrogen-cooled flask was applied. As the volatility of the analytes involved was very low at 0°C, the most probable explanation for the low recoveries is aerosol formation. By freezing the extraction effluent in a sealed vessel, analyte losses through aerosol formation can be eliminated. As at higher temperatures (*i.e.*, 150°C) the expansion of carbon dioxide produces only a single gas phase and at lower temperatures (*i.e.*, 50°C) a two-phase system of carbon dioxide (410 bar) is produced, the formation of small solute particles (<0.2 μm) will dominate at higher temperatures, and low collection efficiencies may be expected in this instance. The higher recoveries for the carbon dioxide–methanol fluid can be explained by the formation of larger liquid–methanol droplets during expansion, which have larger deposition efficiencies owing to their size and liquid character. As all recoveries with open collection are lower than obtained by the closed-loop collection method, the differences are attributed to an improved collection efficiency. Raynor *et al.*[42] also observed the loss of the more volatile components (*e.g.*, naphthalene) during SF decompression and/or solute deposition in a microvalve loop. Hirata and Okamoto[43] noted that, in order to trap polymer additives after decompression of an extract-laden SF, the restrictor had to be connected to two trapping tubes in series, the first trap being empty and the second packed with silica. Most of the analytes were trapped efficiently in the first trap, but about one-third of the analytes were trapped on the silica. McNally and Wheeler[44] initially tried to adsorb sulphonylureas on two guard columns packed with C_{18} and silica. They observed that the major fraction of the analytes was deposited in the back-up collection flask, inserted after the guard columns, rather than in the columns themselves. Schneiderman *et al.*[45] found, in off-line SFE–HPLC studies, that the extraction recoveries of vitamin K_1 dropped

from 95.6% for a milk-based powder formula to less than a few per cent after SFE of a liquid formula. This was probably due to entrainment of water by supercritical carbon dioxide and, consequently, the silica becoming saturated with water and being rendered ineffective for trapping of vitamin K_1.

3.4. Monitoring of extracts

Coupling of SFE with analytical techniques can be performed in both the off-line and on-line modes. In order to optimize on-line SFE procedures, it has been recommended[46] that first the recoveries of the analytes should be measured by means of off-line SFE. In this way, parameters such as (i) pressure and temperature, (ii) polarity of the SF, (iii) volume of the SF per unit time, (iv) volume and dimensions of the extractor, (v) extraction time and (vi) amount of the sample can be optimized.

SFE can be coupled with a variety of detection and separation techniques. One of the oldest and, by modern standards, crudest techniques for determining the extraction yield is a gravimetric analysis, in which the mass of the extract and the mass of the sample are compared with each other[6,47]. Using this technique, Krukonis[6] illustrated the increased dissolving power of SFs at higher densities. SFE of ground ginger using carbon dioxide at 50°C produced a 9% yield at 335 bar, and only a 1% yield at 100 bar. The problem in using gravimetric analysis is that, intrinsically, it is an off-line process, which means extra sample manipulations and thus an increased analysis time and higher costs. A number of dedicated SFE instruments are now commercially available either as stand-alone devices or coupled to another analytical instrument whereby on-line monitoring of the SFE procedure is performed by means of ultraviolet (UV) or flame ionization detection (FID).

Monitoring by UV detection requires the presence of a chromophore in the extracted analytes. This illustrates one of the other advantages of using carbon dioxide as the SF as it is transparent down to *ca.* 190 nm. Wright *et al.*[48] used a closed-loop system with recirculation and on-line UV detection to monitor the effects of ultrasound during the extraction of chrysene from adsorbents or of caffeine from roasted coffee beans. They observed an enhanced extraction rate for caffeine, probably caused by inducing a convective flow through the pores of macroporous materials. Ultrasound with a frequency of 20 kHz did not improve the desorption of chrysene from the micropores of the adsorbents.

By using absorbance detection, in contrast to FID, spectra or functional group information can be obtained[49]. A fibre-optic monitor operating between 400 and 750 nm has been applied for the on-line detection of a blue dye in olive oil, solubilized by supercritical carbon dioxide. As fibre-optic monitor systems are also available for wavelengths in the UV and (near-)infrared range this technique may become increasingly important in coming years.

In addition to UV detection, mass spectrometric (MS) detection is a highly valuable alternative for obtaining structural information. The use of a direct fluid injection interface for MS detection was reported by Smith *et al.*[36] for both qualitative and quantitative solubility measurements of complex mycotoxins of the trichothene group in supercritical carbon dioxide or nitrous oxide. Kalinoski *et al.*[50] used on-line SFE with chemical ionization MS detection and collision-induced dissociation tandem MS (MS–MS) for the rapid identification of ppm levels of several trichothene mycotoxins with minimum sample handling. A limitation of SFE–MS is

the possible overloading of the mass spectrometer with co-extracted compounds when complex samples are analysed[50]. The result is that often sophisticated techniques such as tandem MS may be necessary to obtain the required selectivity and sensitivity. A cheaper and more attractive alternative is to perform some form of chromatography between extraction and detection. This coupling of SFE with various chromatographic techniques will be discussed extensively in the following sections.

4. COUPLING OF SFE WITH CHROMATOGRAPHIC TECHNIQUES

4.1. SFE–TLC

In 1976, Stahl and co-workers[27,51–55] developed a mini-extraction apparatus for the desorption of an SF extract on a moving thin-layer chromatographic (TLC) plate. Both carbon dioxide and nitrous oxide were used as SFs and a wide range of naturally occurring materials (*e.g.*, coffee, dye mixtures, seeds, sage, leaves, ginger, flowers, pepper, chilies, hops, marijuana, vitamin oils and alkaloids) were studied. The apparatus (Fig. 1) consisted of a thermostated diaphragm compressor to attain the desired pressure for the SF[27]. Subsequently the SF flowed into a micro-extraction autoclave, the exit of which was sealed with a cut-off valve. After opening this valve the SF flowed via a narrow capillary onto the moving TLC plate, which was held horizontally at a distance of 1–5 mm from the capillary tip. Extraction of the sample was started at 70 bar and a fixed volume of SF was allowed to flow through. After moving the plate, the pressure was increased stepwise by 5, 10 or 20 bar and the sample was again extracted with the same volume of SF. By comparing zone intensities it was possible to observe whether an increase in the pressure resulted in more or less extraction of an analyte (Fig. 2).

On-line SFE–TLC provides a rapid and simple insight into the extraction performance. Its strength is that the extract is deposited on a plate, which means that detection is a static process. Both one- and two-dimensional chromatography can be performed, *i.e.*, SFE can be combined with a development of the TLC plate in one or two directions, after which the components of interest can be detected on or isolated from the support material for further study (Fig. 2). Limitations of SFE–TLC are that quantification is difficult and that the stability of components on the support

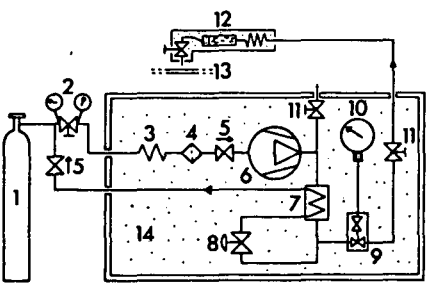

Fig. 1. Schematic diagram of the apparatus for fluid extraction coupled directly with TLC. 1 = Steel cylinder; 2 = reducing valve; 3 = pre-heating coil; 4 = filter; 5 = check valves; 6 = diaphragm compressor; 7 = heat exchanger; 8 = back-pressure regulator; 9 = damping parts; 10 = precision manometer; 11 = shut-off valves; 12 = micro-autoclave for extraction; 13 = TLC receiving layer; 14 = thermostatically controlled container. (Reprinted with permission from ref. 27.)

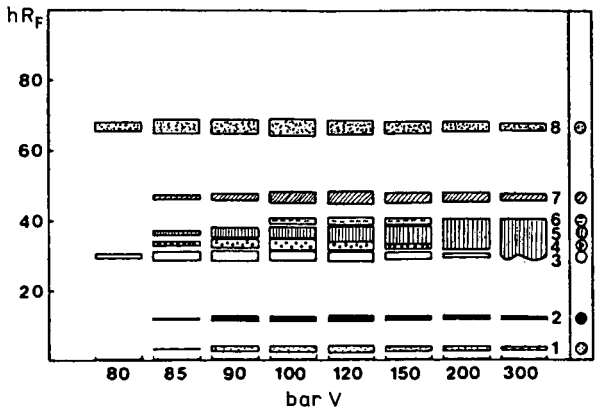

Fig. 2. Thin-layer chromatogram after fluid extraction of a vitamin oil mixture (200 μg each). 1 = Cholesterol; 2 = vitamin D_3; 3 = vitamin K_3; 4 = α-tocopherol; 5 = triglyceride; 6 = vitamin A acetate; 7 = α-tocopheryl acetate; 8 = steryl ester. (Reprinted with permission from ref. 27.)

material or in the presence of oxygen may be a problem. Further, the resolution of TLC is low compared with that of HPLC, GC and SFC, and at high pressures (> 30 bar) problems are encountered such as stripping of the support material by the SF caused by the increased velocity of the expanding fluid.

4.2. SFE–HPLC

4.2.1. Off-line. Various off-line SFE–HPLC analyses have been reported[37,39,56–59]. The effect of different extraction parameters on the amount of caffeine extracted from roasted coffee beans using supercritical carbon dioxide was studied by Sugiyama *et al.*[56]. A closed-loop SFE system with recycling was applied. The trap column was packed with activated carbon and the trapped analytes were eluted, in the off-line mode, with methanol–water (55:45, v/v). Finally an aliquot was injected into the HPLC system. In Fig. 3 the effects of various parameters on the extraction yield are illustrated. The amounts of caffeine extracted are represented as percentages of the amount extracted with hot water, *i.e.*, as percentages of the caffeine level in drinking coffee. The recovery increased with increasing extraction pressure and time, and decreased rapidly with increasing temperature. Above 60°C caffeine was hardly extracted, owing to the diminished solubility of the analyte in carbon dioxide as a result of a decrease in density. Furthermore, reduction of the percentage of water resulted in a decreased recovery, which is in good agreement with other data suggesting that water is essential for the mass transfer of caffeine when carbon dioxide is used as the extraction fluid[11]. High recoveries were found when using a pressure of 200 bar, a temperature of 48°C, 20% of water in the SF and an extraction time of 60 min.

Schneiderman *et al.*[45] extracted vitamin K_1 (phylloquinone) from commercial soy protein-based and milk-based powdered infant formulas using supercritical carbon dioxide at 544 bar and 60°C. Quantitative extraction required only 15 min, whereafter the SF was depressurised, the extracted vitamin K_1 trapped in a short tube packed with silica and eluted off-line with a mixture of dichloromethane and acetone. After removal of the solvent, the residue was dissolved in the eluent and determined

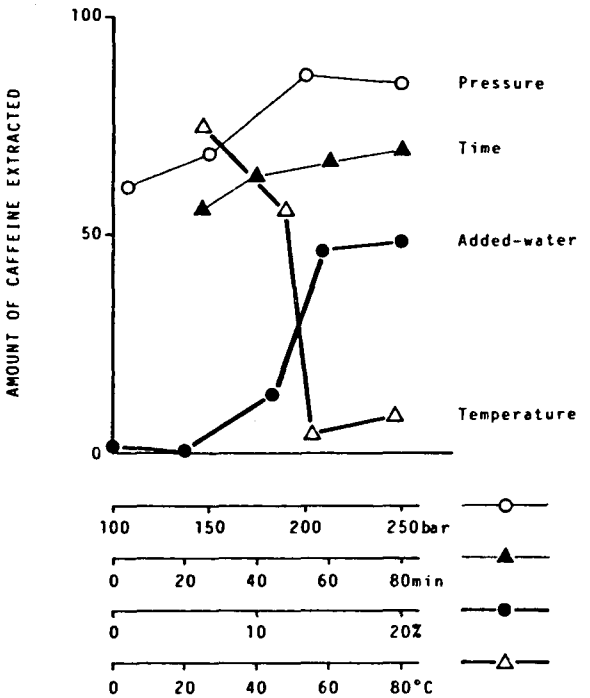

Fig. 3. Percentage of caffeine extracted from roasted coffee beans under various conditions with hot water. ○ = Various pressures with the added water, temperature, and extraction time constant at 20%, 48°C and 60 min, respectively; ▲ = various extraction times with other parameters constant at 150 bar, 20% and 48°C; ● = various amounts of water added to coffee powder with other parameters constant at 150 bar, 48°C and 60 min; △ = various temperatures with other parameters constant at 150 bar, 60 min and 20%. The temperature and the amount of added water have significant effects on the extraction, as shown by the heavy lines. (Reprinted with permission from ref. 56.)

by reversed-phase HPLC with electrochemical detection[45]. The minimum detectable amount was 80 pg and the linear dynamic range was at least five orders of magnitude. The recovery of vitamin K_1 from a milk-based powder was 95.6% with a relative standard deviation (R.S.D.) of 7.4% and from a soy protein-based product 94.4% with an R.S.D. of 6.5%. The same group[57] used the same technique, with comparable results, for the determination of anthraquinone from Kraft paper and pine plywood sawdust, and vitamin K_3 in rat feed[58].

Hirata and Okamoto[43] extracted polymer additives from polyethylene and polypropylene using supercritical carbon dioxide at 250 bar and 35°C. After decompression the analytes were collected in a microtrap filled with silica, held at 60–80°C to maintain a constant flow-rate, and subjected to microcolumn HPLC.

Ndiomu and Simpson[59] used SFE with carbon dioxide to isolate morphine and quinine from various plant materials. The recoveries were determined using off-line HPLC. The extraction was performed by heating of a sealed extractor which had been filled with a certain amount of dry-ice. The results compared favourably (higher recoveries in less time) with those obtained by extractions with subcritical methanol and tetrahydrofuran and organic Soxhlet extractions. Solid-phase extractions, *e.g.*, of

blood samples spiked with 200 μg/ml of morphine, were compared with SFE in terms of percentage recovery. For ten replicate determinations with SFE, the average recovery was 96.7% (R.S.D. 3.2%), whereas the average recovery with solid-phase extraction was 92.2% (R.S.D. 4.0%). However, the time scale for the SFE analysis of the serum samples was excessive, because the aqueous nature of the serum samples first necessitated freeze-drying of the samples for 12 h. Supercritical carbon dioxide was not suitable for the efficient extraction of caffeine from kola nuts under the applied conditions.

Symmetrical triazine herbicides have been extracted from river sediment by supercritical carbon dioxide[60]. The extraction was performed in a 0.57-ml cartridge using a pressure of 230 bar and a temperature of 48°C. The extraction of 500 mg of sample was complete in about 30 min and the analytes were trapped via a capillary restrictor (30 cm x 25 μm I.D.) and analysed by reversed-phase HPLC using UV detection at 225 nm. The recoveries were in excess of 90% in the ppm–ppb[a] range.

Ehntholt *et al.*[61] studied the isolation and concentration of 23 compounds in the ppb range from aqueous samples with supercritical carbon dioxide. The analytes were dissolved in acetone and diluted with an aqueous solution containing sodium hydrogencarbonate, calcium sulphate and calcium chloride. The extractions were performed at 173 bar and 45°C and the extracts were analysed via off-line HPLC or GC. The recoveries for the various solutes were different. For biphenyl, a neutral and relatively non-polar solute, it was 23.4%; for methyl isobutyl ketone, as representative of aldehydes and ketones, 17.3%; for 2,4-dichlorophenol, an acidic phenol, 45.4%; for anthraquinone, an oxygen-containing heterocyclic, 84.6%; and inorganic sodium and calcium salts could not be extracted with this method. Finally, for caffeine, a nitrogen-containing heterocyclic, the recovery was 0%. Probably the low pH of the extraction medium (*ca.* 3) reduces the solubility of the nitrogen-containing solutes in the SF because of protonation. The last example shows that the pH of the extraction medium is an important parameter.

4.2.2. On-line. In 1983, Unger and Roumeliotis[62] described the first coupling device allowing on-line HPLC of SF extracts. The on-line system (Fig. 4) consisted of two high-pressure sample-injection valves connected in series. The first valve operated as a switching valve to the loop and controlled the pressure over a packed microbore column. Two short microbore columns packed with 5-μm LiChrosorb RP-18, positioned between the first and second valves, were used, respectively, to adsorb the analytes over a certain period of time and simultaneously to function as sample loop for the second valve which served as injector for the normal-phase HPLC column, packed with 5-μm LiChrosorb Si 100. The on-line SFE–HPLC system was used to monitor the extraction kinetics of valtrate from *Radix valerianae*. Using an open-loop system with supercritical carbon dioxide at 40°C and 96 bar, an exponential decay was observed for the extracted amount of valtrate with time. The extraction was complete in 1 h.

Recently, Nair and Huber[63] described the on-line SFE–HPLC analysis of ground tablets for ibuprofen. The SFE unit consisted of a constant-pressure pump to transfer the carbon dioxide to a preheater, a heated vial containing the sample, a

[a] Throughout this article, the American billion (10^9) is meant.

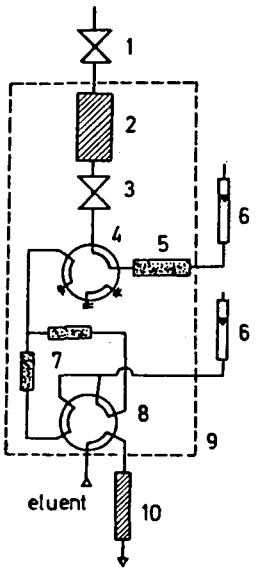

Fig. 4. Schematic diagram of the unit for coupling of SFE and HPLC. 1 = Back-pressure regulator; 2 = extraction vessel; 3 = high-pressure two-way angle valve; 4 = six-port external sample valve; 5 = packed microbore column for release and waste deposit; 6 = rotameter; 7 = microbore columns, serving for deposit and as loop; 8 = sample injector; 9 = thermostat; 10 = HPLC column. (Reprinted with permission from ref. 62.)

fixed-volume injection valve and finally an analytical column. The system mentioned was applied only for qualitative experiments. According to the authors, a fixed-volume recycle loop should be installed in order to obtain quantitative results.

4.3. SFE–GC

4.3.1. Off-line. Schantz and Chesler[22] extracted polychlorinated biphenyls (PCBs) from sediments and polynuclear aromatic hydrocarbons (PAHs) from transformer oil using supercritical carbon dioxide. The extract was trapped on a reversed-phase silica cartridge. Subsequently the trapped analytes were flushed off the cartridge with dichloromethane and, after partial evaporation of the solvent, the analytes were quantified by GC. The results of SFE were comparable to those of a Soxhlet extraction. Under the applied extraction conditions, *i.e.*, density 0.93 g/l, SFE of high-molecular-weight PAHs appeared to be more efficient than Soxhlet extraction (18 and 30% higher extraction values for benzo[*ghi*]perylene and indeno[1,2,3-*cd*]pyrene, respectively). SFE required only 30–60 min, whereas the dichloromethane extraction took 16 h. For more polar compounds the addition of an entrainer, such as methanol, to the SF appeared to be necessary[22].

Hawthorne and Miller[64] described an off-line SFE–GC method using supercritical nitrous oxide with 5% methanol as the entrainer for the extraction of PAHs from environmental solids. The extracted analytes were collected by inserting the outlet restrictor of the SFE system into a vial containing dichloromethane. Quantitative recovery of PAHs from urban dust and of deuterated PAHs from river sediment

and fly ash was obtained within *ca.* 30 min. The recovery of the deuterated PAHs was significantly better than that obtained after 4 h of sonication or 8 h of Soxhlet extraction with either benzene or dichloromethane. The same workers[24] described the rapid and quantitative recovery of PAHs from both solid samples and PAHs adsorbed on Tenax by means of off-line SFE–GC. The results of the class-specific extractions of alkanes and PAHs from diesel-exhaust particulates using different extraction pressures are given in Table 3.

Using a comparable system agrochemicals (*e.g.*, atrazine) and corresponding metabolites in soil samples were analysed[65]. In this study the solubilities of the various analytes in carbon dioxide were calculated using the Peng–Robinson equation of state.

The desorption characteristics of various materials were investigated by Wright *et al.*[25] and Raymer and co-workers[66,67] using off-line SFE–GC. Adsorbents such as XAD-2, polyurethane foam, Spherocarb and Tenax were systematically studied. Wright *et al.*[25] used a modified HPLC pump to pressurize and deliver the extraction fluids. The pump head and check valves were cooled by circulating an ethylene glycol–water mixture (-15°C) through a cooling-jacket. The pressurized fluid was transferred to the high-pressure extraction vessel using 1/16-in. stainless-steel tubing. The extraction vessel allowed operation at pressures of over 400 bar and temperatures higher than 200°C (Fig. 5). The extraction vessel was maintained at elevated temperatures in a GC oven. The transfer line was extended to the bottom of the extraction cell to allow the fluid to move through the sample from the bottom to the top and then to exit the extraction vessel. A stainless-steel frit (0.5–2.0 μm) was placed in the exit port of the extraction vessel to prevent the sample from being flushed out. The

TABLE 3

FRACTIONATION OF ALKANES AND PAHs DURING EXTRACTION WITH SUPERCRITICAL CARBON DIOXIDE OF DIESEL-EXHAUST PARTICULATES

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Species	*Proportion in fraction 1 (%)*[a] *(75 atm CO_2)*	*Proportion in fraction 2 (%)*[a] *(300 atm CO_2)*
n-Alkanes		
Nonadecane (C_{19})	86	14
Eicosane (C_{20})	84	16
Heneicosane (C_{21})	86	14
Docosane (C_{22})	85	15
Hexacosane (C_{26})	85	15
PAHs		
Phenanthrene	28	72
Fluoranthene	9	91
Pyrene	7	93
Benz[*a*]anthracene	ND[b]	>90
Chrysene	9	91
Benzo[*ghi*]perylene	ND	>90

[a] Relative standard deviations were generally <10% for the alkanes and <5% for the PAHs.
[b] Not detected

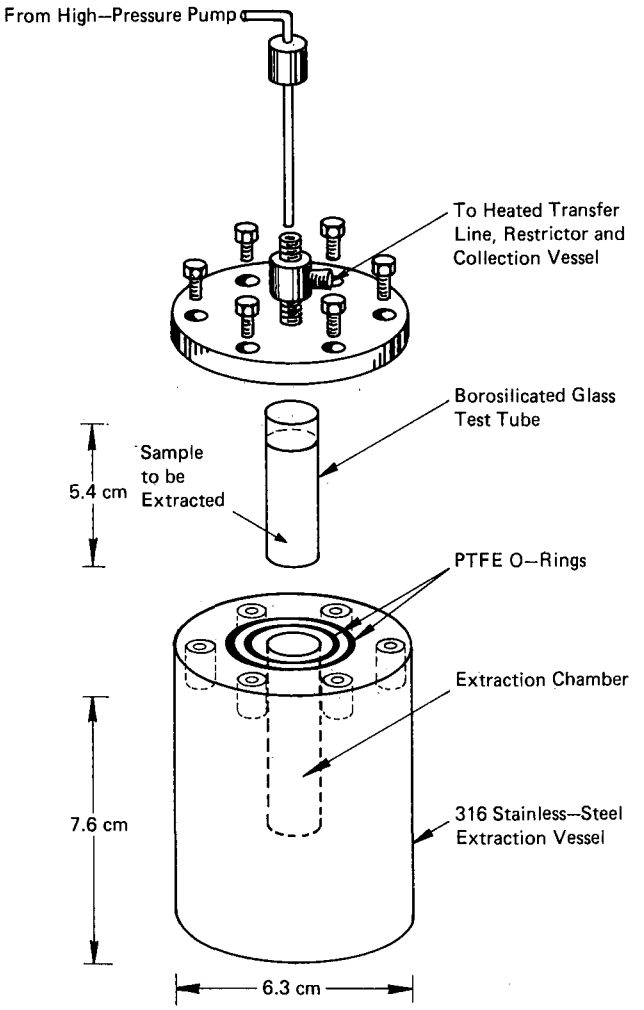

Fig. 5. Design of SFE vessel. (Reprinted with permission from ref. 25.)

extraction effluents were collected by freezing them in a sealed flask cooled in a liquid nitrogen bath. When pure carbon dioxide was used, the 50-μm stainless-steel restrictor tubing was heated by an electrical current to prevent it from freezing during the fluid expansion process in the cooled collection flask[25]. Extraction data for spiked XAD-2 resin obtained by Soxhlet extraction with dichloromethane and by SFE with various fluids are presented in Table 4. With Soxhlet extraction high recoveries were obtained for all model compounds. Similar recoveries were achieved with SFE for the low-molecular-weight compounds, but for high molecular weights, the recoveries diminished progressively. This behaviour was explained by the lower solubility of the higher molecular weight analytes in the SF and the relatively high temperatures and pressures used in this study. SFE with isobutane or with methanol-entrained carbon dioxide provided better overall extraction efficiency than with pure carbon dioxide.

TABLE 4

COMPARISON OF EXTRACTION OF XAD-2 RESIN USING SOXHLET EXTRACTION AND VARIOUS SUPERCRITICAL FLUIDS

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Compound	*Recovery (%)*						
	Soxhlet only[a]	CO_2[b]		*Isobutane*[d]		CO_2–CH_3OH[e]	
		SFE	*Soxhlet*[c]	*SFE*	*Soxhlet*[c]	*SFE*	*Soxhlet*[c]
Chrysene	79	84	0	86	5	88	3
Benzanthrone	86	98	0	110	0	88	0
1-Nitropyrene	93	81	0	70	0	98	0
Dibenzo[*a,i*]carbazole	88	54	22	96	6	97	0
Coronene	81	46	63	93	9	90	5
Decacyclene	85	6	88				

[a] Dichloromethane for 16 h.
[b] Carbon dioxide at 125°C and 400 bar with *ca.* 200 ml of liquid.
[c] Extraction with dichloromethane of the same sample after SFE.
[d] Isobutane at 150°C and 185 bar with *ca.* 300 ml of liquid.
[e] 20 mol-% methanol in carbon dioxide at 130°C and 400 bar with *ca.* 210 ml of liquid.

When entrainers were used, the transfer line was maintained at the same temperature as the oven. These SFEs were accomplished in *ca.* 30–45 min compared with 16 h for the Soxhlet extractions.

In another off-line study, Raymer and co-workers found that SFE was superior to thermal desorption techniques when applying supercritical carbon dioxide for the desorption of hexachlorocyclohexane, a hexachlorobiphenyl, anthracene and parathion from Tenax[66] and polyimide sorbents[67]. All compounds showed recoveries of over 90% from Tenax by SFE, whereas thermal desorption resulted in only a 13% recovery for hexachlorobiphenyl and parathion.

Off-line GC was used by Sugiyama and Saito[47] to compare quantitatively the amounts of components of lemon peel oil obtained by SFE and by cold-pressing. A photographic representation of the lemon peel before and after SFE was also included. Before extraction the oil-containing cells were clearly visible, but after extraction the oil had been drawn out of the cells, which now looked like craters. Obviously, the oil was not simply squeezed out by the pressure of the carbon dioxide, but carbon dioxide had diffused into the oil-containing cells, dissolving the oil and drawing it out of the cells, *i.e.*, the oil was extracted.

4.3.2. On-line. Hawthorne and Miller[24] were the first to couple SFE directly with on-line GC, when they successfully performed a qualitative analysis of automobile-exhaust organics collected on Tenax. Since then, the number of publications involving on-line SFE–GC has continued to increase[20,23,40,48,68–70]. Within this methodology Wright *et al.*[23] reported that several modes of operation are possible, such as quantitative extraction of analytes from a sample matrix, quantitative extraction and concentration of trace analytes and selective extraction at various solvating powers to obtain specific fractions by pressure or density programming.

A modular-design open-loop on-line SFE–GC system was described by Mapelli *et al.*[71]. The system consisted of an oven with an air recirculation system in which the extraction cell and two high-pressure valves were placed. The extraction cell had a volume of 0.4 or 1.5 ml. The coupling was achieved by transferring the contents of the loop to a capillary column by means of a splitting system. The interface was controlled by a heated transfer line, fixed at one end in the top of the extraction module. A fused-silica capillary passed through the heated interface, so that the restrictor penetrated inside. A make-up gas flow was supplied around the restrictor to dilute the decompressed fluid. In this way analyte losses were minimized and even the reconcentration of volatile analytes was possible.

The usefulness of on-line SFE–GC was well demonstrated for the extraction and selective fractionation of PAH standard mixtures[23]. The instrumentation is shown in Fig. 6. It consisted mainly of four sections, *viz.*, a high-pressure pump and extraction cell, a switching valve and interface region, GC with FID and an appropriate microcomputer for complete system automation. A PAH mixture, adsorbed on glass beads, was extracted for 1 min at three progressively increasing pressures and the effluent of each fraction was analysed by temperature-programmed capillary GC prior to the next extraction (Fig. 7). During each GC analysis, the extraction process was continued (*ca.* 75 min) with the effluent being vented to the collection reservoir. In this way, essentially all the material which was soluble at each pressure was extracted from the matrix prior to the next extraction step. Carbon dioxide was used as the extraction fluid. The extractions were performed at 50°C and at densities of 0.23, 0.62 and 0.78 g/ml. The extraction effluent was collected and concentrated on-column using a retention gap (deactivated fused silica, 30 cm × 0.53 mm I.D.) at 30°C, which proved to be adequate to focus the solute injection bands. Collection at higher temperatures or without the retention gap resulted in peak broadening and decreased resolution. Examination of the chromatograms showed that high-resolution separations of three essentially unique fractions of material were obtained. As expected, progressively higher molecular weight material was extracted at higher densities of

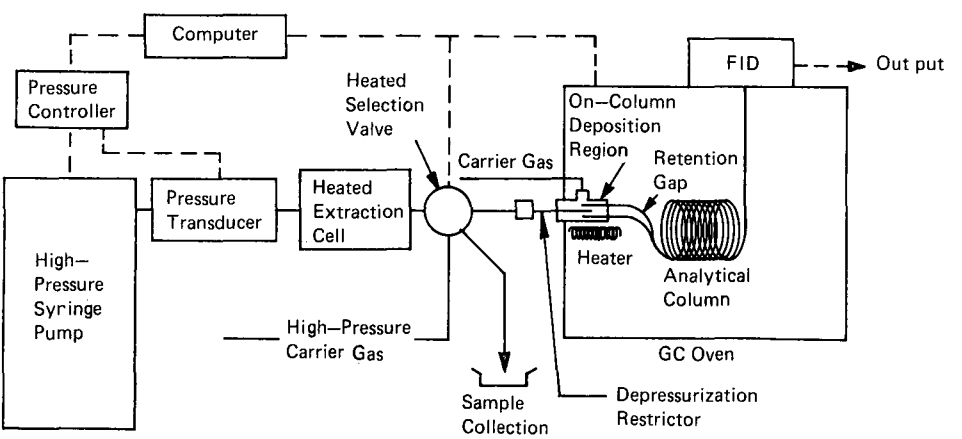

Fig. 6. Schematic of on-line SFE–capillary GC instrumentation. (Reprinted with permission from ref. 23.)

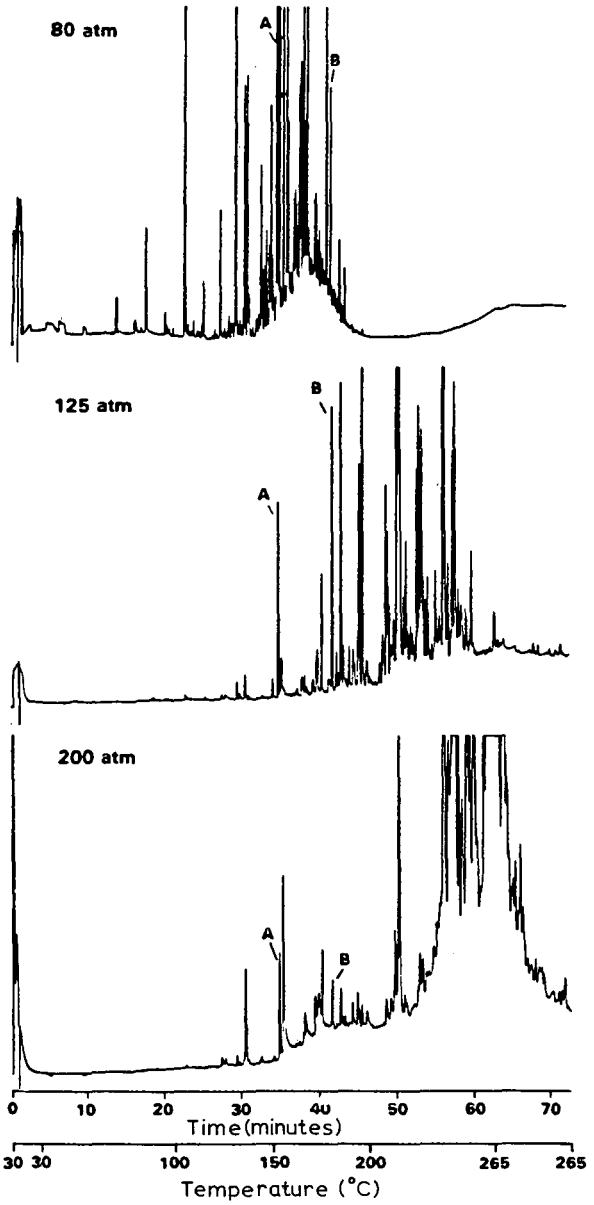

Fig. 7. Capillary gas chromatograms of PAH fractions obtained from supercritical carbon dioxide extraction of a complex matrix at various pressures. Compounds A and B are arbitrarily marked in each fraction to facilitate comparison. (Reprinted with permission from ref. 23.)

the extraction fluid. Although some overlap of components occured in the various fractions, the example demonstrates the potential of SFC–SFE for efficient on-line fractionation.

The same instrumentation was used for the quantitative analysis of orange peel at two different extraction pressures[48] and the potential of the technique was shown by determining PAHs adsorbed on XAD-2 resin.

A similar method for the direct coupling of SFE with GC was used by Hawthorne and Miller[40] for the analysis of environmental samples for PAHs and PCBs. Quantitative studies were performed using FID, electron-capture detection (ECD) and MS. The direct coupling of the SFE vessel with the GC column was accomplished by inserting the SFE outlet restrictor capillary (15 cm × 15–30 μm I.D. × 150 μm O.D.) into the GC column using an on-column injector port. The GC oven was cooled during the extraction to allow thermal focusing of the extracted analytes inside the GC column at the outlet of the SFE restrictor. Restrictors with larger internal diameters (*e.g.*, 30 μm) yielded higher extraction efficiencies in shorter times than restrictors with smaller internal diameters, but internal diameters larger than 30 μm were not practical, because the resulting flow-rates were too high for the pumping system. This meant that a compromise had to be found, because the internal diameter of the restrictor also affected the efficiency of the cryogenic trapping. Nitrous oxide was chosen as the SF in this study, because it is a gas at temperatures which are normally used for cryogenic trapping of organic species in GC columns and because it provided better extraction efficiencies for PAHs than carbon dioxide and ethane[64]. The feasibility of the direct coupling of SFE with GC–FID was confirmed by the analysis of 10 mg cigarette ash, which was extracted for 10 min with supercritical nitrous oxide at 45°C and 300 bar. The extracted species were collected in a wide-bore fused-silica capillary GC column (30 m × 0.32 mm I.D., 1 μm thick film of DB-5), by inserting the outlet restrictor of the extraction cell directly into the GC column via the on-column injector. The GC oven was held at 5°C during the extraction, allowing cryogenic focusing of the analytes at the top of the column. Next, the oven was rapidly heated to 50°C and the GC separation was performed using a temperature programme of 8°C/min to 320°C. Good agreement with the certified values for PAHs in urban dust from the National Bureau of Standards was found (Table 5). The values found for fluoranthene, benz[*a*]anthracene and benzo[*a*]pyrene were slightly higher than the certified values. As the certified values are based on 48 h extractions in a Soxhlet apparatus [both methylene chloride and benzene–methanol (1:1) were used as

TABLE 5

CONCENTRATION OF SELECTED PAHs IN NBS SRM 1649 URBAN DUST

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Compound	*Concentration (μg/g)*[a]	
	Certified	*SFE*
Fluoranthene	7.1 ± 0.5	8.0 ± 0.6
Benz[*a*]anthracene	2.6 ± 0.3	2.9 ± 0.5
Benzo[*a*]pyrene	2.9 ± 0.5	3.2 ± 0.3
Benzo[*ghi*]perylene	4.5 ± 1.1	4.4 ± 0.3
Indeno[1,2,3-*cd*]pyrene	3.3 ± 0.5	3.1 ± 0.2

[a] Data are given as average values ± standard deviations (n = 3).

solvents], the higher values obtained from 60-min SFE may be the result of an increased extraction efficiency.

In more recent on-line SFE–GC studies, Hawthorne *et al.*[68] noted that within certain limits, the influence of the internal diameter of the restrictor is less important than the cryogenic trapping temperature in the chromatographic oven. The chromatograms obtained by on-line SFE–GC analysis of spices, chewing gum, orange peel, spruce needles and cedar wood showed good peak shapes comparable to those obtained by using standard on-column injections. In Fig. 8 the GC–FID trace generated by on-line SFE–GC of rosemary herb is compared with a standard on-column injection of a dichloromethane extract. The on-line SFE–GC took 40 min per sample whereas sonication, concentration and off-line GC took *ca.* 5 h[68].

Recently, on-line SFE–GC was successfully applied to the extraction of PAHs from treated wood, urban dust and river sediment, phenolic species from wood smoke particulates, nicotine from tobacco, biological markers from coal, flavour components from food products[70] and PCBs and PAHs from polyurethane foam sorbents[72]. In general, recoveries of over 95% were achieved in 10–20 min.

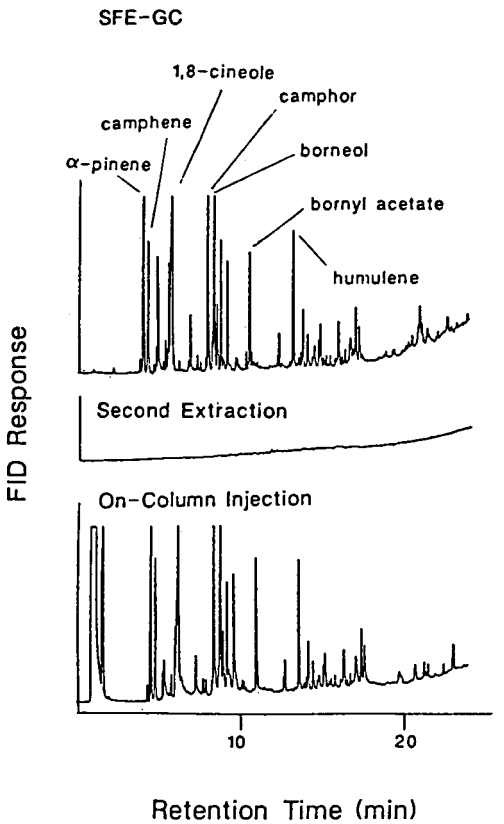

Fig. 8. Comparison of chromatograms generated by using SFE–GC–FID analysis of rosemary herb and standard on-column injection of a dichloromethane extract. The middle chromatogram shows the result of a second SFE–GC–FID analysis of the same sample. (Reprinted with permission from ref. 68.)

Hawthorne *et al.*[23,48,64] have found that a limitation with an on-line SFE–GC interface consisting of a linear fused-silica restrictor, directly inserted into a capillary GC system via an on-column injector or via a T-piece onto a retention gap, is that the restrictor becomes fragile after a few extractions, especially when nitrous oxide is used as the SF. In addition, all of the expanding SF passes through the chromatographic column and all co-extracted components are deposited in the column (or retention gap). Nielen *et al.*[69] described an alternative on-line SFE–GC system that addresses these problems. The transfer line from SFE to GC was an electrically heated (300°C) linear fused-silica capillary that also functioned as a restrictor for the SFE. Injection into the GC column was performed in the split or in the splitless mode. The system was robust and compatible with existing thermal desorption–cold trap equipment. Further, there was no restriction on the choice of GC columns or detectors, as the expanding carbon dioxide could be vented via the GC splitter, while the analytes were trapped in the desorption unit. Its potential for environmental trace analyses was demonstrated by the analysis of Tenax spiked with PCBs at the picogram level. The recoveries were satisfactory (52–63%) and the detection limit for the individual PCBs was 30 pg. Improvement of the detection limits was limited by the presence of background interferences in the GC–ECD system, resulting from the concentration of trace impurities in the 99.999% pure carbon dioxide during SF decompression (cryogenic focusing). The limitations of the technique are that thermally labile compounds cannot be analysed because of the high temperature of the SFE–GC transfer line and that problems are encountered in trapping more volatile compounds. The temperature in the transfer line should be relatively high as the pressure is reduced gradually along the entire length of the column.

An on-line SFE–GC–ECD system for the determination of PCBs was described by Onuska and Terry[73]. In this study the dynamic and static extraction modes were compared. In the dynamic mode the sample was transferred into the extractor, heated and pressurized. When the required pressure had been attained, a valve was switched and the extraction process run according to a previously determined time interval. In the static mode extracts were provided under conditions reaching equilibrium between the analytes in the fluid and in the sample. In order to determine PCBs at trace levels, static extraction was considered advantageous as in this instance the total amount of carbon dioxide passing through the extractor was smaller, resulting in less contamination with impurities.

4.4 SFE–SFC

An obvious advantage of SFE is that it is an ideal way to introduce a sample into an SFC system[21,38,74,75]. Because the injection solvent is the same as the mobile phase[76], the criteria for a successful coupling of different techniques are fulfilled[77], *i.e.*, the output characteristics from the first instrument and the input characteristics of the second instrument are compatible. An additional advantage of on-line SFE–SFC over on-line SFE–HPLC and SFE–GC is that it is unlikely that sample constituents which are insoluble in the mobile phase will be introduced into the column[78].

SFE can be combined with several forms of SFC, *i.e.*, with conventional packed columns (1–4.6 mm I.D.; packed-column SFC), with capillary columns (10–250 μm I.D.; capillary SFC) and, as has been done more recently, with packed capillary columns (200–530 μm I.D., 3–10 μm particles; packed capillary SFC).

4.4.1. On-line SFE–packed-column SFC. Directly coupled laboratory-scale SFE–packed-column SFC was introduced in 1985 by Sugiyama *et al.*[56]. Qualitative on-line SFE–packed-column SFC of powdered coffee beans was performed and monitored by multi-wavelength UV detection, using a high-pressure cell. The separation was performed without any sample pretreatment. Fig. 9 shows a scheme of the SFE–packed-column SFC apparatus. The flow direction during the extraction mode is indicated by the solid line in Fig. 9a. Valve 9/9′ was set in the non-connecting position to make a dead end for the extraction line and at the same time to maintain the pressure over the pre-pressurized columns. Once equilibrium had been reached at the desired extraction pressure, valve 7 was switched to fill sample loop 8. The flow direction during the chromatography is indicated by the solid line in Fig. 9b.

Skelton *et al.*[78] also used a valve-switching scheme to extract solid samples with supercritical carbon dioxide and they introduced the analytes directly onto the SFC column. The viability of the method was demonstrated by the on-line SFE–packed column SFC of paprika. Qualitative comparisons were made between on-line SFE–packed column SFC with UV detection and conventional off-line dichloromethane extraction–packed column SFC for coal and coffee samples. The on-line procedure provides easy sampling, as there is no introduction of solvent into the SFC system because the SF is used both for extraction and as the eluent.

Coupling SFE and packed-column SFC on-line was also described by McNally and Wheeler[44,79]. They applied this configuration for the determination of sulphonylurea herbicides and their metabolites in soil, plant material and cell culture media. Methanol-entrained supercritical carbon dioxide was necessary for the extraction and the separation of the analytes studied. Increasing the flow-rates and the entrainer concentration improved the extraction recoveries. No quatitative data were given for the system. The relatively low temperatures used in the system prevented decomposition of thermolabile compounds and this was a significant advantage over GC methods. A limitation of the open-loop SFE system used was the long equilibration time required if the flow-rate or the concentration of the entrainers had to be adjusted. This is especially disadvantageous in coupled SFE–packed-column SFC systems where the extraction should be carried out at much higher flow-rates and entrainer concentrations than are suitable for packed-column SFC of the polar metabolites. Hence, a compromise has to be chosen and 100% extraction efficiencies should not be expected.

Engelhardt and Gross[80] performed on-line SFE–packed-column SFC-FID. A single-piston reciprocating HPLC pump was used to supply the carbon dioxide for both SFE and packed-column SFC via a T-joint. Finely ground drugs and food were dry-packed into standard HPLC columns of appropriate size. The extractor was then filled with supercritical carbon dioxide and the closed loop allowed a static equilibrium to be reached. After a certain time, the sample loop was filled with the extract by opening a valve. The sample loop could be filled repeatedly with the same extract via the applied closed-loop system. Alternatively, the system could be operated in an open-loop configuration or in a stepwise extraction process, in which the first extract could be vented to waste, and the extraction column refilled with supercritical carbon dioxide and the extraction repeated. In this way the kinetics of the extraction were studied using sample sizes of 1–2 g. The concentration of the extracted solutes reached constant values after 10–15 min. In a similar way, the yield of the extraction was

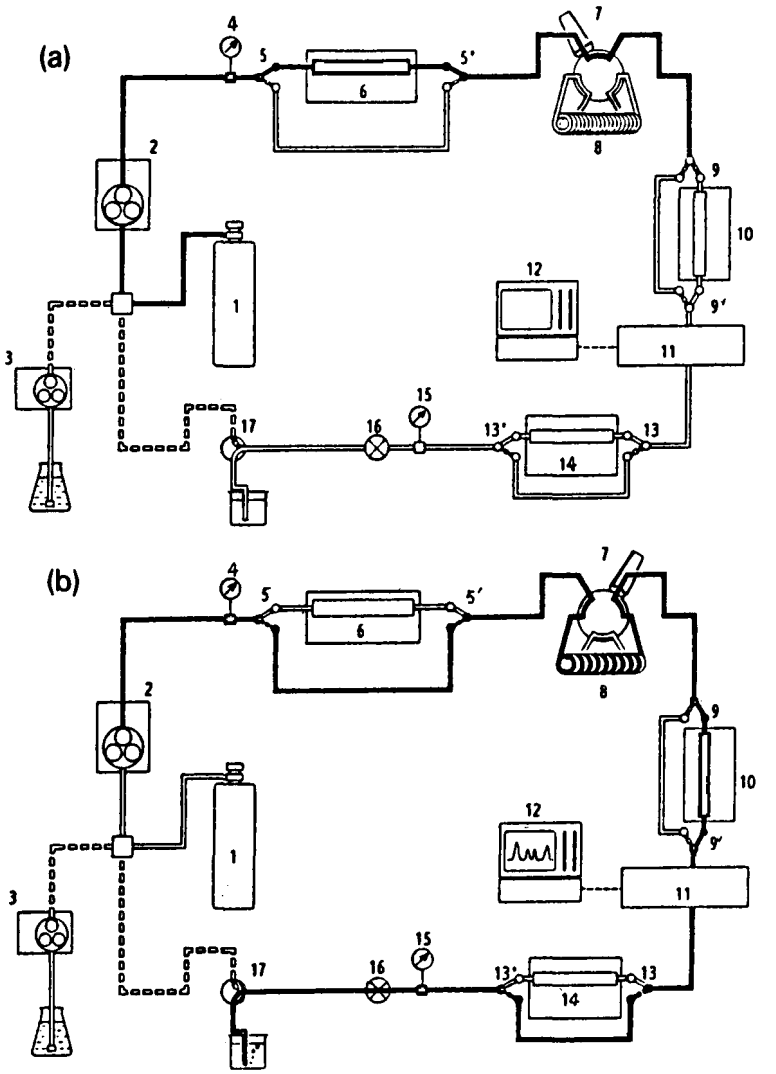

Fig. 9. Hydraulics of directly coupled SFE–SFC for extraction. 1 = Carbon dioxide cylinder; 2 = pump for delivering liquefied carbon dioxide; 3 = pump for delivering modifier solvent; 4 = pressure gauge; 5/5′ = six-way valve; 6 = extraction cartridge, thermostatted in oven; 7 = injector valve; 8 = extract trap loop; 9/9′ = six-way valve; 10 = chromatographic separation column in oven; 11 = highly sensitive multi-wavelength UV detector; 12 = data processor for 11; 13/13′ = six-way valve; 14 = extract trap column in oven; 15 = pressure gauge for monitoring back-pressure; 16 = pressure regulator; 17 = three-way valve. (a) After SFE, the injector (7) is switched to load the extra trap loop (8) with the extract. The injector is then switched back to by-pass the loop for pre-pressurization and equilibration of the separation column (10), while the loop holds the extract. (b) After pre-pressurization and equilibration of the separation column, the injector is switched to inject the extract held in the trap loop into the column. The injector valve in this figure is shown in the position for injection. (Reprinted with permission from ref. 56.)

studied and the amount of carbon dioxide for optimum extraction was determined. The extraction vessel (containing 1.6 g of caraway seed) was filled with supercritical carbon dioxide. A 20-μl aliquot was transferred to the packed-column SFC system and after refilling the extractor, the process was repeated. A plot of the concentration of the residual solutes extracted against the volume of carbon dioxide used in this stepwise extraction process yielded a decreasing curve. The rates of decrease for the various analytes differed significantly. The on-line SFE–packed-column SFC system allowed easy monitoring and control of the kinetics and the yield of the extraction process. SFE–packed-column SFC of *Radix valeriana* was also performed (Fig. 10). Further, a comparison was made between SFE–packed-column SFC and steam distillation–packed-column SFC of curry leaves.

Using the same set-up, Engelhardt and Gross[81] have also shown that non-polar pesticides such as lindane, aldrin and DDT can be selectively extracted from spiked soil (10 ppm of each pesticide) by supercritical carbon dioxide at 138 bar using stepwise extraction. After an equilibration time of 15 min, a 20-μl aliquot was switched to the SFC column with FID. No interfering substances were extracted from the soil. The minimum detectable concentration was about 1 ppm.

On-line SFE–packed-column SFC has been compared with dichloromethane extraction followed by packed-column SFC for double-base propellants by Ashraf-Khorassani and Taylor[82], using both FID and FT-IR detection. SFE with supercritical carbon dioxide (275 bar, 60°C) was performed for 12 h using 100 mg of propellant and a recirculating closed-loop system, while Soxhlet extraction with dichloromethane was performed with 2 g of propellant for 72 h. More than twice as many components were detected via packed-column SFC–FID coupled to SFE as with dichloromethane extraction. The conclusion was that either the SFE process dissolved a larger number of components or that the SFE extract was more concentrated than the dichloromethane extract. Some quantitative experiments should have been performed to study which of these explanations was true.

Ramsey *et al.*[83] evaluated the SFE–packed-column SFC combination for the detection of a small group of veterinary drugs in freeze-dried pig's kidney. During extraction with supercritical carbon dioxide, the drugs were retained by the amino-

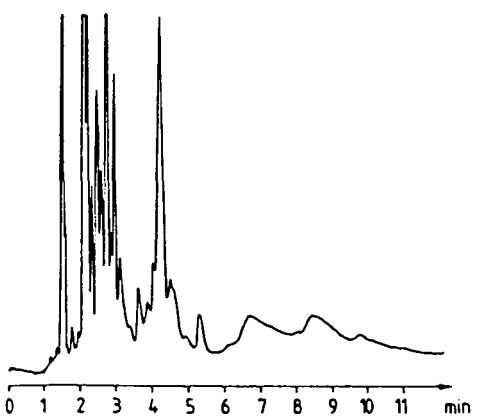

Fig. 10. SFE–SFC of *Radix valeriana*. (Reprinted with permission from ref. 80.)

bonded SFC column, whereas non-polar endogenous material was not retained and passed to waste. The extraction cell was then switched out of the carbon dioxide flow, the mobile phase composition was altered by the addition of methanol and the drugs were eluted. Initial optimization of the SFE–packed-column SFC system with UV detection did not afford sufficient resolution and selectivity to allow detection of the drugs spiked at the 10 mg/kg level. In experiments using SFE–packed-column SFC–MS the intense background of the co-eluting components hindered the analysis. SFE–packed-column SFC–MS–MS was necessary to provide daughter ion spectra virtually free from interferences and to permit the unambiguous detection of drugs at the 10 mg/kg level. Unfortunately, quantitative comparisons between SFE and liquid extraction of both analytes and extraneous material extracted from these biological specimens were not made.

On-line SFE–packed-column SFC has also been described by Niessen *et al.*[84] using a phase-switching system. Plasma samples containing the thermolabile and pH-sensitive cytostatic drug mitomycin C (MMC) were injected onto a short precolumn. After washing with water and drying the precolumn with a stream of nitrogen, the compound of interest was desorbed using 12% methanol in supercritical carbon dioxide and analysed directly by packed-column SFC using the same mobile phase composition. Up to 1 ml of plasma containing 20 ng of MMC was analysed, with typical recoveries of 70% (Fig. 11). The on-line technique was far less time consuming and labour-intensive than its off-line counterpart. Drying of the precolumn appeared to be the rate-determining step. This was necessary, because water becomes entrained

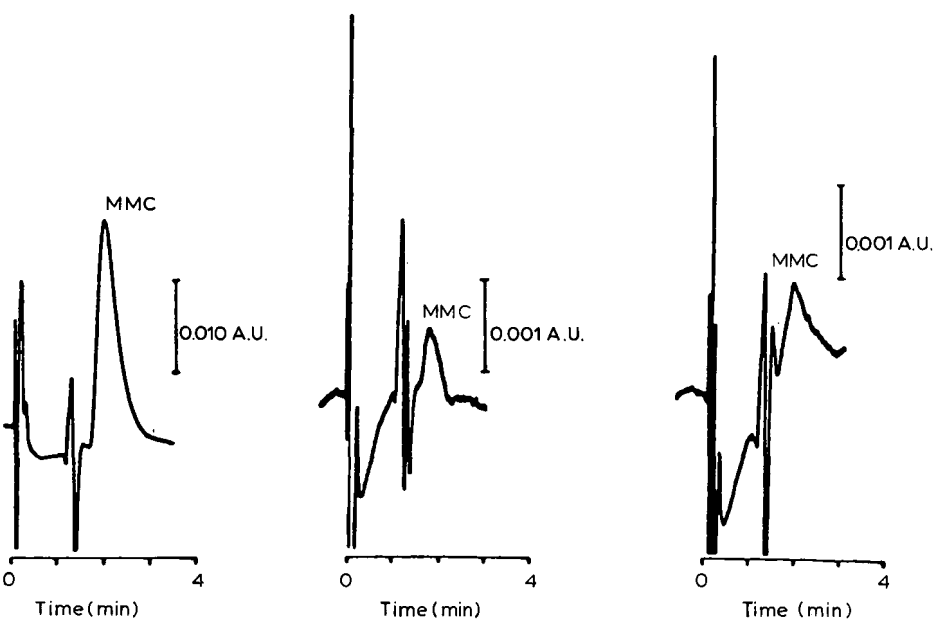

Fig. 11. Chromatograms obtained after on-line liquid–solid extraction of mitomycin C (MMC) from plasma samples. Left, sampling of 20 μl of plasma containing 200 ng of MMC; centre, sampling of 20 μl of plasma containing 20 ng of MMC; right, sampling of 1 ml of plasma containing 20 ng of MMC. (Reprinted with permission from ref. 84.)

in supercritical carbon dioxide and as a result deactivates the SFC column. At room temperature drying times of up to 25 min were not sufficient to remove all the water completely. Raising the temperature to 60°C for 10 min resulted in a significant decrease in the chromatographic signal, probably owing to thermal degradation of MMC. As a compromise 50°C was employed.

Direct SFE of aqueous samples and on-line coupling to packed-column SFC has been performed by Thiebaut *et al.*[85] using a dynamic open-loop system. Aqueous samples were injected directly into a supercritical carbon dioxide stream and extracted in a coil of appropriate length. Water and supercritical carbon dioxide are immiscible and therefore must be separated before detection. The extract-laden SF was separated from the water by means of a phase separator; the effect on the UV signal of SFE with and without phase separation is illustrated in Fig. 12. By trapping the extract-laden SF in a downstream sample loop, it could be diverted to a packed-column SFC system by switching the valve. Phenol and 4-chlorophenol were used as medium-polarity test compounds. The clean-up and extraction of 4-chlorophenol from urine was also shown. The extraction efficiency for the test compounds was over 85%, and the repeatability was 8% (R.S.D.) for the total SFE–phase switching–packed-column SFC system and 4% for both the SFE–phase switching and packed-column SFC systems separately.

Jahn and Wenclawiak[86] described an on-line system using a mini-extractor (85 μl) and a micro-extractor (3–4 μl), which could be coupled on-line with packed-column SFC and used under sub- or supercritical conditions. The mini-extractor was used for on-line SFE–packed-column SFC and the micro-extractor was applied for direct sample introduction.

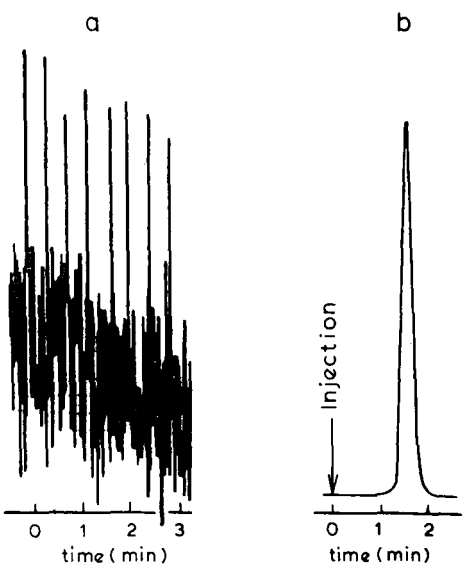

Fig. 12. Dual pump SFE–phase switching–SFC system, (a) without and (b) with phase separator inserted. Sample: 5 μl of water containing 5 μg of phenol. Note that there is a difference in attenuation; the phenol peak in (b) is not visible in (a). (Reprinted with permission from ref. 85.)

4.4.2. On-line SFE–capillary SFC. The direct coupling of SFE to capillary SFC was systematically investigated by Gmür *et al.*[87–89]. The optimization of some important instrumental parameters such as internal diameter and length of the capillary, pressure drop along the column, linear velocity and injection volume was studied. The coupled system was used to analyse natural products such as cheese, butter, coffee, tobacco and camomile. For cheese analyses the coupled technique allowed the simultaneous determination of volatile methyl ketones and non-volatile fatty acids, without any additional sample pretreatment.

An elegant way to ensure the solubility of the analytes in the mobile phase, before they are introduced into an SFC system, is to use the same fluid both as the eluent and as the injection fluid. A sample introduction system for capillary SFC

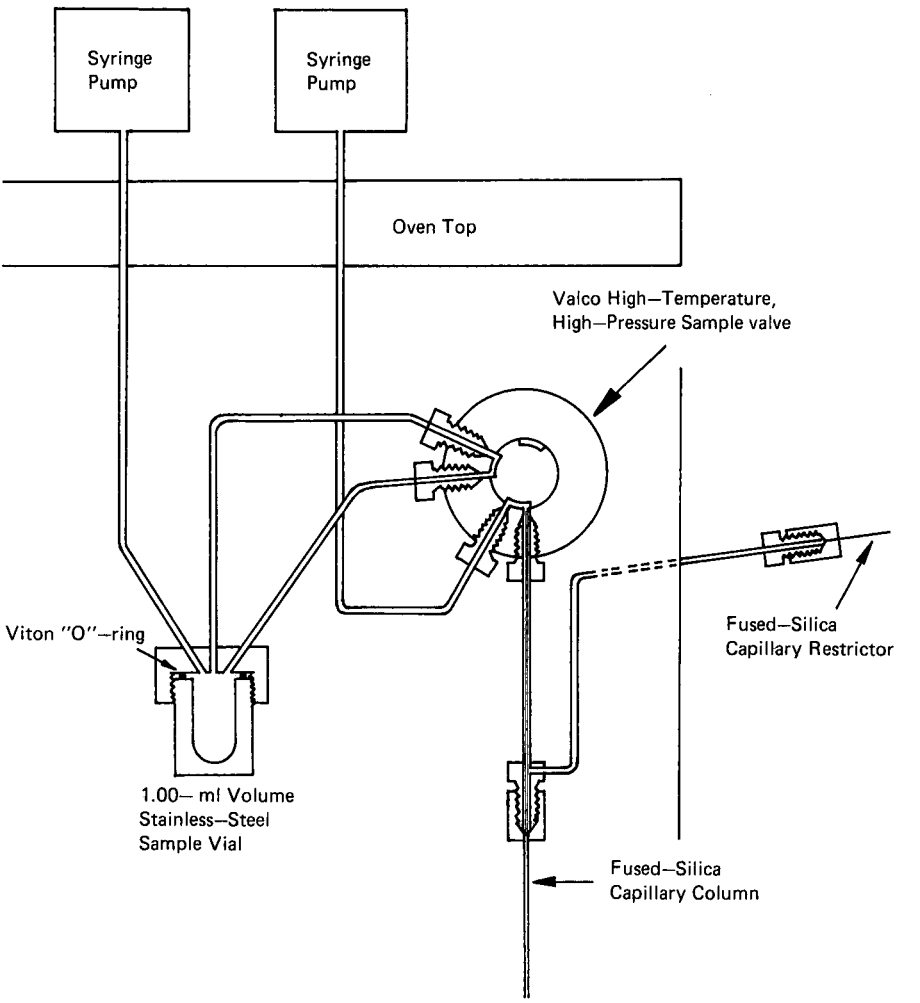

Fig. 13. Schematic diagram of a supercritical fluid injector for SFE–capillary SFC. (Reprinted with permission from ref. 76.)

allowing the dissolution of the sample in the SF before it is introduced into the column was constructed and evaluated by Jackson *et al.*[76]. It consisted of a closed-loop SFE system and injection of the SF was accomplished using a high-temperature, high-pressure sample-loop valve. The valve was mounted inside the oven, with the stand-off handle extending through the oven wall (Fig. 13). A 1.0-ml volume high-pressure stainless-steel sample vial was used as extraction vessel and was connected via two tubes to the valve. A third tube was connected to the syringe pump which served to pressurize the SF in the injector. The analytical column was connected to the valve by means of a glass-lined splitter. The feasibility of SF injection was examined by comparing the results of injections using liquids and fluids[76]. Split injections of the SF solution were found to be more reproducible than split injections of liquids. Further, the solvating capacity of supercritical *n*-pentane as injection solvent was studied by comparing the SF injection of the high-molecular-weight PAH ovalene with injections in two different liquids. The results indicated that supercritical *n*-pentane solvated high-molecular-weight PAHs more rapidly than a few common liquids (Table 6). SF injection introduced over sixteen times as much ovalene into the SFC system as a solution in dichloromethane, and nearly twice as much as a solution in 1,2,4-trichlorobenzene.

The development of an on-line SFE–capillary SFC system with off-line FT-IR detection was recently reported by Raynor *et al.*[42] for the separation and identification of PAHs in coal pitch. An open-loop system was used. The SF extract was decompressed by means of a frit restrictor into the sample cavity of a cooled micro-valve injector, thus depositing the analytes and concentrating them, while the carbon dioxide escaped through the other valve opening. Subsequently, the contents of the loop were switched in-line with the mobile phase of the coupled capillary SFC. Several of the separated analytes were collected on a potassium bromide disc and, after solvent elimination, FT-IR analysis using a microscope accessory was performed (Fig. 14). The spectra obtained showed the power of this detection technique for distinguishing isomers. During on-line SFE–capillary SFC pressure programming was applied to fractionate coal pitch selectively during SFE and to transfer these fractions to the capillary SFC system[42]. The injection valve had to be kept above the critical temperature of the mobile phase, otherwise solutes deposited in the valve after SFE would not be redissolved. Another important aspect was that most samples analysed by capillary SFC were injected using low mobile phase densities. Conse-

TABLE 6

QUANTITATIVE COMPARISON OF LIQUID AND SUPERCRITICAL FLUID INJECTIONS FOR THE DETERMINATION OF OVALENE BY SFC WITH FLUORESCENCE DETECTION

Reprinted with permission from ref. 76.

Solvent	*Peak area*[a]
Dichloromethane (room temperature)	73 ± 13
1,2,4-Trichlorobenzene (room temperature)	674 ± 42
Supercritical *n*-pentane (210°C, 180 p.s.i.)	1177 ± 137

[a] Average of four injections ± standard deviation.

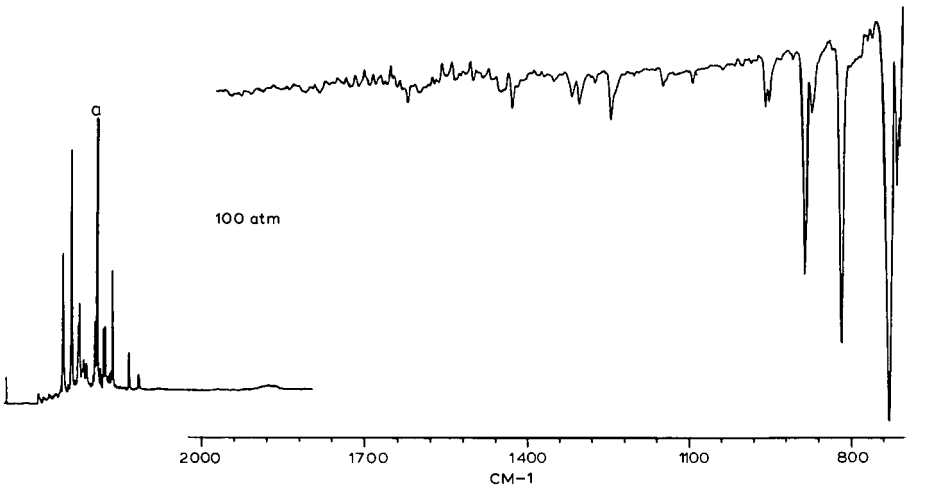

Fig. 14. Left, SFC of coal tar pitch extracted at 100 atm. Right, IR spectrum of peak a indicating the presence of phenanthrene and anthracene which have been co-eluted. (Reprinted with permission from ref. 42.)

quently, certain compounds which were soluble in carbon dioxide at high extraction densities may not have been introduced into the column at the low mobile-phase densities used during injection. An interesting feature is that a difference in density between the SFE and SFC allows samples to be focused on top of the analytical column[90].

With an open-loop system, Anton *et al.*[91] performed rapid qualitative studies of complex materials such as plastics, coffee powder and PAH-contaminated soil using analytical-scale SFE with concurrent capillary SFC. The deposition process did not cause peak broadening.

An SFE–capillary SFC fraction-collection system was developed to perform on-line extraction, separation and fraction collection of biologically important drugs (*e.g.*, ouabain)[92]. The SF extract was decompressed via a linear restrictor and deposited in a deactivated capillary concentrator within a cryogenic trap. The internal diameter of the restrictor is important because larger internal diameters provide higher extraction efficiencies, but lower cryogenic trapping efficiencies. A 25-μm I.D. restrictor seemed to be a good compromise. The internal diameter of the concentrator (150 μm) was slightly larger than the outer diameter of the restrictor (148 μm), allowing tight insertion of the restrictor into the concentrator. This resulted in good chromatographic peak shapes. Fractions of the SFC effluent were collected from a frit restrictor at the column outlet in vials containing a preselected solvent, such as dichloromethane or methanol.

4.4.3. On-line SFE–packed-capillary SFC. On-line SFE–packed-capillary SFC is an interesting development in comparison with SFE–capillary SFC, because of a higher loadability and shorter analysis times. In comparison with SFE–packed column SFC the advantages are a lower pressure drop, higher efficiency (theoretical number of plates) and lower flow-rates, resulting in an easier interfacing with FID or MS instruments.

The use of on-line SFE–packed-capillary SFC was described by Hirata *et al.*[75]. Polyethylene film was extracted with supercritical carbon dioxide and the analytes were trapped on an uncoated fused-silica tubing (15 cm length). By coupling 5-cm sections of this tubing to a packed capillary column and using direct injection, they were able to confirm that the extracts were efficiently trapped in the first 5-cm section, even at an extraction temperature of 65°C. The feasibility of extending the technique to quantitative studies was also demonstrated. Improvements will probably centre on analysing narrower sections of the extracts by controlling the trap temperature and on using coated tubing or even a packed column.

5. CONCLUSIONS

The coupling of SFE with an analytical technique provides the potential for combined sample preparation and analysis. In addition to completely automated operation, rapid analyses and high recoveries can be achieved. Extraction rates often increase by more than an order of magnitude in comparison with Soxhlet extractions and, in general, better extraction recoveries are obtained compared with Soxhlet and thermal desorption techniques. Further, SFE is capable of processing thermolabile compounds, which cannot be desorbed by thermal desorption. Selectivity can be manipulated easily by the wide range of solvent powers available with SFs providing the potential for fractionation of complex samples and isolation of apolar to relatively polar analytes from a variety of matrices. In addition, SFE offers the possibility of sample concentration by decompression of the fluid prior to chromatographic analysis.

Although SFE has a number of advantages over the more classical methods, there are some limitations and problems. For instance, mainly solid samples are handled in SFE systems coupled to separation techniques. However, it will be obvious that for this type of sample this is often the only technique for on-line extraction. The problems associated with extracting aqueous samples have only recently been addressed, and considerable work is still needed in this respect, especially as in a considerable number of studies the samples are still freeze-dried to overcome problems with water. Commercial SFE apparatus is also focusing on the handling of solid samples. Further, mainly qualitative data are available, almost no systematic studies have been performed and the detectabilities are at the ppm–ppb levels.

In comparison with Soxhlet extractions a more complicated set-up is necessary. However, off-line SFE allows the collection of the extracted analytes in a vial containing a suitable organic solvent. In this way the use of complicated interfaces, such as those needed in on-line systems, can be avoided. The major problems in SFE are probably the loss of volatile analytes and blocking of the capillaries due to either precipitation of the extracted analytes or cryogenic cooling of the expanding SF if open collection is performed.

The potential strength of SFE in analytical chemistry is its coupling to other separation techniques such as TLC, HPLC, GC and SFC. SFE–TLC is an elegant and relatively cheap technique when only qualitative data are required. Separation can be improved by applying two-dimensional chromatography. Off-line SFE–HPLC has been applied for the determination of various compounds obtained from solid matrices or liquid matrices after freeze-drying. On-line SFE–HPLC has until now

only been applied to monitor extraction efficiencies. The use of column-switching systems for removing unwanted components in the extraction fluids will allow (semi)-quantitative analyses in the future.

So far, on-line SFE–GC is the most often applied combination. Extractions can be performed at relatively low temperatures and no sample handling or concentration procedures are required between extraction and GC analysis, thus reducing the possibilities for degradation and loss of analyte. The extracted analytes are quantitatively collected, which means that maximum sensitivity can be obtained and hence that the amount of sample needed can be reduced. Class-selective extractions can be achieved by performing multiple SFE–GC analyses at different extraction pressures. Further, on-line SFE–GC requires no modification of the gas chromatograph and there are good possibilities for focusing the extracted analytes at the top of the column. As a result, several companies are now providing SFE units with on-line GC interfaces.

The fastest growing technique in this field is on-line SFE–SFC, because the number of sample manipulations is limited by using the SFE fluid also as the eluent in the subsequent analysis, and because a wide variety of detection devices can be applied.

In general, it may be stated that for uncharged relatively apolar compounds, which can be dissolved in supercritical carbon dioxide, SFE shows several advantages over liquid extraction techniques. However, for more polar analytes the extraction efficiency depends strongly on the extraction conditions (*i.e.*, pressure and temperature) and the addition of an entrainer is often required. The addition of a suitable entrainer or the use of a more suitable SF (*e.g.*, ammonia or nitrous oxide) and the modification of the matrix (*e.g.*, pH) should provide more favourable extraction conditions. However, this makes the interfacing to other techniques, such as GC, more difficult.

Future trends in SFE will probably be the processing of various kinds of matrices by using different SFs and various entrainers, the development of new interfaces for the coupling of techniques, the use of column-switching systems to remove the extraction fluid before the extracted compound is introduced to the actual chromatographic system, miniaturization of SFE systems if small samples have to be analysed and the development of efficient extraction systems allowing larger samples to be extracted and, hence, diminishing the problems in trace analysis caused by impurities present in SFs.

6. SUMMARY

After a brief description of the basic principles of supercritical fluid extraction (SFE), this review extensively discusses the application of SFE via its off-line and on-line coupling to chromatographic techniques, such as thin-layer, high-performance liquid, gas and supercritical fluid chromatography. Aspects such as speed, selectivity, sensitivity, potential for automation and possibilities of fractionation of the supercritical extract are discussed. Further, SFE liquid–liquid and liquid–solid extraction procedures are compared. Until now, SFE has been applied almost exclusively to the extraction of apolar compounds from solid samples, but the method seems also to be attractive for liquid samples. Generally, SFE is more efficient (in terms of extraction times and recoveries) than Soxhlet extractions and more suitable for ther-

molabile compounds. Furthermore, efficient coupling to chromatographic techniques is possible, although much work still has to be done to optimize the necessary interfaces. The extraction of relatively polar compounds is possible only if high densities are used or if modifiers are added to the supercritical fluid. The interfacing with separation techniques is then less simple.

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Physicochemical model of retention for capillary supercritical fluid chromatography

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SUMMARY

The solvating ability of a supercritical fluid mobile phase depends on density and temperature, which in turn govern solute retention. A model is proposed for capillary supercritical fluid chromatography that relates the logarithm of the capacity factor to the logarithm of the reduced density by an empirical equation. This relationship takes into account molecular weight and shape of solute and mobile phase, reduced density, reduced temperature, free energy of solute transfer between phases and film thickness of the stationary phase. Ultimately, each mobile phase and stationary phase combination can be described by a "phase constant" that seems to be dictated by the relative polarizability of the phases. This model has so far been proven valid for a representative group of polyaromatics.

INTRODUCTION

Unlike with gas chromatography (GC) and liquid chromatography (LC), where various retention models have been extensively developed for years, supercritical fluid chromatography (SFC) suffers from the lack of useful models for solute retention. Physicochemical models with a firm theoretical basis covering a variety of analytically useful separation conditions in SFC are necessary not only to separate and delineate the mobile phase and stationary phase contributions to retention, but also to offer insight into the interactions between the two phases and the solutes[1–4]. In modern SFC, the practice of separations is clearly far ahead of the theory to explain them.

Over the years, several models have been suggested to explain retention. Schoenmakers[5] proposed a rigorous thermodynamic model requiring the use of the Lee and Kesler equation of state[6], which covers a wide range of reduced pressures and temperatures. He then evaluated his model using data found in the literature by Van Wasen *et al.*[7]. However, the model does not yield a quantitative estimate for the capacity factor, k', and it requires the use of complex algebraic equations.

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The most popular model proposed to date has been the assumption of a linear log k' *vs.* ρ (density) relationship, ascribed to by many researchers in the field[7–13]. However, in the cases where plots of log k' *vs.* ρ over a wide density range are shown, the curves are clearly non-linear. One common cause of this misrepresentation is that many of the curves are plotted over a very narrow range of densities[10,13], which is misleading.

Martire and Boehm[14] presented a unified theory of GC, LC and SFC in partition chromatography based on a fluid-lattice model. With respect to SFC, they showed that the reduced temperature and reduced density are the natural descriptors for the effect of the mobile phase on retention. This is logical since the reduced parameters are the only way to relate completely dissimilar fluid phases. The reduced density was believed to be involved in a quadratic relationship describing retention. The authors derived an equation to explain the retention of a series of *n*-alkanes and then attempted to fit literature experimental data to their model with moderate success. However, the main drawback of the quadratic relationship was that the coefficients were specific for a particular set of solutes in a specific system, and could not be further generalized to other SFC systems.

Although there exist various theoretical treatments of retention in SFC, there is a dearth of complete, informative experiments available to confirm or refute any claims made in the literature. The aim of this study was to investigate retention of a series of polyaromatics on all combinations of four mobile phases and three stationary phases of varying polarity. It was also hoped that from this database would emerge a logical, empirical relationship to adequately describe retention in SFC.

THEORY

The solubility in fluid systems at low density, and consisting of weak intermolecular interactions, can be described[15] by a linear relationship between the logarithm of the fraction of the solute that is present in the mobile phase, X_M, and the density at constant temperature, ρ_T by

$$\log X_M = b\rho_T + c \tag{1}$$

where b and c are coefficients dependent on the properties of the fluid at that particular temperature. The quantity X_M, which is also referred to as the solute distribution in the mobile phase, is defined by

$$X_M = n_M/(n_M + n_S) \tag{2}$$

where n_M and n_S are the moles of solute in the mobile phase and stationary phase, respectively.

The relationship in eqn. 1 has been corrected by a higher order term in order to compensate for deviations from linearity, so that

$$\log X_M = a\rho_T^2 + b\rho_T + c \tag{3}$$

where a is another constant dependent on the fluid and temperature. Eqn. 3 is a valid

correction to eqn. 1, but it has been found that at very low pressures the correction becomes unnecessary. However, Franck[15] predicted that at higher densities, solubility in the fluid phase would require an empirical logarithmic relationship in order to account for the stronger intermolecular interactions between solvent and solute. This is described by

$$\log X_M = b' \log \rho_T + c' \tag{4}$$

where b' and c' are once again constants that depend on the fluid and temperature. In order to establish the validity of eqn. 4 as model of solvation in SFC, it is necessary to identify the factors that contribute to the coefficients b' and c'.

Importantly, eqn. 4 must be further modified so that it can be used to describe any supercritical fluid in SFC, and thereby allow the different fluids to be directly compared with one another. This is accomplished by replacing the density with the reduced density at constant temperature, $\rho_{R(T)}$, which is defined by

$$\rho_{R(T)} = \rho_T/\rho_c \tag{5}$$

where ρ_c is the critical density of the fluid of interest. Eqn. 4 can now be rewritten in its new form

$$\log X_M = b' \log \rho_{R(T)} + c'' \tag{6}$$

It should be noted that the alteration of the abscissa scale by a constant amount will produce the same slope as in eqn. 4, but the intercept will change, as indicated by the new constant, c''.

At this point, eqn. 6 must take on a form that relates solubility to retention in SFC. By definition,

$$k' = X_S/X_M = n_S/n_M \tag{7}$$

where X_S is the solute distribution ratio in the stationary phase (*cf.*, eqn. 2), and k' is the capacity factor, which can be related to retention by

$$k' = (t_R - t_0)/t_0 \tag{8}$$

where t_R, t_0 are solute and void-volume retention times, respectively. The logarithmic dependence is simply introduced by

$$\log k' = \log X_S - \log X_M \tag{9}$$

Eqn. 9 is the first and most important step in actually separating the mobile phase from the stationary phase contribution to retention. If we substitute eqn. 6 into eqn. 9, this results in

$$\log k' = \log X_{S(T)} - c''_{(T)} - b' \log \rho_{R(T)} \tag{10}$$

where all the terms in the above equation are dependent on temperature. Eqn. 10 is the first step in evaluating the relationship between mobile phase density and retention in capillary SFC. In reality, if the concentration of solute in the mobile phase is varied due to a corresponding density increase, the concentration in the stationary phase must also vary, although not to the same extent as the former. The major assumption being made is that X_S exhibits its own distinct density dependence, similar to that of X_M (*cf.*, eqn. 6); *i.e.*, log X_S is linearly related to log $\rho_{R(T)}$ by

$$\log X_S = a' \log \rho_{R(T)} + d'' \tag{11}$$

Hence a combination of eqn. 10 with eqn. 11 results in

$$\log k' = d''_{(T)} - c''_{(T)} + (a' - b') \log \rho_{R(T)} \tag{12}$$

The immediate consequence of assuming this linear variation of log X_S with log $\rho_{R(T)}$ is the introduction of a new constant, $d''_{(T)}$, which is analogous to the $c''_{(T)}$ term for the mobile phase, where both constants are dependent on temperature, but independent of density. It is evident from eqn. 12 that a straight line should be obtained from a plot of log k' *vs.* log $\rho_{R(T)}$. This implies that the slope of such a curve will contain a contribution from the variation of X_S with reduced density, denoted by the constant, a'. In practice, the slope of the log–log plot is found to be a negative value; this implies that $a' \ll b'$ in the temperature and density range studied. Unfortunately, it may not be possible to specifically distinguish the mobile phase contribution from that of the stationary phase. For the sake of simplicity, in the remainder of the paper the $(a' - b')$ term in eqn. 12 will be referred to as e' in the following manner:

$$\log k' = d''_{(T)} - c''_{(T)} + e' \log \rho_{R(T)} \tag{12}$$

EXPERIMENTAL

Apparatus

The instrument used for all experiments was a Model 602 capillary and packed-column SFC system (Lee Scientific, Salt Lake City, UT, U.S.A.). The syringe pump assembly has a capacity of 175 ml and is surrounded by a cooling jacket through which cold water is circulated in order to fill with the desired mobile phase. The cooling water was supplied by an RTE-110 refrigerated bath circulator (Neslab Instruments, Portsmouth, NH, U.S.A.). The water temperature was maintained between 5 and 10°C during the filling process; however, the bath was shut off when the fill was complete in order to allow expansion of the mobile phase at room temperature.

A pressure-relief valve system and fluid manifold system containing basic alumina (80–200 mesh, Brockman activity I) was placed between the gas cylinder and the pump in order to trap organics, particles and water. In addition, a charcoal filter was situated prior to the alumina trap in order to further ensure the trapping of organic impurities.

Injections were performed by a pneumatically operated injection valve (Valco, Houston, TX, U.S.A.) with a 200-nl sample loop. A variable length (6 to 8 cm) of 10 μm I.D. fused silica was used to split the sample upon injection. The split ratio was approximately 10:1. Injection time ranged between 0.06 and 0.10 s.

A flame ionization detection (FID) system was used for all experiments except those performed with SF_6. This is due to the fact that upon combustion, SF_6 produces HF, which eventually erodes the collector electrode of the detector. To circumvent this problem, an ultraviolet detector (UVIDEC-100 II, Jasco, Easton, MD, U.S.A.) was placed prior to the FID system and configured in the following way: the end of the 50 μm I.D. column was removed from the oven through an existing exit port and attached by epoxy glue to approximately 2 cm of 250 μm I.D. fused silica that had a 1-cm section of its polyimide coating burned away. The other end of this detector cell was connected by epoxy glue to the distal end of a 50 μm I.D. frit restrictor (Lee Scientific) which was also brought outside of the oven by way of the same exit port in the oven side. The frit end of the restrictor sat inside the heated FID system (325°C) as in all of the experiments with other mobile phases, except the flame was not lit. This arrangement is desirable because the column and restrictor configurations are essentially the same for both detection strategies. The absorbance at 254 nm was observed for all injections.

Three 50 μm I.D. capillary columns were obtained from Lee Scientific. The columns and their specifications are summarized in Table I. The four mobile phases chosen were supercritical fluid grade carbon dioxide, nitrous oxide, sulfur hexafluoride (Scott Specialty Gases; Plumsteadville, PA, U.S.A.) and xenon (Air Products, Shelbyville, IN, U.S.A.).

TABLE I

50 μm I.D. COLUMNS USED IN THESE STUDIES

Column	*Stationary phase*	*Film thickness (μm)*	*Length (m)*
A	SB-Methyl-100%	0.50	10
B	SB-Biphenyl-30%	0.15	15
C	SB-Cyanopropyl-50%	0.15	10

The solutes studied were naphthalene, biphenyl, fluorene, phenanthrene, fluoranthene, pyrene, *para*-terphenyl and chrysene. All were obtained from Aldrich (Milwaukee, WI, U.S.A.). The two polyphenyls were included in order to verify the assumption that their retention is governed by a different mechanism than that of the polynuclear aromatic hydrocarbons. The solvent used to dilute these compounds was methylene chloride (Mallinckrodt, Paris, KY, U.S.A.). The concentration of each solute was between 4 and 10 mg/ml.

Procedures

The procedures consisted of replicate injections (usually three) of a mixture of the solutes at the desired temperature and density. Two sets of data were collected for every mobile phase and stationary phase combination. One set consisted of fixing the density and investigating retention as a function of temperature. The other set involved varying the density under constant-temperature conditions.

The mobile phase filling procedure was straightforward for all gases except xenon. In this instance, the xenon gas had to be trapped in a stainless-steel coil configuration by immersing the coils in liquid nitrogen for several minutes. Subse-

quently, the tank valve was closed, the liquid nitrogen removed, and the coils allowed to warm up to room temperature. In this way, the xenon was forced through a one-way valve into the cooled pump housing. The procedure was repeated five or six times, or until the pressure in the pump read 45 atm. At this time, the cooling water was shut off and the xenon allowed to expand to room temperature. This method of filling generated an initial fill-pressure of approximately 70 atm, as opposed to approximately 20 atm without the trap.

The densities of CO_2 and N_2O were provided directly by the Lee Scientific Software, which utilizes the theory of corresponding states for all calculations. However, since no such data were available for Xe and SF_6, this entailed the use of an iterative program based on the Lee–Kesler equation of state for a compressible fluid[6]. The densities calculated with this program for CO_2 and N_2O agreed with those from the instrument software to within 5%.

All data were collected by a Perkin-Elmer (Norwalk, CT, U.S.A.) Sigma 10 integrator. The peak retention times were used to calculate capacity factors for each solute. The dead volume retention time (t_0) was assumed to be the elution time of the solvent, methylene chloride.

RESULTS AND DISCUSSION

Slope

Only a few studies in the literature have given the log k' *vs.* log ρ_R model any credence[7,9,16]. A first step in the evaluation of the proposed log–log relationship is to examine the experimental evidence to see if other models can account for the observed behavior. Hence, for each set of data at constant temperature, the log k' *vs.* ρ plot was constructed in order to test the linearity of retention with density. Fig. 1 is

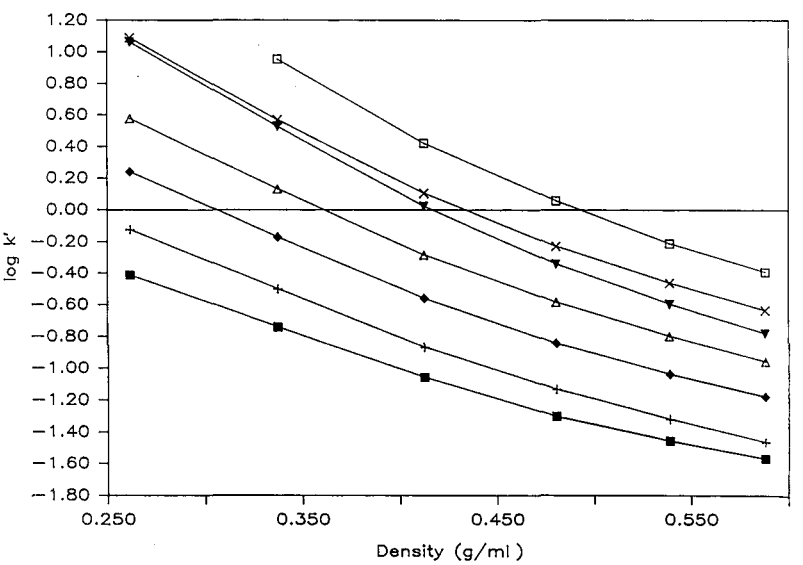

Fig. 1. Retention behavior with density: nitrous oxide and column B. ■ = Naphthalene; + = biphenyl; ◆ = fluorene; △ = phenanthrene; × = pyrene; ▼ = *p*-terphenyl; □ = chrysene.

a representative example of such a plot; it is clear that deviations from linearity occur. Furthermore, the very shape of the curve appears to suggest a logarithmic dependence. It should be noted that this same curve shape was found for every plot of this type.

A quadratic dependence was also considered as a means of explaining the non-linearity of the curve. The model would only be valid in the event that a minimum in the curve exists at high densities[14]. To date, no experimental data points have been found in the literature that pass through a minimum in the curve, although the minimum may exist at an "imaginary" density. No minimum was detected for any of the solutes studied in our laboratory; invariably, retention continued to decrease as density was increased.

Fig. 2 is a log–log plot of the same data as in Fig. 1. These results were encouraging in that the log–log relationship was more successful than the other two models in producing a straight-line relationship between retention and density, even though the densities do not span a very wide range. It is important to point out that the lines connecting the data points in Fig. 2 arise from the linear regression calculated for the data sets, with a correlation coefficient of 0.999 or better. Although Fig. 2 represents retention for seven solutes at a particular temperature on a specific mobile phase–stationary phase combinaton, similar plots were obtained for 8 solutes at 4 other temperatures, on 11 other mobile phase–stationary phase systems; in total, 480 such straight line log–log relationships were produced during the course of this work. However, it was still deemed necessary to attach physical and chemical significance to the slopes and intercepts of these log–log plots rather than to adopt the model by default.

The log–log relationship proposed by Franck[15] and extended to eqn. 6, predicts a monotonic increase in solute solubility with fluid density. Hence, the choice of this model must be justified in light of recent evidence that at very high fluid densities, the

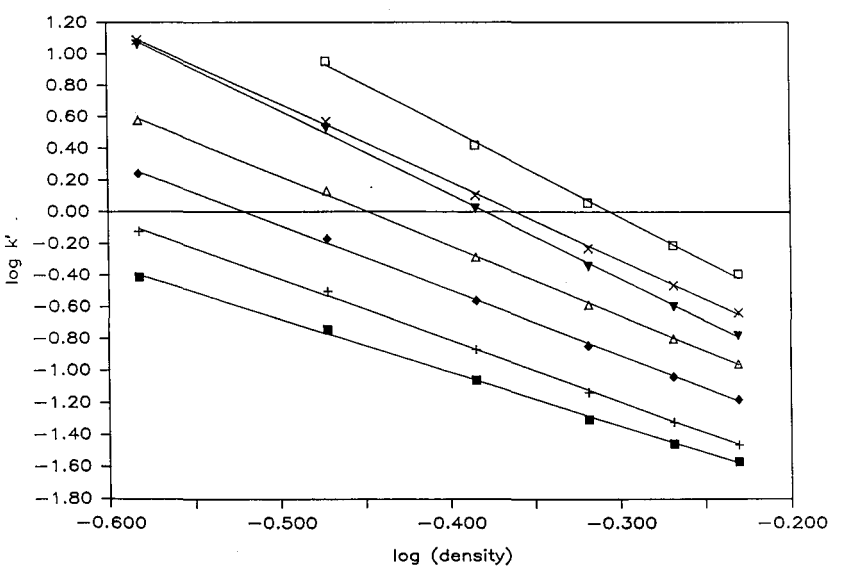

Fig. 2. Retention behavior with log (density): system and solutes as in Fig. 1.

TABLE II

EXAMPLES OF SLOPES AND INTERCEPTS OBTAINED FROM THE LOG–LOG PLOT (EQN. 12)

Solute	*Column A and CO_2*				*Column B and N_2O*				*Column C and SF_6*			
	Slope		*Intercept*		*Slope*		*Intercept*		*Slope*		*Intercept*	
	Temperature (K)		*Temperature (K)*		*Temperature (K)*		*Temperature (K)*		*Temperature (K)*		*Temperature (K)*	
	398	*473*	*398*	*473*	*398*	*423*	*398*	*423*	*423*	*523*	*423*	*523*
Naphthalene	−2.77	−1.77	−0.759	−1.03	−3.06	−2.77	−1.37	−1.45				
Biphenyl	−3.14	−2.02	−0.625	−0.936	−3.56	−2.93	−1.22	−1.32	−2.22	−0.884	−0.813	−1.25
Fluorene	−3.39	−2.21	−0.441	−0.762	−3.75	−3.26	−0.918	−1.05	−2.18	−0.926	−0.407	−0.856
Fluoranthene	−4.04	−2.65	−0.102	−0.473	−4.46	−4.01	−0.406	−0.587	−2.31	–	+0.346	–
Phenanthrene	−3.67	−2.42	−0.279	−0.631	−4.08	−3.61	−0.676	−0.832	−2.30	−0.955	−0.0472	−0.506
Pyrene	−4.08	−2.77	−0.0453	−0.445	−4.55	−4.05	−0.328	−0.505	−2.48	−0.965	+0.390	−0.0724
Chrysene	−4.63	−3.12	+0.133	−0.306	−5.24	−4.50	−0.0515	−0.239				

final predominance of repulsive forces may "squeeze" the solute out of the fluid phase[14,17]. This premise is further supported by the discovery that the infinite-dilution partial molal volume of solute in a high-density fluid ultimately becomes positive[18]. For the purpose of modeling retention in SFC, it is safe to say that we will not be examining densities that are high enough to exhibit this phenomenon, *i.e.* $\rho_R > 1.6$ (ref. 18). In addition, it can be assumed that the high mobile phase velocity associated with a very high density would cause elution of most low-molecular-weight solutes with the void volume, thereby masking any expected repulsive force interaction.

Table II contains a very small representation of data obtained from a linear regression of a $\log k'$ *vs.* $\log \rho_R$ plot. The trends shown in this table were observed for all systems studied and are discussed in detail here.

As temperature is increased for a given solute, the slope increases while the intercept decreases. As solute molecular weight increases at a given temperature, the slope decreases while the intercept increases. However, the most striking feature of the data was that when the slope is divided by the solute molecular weight at a given temperature the result is roughly constant for a pseudo-homologous series of polyaromatics (phenanthrene, pyrene and chrysene). It should be noted that a different constant was obtained for the polyphenyls, biphenyl and *p*-terphenyl, which supports the belief that the two groups are governed by very different modes of retention based on shape. Furthermore, it is clear from Fig. 2 that the slopes of the curves for these compounds deviate significantly from those of the polyaromatic compounds.

In order to prove that these trends were not restricted to our results, we sought comparable systems in the literature as well. Notably, Yonker *et al.*[3] produced retention data at different temperatures for chrysene in CO_2 on an SE-54 stationary phase (column D in Table III). Not surprisingly, the slopes calculated from their data followed the very same trends as ours; in addition, the values for the slopes were in the same range as those calculated for our stationary phases at similar temperatures. The calculated slopes are summarized in Table III.

TABLE III

COMPARISON OF LOG k' *VS.* LOG ρ_R (CO_2) SLOPES WITH LITERATURE VALUES FOR CHRYSENE

Temperature (K)	*Column*			
	A	*B*	*C*	*D (ref. 3)*
373	−5.07	−5.14	−5.38	−4.88 (380.2 K)
398	−4.63	−4.84	−4.90	−4.39 (400.4 K)

It was soon discovered that, in addition to the solute molecular weight, a "shape factor" would be required to account for the distinctly different retention times of the positional isomers, fluoranthene and pyrene. For this reason, the Van der Waals volume (b_v term) was introduced, which could account not only for the size of the solute, but for the shape as well. Since these values are not commonly found in the literature, they had to be estimated based on a procedure detailed by Edward[19] and Bondi[20]. The calculated values (b_v^{sol}) for the eight solutes are shown in Table IV.

TABLE IV

CALCULATED VAN DER WAALS VOLUMES

Based on procedure outlined in refs. 19 and 20.

Solute	*Volume (ml/mol)*
Naphthalene	74.29
Biphenyl	90.60
Fluorene	95.79
Phenanthrene	83.46
Fluoranthene	109.82
Pyrene	109.06
p-Terphenyl	132.84 (max)
Chrysene	125.18

It seemed logical that if size and shape of the solute were vital, then the same should be true for the mobile phase. Therefore, the molecular weight of the mobile phase was included in addition to its Van der Waals volume. These b_v values are tabulated for most gases[21]; however, an approximation from the Van der Waals equation of state was adopted for b_v that makes use of the critical values of the individual mobile phases[22]:

$$b_v = R_1 T_c / 8 P_c \tag{13}$$

where T_c, P_c are the critical temperature and pressure of the given mobile phase, respectively, and R_1 is the gas constant in units of l atm/mol K.

At this point, the slope from eqn. 12, e', was found to be proportional to the following expression:

$$\frac{MW^{sol}\ R_1\ T_c}{MW^{mob}\ b_v^{sol}\ 8 P_c} \tag{14}$$

where MW^{sol} and MW^{mob} are the molecular weight of the solute and mobile phase, respectively. Eqn. 14 represents the status of the empirical equation using only our constant temperature data, and so far lacks a suitable proportionality constant.

The next step was to look at the constant density experiments involving the variation of temperature, which involved a plot of ln k' *vs.* $1/T$. This is a variation of the Van 't Hoff plot from which enthalpy and entropy change are obtainable from slope and intercept, respectively:

$$\Delta H = -(\text{slope}) R_2 \tag{15}$$

$$\Delta S = (\text{intercept} + \ln \beta) R_2 \tag{16}$$

where R_2 is the gas constant in units of cal/mol K, and β is the phase ratio, defined by:

$$\beta = V_M / V_S \tag{17}$$

where V_M and V_S are the volumes occupied by the mobile and stationary phase, respectively. For these systems, the enthalpy and entropy values describe solute transfer *from* the mobile phase *to* the stationary phase. It should be noted that the ΔS values were meaningful only if swelling was neglected and the phase ratio was known. The calculated phase ratios were 25 for column A, and 83 for both columns B and C.

Obviously, the thermodynamics of solute transfer play a crucial role in retention in SFC[23]. In order to verify this fact, ΔH and ΔS were combined for each solute on each system by

$$\Delta G = \Delta H - T\Delta S \tag{18}$$

where ΔG is the free energy of solute transfer from mobile to stationary phase. Table V contains ΔH and ΔS values that exemplify the trends noted for all systems studied. The corresponding ΔG values calculated for two different temperatures are also included in Table V. The following is a summary of the trends observed for ΔG.

(a) For a given solute at constant temperature, ΔG increases (*i.e.*, its absolute value decreases) as density increases. This is explained by the increasing solvating ability of the mobile phase, which makes solute transfer into the stationary phase less favorable.

(b) For a given solute at constant density, ΔG increases as temperature increases. This may be explained by the increase in thermal energy imparted to the system.

(c) At constant temperature and density, ΔG decreases as the solute molecular weight increases. This indicates a lower solubility of the larger, heavier molecules in the mobile phase. In addition, one would expect more negative ΔG values for compounds that are retained longer and would, therefore, have larger partition coefficients.

It was necessary to prove that ΔG is included in the e' term of eqn. 12, by assuming that this slope is proportional to log K, were K is the partition coefficient, defined by:

$$K = k' \beta \tag{19}$$

Proving that ΔG is included in the slope was accomplished by combining eqn. 19 with the following relationship:

$$\log K = \frac{-\Delta G}{2.303 R_2 T} \tag{20}$$

whereby the combination of the two equations results in the following:

$$\frac{-\Delta G}{2.303 R_2 T} = \log k' + \log \beta \tag{21}$$

Subsequently, eqn. 21 was combined with eqn. 12 to yield

$$\frac{-\Delta G}{2.303 R_2 T} = d''_{(T)} - c''_{(T)} + e' \log \rho_{R(T)} + \log \beta \tag{22}$$

TABLE V

THERMODYNAMICS OF SOLUTE TRANSFER AS A FUNCTION OF TEMPERATURE AND REDUCED DENSITY FOR COLUMN B AND CO_2

Solute	*ΔH (kcal/mol) and ΔS (cal/mol K)*				*$\Delta G_{(T)}$ (kcal/mol)*			
	ρ_R = 0.6547		*ρ_R = 0.8712*		*T = 398 K*		*T = 448 K*	
	ΔH	*ΔS*	*ΔH*	*ΔS*	*ρ_R = 0.6547*	*ρ_R = 0.8712*	*ρ_R = 0.6547*	*ρ_R = 0.8712*
Naphthalene	–	–	−5.41	− 9.69	–	−1.55	–	−1.07
Biphenyl	− 7.24	−11.7	−6.19	−10.8	−2.60	−1.91	−2.02	−1.37
Fluorene	− 7.42	−10.6	−6.62	−10.4	−3.21	−2.48	−2.69	−1.97
Fluoranthene	− 9.02	−11.4	−8.00	−11.1	−4.49	−3.57	−3.92	−3.02
Phenanthrene	− 8.15	−10.9	−7.22	−10.6	−3.81	−3.01	−3.27	−2.49
Pyrene	− 9.14	−11.2	−8.13	−11.0	−4.68	−3.75	−4.12	−3.20
Chrysene	−10.6	−13.1	−8.99	−11.6	−5.23	−4.36	−4.78	−3.78

Finally, eqn. 22 was rearranged to the following form:

$$-\Delta G_{(T)} = (2.303R_2T)\,[d''_{(T)} - c''_{(T)} + \log \beta] + (2.303R_2T)e' \log \rho_{R(T)} \quad (23)$$

Hence, a plot of $-\Delta G_{(T)}$ *versus* $\log \rho_{R(T)}$ should result in a straight line with slope $(2.303R_2T)e'$, at a given temperature, T. Based on eqns. 21–23, the e' value obtained in this manner should be identical to that obtained from the $\log k'$ *vs.* $\log \rho_{R(T)}$ plot. The results for three different systems at three nominal temperatures are shown in Table VI; the agreement is very good, even striking in some cases considering that the ΔG values were obtained from a completely different set of experiments (constant density) than those obtained from eqn. 12 (constant temperature).

TABLE VI

COMPARISON OF e' VALUES OBTAINED FROM THE SLOPE OF $-\Delta G_{(T)}$ *VS.* LOG $\rho_{R(T)}$ (EQN. 23) WITH THOSE OBTAINED FROM THE SLOPE OF LOG k' *VS.* LOG $\rho_{R(T)}$ (EQN. 12)

Solute	e'					
	Column A and CO_2, $T = 373$ *K*		*Column B and* N_2O, $T = 448$ *K*		*Column B and* CO_2, $T = 398$ *K*	
	Eqn. 23	*Eqn. 12*	*Eqn. 23*	*Eqn. 12*	*Eqn. 23*	*Eqn. 12*
Naphthalene	−2.89	−3.00	–	−2.70	–	−3.00
Biphenyl	3.31	−3.41	–	−3.17	−3.38	−3.31
Fluorene	−3.69	–	−2.78	−2.96	−3.50	−3.53
Fluoranthene	−4.43	−4.42	−3.58	−3.75	−4.24	−4.23
Phenanthrene	−3.90	−4.01	−3.22	−3.26	−3.79	−3.85
Pyrene	−4.38	−4.47	−3.63	−3.67	−4.29	−4.28
Chrysene	−5.20	−5.07	−4.24	−4.21	−4.99	−4.84

The free energy can now be incorporated into the expression for the slope, e', by multiplying eqn. 14 by the right-side of eqn. 20 to yield

$$\frac{\Delta G\ R_1\ T_c\ MW^{sol}}{8P_c\ 2.303R_2T\ MW^{mob}\ b_v^{sol}} \quad (24)$$

This expression produces a value which is directly proportional to the experimental value of the slope for any solute in any mobile phase at any temperature, except it does not account for the density dependence of the free energy change. As stated earlier, it was not surprising that ΔG is density dependent; however, the key was to quantify this dependence and include it in the expression in eqn. 24. Ultimately, it was determined that the product of the free energy and the reduced density, $\Delta G\ \rho_R$, is a constant for a given system at a given temperature. Consequently, eqn. 24 was further revised to include this dependence:

$$e' = f\frac{\Delta G\ \rho_R\ R_1\ MW^{sol}}{8P_c\ 2.303R_2T_R\ MW^{mob}\ b_v^{sol}} \quad (25)$$

where T_R is the reduced temperature, T/T_c.

A dimensional analysis of eqn. 25 reveals that all the units cancel, leaving the constant, f, as a dimensionless proportionality constant that is characteristic of the mobile phase–stationary phase combination. Hence, we call this value the "phase constant", although we are currently unsure of the exact factors that give rise to it. The average values of the phase constant for the polyaromatic group of solutes, standard deviation and number of points used in the determinations, are listed in Table VII. The values for both CO_2 and N_2O were determined from data sets consisting of 5 temperatures, 3 densities and the series of phenanthrene, pyrene, fluoranthene and chrysene. Although fluoranthene was not expected to fit into the pseudo-homologous series of polyaromatics, it consistently exhibited a comparable phase constant and was included in the calculation of the average f value. The values for xenon were calculated from 3 temperatures, 2 densities and all 4 solutes. For SF_6 with column A, the data set consisted of 5 temperatures, 1 density and all 4 solutes. The combination of SF_6 and column B only consisted of one solute (phenanthrene) at one density because of instrumental difficulties. Finally, for SF_6 with column C, the set consisted of 4 temperatures, 3 densities and 2 solutes, phenanthrene and pyrene. It is apparent that the values for all systems are within a comparable range, except for xenon. The unique results for xenon may be related to the fact that it is a monoatomic molecule of unusually high polarizability[24,25].

TABLE VII

AVERAGE "PHASE CONSTANT" (f) CALCULATED FOR SOLUTES IN THE POLYAROMATIC SERIES OF PHENANTHRENE, PYRENE, FLUORANTHENE AND CHRYSENE

Mobile phase	*Column*	*Phase constant ± S.D.*	*n*
CO_2	A	1.58 ± 0.08	60
	B	1.31 ± 0.06	60
	C	1.34 ± 0.05	55
N_2O	A	1.70 ± 0.09	60
	B	1.55 ± 0.07	48
	C	1.53 ± 0.06	45
Xe	A	4.43 ± 0.35	24
	B	3.87 ± 0.16	24
	C	4.44 ± 0.23	21
SF_6	A	1.50 ± 0.06	20
	B	1.22 ± 0.06	5
	C	1.13 ± 0.17	24

As mentioned earlier, it is very likely that the effect of density on X_S is included in the a' term in eqn. 11. Since there are no stationary phase factors explicitly included in eqn. 25, it follows that f contains factors related to the amount and composition of the stationary phase coated on the column wall. An interesting point to note, with respect to CO_2 and N_2O, is that the phase constants on the biphenyl-containing (column B) and cyanopropyl-containing (column C) stationary phases are not significantly different from one another. This may be proof that the polarity and/or the polarizability of the stationary phase is one of the dominant factors in the phase

constant, accompanied by the specific interaction between a given mobile phase and a given stationary phase. However, in the case of xenon, it seems that the f values for columns A and C are comparable, although the standard deviations are substantial. In this particular case, it may be assumed that xenon undergoes a unique interaction with all three stationary phases due to its size and polarizability.

Another dominant factor implicit in the phase constant, f, may be the swelling of the stationary phase caused by uptake of the mobile phase[26–28]. Specifically, since column A has a larger film thickness than the two more polar columns, the difference between the respective phase constants may indeed be a reflection of swelling effects, or simply a greater sample capacity exhibited by the phase of greater film thickness. Closer examination of the values in Table VII reveals that the ratio of f for column A to that of column B is between 1.1 and 1.2 for all four mobile phases. This may be related to the fact that column B only contains 30% as much unswollen stationary phase as column A. Further investigation is clearly warranted in this area.

The precision in the constant for a given system is surprisingly good, considering the numerous sources of error associated with the development of this empirical equation. First, there is a large uncertainty associated with the determination of ΔG from the Van 't Hoff plot due to the expected error in the slope and intercept from the linear regression. In addition, ΔG is dependent on knowing the true value of β, which is assumed to be invariant with temperature and density for a thin-film capillary column[26].

Other sources of error in the phase constant can be attributed to the estimation of the Van der Waals volume of the solute, and an error in the calculation of the "true" critical density, which is an iterative process based on an equation of state, and may not be truly representative of the experimental value. Finally, there exists a source of experimental error in maintaining a constant density. Although the conditions remain relatively stable, there will always be fluctuation around the desired value, which will detract from the precision of the resulting retention data.

Table VIII demonstrates that the other solutes, naphthalene, biphenyl and fluorene, do not produce the same constant and must belong to different series. This is only surprising in the case of naphthalene, which should fit into the polyaromatic series of Table VII. This anomalous behavior is attributed to an exaggerated error in peak retention for the smallest molecule, which often coelutes with the solvent peak. Another possible source of error is the use of methylene chloride for the determination of t_0, because even this small, volatile molecule will exhibit some retention during elution. A more accurate measure of t_0 may be obtained from extrapolation of homologous series retention measurements or alternatively, from an injection of methane gas[29].

Examination of the "phase constants" in Table VIII reveals that most of the calculated values are between 1.75 and 2.45, with the exception of the results for xenon. Once again, it is apparent that those f values are considerably higher than those of the other systems, *i.e.*, between 5 and 8. Unfortunately, there are not enough values on any given system to permit a detailed comparison of behavior based on the combination of mobile phase and stationary phase.

Intercept

We have so far ignored the intercept of the $\log k'$ *vs.* $\log \rho_{R(T)}$ plot. The intercept

TABLE VIII

AVERAGE "PHASE CONSTANT" (f) CALCULATED FOR OTHER SOLUTES STUDIED

Mobile phase	*Column*	*Solute*	*Phase constant ± S.D.*	*n*
CO_2	A	Naphthalene	2.22 ± 0.14	12
		Biphenyl	2.13 ± 0.11	12
		Fluorene	1.95 ± 0.05	8
	B	Naphthalene	2.45 ± 0.23	5
		Biphenyl	2.13 ± 0.12	15
		Fluorene	1.75 ± 0.04	15
	C	Fluorene	2.05 ± 0.12	12
N_2O	A	Naphthalene	2.32 ± 0.06	12
		Biphenyl	2.28 ± 0.06	12
		Fluorene	2.11 ± 0.14	15
	B	Fluorene	2.06 ± 0.19	15
SF_6	A	Naphthalene	2.45 ± 0.08	5
		Biphenyl	2.21 ± 0.10	5
		Fluorene	1.85 ± 0.05	5
	C	Biphenyl	2.31 ± 0.23	12
		Fluorene	1.63 ± 0.20	12
Xe	A	Naphthalene	7.25 ± 1.22	6
		Biphenyl	6.53 ± 0.85	6
		Fluorene	5.51 ± 0.44	6
	B	Fluorene	5.30 ± 0.26	6

TABLE IX

COMPARISON OF LOG k'_0 OBTAINED EXPERIMENTALLY AND FROM THE INTERCEPT OF THE LOG–LOG PLOT FOR TWO SOLUTES AT TWO TEMPERATURES

Solute	*Mobile phase*	*Column*	*398 K*		*423 K*	
			Experimental	*Intercept*	*Experimental*	*Intercept*
Pyrene	CO_2	A	−0.045	−0.092	−0.25	−0.17
		B	−0.14	−0.14	−0.37	−0.33
		C	−0.17	−0.15	−0.42	−0.37
	N_2O	A	−0.16	−0.22	−0.36	−0.31
		B	−0.33	−0.34	−0.53	−0.51
		C	−0.37	−0.32	−0.63	−0.57
Fluorene	CO_2	A	−0.48	−0.44	−0.62	−0.54
		B	−0.81	−0.80	−0.98	−0.96
		C	−0.94	−0.90	−1.14	−1.05
	N_2O	A	−0.58	−0.50	−0.69	−0.64
		B	−0.94	−0.92	−1.10	−1.05
		C	–	−1.05	–	−1.21

contribution should theoretically come from the solubility of the solute in the mobile phase and the stationary phase, and perhaps other unknown contributions. In this way, the intercept can be isolated quite easily when $\rho_R = 1$, *i.e.* when $\rho = \rho_c$; at this point, log k' should equal the intercept of the log–log plot. Therefore, we refer to this value as the "critical-density point", log k'_0. Martire and Boehm[14] proposed a similar concept in their study of retention in SFC; however, their term, called ln K^0, represented the "zero-density point", corresponding to ideal GC. We believe that our interpretation reflects the true situation in SFC. In order to verify the assumption, a series of injections was performed at the estimated critical density, *i.e.*, at several points on the critical isotherm, for several systems. Table IX contains the comparison of measured and linearly extrapolated log k'_0 values for two nominal solutes, pyrene and fluorene, although similar results were found for all solutes studied. The agreement is strikingly good, and any discrepancies between the two methods are believed to be due to variations in pump pressure during the run, and from the error in choosing the critical density. The final form of the empirical equation is presented here, combining intercept and slope terms derived above:

$$\log k' = \log k'_0 + f\frac{\Delta G\ \rho_R\ R_1\ \mathrm{MW}^{sol}}{8P_c\ 2.303R_2\ T_R\ \mathrm{MW}^{mob}\ b_v^{sol}} \log \rho_{R(T)} \tag{26}$$

where

$$\log k'_0 = d''_{(T)} - c''_{(T)} \tag{27}$$

at a given point on the critical isotherm. The value obtained for the intercept is normally negative at high temperatures, but often positive at low temperatures, depending on the molecular weight of the solute. This is very interesting because it implies that the relative contributions of d'' and c'' are dependent on solute size as well as temperature. From these investigations, it was found that elution of the largest solute (chrysene) at low temperatures consistently produces the most positive intercept, whereas elution of the smallest solute (naphthalene) at higher temperatures produces the most negative intercept (see Table II). These observations can be rationalized by considering that the heavier molecules exhibit low solubility in the mobile phase, especially at low temperature, and therefore possess a dominant d'' term. Conversely, the lighter solutes will be more soluble in the mobile phase, and therefore possess a dominant c'' term. Identification of the d'' and c'' constants can be made by considering the definition of log k'_0 (*cf.*, eqn. 9) where

$$\log k'_0 = \log n_{S(0)} - \log n_{M(0)} \tag{28}$$

Eqn. 28 is, by definition, simply the amount of solute dissolved in each phase at a given point on the supercritical isotherm, and the subscript (0) denotes the critical density condition. The final equation that describes polyaromatic solute retention results from the substitution of eqn. 28 into eqn. 26.

CONCLUSION

A log k' *vs.* log ρ_R relationship was chosen to describe retention in SFC. The resultant empirical equation is a comprehensive and potentially useful model of retention. Although this model has only been proposed for a capillary column system, it seems likely that the theory would be valid for packed columns as well. Experimental data obtained with polynuclear solutes are in excellent agreement with eqn. 26, which indicates that the actual retention of a group of chemically related compounds may be predictable. In addition, the investigation of the polyphenyl compounds demonstrated that different classes of compounds indeed exhibit different retention mechanisms, as one would expect. Importantly, although most of the SFC practiced today is done with density programming, this does not represent a fundamental problem in our derivation. If the variation of density with time is known, then the prediction of retention under programming conditions should essentially be a simple mathematical problem.

As yet, the main drawback of our equation is that the "phase constant", f, must be pre-determined for a given mobile phase–stationary phase combination because of the presently unknown factors that give rise to it. In addition, since the equation has been developed for a specific group of polyaromatics, it is unknown at this time how the equation should be modified to describe the retention of other compound classes. There is also a minor difficulty in adapting the equation to modified mobile phases, because the critical temperature, pressure and density, must be known in order to use the equation. Hence, there is a need to develop algorithms that are capable of determining the critical conditions of mixed mobile phases with reasonable accuracy.

Finally, it must be pointed out that the theory cannot be logically extrapolated to GC or LC, as it is by no means an attempt at providing a unified view of chromatography; however, the derived equations make sense in the generalized concept of SFC and will be continually developed in order to encompass a broader range of solutes and phases in order to be truly useful. For example, work is now underway to test this theory on the retention of polar compounds.

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CHROM. 22 250

Isocratic networks in supercritical fluid chromatography

III[a]. Dependence of capacity factor, selectivity and resolution on temperature, pressure, density and free volume of pentane as shown in multi-dimensional plots

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SUMMARY

A plotting program for drawing three-dimensional graphs in supercritical fluid chromatography was employed which allows a fourth variable to be represented by colours. The graphs contain two physical parameters of the mobile phase pentane, *e.g.*, temperature and pressure or temperature and density, and, on the same plot, one or two chromatographic parameters, *e.g.*, capacity factor, selectivity or resolution of polycyclic aromatic hydrocarbons as the analyte. The plotting program is useful for showing interrelations between physical and chromatographic parameters on the one hand, and between chromatographic parameters themselves on the other.

INTRODUCTION

In preceding papers[1–11], we have reported on the dependence of chromatographic parameters, such as retention, selectivity, plate number and resolution, on the physical parameters temperature and pressure for pure, one-component mobile phases on bare, unmodified silica. All these studies were performed with an analyte test mixture composed of the polycyclic aromatic hydrocarbons naphthalene, anthracene, pyrene and chrysene. In some instances, the dependence of chromatographic parameters on the density and free volume of the mobile phase was also studied[6,9,12,13]. In general, the behaviours of different mobile phases such as alkanes, ethers, carbon dioxide, nitrous oxide and trifluoromethane were found to be very similar.

For pentane as the mobile phase, the network of available data was dense enough to allow three-dimensional plotting[5,7]. These three-dimensional plots yield an overall impression of the dependence of capacity factors[5], selectivity[7], plate number[7] and resolution[5] on both pressure and temperature over the pressure and temperature

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ranges studied. Hence, they can guide the chromatographer towards the optimization of a separation, as long as the pressure–temperature dependence of the chromatographic behaviour of the sample and of the column under investigation resembles that of the test mixture and the test column.

In this paper we report the use of a computer program that is capable not only of three-dimensional regression of data and of forming a regular grid from irregularly placed experimental data, in addition to smoothing of the grid, but also permits colour shading of the three-dimensional contour[14]. The last feature can be used for facilitating the approximate reading of data from the plots, or for adding additional information by superimposing data of a second z-variable in the form of coloured bands on the three-dimensional contour.

EXPERIMENTAL

The supercritical fluid chromatographic (SFC) apparatus, including the UV detector, the columns and the chemicals, have been described previously[5]. The mobile phase was pentane, the stationary phase unbonded LiChrosorb Si 60 (10 μm) and the analyte mixture consisted of naphthalene, anthracene, pyrene and chrysene (PAH-1). Heptane, which gave a small but definite response in the UV trace, was used as an inert compound for measuring dead times. The liquid volume flow-rate of the pumps at ambient temperature was maintained at 1 ml/min, and confirmed by measurement at the outlet of the apparatus.

The capacity factor of chrysene, k'(C), and the selectivity between pyrene and chrysene, α(PC), were calculated as usual by means of the equations

$$k'(\mathrm{C}) = [t_\mathrm{r}\,(\mathrm{C}) - t_0]/t_0 \tag{1}$$

$$\alpha(\mathrm{PC}) = [t_\mathrm{r}\,(\mathrm{C}) - t_0]/[t_\mathrm{r}\,(\mathrm{P}) - t_0] \tag{2}$$

where t_r (C) and t_r (P) are the retention times of chrysene and pyrene, respectively, and t_0 is the dead time. As a measure of separation quality, the average resolution $R_\mathrm{m}^{*1,5}$ for the three peak pairs of PAH-1 was chosen. The resolution values for the individual pairs were calculated as described previously[5].

The free volume, f_r, is defined[12] relative to the volume required for the close packing of the mobile-phase molecules in the crystalline state, *i.e.*, as a reduced parameter according to the equation

$$f_\mathrm{r} = (V_{p,T} - V_0)/(V_0) = (V_{p,T}/V_0) - 1 = \rho_0/\rho_{p,T} - 1 \tag{3}$$

where V_0 is the specific volume of the crystalline state at the melting temperature, $V_{p,T}$ the specific volume of the gaseous mobile phase at the pressure p and the temperature T of the chromatographic experiment and ρ_0 and $\rho_{p,T}$ are the corresponding densities.

The regression and plotting program (Unimap; Uniras European Software Contractors, Düsseldorf, F.R.G. and Lyngby, Denmark) used in this work offers a number of possibilities. Different interpolation methods can be selected to transform a set of chromatographic data which is not regularly distributed with respect to the physical parameters, *e.g.*, temperature and pressure, to a set which is regularly

distributed, that is, which forms a regular network or grid. Here a bilinear interpolation was used to form the regular network. In all cases shown, a grid was chosen which, first, adheres as closely as possible to the experimental data points available, and second exhibited nodes (crossing points) not more numerous than the number of data points. Additionally, a low smoothing level was selected which was based on quadratic interpolation. The smoothing consisted of improving the estimate of the bilinear interpolation by computing gradients at the points and using quadratic interpolation, and finally by refining the values by distance weighting methods. For a given set of original data, different interpolation methods and different smoothing levels were compared with each other and with the original data. It was ascertained that the graph represented the original untreated data within a reasonable range of experimental error. The three-dimensional plots, for which any viewpoint, *i.e.*, perspective, can be selected, show the *z*-parameter as a three-dimensional contour in space, on which coloured contour bands can be drawn to allow an easier reading of the *z*-parameter. Alternatively, it is possible to present two different *z*-parameters simultaneously. In this instance, the three-dimensional contour represents one *z*-parameter, leaving the information conveyed by the coloured bands for the second *z*-parameter.

For a pretreatment of the irregularly distributed original data points[15], to adapt them beforehand to the regular interpolation grid, it was of advantage first to plot these experimental data, *e.g.*, k' *versus* p and k' *versus* T. From these curves, the data were read which directly fitted the physical parameters in the selected regular grid. For the density-based plots, the experimental p–T pairs were converted to densities using data tables[15].

RESULTS AND DISCUSSION

Chromatographic parameters such as retention, selectivity and resolution have been shown to depend strongly on both temperature and pressure. This is found again in three-dimensional plots for the simultaneous dependence on temperature and pressure of the capacity ratio of chrysene (not shown), of the selectivity between pyrene and chrysene (Fig. 1) and of the average resolution (not shown), all of them calculated from the chromatograms for the PAH-1 test mixture. For each of these three chromatographic variables, a distinct maximum is seen above the critical temperature of the mobile phase [T_c (pentane) = 196.5°C], whereas a minimum is formed below T_c. Although the height of the maximum of k'(C) is greatly reduced if the pressure is above p_c [p_c (pentane) = 33.7 bar], the corresponding maximum for the selectivity between pyrene and chrysene, α(PC), is still considerable. At temperatures below T_c, α(PC) rises strongly again, even at pressures above p_c. This is in contrast to the selectivity between naphthalene and anthracene or that between anthracene and pyrene[7], but it corresponds to results obtained with enantiomer separations[16,17], where below T_c the selectivity was found to increase with decreasing temperature of the liquid mobile phase. Nevertheless, Fig. 1 shows that at low pressures the highest values for α(PC) occurs in the region above T_c.

Fig. 1 demonstrates that the three-dimensional graphs are useful not only for gaining a qualitative overview of the behaviour of a chromatographic variable over a given pressure–temperature range, but also for obtaining easier readings from the

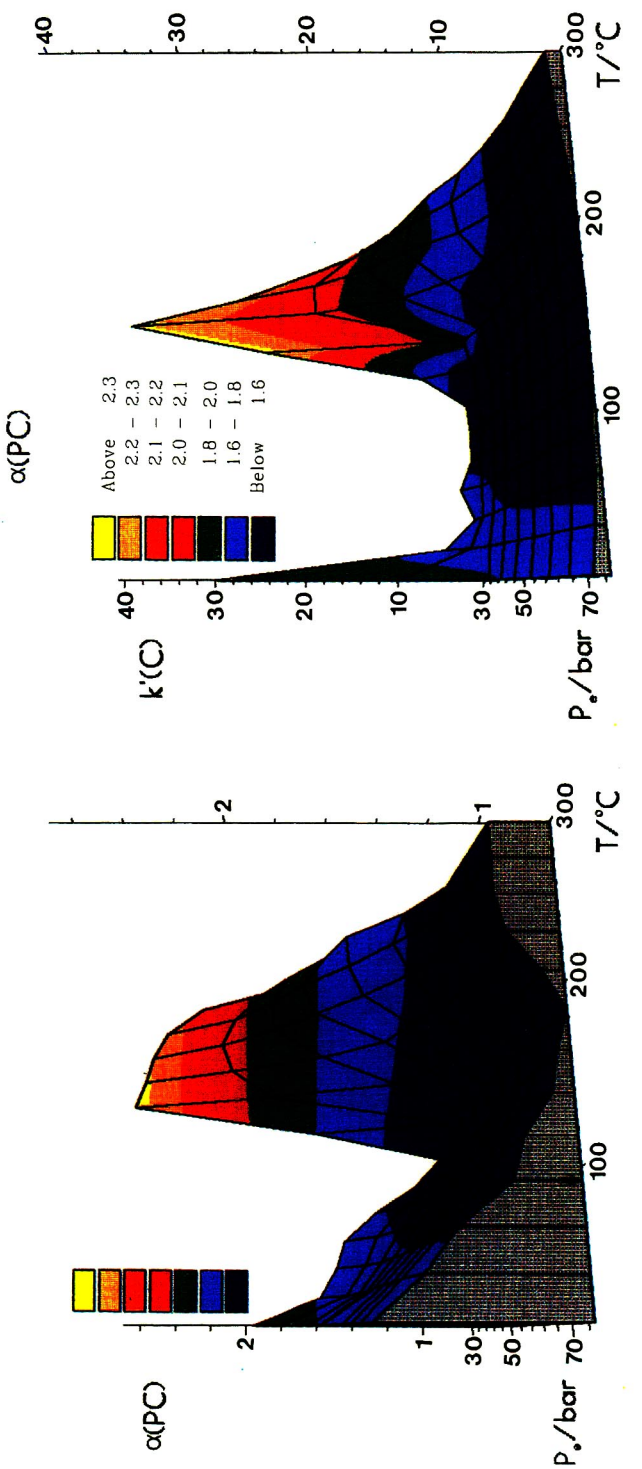

Fig. 1. Three-dimensional network plot for the selectivity between pyrene and chrysene, α(PC), *versus* column exit pressure, p_e, and temperature, T. The α(PC) are shown both as one of the three axes (*z*-axis) and as colour bands (key to colours as given), the latter facilitating the reading of numerical values from the plots.

Fig. 2. Three-dimensional network plot for the capacity factor of chrysene, k'(C), *versus* p_e and T. The corresponding selectivity between pyrene and chrysene, α(PC), is shown as colour bands.

graphs by means of the colour bands. Nevertheless, the main benefit of such three-dimensional graphs is not to allow readings of data, but rather to show instantly general, qualitative features about the interrelations between physical and chromatographic parameters. It should be emphasized, however, that the chromatographic data will depend not only on the physical conditions of the mobile phase such as temperature and pressure, but also on the analyte and on the stationary phase material, although the general behaviour may often resemble fairly closely that shown here.

The possibility of plotting two different z-variables simultaneously is demonstrated by Fig. 2. One z-variable is displayed as the three-dimensional contour, while the other is superimposed on that contour by coloured bands. Thus, interrelations between two chromatographic variables can be made directly visible. Fig. 2 shows such an interrelation between k'(C) and α(PC). Similarities and differences of the behaviour of these two variables as functions of p and T become obvious from this diagram. There are maximum values for both k'(C) and α(PC) above T_c. These maxima become lower and are shifted to higher temperatures with increasing pressure. A broad α(PC) maximum compared with the sharp maximum for k'(C) and differences in behaviour at high pressures and low temperatures can also be seen.

The dependence of retention on density and temperature is shown in Fig. 3, on reduced density and reduced temperature in Fig. 4 and on free volume and reduced temperature in Fig. 5, all in the temperature region above T_c. The retention k' decreases monotonously with increasing temperature (at constant density), with increasing density (at constant temperature) and with increasing reduced density. The free volume shows a basically similar behaviour, although, as expected, in the opposite direction of the density or the reduced density. The use of the free volume instead of the density has the advantage, however, that different mobile phases can be compared directly. In comparison with the reduced density, the free volume gives a more realistic measure of the space between molecules. Together with the structure, configuration and conformation of the mobile-phase molecules, this factor determines the chromatographic properties of the mobile phase at a given temperature. High free volumes give rise to decreasing solvent strength of the mobile phase. Higher temperatures lead to the opposite effect. Thus, k'(C) rises with increasing free volume (at constant temperature) and with decreasing temperature (at constant free volume). The qualitative information in these three plots is similar, because the physical parameters are related to each other.

The dependences of selectivity and resolution on density and temperature are illustrated in Figs. 6 and 7, respectively. The selectivity between pyrene and chrysene shows similar features to the retention of chrysene (Fig. 3). This is reasonable, because these compounds closely resemble each other in chemical structure and molecular weight. Hence the influence of varying the density of the mobile phase and the temperature on the k' values of the two compounds is expected to be similar. The resolution, on the other hand, shows a maximum with respect to temperature. Whereas retention and selectivity are influenced by thermodynamics alone, resolution is also influenced by kinetic effects. These kinetic effects, which determine band broadening, differ in their dependence on pressure and temperature from the thermodynamics effects. Temperature-dependent maxima of resolution at constant density have also been observed previously using propane[9] and carbon dioxide[13] as mobile phases.

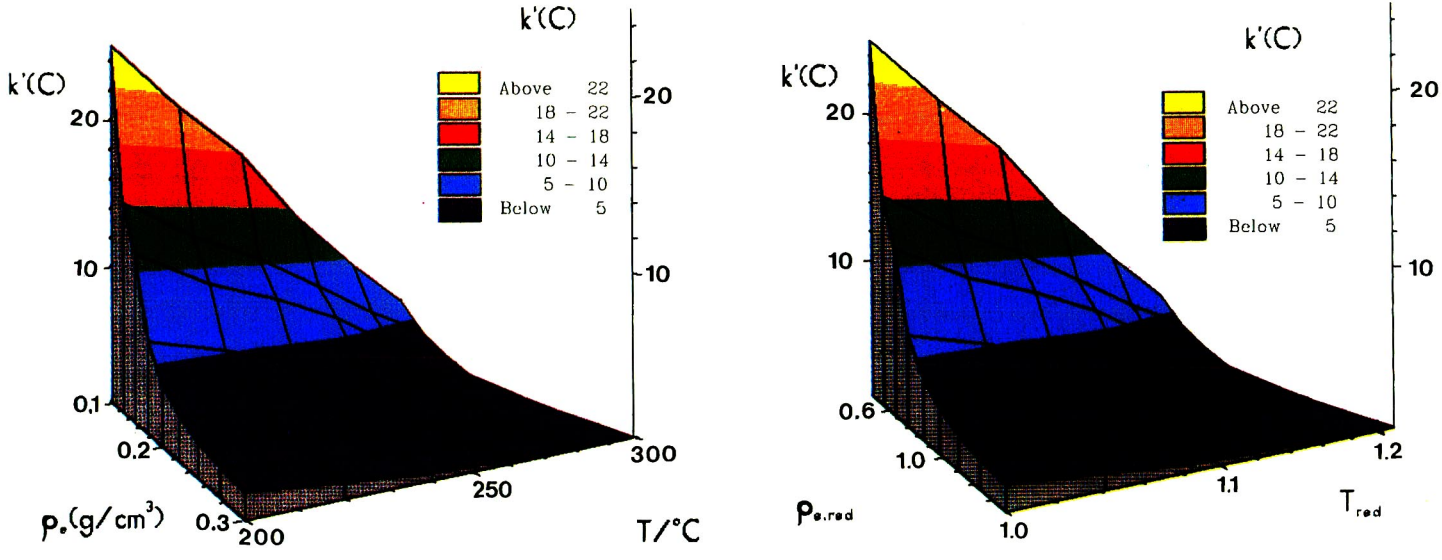

Fig. 3. Three-dimensional network plot for k'(C) *versus* the density at the column exit, ρ_e, and temperature, T. The k'(C) are shown both as one of the axes and as colour bands.

Fig. 4. Plot of k'(C) *versus* the reduced density at the column exit, $\rho_{e,red}$, and the reduced temperature, T_{red}. The k'(C) are seen both as one of the axes and as colour bands.

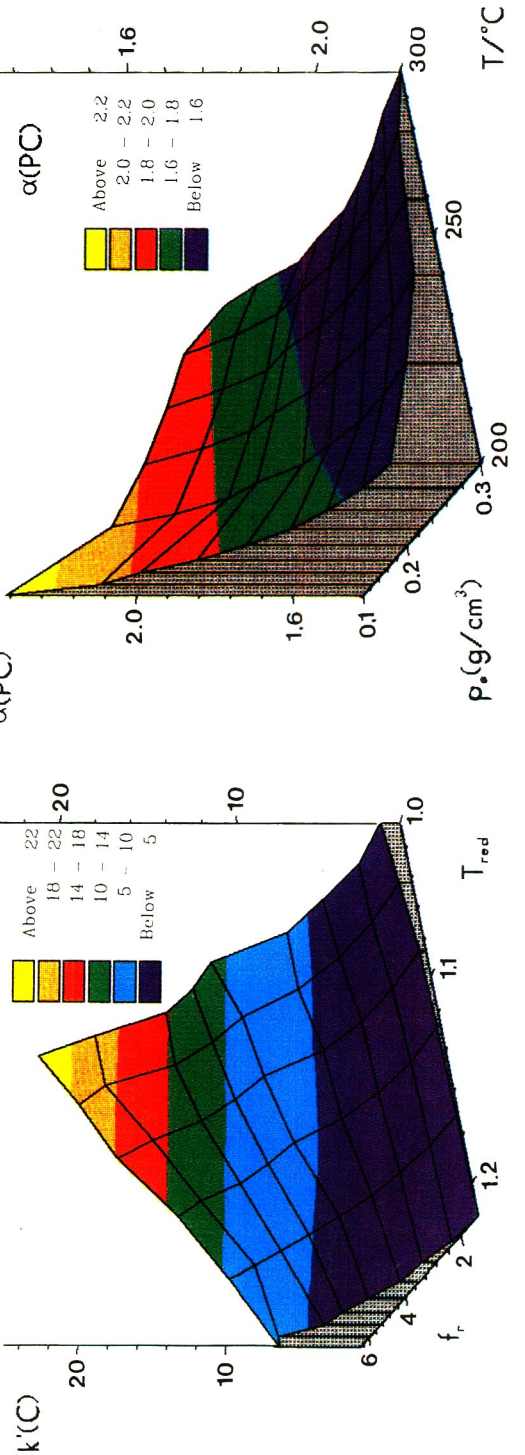

Fig. 5. Plot of k'(C) *versus* the reduced free volume, f_r, and the reduced temperature, T_{red}. The k'(C) are given both as one of the axes and as colour bands.

Fig. 6. Plot of α(PC) *versus* ρ_e and T. The α(PC) are shown both as one of the axes and as colour bands.

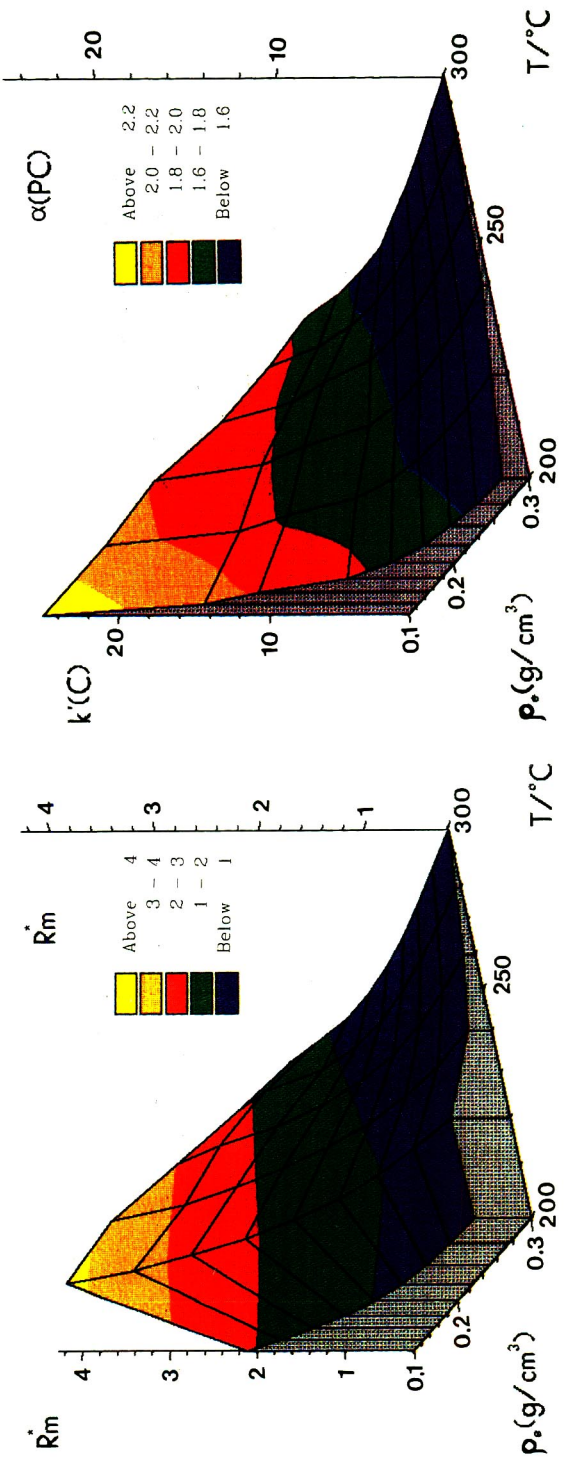

Fig. 7. Plot of R_m^* *versus* ρ_e and T. The R_m^* are seen both as one of the axes and as colour bands.

Fig. 8. Three-dimensional network plot for k'(C) *versus* ρ_e and T. The corresponding α(PC) are given as colour bands.

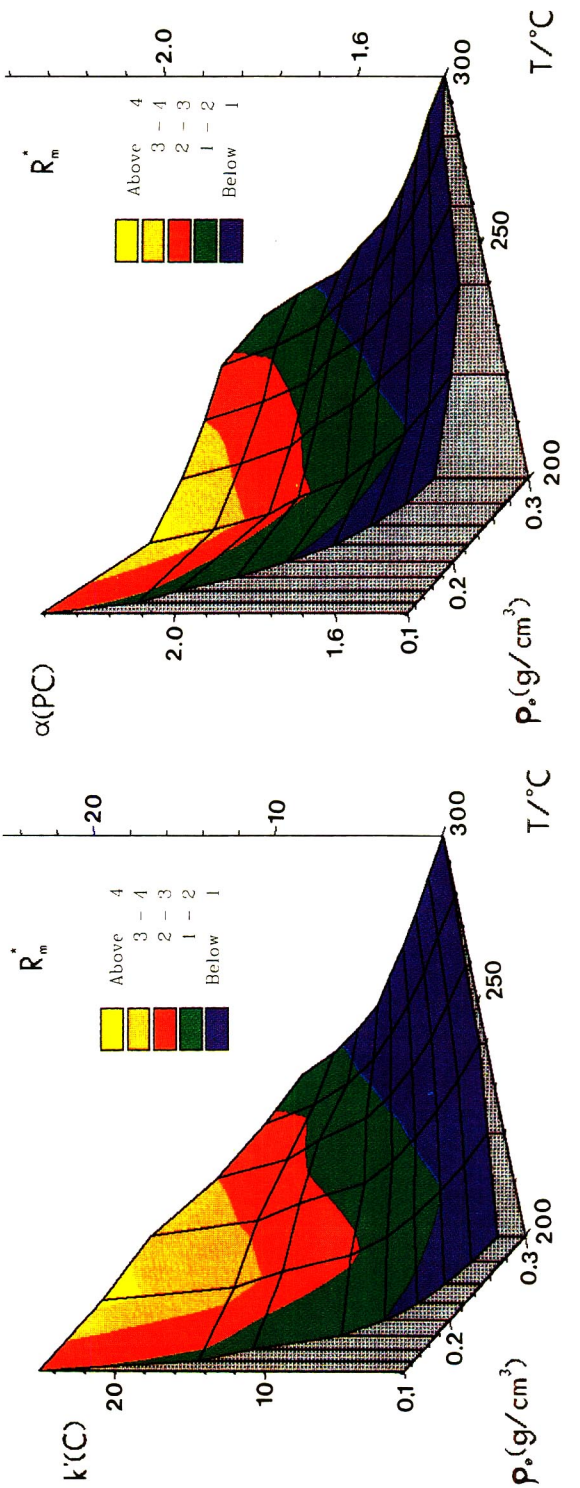

Fig. 9. Plot of k'(C) *versus* ρ_e and T. The corresponding R_m^* are shown as colour bands.

Fig. 10. Plot of α(PC) *versus* ρ_e and T. The corresponding R_m^* are seen as colour bands.

The similarities between the density–temperature dependence of retention and selectivity are clearly evident from Fig. 8, which shows k'(C) as the three-dimensional contour and α(PC) as coloured bands. Also directly visible are the strong differences in behaviour between retention and resolution (Fig. 9) and between selectivity and resolution (Fig. 10), showing R_m^* as the coloured bands. The coloured bands are shaped as irregular half-circles and, therefore, do not behave like contour lines with respect to height, as approximated, for instance, in Fig. 8.

CONCLUSION

The applications of the plot program presented here demonstrate that this and similar programs can be of value for generating informative three-dimensional plots. Specifically, the possibility of presenting two chromatographic variables in one three-dimensional diagram can be used for demonstrating interrelations between different chromatographic variables.

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Three-dimensional network plots using colours for a fourth variable with binary mixtures of pentane and 1,4-dioxane under sub- and supercritical conditions

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SUMMARY

Three-dimensional contour plots are shown for the capacity ratio of chrysene, k'(C), and the selectivity between pyrene and chrysene, α(PC), in mobile phases composed of pentane and 1,4-dioxane. For isocratic plots, the dependence of k' and α on pressure and temperature is shown, and for isobaric plots the dependence on temperature and mobile phase composition is demonstrated at two constant pressures and at one constant reduced pressure. Additional information is included in the plots by coloured shading of the three-dimensional surface, where the different colour bands represent the different ranges of an additional chromatographic variable. Thus, k'(C) was combined with α(PC) or with the average resolution, R_m, of an aromatic–hydrocarbon standard mixture, the latter two chromatographic variables represented by colour. α(PC) was similarly combined with R_m. Such plots can help in clarifying interrelations between different physical and chromatographic parameters, which is useful for the optimization of separations.

INTRODUCTION

In supercritical fluid chromatography (SFC), changes in temperature, pressure, mobile phase flow-rate and mobile phase composition can be used to influence a chromatographic separation. The aim of these manipulations is to obtain a high resolution in combination with a short analysis time. It is therefore useful to know how capacity factors and resolution vary with changing physical parameters.

It has been observed experimentally that for chromatographic parameters, such as the capacity factor k', the mean resolution R_m and the selectivity α, maxima may occur above the critical point of the mobile phase, depending on the pressure, temperature and mobile phase composition[1–3]. Frequently, but not generally, a similar location of the maxima for capacity factors and resolution was found. A direct correlation of capacity factors with resolution or with selectivity in the same plot,

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comprising the two chromatographic variables on the *z*-axis or as colour, respectively, would help to illustrate the interrelations between the two chromatographic parameters. In this work, an example of such a direct correlation of the two chromatographic parameters is shown for isocratic or isobaric plots employing binary mobile phases composed of pentane and 1,4-dioxane. The plots demonstrate several fundamental features that are useful for the optimization of separations when varying temperature, composition and pressure.

EXPERIMENTAL

The chromatograms were measured on a previously described SFC instrument[4] based on a commercially available high-performance liquid chromatograph. Pretreatment of eluents and chromatographic conditions have also been described in detail elsewhere[5]. All measurements were made at constant volume flow-rate, as delivered by the pumps in the liquid state. The stainless-steel columns (25 cm × 4.6 mm I.D.) were packed with LiChrosorb Si 100 (10 μm). The capacity factor, k', and the selectivity, α, were calculated using the following equations:

$$k' = \frac{t_r - t_0}{t_0} \tag{1}$$

$$\alpha = \frac{k'_j}{k'_i} \quad \text{or} \quad \frac{k'(\text{pyrene})}{k'(\text{chrysene})} \tag{2}$$

The resolution, R_m, was calculated as the arithmetic mean of the resolutions R_{ij} for the hydrocarbon pairs naphthalene–anthracene, anthracene–pyrene and pyrene–chrysene (the four aromatic hydrocarbons were used as a standard test mixture):

$$R_m = \frac{\Sigma R^*_{ij}}{n} \tag{3}$$

where $n = 3$ and where the resolutions of the individual peak pairs R^*_{ij} are given by[6]

$$R^*_{ij} = \frac{f_{ij}}{g_{ij}} + \frac{d_{ij}}{w'_i + w'_j} \cdot \sqrt{\ln 4} \tag{4}$$

The mean resolution R_m was chosen as a measure of the separation quality because of its simplicity and its direct connection with the basic chromatographic parameters k', α and plate number, N. For interpolation and plotting of the three-dimensional network plots, in which a fourth variable is represented by colouring the network, a computer program (Unimap; Uniras European Software Contractors, Lyngby, Denmark and Düsseldorf, F.R.G.) was used, as described in detail elsewhere[7].

Although for a variety of binary fluid media the critical data and their variance with composition have been determined experimentally, no experimental data have been published so far for the pair pentane–1,4-dioxane. Since a knowledge of the critical data is essential for supercritical fluid chromatography, the data were

calculated according to the method of Chueh and Prausnitz[8]. The binary interaction parameter needed for the calculation was estimated to be $k_{ij} = 0.05$, in analogy with similar values for other solvent pairs. The resulting curves are shown in Fig. 1, giving the critical temperature, T_c, and the critical pressure, p_c, as a function of the volume fraction of 1,4-dioxane in pentane. The volume fraction was chosen as a scale in order to facilitate correlation of the calculated critical data with the chromatographic results shown in the subsequent figures.

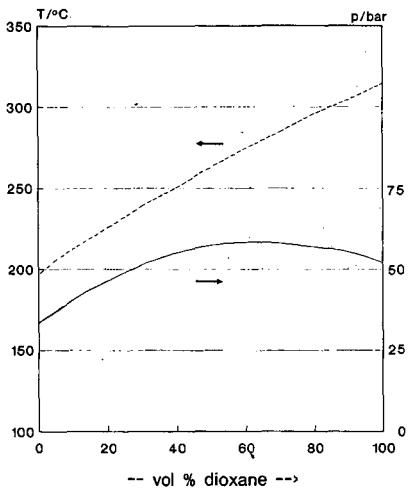

Fig. 1. Critical pressure (full line) and critical temperature (broken line) for pentane–1,4-dioxane calculated by the method of Chueh and Prausnitz[8].

RESULTS AND DISCUSSION

A three-dimensional network plot of two chromatographic *versus* two physical parameters, showing the capacity factor of chrysene, k'(C), as a contour variable (z-axis), depending on pressure, p (bar) (x-axis) and temperature, T (°C) (y-axis), is shown in Fig. 2. As a second z-variable, the mean resolution, R_m, is added as coloured contour bands. The mobile phase was pentane containing 10% of dioxane, for which the exprimental data have been presented previously[9]. The colour scale on the left of Fig. 2 shows the actual mean resolution values corresponding to the colours used in the plot. For both the capacity factor and the resolution a maximum is found fairly close to the mobile phases critical temperature of 212.5°C (*cf.*, Fig. 1). The maximum for the mean resolution is, however, shifted by about 20°C to lower temperatures compared with the maximum for the capacity factor. Going to higher pressures, the maxima for both variables form a "mountain ridge", which slopes downward strongly with higher pressures and which shifts at the same time to higher temperatures. The highest values for both parameters are shown in the region of subcritical pressure ($p_c \approx 40$ bar), where the capacity factor exceeds 70 and the mean resolution reaches values around 8. High absolute values for both parameters may be of interest for the separation of complex mixtures in wich compounds with low capacity factors are combined with

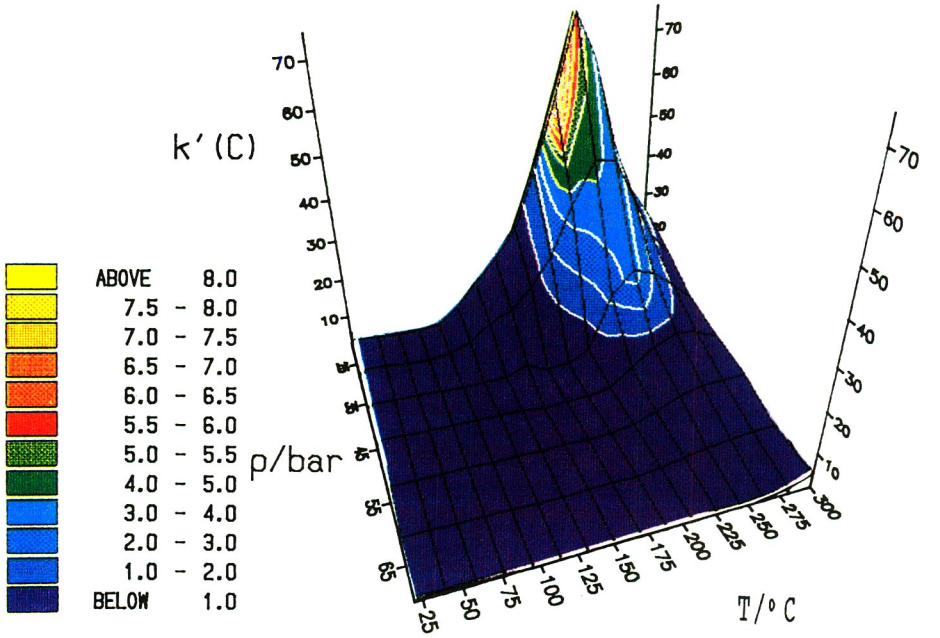

Fig. 2. Capacity factor of chrysene, k'(C), and mean resolution, R_m, as a function of column outlet pressure, p, and temperature, T, for pentane–10% (v/v) 1,4-dioxane.

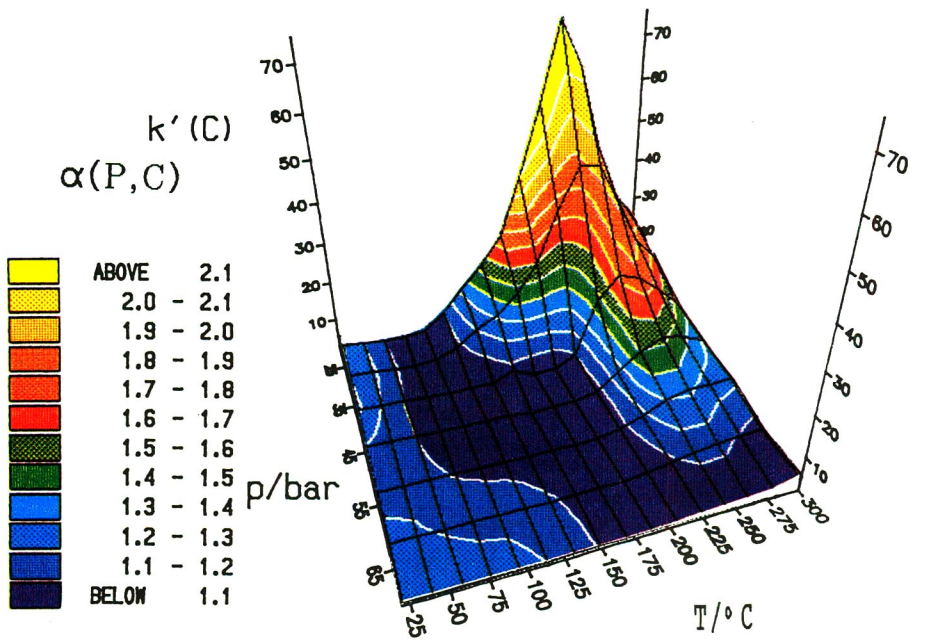

Fig. 3. Capacity factor, k'(C), and selectivity, α(PC), as a function of column outlet pressure, p, and temperature, T, for pentane–10% (v/v), 1,4-dioxane.

compounds with much higher capacity factors. For less difficult separations, much lower resolutions will, of course, be sufficient. The plot demonstrates that for the test mixture used here, resolutions above 1.0 are found over a fairly wide range of capacity factors, depending on temperature and pressure of the mobile phase.

The selectivity, α, between two peaks is also useful for the characterization of chromatographic separations. Fig. 3 again shows the capacity factor, k'(C), as the contour variable but, instead of R_m, the selectivity, α(PC), between pyrene and chrysene as the coloured contour bands. The general trend is the same as in the previous plot. The behaviour of k' and α differs only at low temperatures, where α is found at relatively high values (up to 1.3), as opposed to the very low values of k'.

An increase in selectivity with decrease in temperature has frequently been observed in liquid chromatography (LC) and has also been reported for the supercritical range[10,11]. Further information can be obtained from a plot which correlates selectivity and mean resolution, as presented in Fig. 4. The combination of selectivity, α(PC), as the contour variable and the mean resolution, R_m, as colour information shows that in the low-temperature area a fairly high selectivity can still result in a low mean resolution; the minimum of selectivity with respect to temperature is not reflected in a corrsponding behaviour of the mean resolution. Fig. 4 demonstrates that in the supercritical region the selectivity generally needs to be high for the resolution to exceed unity.

In contrast to the isocratic plots in Figs. 2–4 which are valid for 10% dioxane, Fig. 5 shows an isobaric plot for pentane–dioxane at 20 bar. The variables are the capacity factor, k'(C) (z-axis, contour), the amount of dioxane in the mobile phase, %B (x-axis), the temperature, T(°C) (y-axis) and the mean resolution, R_m (z-axis, colour bands). For both z-variables, maxima at about the same position are observed, the general behaviour of the capacity factor and mean resolution being similar to that described before for Figs. 2–4. For the whole plot, the pentane–dioxane system is below the critical pressure. As shown in Fig. 1, the critical parameters T_c and p_c vary with changing dioxane content, *i.e.*, along the %B axis in the plots. The increase of the critical temperature with increase in the content of the modifier dioxane may explained the observed shift of the "mountain ridge" to higher temperatures on increasing the dioxane content. Fig. 6 shows a plot for the same system at 36 bar. Here, part of the plot is in the supercritical region with respect to both temperature *and* pressure; the highest values for the two z-parameters are found in this supercritical area. However, when compared with Fig. 5 the absolute values of capacity factors and resolutions decrease with increasing pressure. Generally, a low pressure is advantageous for the resolution of complex mixtures of volatile and non-volatile compounds, whereas a higher pressure reduces capacity factors. With respect to the reduced capacity factors, it is observed by comparison of Figs. 5 and 6 that the decrease in capacity factor with increasing pressure is much more pronounced than the corresponding decrease in resolution. For parts of the plot in Fig. 6 the resolution still exceeds 2, *i.e.*, it may be high enough for the separation of not too complex mixtures.

Figs. 7 and 8 show plots for an estimated reduced pressure $p_r = p/p_c$ of 1.09 for the pentane–dioxane system. This means that the pressure is above p_c for the whole area of the plots. The experiments were carried out such that the pressure was increased according to the increase in the critical pressure, p_c, with composition. However, the low-temperature parts of the plots are still subcritical with respect to temperature (*cf.*,

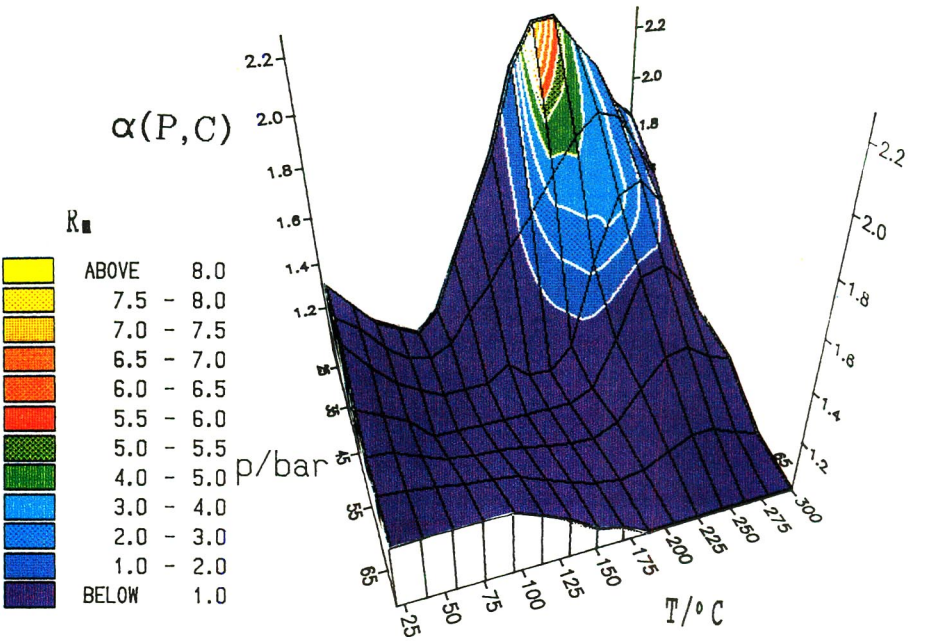

Fig. 4. Selectivity, α(PC), and resolution, R_m, as a function of column outlet pressure, p, and temperature, T, for pentane–10%, (v/v) 1,4-dioxane.

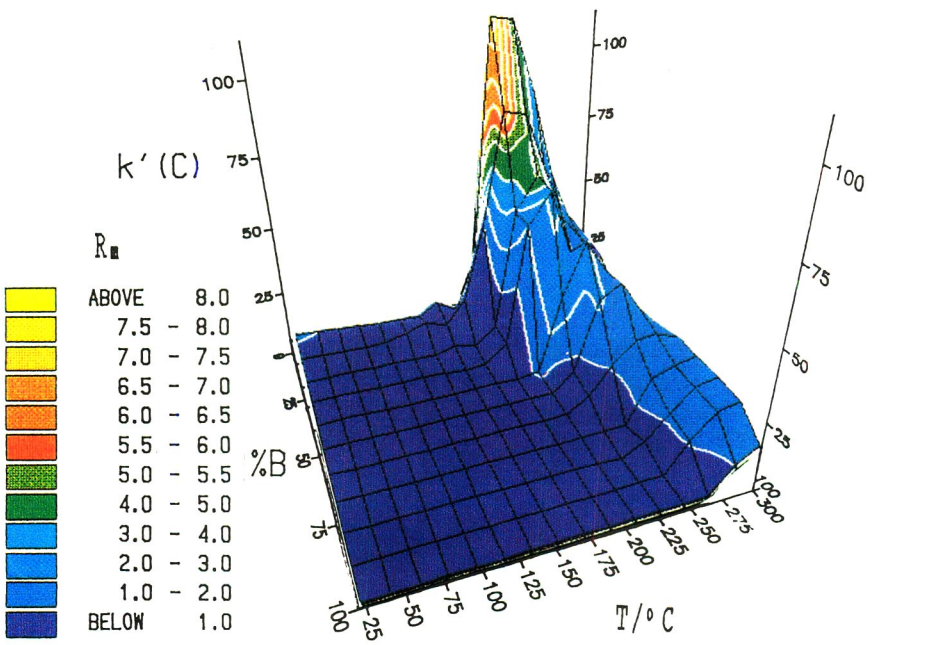

Fig. 5. Capacity factor, k'(C), and resolution, R_m, as a function of mobile phase composition, %B (v/v), and temperature, T, for pentane–1,4-dioxane at a constant column outlet pressure of 20 bar.

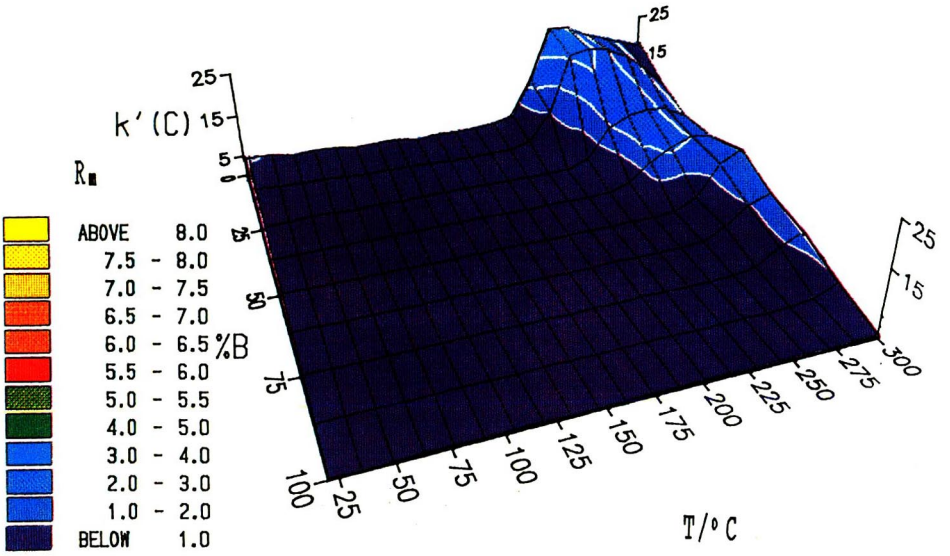

Fig. 6. Capacity factor, k'(C), and resolution, R_m, as a function of mobile phase composition, %B (v/v), and temperature, T, for pentane–1,4-dioxane at a constant column outlet pressure of 36 bar.

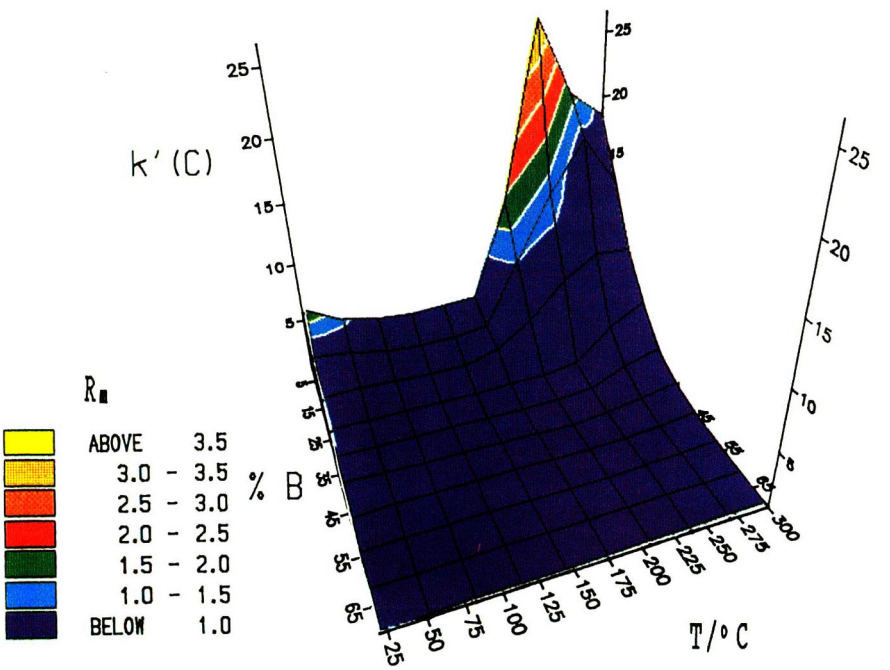

Fig. 7. Capacity factor, k'(C), and resolution, R_m, as a function of mobile phase composition, %B (v/v), and temperature, T, at a constant reduced pressure of $p_r \approx 1.09$.

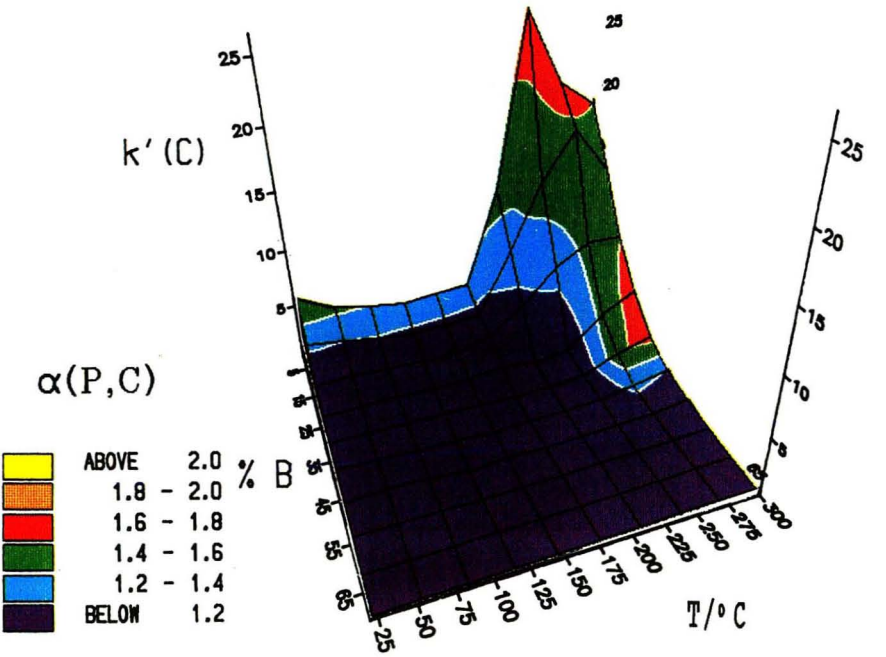

Fig. 8. Capacity factor, k'(C), and selectivity, α(PC), as a function of mobile phase composition, %B (v/v), and temperature, T at a constant reduced pressure of $p_r \approx 1.09$.

Fig. 1). Fig. 7 shows the capacity factor as the contour variable and resolution as colour information for a composition range between 0 and 70% dioxane. Both z-variables show a nearly parallel behaviour with a maximum in the supercritical area. Fig. 8 shows the combination of capacity factor and selectivity for the same composition–temperature range. There is an area (red) with low capacity factors but still relatively high selectivies, as already found to some extent in Fig. 3. A combined plot for selectivities, α(PC), and resolutions, R_m, demonstrates the differences in behaviour between selectivity and resolution (not shown). Whereas the selectivity contour reveals three separate maxima, only two of these coincide with resolution maxima. In the third α-maximum, which is located of *ca.* 20–30% dioxane at high temperatures, selectivity values are seen that are as high as for the two other maxima, but resolution remains below unity. This is due to increasing peak widths yielding reduced plate numbers. This demonstrates that high selectivities do not neccessarily lead to highly resolved peaks.

CONCLUSIONS

The coloured three-dimensional plots, in which two axes represent physical parameters such as pressure and temperature in isocratic plots and temperature and mobile phase composition in isobaric plots, are useful for providing an overview of the dependence of two chromatographic parameters chosen among k', α and R_m from the two named physical parameters. The chromatographic parameters all pass through

maxima with changing temperature. Thereby, for both the isocratic and the isobaric plots, the maxima for k', α and R_m tend to be in similar locations with respect to the physical parameters but they do not usually coincide. The maxima decrease in height with pressure and also with the content of dioxane in the pentane–dioxane binary mobile phase used in this work. Whereas this decrease is very strong for k', it is much less pronounced for R_m. Hence high resolutions can still be obtained with reasonably low capacity factors. The plots indicate the values for the physical parameters which give particularly favourable resolutions at a given capacity factor.

ACKNOWLEDGEMENT

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Comparison of supercritical carbon dioxide and supercritical propane as mobile phases in supercritical fluid chromatography

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SUMMARY

Comparisons of supercritical propane and supercritical carbon dioxide eluents were made on bare silica and octadecylsilane-derivatized silica using substituted and unsubstituted aromatic hydrocarbons as test solutes. The greater elution strength of carbon dioxide relative to propane for these solutes on underivatized silica is indicated to be largely the result of more effective competitive adsorption by carbon dioxide rather than enhanced mobile phase solubility. The effects of the addition of methanol modifier on solute retention were more pronounced in propane for both polar and non-polar solutes. An increase in the retention of polar aromatics on silica was observed with increasing density in methanol modified propane. This is apparently the result of a concomitant increase in the availability of stationary phase adsorption sites.

INTRODUCTION

The choice of a mobile phase solvent in supercritical fluid chromatography (SFC) depends to a large extent on the nature of the samples to be analyzed. Solute retention and selectivity are determined in part by mobile phase solvent strength which is a result of dispersive and specific interactions between solute and solvent. Direct comparisons of mobile phases can be used to gain insight into the nature and relative magnitude of solvent–solute interactions which can be useful for selecting an appropriate mobile phase.

Several comparisons of supercritical fluid mobile phases have been reported in the literature. Leyendecker *et al.*[1] examined the influence of density on the chromatographic behavior of lower alkanes as mobile phases in SFC. Chrysene was eluted more quickly in supercritical pentane than in supercritical propane at the same reduced density and reduced temperature. Lauer *et al.*[2] reported the retention behavior of a number of model compounds in carbon dioxide and in nitrous oxide as

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a function of temperature at equal densities. Leyendecker *et al.*[3] have compared the retention of several aromatic hydrocarbons in a number of SFC mobile phases at equal pressures and at equal reduced pressures. Retention results obtained at equal pressures varied considerably from those obtained at equal reduced pressures as was anticipated based on the differences in solvent critical pressures. Wright *et al.*[4] compared capacity factors of a polarity test mixture in supercritical carbon dioxide, nitrous oxide and ethane at equal reduced temperatures and reduced pressures. Leyendecker *et al.*[5] compared the capacity factors of aromatic hydrocarbons in supercritical diethyl ether and dimethyl ether under isobaric conditions.

Since comparisons of mobile phases may be conducted in a number of different ways and because the experimental parameters chosen for a comparison can greatly alter solute retention, care must be taken in drawing conclusions as to the relative solvent strength of the mobile phases. For instance, a comparison made at equal reduced temperatures of two mobile phases which have different critical temperatures may require greatly different operating temperatures. Differences in solute retention measured under these conditions may be due in part to differences in solute volatility resulting from variations in operating temperature rather than differences in the solvating strengths of the mobile phases. Since the solvating strength should depend on the distance between molecules, comparisons made at equal density should be more informative than those based on equal pressure. However, equal densities do not account for differences in molecular weights which may influence solvent strength. Klesper *et al.*[6] have suggested comparisons of mobile phases at equal free volumes in order to best evaluate the relative dissolution power of the solvents. In their report, the free volume, V_f, was calculated from the fluid density at pressure p and temperature T, $\rho_{p,T}$, and from the reference state density, ρ_0, of the crystalline state at the melting point and atmospheric pressure:

$$V_f = \frac{\rho_0}{\rho_{p,T}} - 1 \tag{1}$$

A comparison at equal free volumes is similar to one based on equal reduced densities. As an experimental consideration, it should be noted that equal reduced pressures do not necessarily result in equal reduced densities for two fluids at either the same temperature or at the same reduced temperature. In the report by Klesper *et al.*[6], capacity factors of chrysene and pyrene measured on a LiChrosorb Si 100 column were found to be much larger in supercritical carbon dioxide than in supercritical pentane at equal free volumes and equal reduced temperatures. However, on the same column at equal free volumes and equal temperatures, the capacity factor of pyrene was found to be approximately the same in both solvents as reported by Schmitz[7]. Capacity factors for pyrene determined at equal free volumes in supercritical carbon dioxide and in several supercritical alkane eluents were found to fall on a common curve when plotted *versus* temperature. These similarities decreased at higher free volumes (lower densities), but good correlation was found[7] at free volumes as high as 1.7. In a comparison of the same solvents at equal densities, the capacity factor of chrysene was found to be much larger in carbon dioxide than in propane at the same temperature. This would be anticipated based on the previous results since the density of carbon dioxide is more than twice that of propane at equal free volumes.

In this report, the retention behavior of several substituted and unsubstituted aromatic hydrocarbons is presented in supercritical carbon dioxide and in supercritical propane on silica and octadecylsilane (ODS)-derivatized silica stationary phases. Aromatic hydrocarbons with polar functionalities were included in order to examine differences in the specific interactions of the solvents with various functional groups. The effects of a small amount of methanol modifier on the solvent strength of each mobile phase was also determined and adsorption isotherm measurements were made for methanol in each solvent in an attempt to correlate relative modifier surface coverage with the retention data.

EXPERIMENTAL

The experimental set-up for the measurement of solute capacity factors is illustrated in Fig. 1. Methanol was mixed with the solvents by adding a known volume to the syringe reservoir of a Varian 8500 syringe pump and filling the balance with the mobile phase solvent. The volume percent modifier was calculated from the total reservoir volume during pressurization at the point at which the operating pressure was obtained. The determination of adsorption isotherms was carried out in the same manner as described previously[8]. For both types of measurements, additional safety precautions were implemented to minimize the possibility of propane combustion. A steady flow of argon at *ca.* 100 ml/min was introduced into the column oven to prevent build-up of an explosive atmosphere in the event of column leakage. The propane effluent was collected after it passed through the back pressure regulator by allowing it to expand into a 20-lbs. refillable propane cylinder (Charmglow). A 4 cm × 4.6 mm I.D. column packed with 60–200-mesh silica (Fisher) was placed in line from

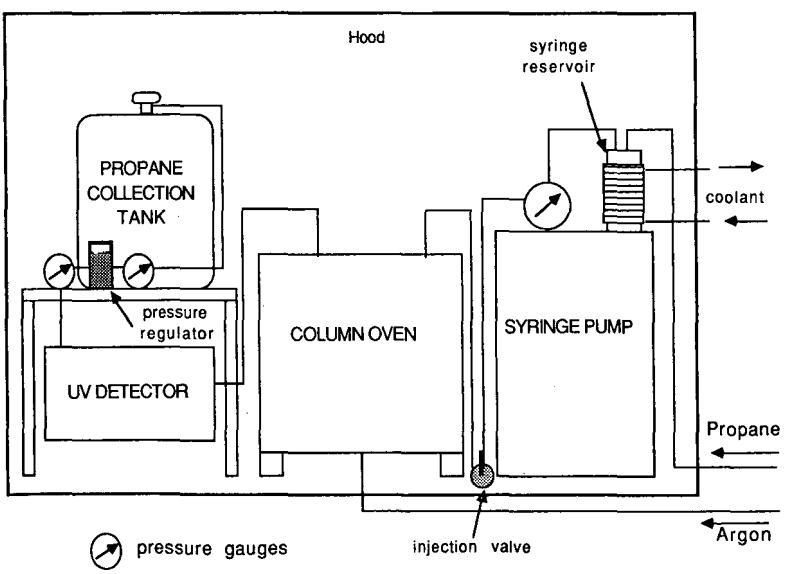

Fig. 1. Experimental set-up for retention studies.

the fluid cylinders to the solvent pump to remove impurities from the solvents. Identical conditions were maintained for carbon dioxide with the exception that the carbon dioxide effluent was vented into the hood rather than collected.

The underivatized silica column was 10 cm × 4.6 mm I.D. and packed with Whatman Partisil-10. The ODS-derivatized silica column was 4 cm × 4.6 mm I.D. and packed with Whatman Partisil-10 chlorotrimethylsilane endcapped ODS-2. Free volumes were calculated from eqn. 1 using values of ρ_0 of 0.75 g/ml for propane and 1.56 g/ml for carbon dioxide[9]. The carbon dioxide was supercritical fluid grade from Scott Speciality Gases. The propane was C. P. grade and also obtained from Scott. Mobile phase densities were calculated from published temperature–pressure–density relationships for propane[10] and carbon dioxide[11].

All capacity factor measurements were made in the usual manner from the retention time, t_R, and the column dead time, t_0, determined using benzene[a]

$$k' = \frac{(t_R - t_0)}{t_0} \tag{2}$$

and were reproducible to ± 1.5%. All measurements were made at 100°C.

The solutes used in this study are listed in Fig. 2. The solutes are numbered in the same order throughout this paper.

RESULTS AND DISCUSSION

Capacity factors on underivatized silica

The capacity factors for solutes 1–7 measured on the underivatized silica column (Partisil 10) in carbon dioxide and in propane at equal free volumes of 1.55 are shown graphically in Fig. 3. A free volume of 1.55 corresponds to a propane density of 0.29 g/ml and a carbon dioxide density of 0.61 g/ml. The capacity factors of solutes 8–11 were too large to be measured in propane under these conditions. In contrast to the results of Schmitz[7], pyrene (solute 2) exhibited significantly greater retention in propane than in carbon dioxide. As shown in Fig. 3, the capacity factors measured in propane are more than twice those measured in carbon dioxide. Solvent selectivities for each solute were determined from capacity factor ratios

$$\alpha_{CO_2/C_3H_8} = \frac{k'_{CO_2}}{k'_{C_3H_8}} \tag{3}$$

[a] The retention time of benzene decreased slightly in propane on the underivatized silica column with the addition of 1% methanol modifier indicating that benzene was not unretained in unmodified propane under these conditions. The retention time of hexane measured at 200 nm in unmodified propane on this column was found to be slightly less than that of benzene measured under the same conditions and to correspond with the retention time of benzene in methanol-modified propane. This value was used as a measure of t_0 for unmodified propane on the bare silica column.

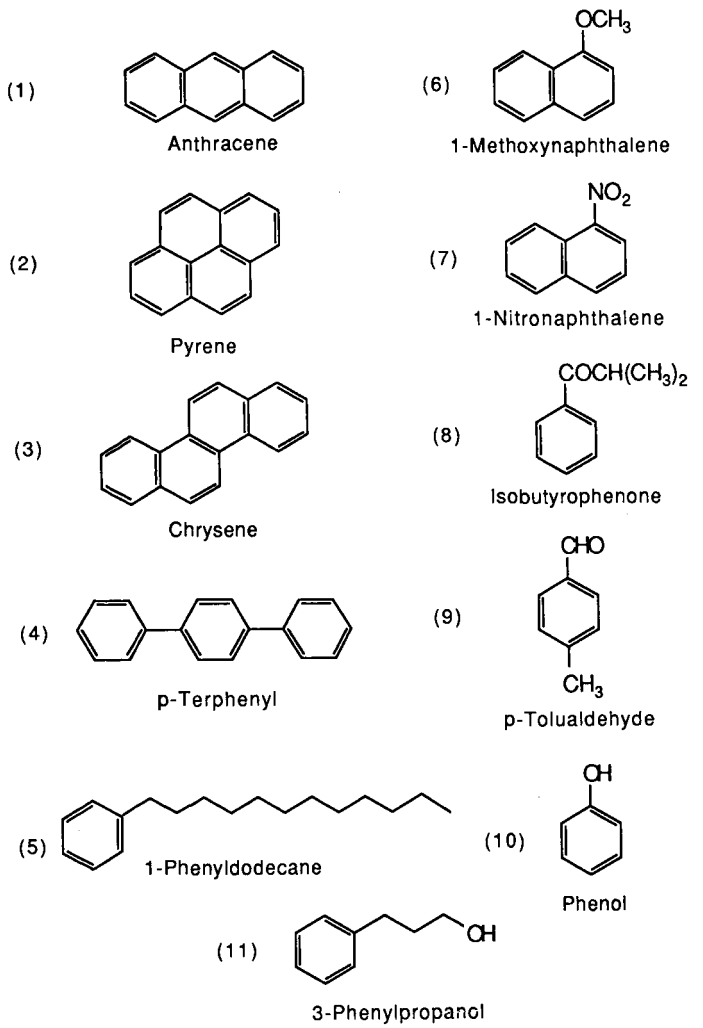

Fig. 2. Test solutes.

and are listed in the first column of Table I. The greatest relative difference in retention is seen for nitronaphthalene (solute 7) which suggests that specific interactions are an important factor in the greater elution strength of carbon dioxide. Among the unsubstituted polycyclic aromatics, solutes 1–4, *p*-terphenyl (solute 4) exhibited the greatest relative difference in retention and pyrene (solute 2) the smallest difference. The polarizabilities of the unsubstituted aromatics were calculated according to the method of Miller and Savchik[12] and are listed in Table II. Van der Waals volumes were calculated using the method of Bondi[13] and are also listed in Table II. A strong correlation was found between solute polarizabilities and the logarithms of the capacity factors in carbon dioxide as indicated by the correlation coefficient (r = 0.990) for a linear least squares fit to the data. Poor correlation (r = 0.786) was

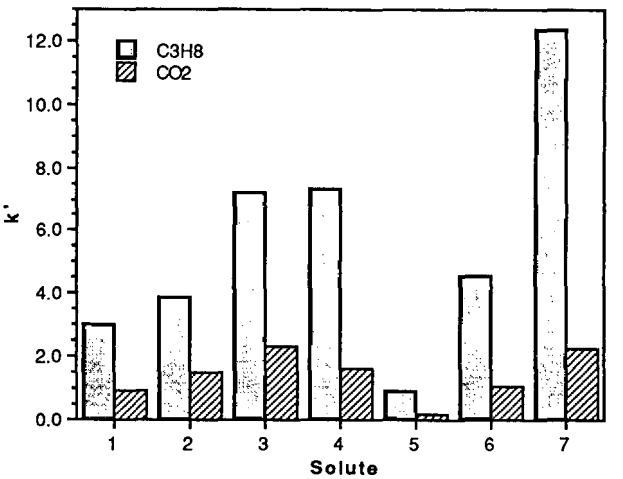

Fig. 3. Capacity factors (k') in propane and in carbon dioxide at equal free volumes of 1.55 on underivatized silica.

TABLE I

SOLVENT SELECTIVITIES AT 100°C

Stationary phase	Partisil-10	Partisil-10	ODS-2	Partisil-10	ODS-2
V_f (CO_2)	1.55	3.25	1.55	1.55	1.55
V_f (C_3H_8)	1.55	0.88	1.55	1.55	1.55
Methanol (%g/ml)	0.0	0.0	0.0	1.0	1.0
Solute	α_{CO_2/C_3H_8}				
1	0.311	1.02	2.07	0.813	1.37
2	0.392	1.25	2.73	0.882	1.76
3	0.323	1.26	2.89	0.804	1.86
4	0.223	0.849	2.81	0.969	1.83
5	0.217	1.59	6.50	1.86	1.83
6	0.238	0.660	1.59	0.671	1.18
7	0.181	0.505	0.844	0.494	0.710
8	–	0.316	0.691	0.348	0.974
9	–	0.356	0.417	0.292	0.524
10	–	0.310	0.416	0.246	0.327
11	–	–	0.558	0.246	0.476

TABLE II

PHYSICAL CONSTANTS OF UNSUBSTITUTED POLYCYCLIC AROMATICS

Solute	*Molecular weight*	*Polarizability* ($Å^3$)	*Van der Waals volume* (cm^3/mol)
Anthracene	178.2	25.8	99.56
Pyrene	202.3	30.1	109.0
Chrysene	228.3	33.5	125.2
p-Terphenyl	230.3	29.7	131.8

observed between these measurements in propane. Conversely, a good correlation was observed between the solute Van der Waals volumes and the logarithms of the capacity factors in propane ($r = 0.985$) but not in carbon dioxide ($r = 0.774$). Although the exact nature of the above correlations canot be determined from these results, a difference in solute molecular interactions in these two solvents is indicated.

In an attempt to compare the two mobile phases under iso-eluotropic conditions, the density of the carbon dioxide mobile phase was lowered and the density of the propane mobile phase was raised until roughly equivalent capacity factors were obtained on the average for the unsubstituted aromatic hydrocarbons as shown in Fig. 4a. The trend in solvent selectivities for solutes 1–7 at equal free volumes is shifted as at

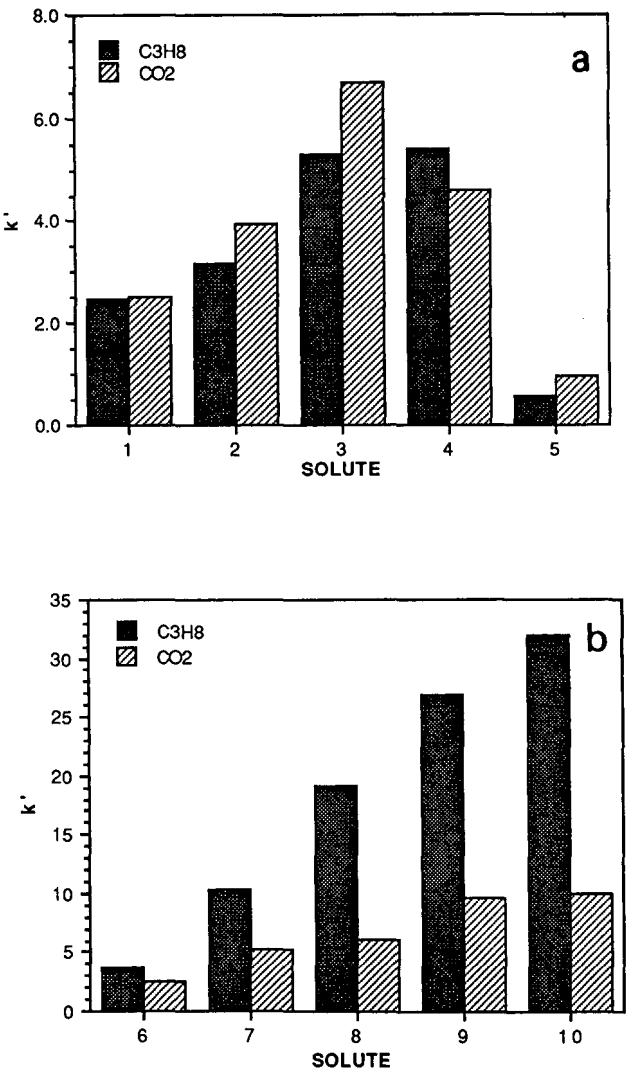

Fig. 4. Capacity factors in propane at 0.40 g/ml and in carbon dioxide at 0.48 g/ml on underivatized silica: (a) solutes 1–5 and (b) solutes 6–10.

these new densities as can be seen in the second column of Table I. Chrysene (solute 3) has the largest value of solvent selectivity of the unsubstituted aromatics with shorter retention in propane than carbon dioxide. *p*-Terphenyl (solute 4) still exhibits shorter retention in carbon dioxide than in propane under these conditions resulting in a much smaller value of solvent selectivity. Phenyldodecane (solute 5) exhibited the largest change in solvent selectivity with changing density showing a sharp increase in retention in carbon dioxide with decreasing density. The capacity factors of the polar-substituted aromatics at these same densities are plotted in Fig. 4b. 3-Phenylpropanol (solute 11) was not eluted in propane at this density. Unlike the unsubstituted aromatics, the capacity factors of these solutes are much smaller in carbon dioxide than in propane. This result is not unexpected since, unlike propane, carbon dioxide possesses bond dipoles which should increase its specific interactions with the more polar solutes. As indicated by the solvent selectivity values in the second column of Table I, the capacity factors of these solutes in carbon dioxide relative to propane tend to decrease as the retention of the solutes increases. Apparently, as the magnitude of the specific interactions of the solutes with the polar adsorption sites on the silica surface increases, the greater chemical effect of carbon dioxide relative to propane becomes more pronounced.

ODS column at equal free volumes

Capacity factors for all of the test solutes were measured in carbon dioxide and in propane at an equal free volume of 1.55 on an ODS-derivatized column. These capacity factors are shown graphically in Fig. 5. In contrast to the results shown in Fig. 3, which were obtained under these same conditions on the underivatized column, the retention of solutes 1–6 is much shorter in this case in propane than in carbon dioxide. The greater elution strength of propane indicates a greater distribution of these solutes into the mobile phase from the alkyl-bonded stationary phase. It can be assumed that this is the result of greater solubility of these solutes in propane relative to carbon

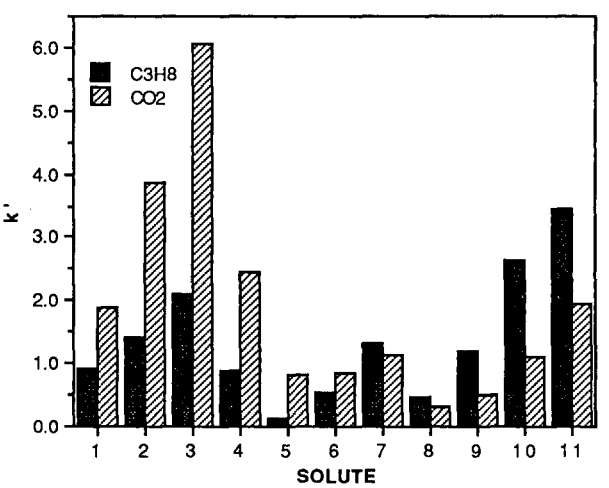

Fig. 5. Capacity factors in propane and in carbon dioxide at equal free volumes of 1.55 on ODS-derivatized silica.

dioxide since the potential for competitive adsorption is essentially eliminated by chemical derivatization of the silica surface. On the other hand, this argues that the greater eluent strength of carbon dioxide for these solutes on the underivatized silica column is the result of its ability to interact more strongly with the stationary phase surface and not the result of greater solubility of the solutes in the mobile phase. Carbon dioxide can apparently interfere more effectively with solute adsorption onto the silica surface thus reducing retention by competitive adsorption or through a mechanism of the type described by Snyder and Glajch[14] as site-competition delocalization. Therefore, the nature of the stationary phase must be considered in making comparisons of mobile phase solvent strength. The results reported by Schmitz[7] which indicated equal capacity factors for pyrene (solute 2) in carbon dioxide and in propane at equal free volumes on a LiChrosorb Si 100 column apparently represent an intermediate case in which competitive adsorption by carbon dioxide just balances the greater solubility of pyrene in propane.

As indicated by the mobile phase selectivities in the third column of Table I, phenyldodecane (solute 5) exhibits a much greater solubility in propane than in carbon dioxide as compared to the other solutes. This may be the result of the greater aliphatic character of this solute providing for better solubility in the hydrocarbonaceous solvent, however the same selectivity difference is not seen on the underivatized column. This might be explained by more effective competitive adsorption of carbon dioxide with this solute as compared to the unsubstituted polycyclic aromatics which would result in a smaller value of solvent selectivity for phenyldodecane than would otherwise be anticipated. The solvent selectivities of solutes 1–4 are similar and result in a good correlation between the corresponding capacity factors in each solvent ($r = 0.989$). This suggests similar types of molecular interactions of these solutes in the two solvents in contrast to the differences observed on the underivatized silica column.

In comparison to solutes 1–5, the polar-substituted aromatics exhibit shorter retention in carbon dioxide than in propane on the ODS column with the exception of methoxynaphthalene (solute 6) as also illustrated in Fig. 5. This is expected based on the potential for greater specific interactions between the polar functionalities of the substituted aromatics and the bond dipoles of carbon dioxide. It also suggests that the shorter retention of these solutes in carbon dioxide relative to propane on the underivatized silica column is not solely the result of more effective competitive adsorption.

Column selectivities

Another interesting comparison can be made by examining the relative change in retention of the solutes in each mobile phase as a function of column type. Column selectivities were calulated from the capacity factors of the solutes on the underivatized Partisil-10 column and the ODS column:

$$\alpha_{\text{Partisil}-10/\text{ODS}} = \frac{k'_{\text{Partisil}-10}}{k'_{\text{ODS}}} \tag{4}$$

As indicated in Table III, the retention of solutes 1–5 in carbon dioxide is greater on the underivatized column as compared to the ODS column. The opposite is true in

TABLE III

COLUMN SELECTIVITIES IN CARBON DIOXIDE AND IN PROPANE AT $V_f = 1.55$

Solute	$\alpha_{Partisil\text{-}10/ODS}$	
	Carbon dioxide	*Propane*
1	0.502	3.34
2	0.395	2.75
3	0.386	3.46
4	0.672	8.48
5	0.256	7.66
6	1.28	8.56
7	1.99	9.28
8	9.78	–
9	14.5	–
10	4.48	–
11	12.2	–

propane. This supports the idea that competitive adsorption plays an important role in determining the retention of solutes 1–5 on the underivatized column. If solubility of these solutes in carbon dioxide was the only contribution to its greater elution strength relative to propane, then carbon dioxide should compete more effectively for solute distribution with the hydrocarbon-ODS phase than does propane. As a result, the retention of these solutes in carbon dioxide should decrease, as in propane, rather than increase on going from the underivatized to the ODS-derivatized column.

In contrast, solutes 6 and 7, methoxynaphthalene and nitronaphthalene, exhibit shorter retention on the ODS column relative to the underivatized column in both carbon dioxide and in propane. Unlike solutes 1–5, the interaction energy of these polar solutes with the hydrophobic bonded phase is apparently less than their interaction energy with the underivatized silica surface in both of these solvents.

Modifier effects on underivatized silica

A further comparison of propane and carbon dioxide was made by examining the effects on solute retention of the addition of 1.0% g/ml methanol to each mobile phase at equal free volumes of 1.55 on both the underivatized and ODS-derivatized columns. The effect of modifier addition on free volume is small at this concentration and was neglected.

Using the underivatized silica column, the addition of methanol to carbon dioxide resulted in a relatively small decrease in the capacity factors of solutes 1–5 in comparison to unmodified carbon dioxide as shown in Fig. 6a. The polar substituted aromatics, on the other hand, exhibited substantial decreases in retention, particularly in the case of isobutyrophenone (solute 8) and tolualdehyde (solute 9). The large percentage decrease in retention for these solutes relative to methoxynaphthalene (solute 6) and nitronaphthalene (solute 7) may be the result of stronger localized interactions of the solutes with the silica surface which would magnify the observed effect on retention resulting from displacement of the solutes from active sites by methanol. The smaller percent change in retention seen for phenol (solute 10) may be

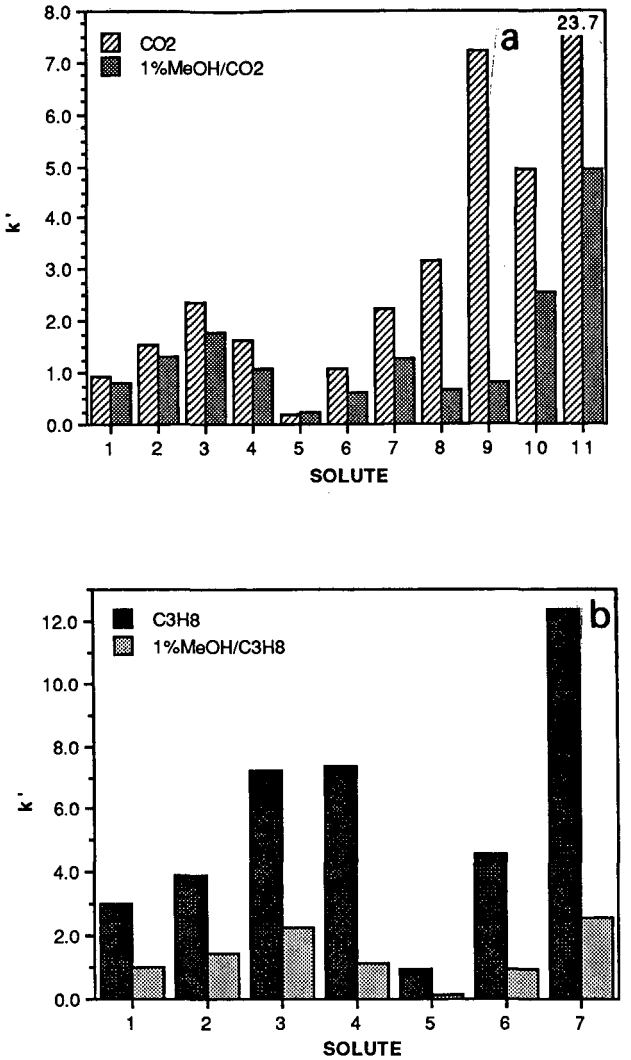

Fig. 6. Capacity factors in unmodified and methanol (MeOH)-modified solvents at equal free volumes on underivatized silica: (a) carbon dioxide and (b) propane.

due to the ability of this solute to displace rather than compete with methanol for adsorption sites[15].

In propane on this same column, solutes 1–5 exhibit much larger decreases in retention with the addition of methanol, as shown in Fig. 6b, than was observed in carbon dioxide. Since the solubility of these solutes was indicated to be greater in propane than in carbon dioxide at these conditions, greater enhancement of mobile phase solubility in propane relative to carbon dioxide with the addition of modifier is unlikely. Therefore, for propane, competitive adsorption by methanol appears to be a more important factor for reducing solute retention. This follows from the previous

assumption that carbon dioxide competes more effectively with solutes 1–5 for adsorption sites without the aid of a modifier. Therefore, the effect of the modifier on the retention of these solutes in carbon dioxide due to competitive adsorption should be less pronounced, as was observed. In addition, it was determined from adsorption isotherm measurements that the stationary phase concentration of methanol was greater in propane than in carbon dioxide by nearly a factor of two at equal free volumes. Therefore, the effects of the modifier due to competitive adsorption should be enhanced in propane relative to carbon dioxide because of a greater concentration of modifier on the surface. For more polar solutes, such as nitronaphthalene (solute 7)

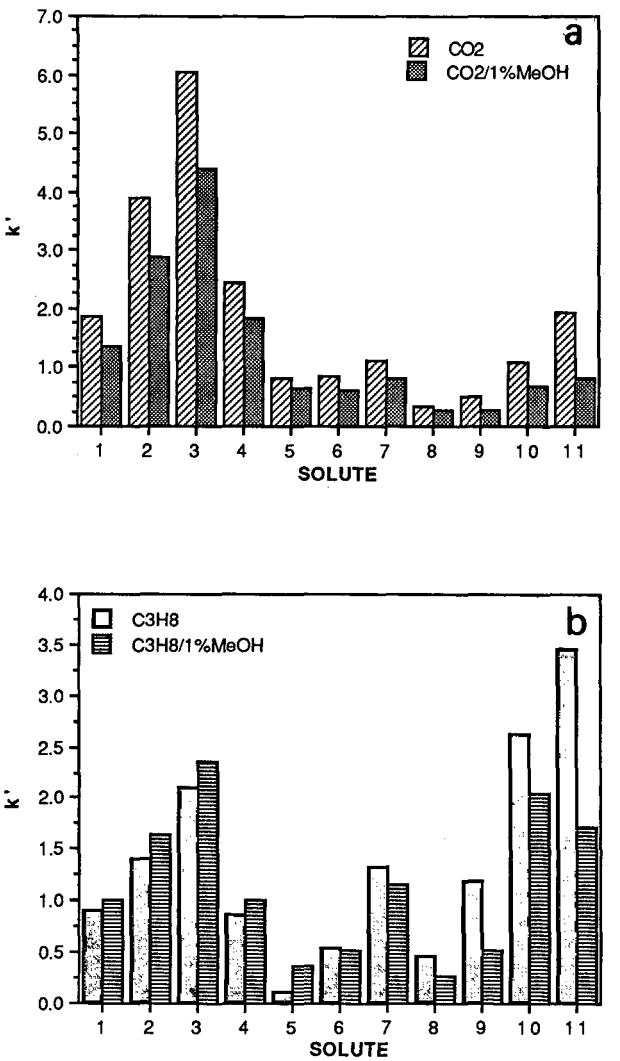

Fig. 7. Capacity factors in unmodified and methanol-modified solvents at equal free volumes on ODS-derivatized silica: (a) carbon dioxide and (b) propane.

which exibited greater solubility in carbon dioxide than in propane based on the results obtained on the ODS column, a greater enhancement of mobile phase solubility in propane relative to carbon dioxide as a result of modifier addition should also be expected.

Comparison of the solvent selectivities in Table I for the solutes in methanol-modified (fourth column) and -unmodified fluids (first column) at equal free volumes on the underivatized column indicates a much larger increase in solvent selectivity with the addition of modifier for phenyldodecane (solute 5) relative to the other solutes. This result is similar to that obtained in the earlier comparison of solvent selectivities for phenyldodecane on the underivatized and ODS-modified columns, and is consistent with the earlier argument that phenyldodecane competes with carbon dioxide for adsorption sites less effectively than the other solutes.

Modifier effects on ODS-derivatized silica

The effects on solute retention of the addition of methanol modifier to carbon dioxide on an ODS-modified silica column are illustrated in Fig. 7a. As indicated by the solvent selectivities of the modified and unmodified solvents on the ODS column listed in Table IV, the largest relative decrease in retention in carbon dioxide occurs for solutes 9–11. This is likely the result of a greater enhancement of their mobile phase solubility relative to the other solutes which is expected based on the ability of solutes 9–11 to interact strongly with methanol through hydrogen bonding.

A similar effect is observed for these solutes with the addition of methanol to propane on the ODS column as illustrated in Fig. 7b. In contrast to the results obtained in carbon dioxide, however, isobutyrophenone (solute 8) and tolualdehyde (solute 9) exhibit considerably larger changes in retention in propane with the addition of modifier on the ODS column as indicated in Table IV. This may result because of the poorer solvent strength of propane relative to carbon dioxide which would magnify the contribution of the modifier to mobile phase solubility.

TABLE IV

SELECTIVITIES OF MODIFIED AND UNMODIFIED SOLVENTS ON UNDERIVATIZED AND ODS-DERIVATIZED SILICA

Solute	$\alpha_{CO_2/CO_2/MeOH}$		$\alpha_{C_3H_8/C_3H_8/MeOH}$	
	Partisil-10	*ODS*	*Partisil-10*	*ODS*
1	1.18	1.37	3.08	0.903
2	1.18	1.34	2.66	0.865
3	1.30	1.38	3.24	0.889
4	1.53	1.33	6.67	0.867
5	0.916	1.24	7.85	0.347
6	1.77	1.39	4.99	1.03
7	1.76	1.36	4.81	1.15
8	4.79	1.18	–	1.67
9	8.84	1.83	–	2.31
10	1.92	1.64	–	1.29
11	4.78	2.37	–	2.02

A difference in the effect of the modifier on the retention of solutes 1–5 in propane and in carbon dioxide is also observed in Fig. 7a and b. With the addition of methanol, the capacity factors of these solutes are reduced in carbon dioxide but are increased in propane. This suggests an enhancement of solubility in carbon dioxide and a reduction of solubility in propane with the addition of modifier. If the masking of residual adsorption sites by the modifier on the ODS column contributed significantly to the reduction of retention of these solutes in carbon dioxide, then a reduction in retention would also be anticipated in propane. The increased retention in propane is most significant for phenyldodecane (solute 5), which exhibits nearly a three-fold change in retention. Once again, methoxynapthalene (solute 6) appears to be intermediate in its response exhibiting almost no change in retention.

The solvent selectivities for the modified solvents on the ODS column are given in the fifth column of Table I. Although the capacity factors of solutes 1–5 increased in propane and decreased in carbon dioxide relative to the unmodified solvents, the capacity factors are still larger in carbon dioxide. However, the solvent selectivities were reduced by *ca.* 35% for solutes 1–4 and by over 70% for phenyldodecane (solute 5) in comparison to the selectivity obtained in the unmodified solvents on the same column. Of the polar-substituted aromatics, nitronaphthalene (solute 7), phenol (solute 10), and 3-phenylpropanol (solute 11) exhibited an increase in the difference in retention in the two solvents on the ODS column with the addition of modifier.

The difference in the modifier effect on each solvent on the underivatized and ODS-derivatized columns can be observed by comparing the selectivities of the modified and unmodified solvents which were given in Table IV. In carbon dioxide and in propane on the ODS column, solutes 1–4 exhibit approximately the same value of modifier selectivity. In contrast, these values vary considerably for these solutes on the underivatized column. This suggests that the differences in modifier selectivity for these solutes on the underivatized column result from differences in competitive adsorption with the modifier rather than changes in their relative mobile phase solubilities.

Comparison of ODS and methanol-modified silicas

As shown in the previous results, the capacity factors of the polar-substituted aromatics were found to decrease substantially in both solvents on underivatized silica with the addition of a small amount of methanol modifier. This was further indicated to be the result of competitive adsorption and to some extent enhanced mobile phase solubility. The ability of the methanol modifier to effectively reduce solute–stationary phase interactions by masking adsorption sites on the surface can be examined by comparing the retention results in methanol-modified solvents on the underivatized column with the results obtained in the unmodified solvents on the ODS column. These results are compared for propane in Fig. 8b. It is clear that even with the additional solvent strength afforded by the presence of the modifier, the underivatized silica surface covered at more than 90% of its maximum methanol concentration still interacts much more strongly with these solutes than does the ODS-bonded phase. A similar result is obtained for carbon dioxide, as shown in Fig. 8a, although the difference in retention is much smaller.

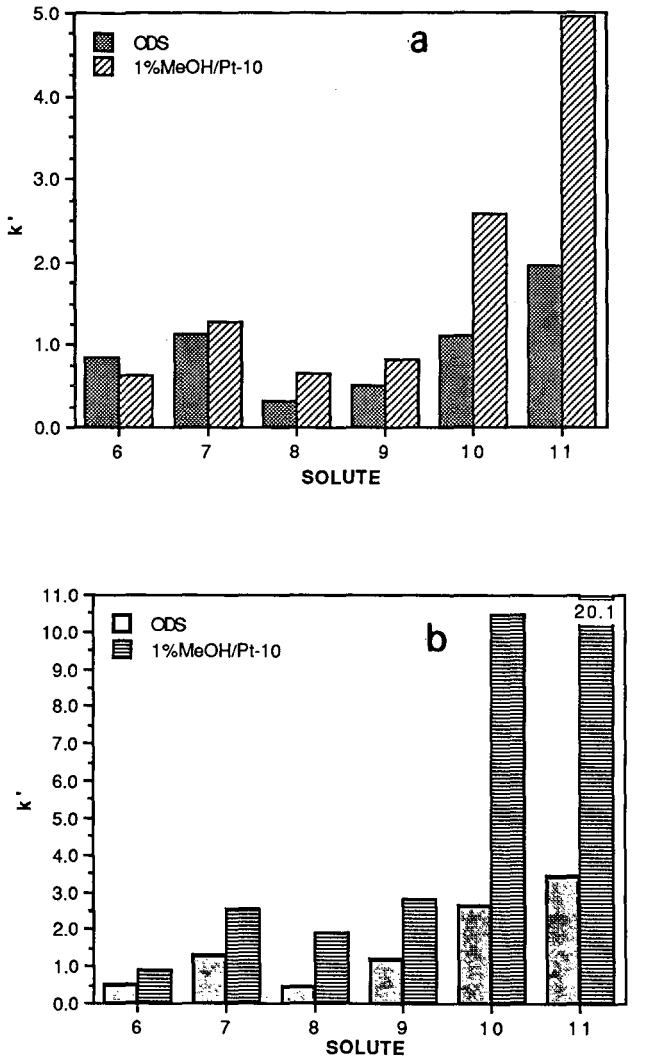

Fig. 8. Capacity factors in unmodified solvents on ODS-derivatized silica, and in modified solvents on underivatized silica: (a) carbon dioxide and (b) propane. Pt-10 = Partisil-10.

Density effects in methanol-modified solvents

A final comparison was made to examine the effects of increased mobile phase density on the retention of the test solutes in the methanol-modified solvents on the underivatized silica column. The retention results in carbon dioxide with 1.0% methanol modifier are illustrated in Fig. 9a. The capacity factors of all of the solutes decrease with increasing density. The relative change in retention is approximately the same for all solutes, as indicated by the density-selectivity values given in Table V. The effect of increased interactions in the mobile phase resulting from increased density appears to be nearly the same for all solutes.

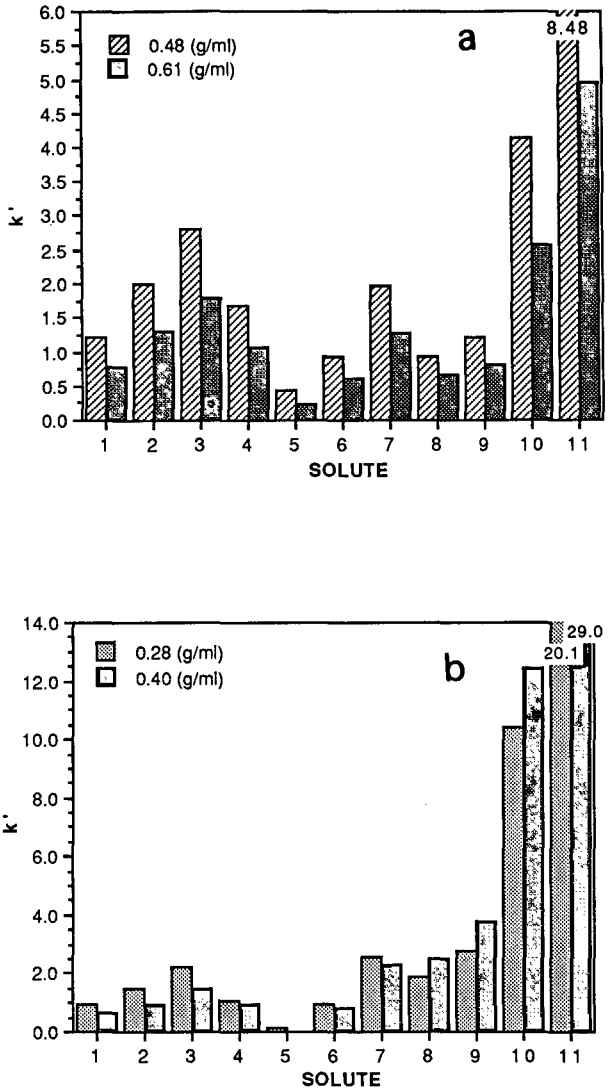

Fig. 9. Capacity factors in methanol-modified solvents at two densities on underivatized silica: (a) carbon dioxide and (b) propane.

In propane, the capacity factors of solutes 1–7 also decrease with increasing density as shown in Fig. 9b. In this case, however, the density selectivity values vary considerably for each solute. In addition, solutes 8–11 exhibit an unexpected increase in retention with increasing density. Since the methanol concentration is held constant, and the interactions of the mobile phase increase with increasing density, the increased retention of solutes 8–11 must be due to increased interactions of the solutes with the silica surface. A comparison of the adsorption isotherms of methanol on the same column was made in order to determine the extent of the change in modifier surface

TABLE V

DENSITY SELECTIVITIES OF METHANOL-MODIFIED SOLVENTS ON UNDERIVATIZED SILICA

Densities 0.28, 0.40, 0.48 and 0.61 g/ml are indicated in parentheses.

Solute	$\alpha_{CO_2(0.48/0.61)}$	$\alpha_{C_3H_8(0.28/0.40)}$
1	1.54	1.40
2	1.54	1.50
3	1.57	1.51
4	1.57	1.20
5	1.90	–
6	1.52	1.17
7	1.55	1.11
8	1.42	0.761
9	1.50	0.738
10	1.62	0.841
11	1.71	0.694

coverage. The isotherms are given in Fig. 10. The methanol concentration on the adsorbent from propane was calculated to decrease by 23% with increasing density. The resulting increase in the availability of adsorption sites apparently more than compensates for the increase in mobile phase solubility of these solutes which results from increased density.

In carbon dioxide, the methanol concentration on the adsorbent changes less with decreasing density, increasing by 13% with an absolute change in surface

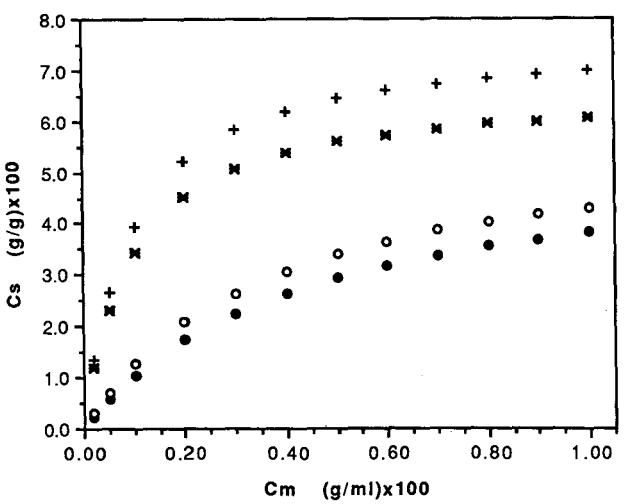

Fig. 10. Methanol adsorption isotherms at 100°C on underivatized silica from carbon dioxide and from propane at two densities. + = Propane, 0.28 g/ml ($V_f = 1.55$); ✻ = propane, 0.40 g/ml; ○ = carbon dioxide, 0.48 g/ml; ● = carbon dioxide, 0.61 g/ml ($V_f = 1.55$); Cs = stationary phase concentration, Cm = mobile phase concentration.

coverage *ca.* one third of that calculated from propane. This, in combination with the greater solvent strength of carbon dioxide for these solutes that was indicated previously, may explain why a reversal in the anticipated elution strength is not observed in carbon dioxide.

CONCLUSIONS

The results presented herein indicated a number of differences in the factors that influence solute retention in carbon dioxide and propane mobile phases and in the effects of modifier addition on these solvents:

(1) The logarithms of the capacity factors of unsubstituted polycyclic aromatic hydrocarbons exhibited a good correlation with molar volume in propane and with polarizability in carbon dioxide on an underivatized silica column. This suggests a difference in the nature of the molecular interaction of these solutes in each of these solvents.

(2) At equal free volumes and equivalent temperatures, carbon dioxide and propane were found to have different solvent strengths for unsubstituted aromatic hydrocarbons which varied with the nature of the adsorbent surface.

(3) Although studies on a non-polar bonded phase column indicated greater solubility of unsubstituted aromatic hydrocarbons in propane, carbon dioxide exhibited a greater elution strength for these solutes on silica. This was attributed to its ability to compete more effectively with the solutes for non-specific adsorption sites on the silica surface.

(4) The greater elution strength of carbon dioxide for polar substituted aromatics was indicated to be the result of greater mobile phase solubility as well as more effective competitive adsorption onto silica.

(5) Although earlier results[16] indicated that unsubstituted aromatics do not compete with methanol for direct adsorption onto surface silanols, the modifier effect in carbon dioxide on underivatized silica appears to be largely the result of displacement of these solutes from stationary phase surface by the modifier. Both surface interactions and changes in mobile phase solvent strength appear to contribute to the modifier effect for more polar solutes.

(6) The modifier effect was more pronounced for both types of solutes in propane as a result of propane's inability to compete with the solutes for stationary phase adsorption sites and to solvate more polar solutes in the mobile phase without the aid of a modifier. The retention dependence of the more polar solutes on competitive modifier adsorption was sufficient in propane to result in an increase in retention with increasing mobile phase density due to the accompanying decrease in modifier stationary phase coverage.

The exact nature of the interactions of non-localizing solutes with the surface of silica is unclear. Although non-localized adsorption is apparently the case for unsubstituted aromatic hydrocarbons, it does not preclude effects on solute retention that results from competitive adsorption by the mobile phase solvent and modifier. This supports the model of Snyder and Glajch[14,17] which suggests that both localizing and non-localizing solutes should exhibit a retention dependence on the concentration of adsorbed modifier. Mobile phase contributions, however, still appear to play a role in the modifier effect. The nature of the interaction between carbon dioxide and the silica surface should be investigated further.

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Sorption isotherms of mobile phase components in capillary supercritical fluid chromatography

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SUMMARY

Tracer pulse chromatography–mass spectrometry (TPC–MS) was used to study the sorption isotherms of carbon dioxide with a bonded and cross-linked SE-54 stationary phase in capillary supercritical fluid chromatography. These data were compared and contrasted with previously reported absorption isotherm studies for 2-propanol in 2-propanol–CO_2 binary solvents using TPC–MS. Isotherms were determined as a function of density (pressure) and temperature. Results for CO_2 demonstrate an initial increase in the surface excess isotherm with increasing pressure, a maximum being reached, followed by a decrease in the amount adsorbed with further increases in pressure.

INTRODUCTION

The experimental study of stationary phase solvation by supercritical fluids has been limited. Pioneering studies by Sie *et al.*[1] in 1966 demonstrated that CO_2 was soluble in squalane and slightly soluble in glycerol-coated stationary phases at 40°C. The study of stationary phase solvation lapsed until the report of Springston *et al.*[2] in 1986. These authors reported the swelling of non-extractable polymer film stationary phases in supercritical fluids. The determination of the adsorption isotherms of ethyl acetate modifier in supercritical CO_2 on silica was studied by Lochmüller and Mink[3] in 1987. These workers reported stationary phase saturation coverage of the silica surface at approximately 1% (g/ml) ethyl acetate in CO_2.

Studies of supercritical fluid mobile phase component adsorption isotherms using tracer pulse chromatography–mass spectrometry (TPC–MS) have been reported by Strubinger and Parcher[4], Selim and Strubinger[5,6], and Yonker and Smith[7]. Strubinger and Parcher[4] have studied the surface excess adsorption isotherms of supercritical carbon dioxide on packed columns with octadecyl silica stationary phases. Selim and Strubinger[5,6] have studied the absorption isotherms of supercritical pentane and methanol-modified supercritical pentane using bonded polymeric stationary phases in capillary supercritical fluid chromatography (SFC). Yonker and Smith[7] studied the surface excess adsorption isotherms of 2-propanol from super-

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critical 2-propanol–CO_2 binary solutions using a bonded polymeric stationary phase in capillary SFC as a function of density, temperature and modifier mole fraction.

The study of stationary phase swelling or solvation and the isotherm determination of mobile phase components could lead to a greater insight into the physicochemical processes governing the retention mechanism in SFC. The effects of small amounts of modifiers in supercritical mobile phases and their effects on solute retention have been well documented[8–11]. Whether these modifiers interact with the stationary phase covering active sites, modify the bound polymeric stationary phase, or simply alter the mobile phase solvating power remains to be elucidated. Spectroscopic studies of pure and binary supercritical fluid mobile phases have demonstrated many interesting results concerning possible intermolecular interactions in the fluid phase[12–14]. The study of stationary phase solvation is more complex and dependent on the direct determination of the adsorption isotherms of the mobile phase components into the bound polymer.

The purpose of this paper is to present results obtained using TPC–MS to determine the surface excess adsorption isotherms of supercritical CO_2 in capillary SFC. The results for pure CO_2 are contrasted with similar studies using a 2-propanol–CO_2 binary solvent system with the same stationary phase under similar experimental conditions.

EXPERIMENTAL

SFC–MS system

The SFC–MS system has been described in detail in earlier work[15]. The effluent from the capillary column was introduced into a splitter directing the flow to either the MS ion source or into a second restrictor which controlled the flow-rate through the capillary column. Through appropriate adjustment of both restrictors one could accurately control the flow-rate through the column, while keeping the pressure in the MS ion source region sufficiently low for electron impact ionization. This splitter interface for the MS system is an ideal compromise allowing maximum experimental flexibility when using gases (*e.g.*, argon and CO_2) as solute probe molecules.

Materials and column

SFC-grade CO_2 was obtained from Scott Specialty Gases (Plumsteadville, PA, U.S.A.). Oxygen-labeled carbon dioxide ($C^{18}O_2$, mol.wt. 48.0) was obtained from Cambridge Isotope Labs. (Woburn, MA, U.S.A.) and was mixed in a gas mixture of non-labeled CO_2 and argon. The argon was used to determine the void volume of the capillary column. Therefore, from a 0.2-μl injection of this gas mixture one could simultaneously determine the retention time of an unretained component t_0 (argon) and the retention time t_R ($C^{18}O_2$) at the system pressure and temperature.

The capillary column, 7.5 m × 100 μm I.D., was prepared in our laboratory. The column was coated by using a pentane solution of the SE-54 stationary phase and was cross-linked twice with azo-*tert.*-butane (Alfa, Danvers, MA, U.S.A.), which prevents extraction of the bound polymer by supercritical CO_2. The stationary phase had a calculated film thickness of approximately 1.0 μm.

Distribution isotherms

The TPC–MS technique used in this study is based upon the detection of an isotopically labeled solvent molecule ($C^{18}O_2$) as it elutes from the capillary column. The surface excess isotherm (the excess amount of sorbate present on the surface beyond that corresponding to the bulk density of the gas phase above the surface) can be determined from a simple chromatographic experiment that measures the retention time of the tracer pulse. The assumptions made when applying the TPC–MS technique to isotherms studies are: (i) there is fast equilibration between the mobile and stationary phase; (ii) there are no isotope effects; (iii) the pressure drop across the column should be low, and (iv) the accurate determination of the time of an unretained component. This technique, using a tracer pulse to study adsorption isotherms, was first described by Helfferich and co-workers[16,17]. As the amount of carbon dioxide sorbed into the stationary phase increases, so does the retention time of the isotopically labeled $C^{18}O_2$. The isotherm data for $C^{18}O_2$ in the bound SE-54 polymeric stationary phase were determined at 45, 65, 85, 110 and 130°C and for pressures ranging from 65 to 220 atm. The amount of adsorbate in the stationary phase (N_i^{sp}) for the TPC–MS technique is related to

$$N_i^{sp} = V_n Y_i P/ZRT \tag{1}$$

where V_n is the net retention volume, Y_i is the mole fraction of the solvent molecule under study (in this case $Y_i = 1$), P is the system pressure, Z is the compressibility of the fluid (determined through an appropriate equation of state), R is the gas constant and T is the system temperature[7,18]. From the net retention time of the labeled CO_2 and argon, the net retention volume for the isotope can be calculated:

$$V_n = [(t_R - t_0)/t_0]\, \pi R^2 L \tag{2}$$

where R is the column radius and L is the column length. The term $\pi R^2 L$ is the geometric volume of the capillary column. The use of column radius instead of free radius ($R - d_f$) contributes less than a 4% difference to the volume determination and negates the need to determine the film thickness (d_f) variation as conditions changed. Therefore, using eqn. 1, knowing the pressure, temperature, and calculating the compressibility of supercritical CO_2, one can calculate the number of moles of CO_2 sorbed into the bonded polymeric stationary phase. Estimated precision (relative standard deviations, R.S.D.) on the retention time for the gases and the calculation of the number of moles of CO_2 sorbed are 2.0 and 18%, respectively. Dividing the number of moles of CO_2 absorbed into the stationary phase by the weight in milligrams of the polymer cross-linked in the capillary column, one can compare results between different column types and different stationary phase film thicknesses.

RESULTS AND DISCUSSION

The study of sorption at high pressures is an important area leading to a greater insight into the physicochemical processes and intermolecular interactions between molecules and surfaces. There have been few studies to date involving dense gases above their critical conditions on simple adsorbent surfaces[19–22]. The extension of this

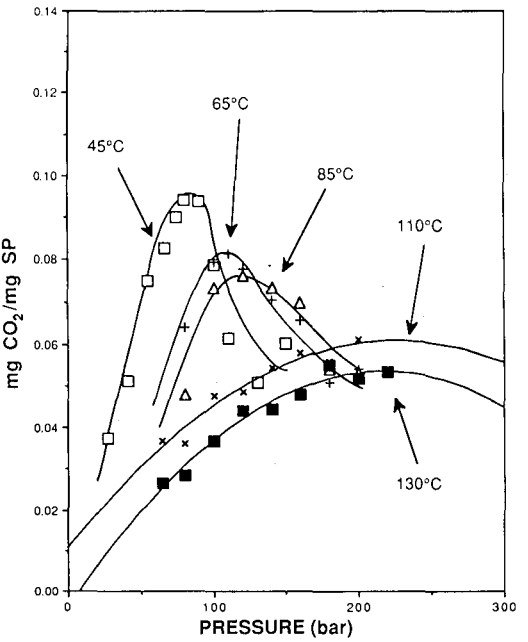

Fig. 1. Plot of mg CO_2/mg stationary phase (SP) *versus* pressure of supercritical CO_2 for temperatures of (□) 45, (+) 65, (△) 85, (×) 110 and (■) 130°C.

area of research to chromatographically interesting phases and surfaces presents an important opportunity. TPC–MS allows one to study the role of fluid pressure, temperature and modifier mole fraction on the physicochemical processes relevant to stationary phase solvation in SFC.

The data for the sorption isotherms for CO_2 on the SE-54 stationary phase are presented in Fig. 1. For the temperatures of 45, 65 and 85°C, maxima in the isotherms are seen at different experimental pressures. At 110 and 130°C the maxima in the isotherms are just being reached or lie at slightly higher pressures, due to the lower density of CO_2 at these temperatures. Similar behavior for "surface excess" adsorption isotherms has been reported by Findenegg and co-workers[19,20] and Hori and Kobayashi[21]. This behavior can qualitatively be thought of as the density difference between the bulk fluid phase and the absorbed phase (surface excess). This differential value will reach a maximum with increasing pressure of the supercritical fluid. Upon increasing the pressure, one gradually approaches the limit in which the density of the bulk fluid equals that of the absorbed phase, at which point the surface excess becomes zero. Therefore, the behavior of the isotherms in Fig. 1 follows qualitative expectations. The swelling factor (defined as the relative expansion of the stationary phase volume[2]) of SE-54 using supercritical CO_2 at 45°C is *ca.* 0.4, which is comparable to the values reported by Springston *et al.*[2] using supercritical butane and SE-54. As a function of temperature, the amount sorbed is seen to decrease with increasing temperature. The increased thermal energy added to the system most likely accounts for this behavior. Similar results as a function of pressure (density) were reported

previously[7] for the CO_2–2-propanol binary supercritical fluid system on SE-54. In this case, the absorption isotherms for 2-propanol also decreased with increasing pressure of the binary supercritical fluid as shown in Fig. 2. The data in Fig. 2 were all obtained at 110°C; it is interesting to note that the absorbed amount of 2-propanol in the stationary phase is approximately 3 to 4 times greater than the amount of CO_2 at similar densities. Carbon dioxide appears to be partitioning into the bonded polymeric stationary phase, similar to that seen for the 2-propanol. The data in Fig. 2 do not take into account any possible synergistic effects due to 2-propanol and CO_2 solvation of the stationary phase, which could lead to a greater amount of CO_2 for the binary fluid system as compared to that seen for pure CO_2. However, the conclusions drawn from Fig. 2 suggest that 2-propanol interacts more effectively with the SE-54 polymeric stationary phase than CO_2. With a single-fluid system, such as CO_2, a larger pressure range can be investigated as compared to a binary fluid, where the pressure region for a two-phase system must be avoided when performing these experiments.

A more effective means of presentation of the data in Fig. 1 is to plot the amount absorbed against fluid density and reduced density. The replot of these data is shown in Fig. 3. Blümel *et al.*[19] have reported that the isotherms at supercritical temperatures usually pass through a maximum for a density (ρ) between $0.5\,\rho_c < \rho < 1.0\,\rho_c$ (where ρ_c is the critical density). The surface excess isotherms for CO_2 also reach their maximum value in this range as shown in Fig. 3.

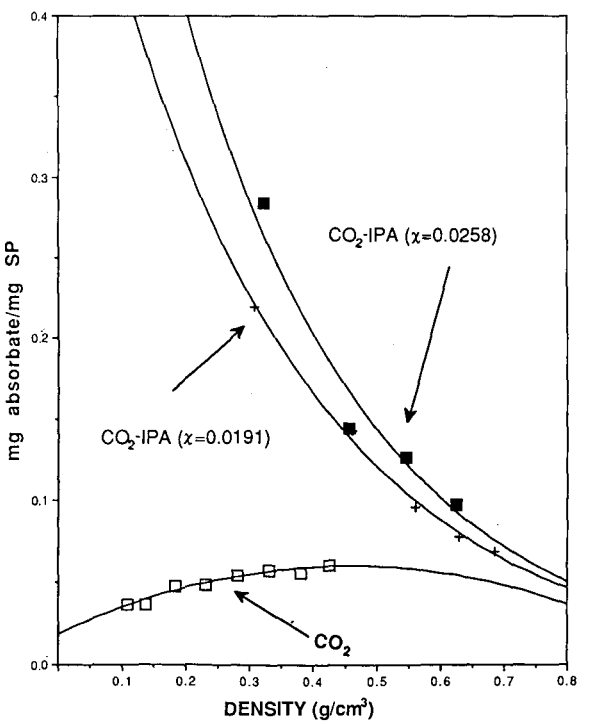

Fig. 2. Plot of mg absorbate/mg stationary phase (SP) *versus* density at 110°C for pure CO_2 (□) and for CO_2–2-propanol (IPA) in the binary supercritical fluid systems for CO_2–2-propanol at (+) 0.0191 mole fraction and (■) 0.0258 mole fraction.

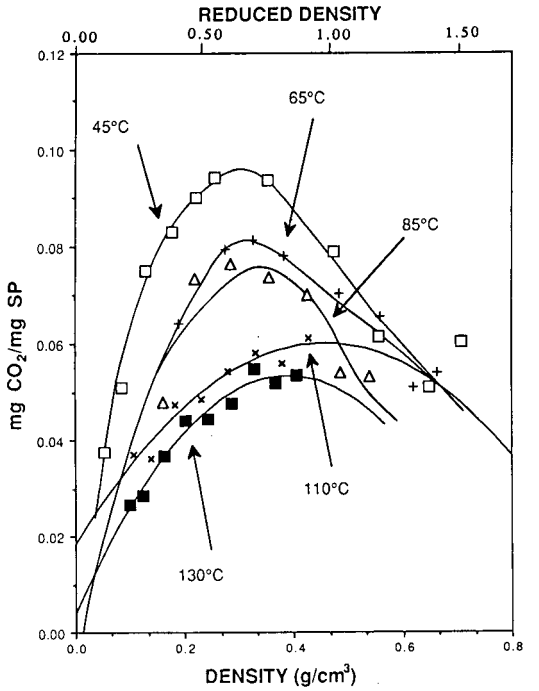

Fig. 3. Plot of mg CO_2/mg stationary phase (SP) *versus* density and reduced density for the temperatures of (□) 45, (+) 65, (△) 85, (×) 110 and (■) 130°C.

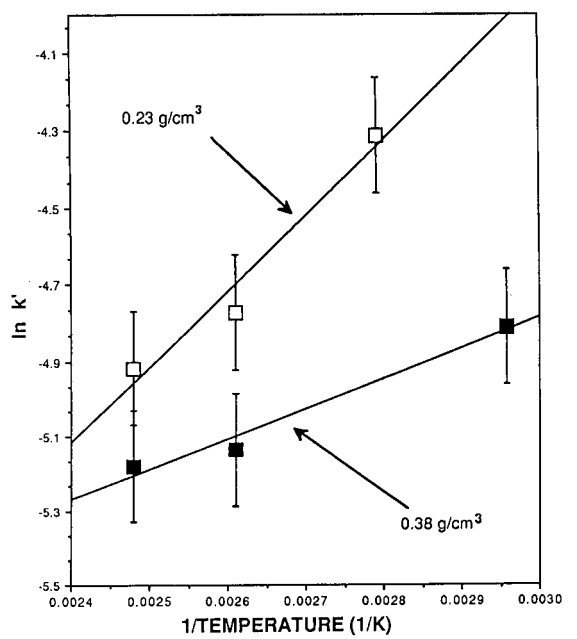

Fig. 4. Plot of ln k' for $C^{18}O_2$ *versus* reciprocal temperature at a constant density of (□) 0.23 g/ml and (■) 0.38 g/ml for supercritical CO_2.

The enthalpy for CO_2 sorption in the polymeric stationary phase can be obtained from the TPC–MS experiment. The retention factor, k', for the $C^{18}O_2$ molecule can be obtained experimentally. The retention time difference for $C^{18}O_2$ is very small in this system compared to argon, therefore, k' values will be quite small and prone to an experimental error of *ca.* 10 to 20% R.S.D. One can still obtain an approximate value for the sorption enthalpy for CO_2 at constant mobile phase density and compare it to isosteric enthalpies (determined at constant volume) reported in the literature. Van 't Hoff plots for the natural logarithm of the retention factor for $C^{18}O_2$ *versus* reciprocal temperature are shown in Fig. 4. The heats of sorption for CO_2 determined from this technique are approximately -4 kcal/mol (density of 0.23 g/ml) and approximately -2 kcal/mol (density of 0.38 g/ml). The isosteric heats of adsorption for propane, *n*-butane and acetone on graphitized carbon black were -5.6, -7.7 and -7.5 kcal/mol, respectively[23]. The isosteric enthalpy of krypton on graphitized carbon black reported by Blümel *et al.*[19] was -2.95 kcal/mol, whereas Ross *et al.*[24] obtained a value of -3.20 kcal/mol. Comparing the enthalpy of CO_2 sorption with enthalpies values reported for retention in SFC and gas chromatography provides an interesting contrast. Yonker and Smith[25] have reported enthalpies of transfer of heptadecane in SE-54 with supercritical CO_2 of -8.6 kcal/mol (density of 0.30 g/ml), -6.5 kcal/mol (density of 0.40 g/ml), and -3.9 kcal/mol (density of 0.50 g/ml). Lauer *et al.*[26] have reported average enthalpy values of -6.0 kcal/mol (density of 0.80 g/ml) with supercritical CO_2 using a PRP-1 packed column. Meyer *et al.*[27] and Martire *et al.*[28,29] have reported enthalpies of transfer for the *n*-alkanes of hexane, heptane and octane of -6.8 to -9.1 kcal/mol using *n*-tetrocosane as the stationary phase with gas chromatography (temperature range was 76–88°C). The values obtained for the enthalpy of CO_2 sorption from k' determinations at the two densities follow the trend reported by Yonker and Smith for the enthalpies of transfer as a function of density in supercritical CO_2, but the absolute values of the enthalpy of sorption is less than the enthalpy of transfer in both SFC and gas chromatography. In fact, the enthalpy of sorption values obtained do not vary greatly from the isosteric enthalpies reported for krypton on graphitized carbon black. This could point to a possible conclusion that in this case CO_2 is behaving in a manner similar to krypton adsorption on carbon black. The sorbed CO_2 is not interacting with the polymer; it is forming solvent multilayers on the polymer surface. Further improvements of k' determinations for the sorbed species is desirable to obtain more accurate enthalpy determinations which will aid in data interpretation.

CONCLUSIONS

Implications of these studies for understanding the retention process for SFC are significant. The TPC–MS technique can be used to determine sorption isotherms under supercritical fluid conditions. This presents the opportunity to study the intermolecular interactions between a molecule and the surface directly and investigate their effects on retention in SFC. The sorption isotherm for CO_2 showed maxima in the reduced density region of 0.5 to 1.0, which is consistent with studies reported in the literature for other systems[19]. As pressure (density) increased the amount of CO_2 sorbed decreased. The excess amount of CO_2 associated with the polymeric stationary phase was determined to be less than that seen for 2-propanol using the binary

supercritical fluid of 2-propanol–CO_2. The amount of CO_2 associated with the stationary phase decreased as a function of temperature over the temperature range used in this study. Sorption enthalpies for CO_2 were determined from the measured retention factors for the isotopically labeled CO_2. These values were shown to compare favorably with isosteric heats of adsorption for krypton reported in the literature[19,24].

Further studies need to be undertaken involving the role of fluid modifiers and solvation of the bound polymeric stationary phase, to define any synergistic effects between the modifier and dense gas relating to an enhanced sorption isotherm. The distinction between solvent multilayers on the polymer surface or the intercalation of the solvent into the bound polymer, and their roles in determining solute retention in SFC, needs to be better understood. The TPC–MS technique presents an important opportunity for studying these questions and other solvation models rapidly and accurately. Our aim is to extend these methods to high-temperature systems of much more highly solvating polar fluids.

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Retention and selectivity in supercritical fluid chromatography on an octadecylsilyl-silica column

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SUMMARY

The changes in retention and selectivity of a series of model test compounds on an octadecylsilyl-silica column in supercritical fluid chromatography have been examined at different column pressures. The effects of the addition of modifiers to the carbon dioxide eluent have been compared using retention indices based on the alkyl aryl ketone scale. Without modifier, polar hydrogen-bonding analytes, such as alcohols and phenols, were highly retained, but their relative retention times were considerably reduced as the proportion of modifier increased. Aliphatic amines appeared to interact with free silanols on the surface on the stationary phase in the absence of modifiers and their retention changed markedly on the addition of small proportions of modifier. However, the relative retentions of more weakly basic aromatic amines were unaltered by modifier.

INTRODUCTION

Supercritical fluid chromatography (SFC) has been undergoing a considerable growth in the last few years[1–3] and is now moving from the research to the industrial laboratory as commercial equipment becomes available and as more applications are demonstrated for petrochemicals, agrochemicals and pharmaceuticals and interest is starting to be expressed in its application for official methods[4]. It has considerable potential as an alternative instrumental analytical technique complementing high-performance liquid chromatography (HPLC) and gas–liquid chromatography (GLC). In particular it can offer a different selectivity to the other techniques and thus may enable difficult separations to be achieved. As with HPLC there is the possibility of altering the composition of the eluent either by using a different supercritical fluid or, more commonly, by the addition of modifiers. However, as yet the mechanisms and selectivity of the retention in single eluents are not fully understood and the influence of the modifier has not yet been examined in detail.

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As well as the composition of the mobile phase, the nature of the stationary phase and the operating conditions can have a significant effect on the separation. In order for SFC to be fully accepted, it must be possible to reproduce the conditions of a separation in different laboratories. However, this may be more difficult than in other chromatographic techniques, because the separation power of the eluent is very dependent on the density of carbon dioxide and hence on the temperature, mean pressure, and pressure drop across the column[5]. Whereas the first of these is easy to define and control, it will be more difficult to accurately reproduce pressures in different laboratories. Even if standardised gauges are used, the mean pressure in the column will depend on the resistance of the column to flow, as well as on the pressure applied by the pump.

In addition, for many analytes it has been found necessary to add a modifier, such as methanol or acetonitrile, to the carbon dioxide eluent in order to obtain reasonable retention times and good peak shapes[6–10]. It is difficult to reproducibly prepare mixed eluents in defined proportions for packed columns using a dual-pump system, because the compressibility of the carbon dioxide means that its mass flow-rate is determined by the pressure and temperature in the pump head, rather than just by the flow-rate setting, whereas the modifier flow should be independent of the pressure[9]. Related problems in capillary SFC using syringe pumps have been discussed in a study of the separation of sulphonamides[10]. Even the use of premixed cylinders of eluent is not a solution as it is reported that the composition leaving the cylinder changes as the cylinder's contents are consumed[11].

As many of these changes in operating conditions affect all analytes to a similar extent, relative measurements of retention compared to a standard or standards might give better reproducibility and enable results from different instruments or laboratories to be compared. This concept forms the basis of the use of retention indices in GLC and HPLC[12] and in previous work we have examined its application to SFC on polystyrene–divinylbenzene (PS–DVB) packed columns[13] and for the separation of barbiturates[14,15] and benzodiazepines[9] on PS–DVB and octadecylsilyl (ODS)-silica packed columns. On the PS–DVB column it was found that the retention indices of a series of model test compounds were largely independent of the temperature and pressure of the separation but were critically dependent on the modifier proportion. Large relative retention changes with the proportion of modifier were also observed in the studies of the drug compounds on both PS–DVB and ODS-silica based columns.

The present study reports the changes in the selectivity and relative retentions of a set of model aromatic test compounds on an ODS-silica stationary phase with operating conditions and the potential application of retention indices to improve the reproducibility of recorded separations. The different behaviour of the model compounds on the addition of modifiers should also give an indication of the types of interactions that are taking place.

EXPERIMENTAL

Chemicals

n-Alkanes from decane to tetracosane, alkyl aryl ketones from valerophenone to octadecanophenone and test compounds were laboratory grade from a range of suppliers. Carbon dioxide was industrial grade (99.98%) from British Oxygen

Company (Brentford, U.K.) and modifiers were HPLC grade from FSA Scientific Apparatus (Loughborough, U.K.).

Equipment

The separations were carried out using a packed-column chromatograph, consisting of a Jasco BIP-1 pump (Hachioji City, Tokyo, Japan) with cooled check valves and pumping head, operating under constant-pressure conditions, a Pye Unicam-XPS pump for the addition of modifier, a Rheodyne (Cotati, CA, U.S.A.) 7125 injection valve with a 20-μl loop and a Pye 104 gas chromatographic oven (Pye Unicam, Cambridge, U.K.). The analytes were detected using the flame ionisation detector on the Pye 104 or at 254 nm with an ACS 750/12 variable-wavelength ultraviolet spectrophotometric detector (Applied Chromatography Systems, Macclesfield, U.K.) fitted with a high-pressure flow cell. A crimped tube was used as a restrictor. The samples were separated on a 250 × 4.6 mm I.D. column packed with Ultrasphere ODS 5-μm particles (Beckman, High Wycombe, U.K., batch No. T608035) and peaks were recorded using a Hewlett-Packard (Winnersh, U.K.) 3390 integrator.

Method

Samples of the test compounds and retention index standards in methanol were injected onto the column and eluted with supercritical carbon dioxide at 60°C and different applied pressures. Methanol was added as required. A sample of acetone was used as the column void volume marker.

Capacity factors were calculated as $k' = (t_R - t_0)/t_0$ (where t_R is retention time and t_0 column void volume) and the mean of triplicate injections was used in calculations. Retention indices based on the alkyl aryl ketones were calculated as described for HPLC[16] by fitting log k' against carbon number × 100 for the standards to a linear correlation using a least-squares routine and then interpolating the log k' values for the test compounds.

RESULTS AND DISCUSSION

The mechanism of retention on bonded-phase columns in SFC is not fully understood, but for a homologous series of compounds the retention increases logarithmically with carbon number in a similar manner to separations by GLC or reversed-phase HPLC[12]. A number of series of homologous organic compounds could potentially be used as retention index scales. The *n*-alkanes are already widely accepted for GLC, but because of the need for a more polar analyte with a chromophore, the alkyl aryl ketones and alkan-2-ones have been used in HPLC[12]. The former have been used as retention index standards in our earlier studies on SFC using a PS–DVB column[13] and the alkan-2-ones have been reported to show linear behaviour in SFC on an ODS column[17]. Thus, either set of homologues could potentially be used as a retention index scale. There are also isolated reports on retention being expressed as retention indices in SFC using the *n*-alkanes[18].

When *n*-alkanes, from decane to tetracosane, and alkyl aryl ketones, from valerophenone to octadecanophenone, were examined by SFC on an ODS-silica (Ultrasphere ODS) column (Table I), both sets of compounds were retained with

similar retention times. Comparable retention times were also found for a number of model aromatic test compounds containing different functional groups and for four homologous alkylbenzenes (toluene to *n*-butylbenzene). Two polar model compounds, benzamide and benzoic acid, were not eluted from the column. Benzylamine was highly retained and was not eluted at 1665 p.s.i. At higher pressures it gave tailing peaks, but a less basic aromatic amine, N-propylaniline, was readily eluted with symmetrical peaks at all the pressures examined. The peaks for the hydroxy

TABLE I

CAPACITY FACTORS OF ALKYL ARYL KETONES, *n*-ALKANES, ALKYLBENZENES AND TEST COMPOUNDS AT DIFFERENT COLUMN PRESSURES

Conditions: column, Ultrasphere ODS; eluent, carbon dioxide; temperature, 60°C.

Compound	*Capacity factor*			
	Mean column pressure (p.s.i.)			
	1665	*1950*	*2160*	*2470*
Alkyl aryl ketones				
Valerophenone	2.39	1.43	1.00	0.81
Hexanophenone	2.91	1.69	1.16	0.95
Heptanophenone	3.58	2.02	1.37	1.10
Octanophenone	4.45	2.43	1.62	1.30
Decanophenone	7.04	3.62	2.21	1.84
Dodecanophenone	10.81	5.26	3.25	2.52
Tetradecanophenone	16.95	7.65	4.53	3.47
Hexadecanophenone	26.28	11.10	6.30	4.74
Octadecanophenone	40.05	15.66	8.57	6.32
n-Alkanes				
Decane	0.97	0.62	0.48	0.43
Dodecane	1.70	1.01	0.77	0.66
Tetradecane	2.83	1.56	1.15	0.97
Hexadecane	4.56	2.37	1.66	1.37
Octadecane	7.20	3.46	2.34	1.90
Eicosane	11.25	4.99	3.25	2.57
Docosane	16.92	7.15	4.43	3.48
Tetracosane	25.56	10.19	6.15	4.68
Alkylbenzenes				
Toluene	0.46	0.34	0.28	0.21
Ethylbenzene	0.64	0.46	0.36	0.28
n-Propylbenzene	0.85	0.59	0.46	0.36
n-Butylbenzene	1.12	0.76	0.58	0.46
Test compounds				
Nitrobenzene	1.02	0.66	0.48	0.36
Benzaldehyde	1.14	0.80	0.56	0.47
Methyl benzoate	1.24	0.82	0.62	0.51
N-Propylaniline	2.43	2.12	1.16	1.00
p-Cresol	2.63	1.71	1.35	1.08
Benzyl alcohol	4.62	2.66	1.84	1.47
Benzylamine	–	7.01	4.83	3.95

compounds, *p*-cresol and benzyl alcohol, both tailed badly and the retention times varied with sample loading on the column.

These results contrast with reversed-phase (RP) HPLC on an ODS-silica column, where the non-polar alkanes would be much more retained than any of the substituted compounds and the polar hydroxy and amino compounds would be rapidly eluted. However, the present results are similar to those found for SFC on a PS–DVB column[13], although in that case, at similar elution pressures, the analytes were more retained. On the PS–DVB column the first members of the alkyl aryl ketone series (acetophenone to butanophenone) were resolved from the solvent front, whereas on the ODS-silica column they were virtually unretained suggesting a weaker retention by the column. This difference between the relative retention on the ODS-silica and PS–DVB column materials was also observed by Morin *et al.*[17].

There was a linear relationship for both the alkyl aryl ketones and the *n*-alkanes between log k' and the carbon number (Table II, Fig. 1). The slopes of the two sets of standards are different, suggesting that the methylene increment differs for the two sets of compounds. However, because the *n*-alkanes can only be detected using a flame ionisation detector, they are impractical for use in the presence of an organic modifier when spectroscopic detection has to be used. Consequently, the alkyl aryl ketones have been adopted as the retention index scale in the rest of the study. When these compounds were examined previously on a PS–DVB column, propiophenone was found to be anomalous and to have a higher retention index (I = 938–960) than its nominal value of 900 units[13]. However, as noted above, the smaller alkyl aryl ketones were unresolved in the present study and not included in the set of standards. However, during the examination of the barbiturates, a wider range of ketones was examined individually on the ODS-silica column. On elution with carbon dioxide containing 4.2 and 8.4% methanol propiophenone behaved as expected (I = 902 and 888, respectively), but acetophenone was more rapidly eluted (I = 726 and 722) than its nominal value of 800 units[15].

TABLE II

CORRELATION COEFFICIENTS BETWEEN LOG k' AND RETENTION INDICES (CARBON NUMBER × 100) FOR ALKYL ARYL KETONES AND *n*-ALKANES

Conditions as in Table I.

Compound	*Mean column pressure (p.s.i.)*	*Correlation coefficient*	*Slope* $\times 10^{-4}$	*Intercept*
Alkyl aryl ketones				
Valerophenone–octadecanophenone	1665	0.9999	9.51	−0.676
	1950	0.9999	8.10	−0.742
	2160	0.9998	7.28	−0.808
	2470	0.9998	6.94	−0.855
n-Alkanes				
Decane–tetracosane	1665	0.9986	10.01	−0.980
	1950	0.9984	8.60	−1.030
	2160	0.9978	7.77	−1.052
	2470	0.9978	7.31	−1.059

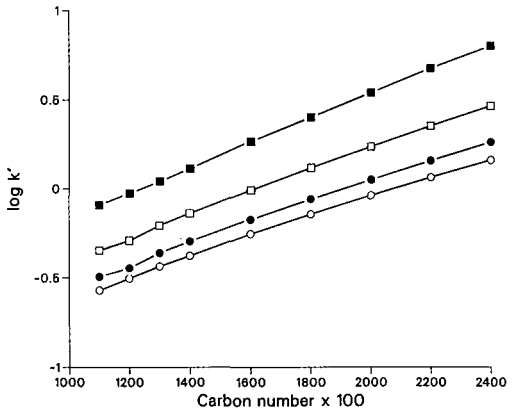

Fig. 1. Relationship between log k' and carbon number × 100 for alkyl aryl ketones on an Ultrasphere ODS column with different proportions of methanol (%, w/w) as modifier in the eluent. ■ = 0%; □ = 4.0%; ● = 8.3%; ○ = 12.7% methanol. Column: Ultrasphere ODS 5-μm, 2470 p.s.i., 60°C.

Retention indices

Using the linear relationship for the alkyl aryl ketones (valerophenone to octanophenone) the retention indices of all the analytes were determined (Table III). As expected, all the alkyl aryl ketones gave retention indices close to their nominal values (carbon number × 100).

The retention indices of the model compounds were often markedly different from those on the PS–DVB column[13]. Nitrobenzene was much lower (I = 583 at 2470 p.s.i. and 60°C compared to 946 at 2515 p.s.i. on the PS–DVB column). In contrast, the retention of benzyl alcohol was much larger (I = 1473 compared to 904), whereas N-propylaniline (I = 1230 compared to 1123) and benzylamine (I = 2089 compared to 1891) were relatively similar on the two columns. Similar differences in relative retentions between these columns were also found by Morin *et al.*[17]. Compared to acetophenone, nitrobenzene had a much higher capacity factor on PRP-1 (PS–DVB) column suggesting that there are particularly strong π–π interactions which are absent on an ODS column.

The retention indices of the polar compounds on the ODS-silica column were also much larger in SFC than in RP-HPLC. The retention on an Hypersil ODS column with methanol–water (50:50)[16] can be compared with the SFC separation at 1950 p.s.i. (in parenthesis) for the polar analytes; *p*-cresol, 807 (1203); benzyl alcohol, 720 (1440); and N-propylaniline, 989 (ref. 19) (1317). In contrast, the retention index values for the less polar compounds were larger on RP-HPLC than on SFC (in parenthesis): nitrobenzene, 843 (690); methyl benzoate, 909 (809); toluene, 1010 (337). These changes emphasize the different selectivities of SFC and RP-HPLC separations. For homologues the two systems show a similar behaviour of increasing retention with molecular size (as in reversed-phase chromatography), but an almost complete reversal of retention characteristics with regard to the polarity of the analytes. Whereas in RP-HPLC polar compounds are rapidly eluted, in SFC with carbon dioxide the polar compounds are retained and in some cases cannot be eluted. This marked change was also observed in the study of the benzodiazepines and a nearly complete reversal of elution order on similar columns was observed in the two techniques[9].

TABLE III

RETENTION INDICES OF ANALYTES BASED ON ALKYL ARYL KETONE STANDARDS

Conditions as in Table I. Based on valerophenone to octadecanophenone scale.

Compound	*Retention index*			
	Mean column pressure (p.s.i.)			
	1665	*1950*	*2160*	*2470*
Alkyl aryl ketones				
Valerophenone	1109	1107	1109	1100
Hexanophenone	1199	1197	1199	1195
Heptanophenone	1294	1293	1295	1291
Octanophenone	1393	1391	1397	1395
Decanophenone	1602	1615	1582	1613
Dodecanophenone	1798	1806	1812	1809
Tetradecanophenone	2004	2006	2009	2008
Hexadecanophenone	2204	2205	2206	2204
Octadecanophenone	2397	2390	2390	2383
n-Alkanes				
Decane	695	657	673	699
Dodecane	954	921	949	972
Tetradecane	1186	1154	1191	1210
Hexadecane	1405	1377	1410	1427
Octadecane	1613	1580	1617	1630
Eicosane	1816	1777	1812	1821
Docosane	2003	1970	1996	2011
Tetracosane	2191	2160	2191	2195
Alkylbenzenes				
Toluene	355	337	348	246
Ethylbenzene	506	496	501	428
n-Propylbenzene	634	628	643	583
n-Butylbenzene	764	765	784	740
Test compounds				
Nitrobenzene	721	690	666	583
Benzaldehyde	772	795	763	753
Methyl benzoate	809	809	826	803
N-Propylaniline	1116	1317	1196	1230
p-Cresol	1163	1203	1288	1281
Benzyl alcohol	1410	1440	1473	1473
Benzylamine	–	1959	2048	2089

Similar large differences between RP-HPLC and SFC were also reported by Wheeler and McNally[20], who found that compared to substituted benzenes such as nitrobenzamides, polynuclear aromatic compound had much longer retentions on RP-HPLC, but were rapidly eluted by SFC with 5 or 2% methanol in carbon dioxide.

Sensitivity of retention and retention indices to changes in operating conditions

To be valuable as a source of robust retention data, the retention indices of analytes should be largely independent of the small variations in operating conditions that can occur between laboratories or between replicate separations on the same

column. The marked changes in capacity factors with mean column pressure (Table I) emphasise the importance of pressure as well as temperature in SFC. Although the latter is reasonably reproducible between laboratories, pressure differences from nominated values are likely to be significant.

In contrast, the retention indices of most of the test compounds showed only small variations with the changes in pressure. Some of the apparent differences at 2470 p.s.i. may be due to the uncertainty involved in the measurement of very small retention times and particular problems will be experienced in the accuracy of the extrapolated values lower than 1100 (valerophenone). Even in these cases the changes are only moderate. More significant changes were noted for nitrobenzene, which decreased, and the polar analytes, *p*-cresol, N-propylaniline and benzylamine, which increased with increasing pressure.

TABLE IV

EFFECT OF PROPORTION OF METHANOL AS A MODIFIER ON THE CAPACITY FACTORS

Conditions: column, Ultrasphere ODS; eluent, carbon dioxide plus methanol; temperature, 60°C; mean column pressure, 2470 p.s.i.; UV detection, 254 nm.

Compound	*Capacity factor*			
	Methanol (%, w/w)			
	0	*4.0*	*8.3*	*12.7*
Alkyl aryl ketones				
Valerophenone	0.81	0.45	0.32	0.27
Hexanophenone	0.95	0.51	0.36	0.31
Heptanophenone	1.10	0.62	0.44	0.37
Octanophenone	1.30	0.73	0.51	0.42
Decanophenone	1.84	0.99	0.67	0.56
Dodecanophenone	2.52	1.31	0.88	0.72
Tetradecanophenone	3.47	1.73	1.23	0.92
Hexadecanophenone	4.74	2.26	1.45	1.16
Octadecanophenone	6.32	2.92	1.84	1.45
Alkylbenzenes				
Toluene	0.21	0.24	0.23	0.24
Ethylbenzene	0.28	0.29	0.27	0.29
n-Propylbenzene	0.36	0.34	0.31	–
n-Butylbenzene	0.46	0.42	0.34	0.34
Test compounds				
Nitrobenzene	0.34	0.30	0.23	0.19
Benzaldehyde	0.47	0.23	0.18	0.16
Methyl benzoate	0.51	0.29	0.22	0.20
N-Propylaniline	1.00	0.48	0.36	0.30
p-Cresol	1.08	0.31	0.04	0.09
Benzyl alcohol	1.47	0.24	0.15	0.14
Benzylamine	3.95	1.07	0.69	0.54
Benzoic acid	–	0.55	0.38	0.25
Benzamide	–	0.75	0.24	0.13

Effect of the addition of modifiers to the mobile phase

As noted earlier, for many polar compounds peak shapes and retentions in SFC are greatly altered by the addition of small percentages of an organic modifier to the mobile phase. Some authors have suggested that for packed columns this occurs by a dynamic coating of free silanols on the surface of the stationary phase[8,21] as well as changes in the overall elution strength of the eluent. The capacity factors of the alkyl aryl ketones, alkylbenzenes and test compounds were therefore determined using constant pressure and temperature but with increasing proportions of methanol (Table IV) or acetonitrile (Table V) in the mobile phase. In both cases the retention times decreased markedly on the addition of modifier with acetonitrile showing the greater effect. Even with small proportions of methanol, benzamide and benzoic acid could now be eluted with only slight peak tailing. However, these compounds still gave broad tailing peaks with acetonitrile as modifier and their retentions could not be

TABLE V

EFFECT OF PROPORTION OF ACETONITRILE AS A MODIFIER ON THE CAPACITY FACTORS

Conditions: column, Ultrasphere ODS; eluent, carbon dioxide plus acetonitrile; temperature, 60°C; mean column pressure, 2470 p.s.i.; detection at 254 nm. Benzoic acid and benzamide gave broad peaks and retentions could not be measured.

Compound	*Capacity factor*			
	Acetonitrile (%, w/w)			
	0.0	*3.7*	*7.1*	*13.4*
Alkyl aryl ketones				
Valerophenone	0.81	0.41	0.26	0.19
Hexanophenone	0.95	0.49	0.31	0.23
Heptanophenone	1.10	0.57	0.37	0.27
Octanophenone	1.30	0.66	0.43	0.32
Decanophenone	1.84	0.90	0.57	0.43
Dodecanophenone	2.52	1.20	0.75	0.56
Tetradecanophenone	3.47	1.59	0.94	0.72
Hexadecanophenone	4.74	2.07	1.23	0.91
Octadecanophenone	6.32	2.67	1.55	1.14
Alkylbenzenes				
Toluene	0.21	0.21	0.21	0.19
Ethylbenzene	0.28	0.25	0.25	0.23
n-Propylbenzene	0.36	0.31	–	–
n-Butylbenzene	0.46	0.38	0.30	0.28
Test compounds				
Nitrobenzene	0.36	0.24	0.15	0.10
Benzaldehyde	0.47	0.20	0.13	0.10
Methyl benzoate	0.51	0.26	0.18	0.15
N-Propylaniline	1.00	0.50	0.34	0.28
p-Cresol	1.08	0.36	0.18	0.10
Benzyl alcohol	1.47	0.35	0.19	0.10
Benzylamine	3.95	0.25	0.16	0.13

TABLE VI

EFFECT OF PROPORTION OF METHANOL AS A MODIFIER ON THE RETENTION INDICES

Conditions as in Table IV. Based on valerophenone to octadecanophenone scale.

Compound	*Retention index*			
	Methanol (%, w/w)			
	0.0	*4.0*	*8.3*	*12.7*
Alkyl aryl ketones				
Valerophenone	1100	1083	1080	1065
Hexanophenone	1195	1171	1163	1185
Heptanophenone	1291	1307	1309	1306
Octanophenone	1395	1414	1420	1412
Decanophenone	1613	1623	1627	1631
Dodecanophenone	1809	1821	1824	1825
Tetradecanophenone	2008	2101	2011	2015
Hexadecanophenone	2204	2196	2194	2194
Octadecanophenone	2383	2374	2370	2367
Alkylbenzenes				
Toluene	246	633	823	958
Ethylbenzene	428	771	955	1110
n-Propylbenzene	583	898	1062	–
n-Butylbenzene	740	1031	1127	1249
Test compounds				
Nitrobenzene	583	809	820	797
Benzaldehyde	753	630	651	639
Methyl benzoate	803	771	793	821
N-Propylaniline	1230	1124	1170	1154
p-Cresol	1281	813	493	193
Benzyl alcohol	1473	656	535	503
Benzylamine	2089	1679	1650	1604
Benzoic acid	–	1214	1200	1015
Benzamide	–	1430	849	473

readily measured. The polar hydroxyl-containing analytes gave good peak shapes with both modifiers.

There was again a linear relationship between carbon number and log k' for the alkyl aryl ketones (Fig. 1). The reduced methylene selectivity indicated by the flatter curves with increasing organic modifier content was also noted by Yonker and co-workers[22,23] in studies of alkyl aryl ketones using a capillary column with carbon dioxide containing 2-propanol as a modifier.

Using these results the corresponding retention indices were calculated for the methanol (Table VI) and acetonitrile eluents (Table VII). With this column the retention indices of the alkylbenzenes increased markedly with both modifiers (Figs. 2 and 3). A similar but small shift to higher retention indices was also seen on the PS–DVB column with increased modifier. The retention indices of most of the less polar model compounds, methyl benzoate, nitrobenzene and benzaldehyde, increased slightly or remained relatively constant. At the higher proportions of modifier many of

TABLE VII

EFFECT OF PROPORTION OF ACETONITRILE AS A MODIFIER ON THE RETENTION INDICES

Conditions as in Table V. Based on valerophenone to octadecanophenone.

Compound	*Retention index*			
	Acetonitrile (%, w/w)			
	0.0	*3.7*	*7.1*	*13.4*
Alkyl aryl ketones				
Valerophenone	1100	1075	1056	1046
Hexanophenone	1195	1192	1187	1179
Heptanophenone	1291	1304	1307	1310
Octanophenone	1395	1405	1421	1424
Decanophenone	1613	1620	1633	1641
Dodecanophenone	1809	1820	1831	1838
Tetradecanophenone	2004	2013	2000	2018
Hexadecanophenone	2204	2197	2196	2190
Octadecanophenone	2383	2374	2369	2355
Alkylbenzenes				
Toluene	246	592	906	1023
Ethylbenzene	438	730	1033	1185
n-Propylbenzene	583	880	–	–
n-Butylbenzene	740	1012	1163	1313
Test compounds				
Nitrobenzene	583	693	634	601
Benzaldehyde	753	582	561	573
Methyl benzoate	803	765	587	845
N-Propylaniline	1230	1209	1249	1326
p-Cresol	1281	989	771	594
Benzyl alcohol	1473	962	807	587
Benzylamine	2089	726	710	749

the retention times are very small and measurement and calculation uncertainties from 20 to 50 units are estimated to be present in these results, particularly for compounds with short retention times or extrapolated indices.

Of the two basic compounds the retention index of N-propylaniline remained virtually constant, in contrast to benzylamine which suffered an initial large change but remained constant upon further addition. These results suggest that the more basic aliphatic amine group was interacting with free silanol groups on the surface of the silica and that this effect was removed by the initial addition of methanol. Smaller proportions may have the same effect but this could not be tested in the present equipment. This observation agrees with work carried out elsewhere[24]. The subsequent lack of change on increased addition of methanol agrees with the study on the PS–DVB column (which lacks the silanol effect) where the retention indices of both amines were virtually unaltered by the addition of modifier[13].

The retention indices of the other polar compounds, *p*-cresol, benzyl alcohol, benzamide, and benzoic acid, changed considerably with increasing modifier content.

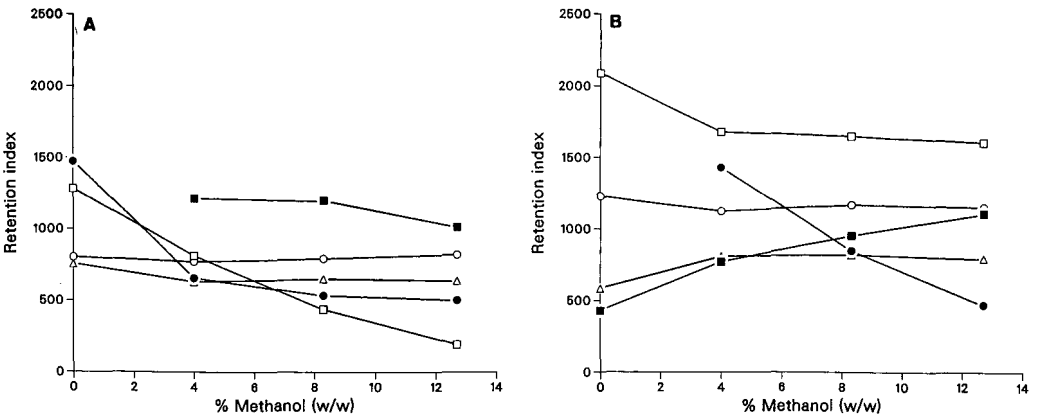

Fig. 2. Effect of the addition of methanol as a modifier on the retention indices (based on alkyl aryl ketones) of test compounds. Conditions: Ultrasphere ODS column; temperature, 60°C; mean pressure, 2470 p.s.i. Compounds: (A): ■ = benzoic acid; □ = *p*-cresol; ● = benzyl alcohol; ○ = methyl benzoate; △ = benzaldehyde. (B): ■ = ethylbenzene; □ = benzylamine; ● = benzamide; ○ = N-propylaniline; △ = nitrobenzene.

p-Cresol changed from 1281 to 193 units in methanol. Large changes for these compounds were also noted on the PS–DVB column[13]. These conclusions suggest that these compounds are not interacting strongly with the column materials, but are interacting poorly with the mobile phase and have a low solubility, which is considerably increased by the addition of modifier. The peak shapes also improved considerably with the addition of modifier. Corresponding changes have been noticed by other workers with polar amide pesticides[20] and in our earlier work with benzodiazepines[9] and barbiturates[15].

Except for the silanol interaction of the basic amines, these changes with modifier proportion do not appear to be primarily related to the stationary phase as

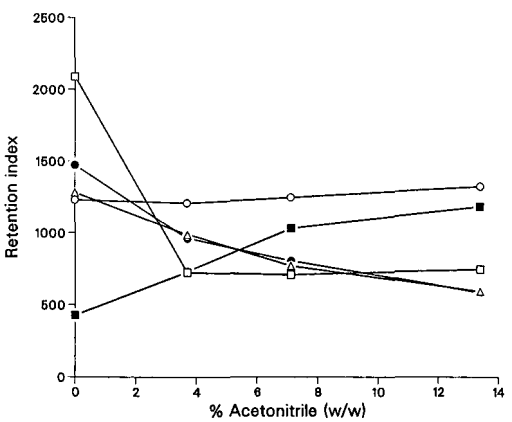

Fig. 3. Effect on the addition of acetonitrile as a modifier on the retention indices (based on alkyl aryl ketones) of test compounds. Conditions as in Fig. 1. Compounds: □ = benzylamine; ○ = N-propylaniline; ● = benzyl alcohol; ■ = ethylbenzene; △ = *p*-cresol.

they are similar on both PS–DVB and ODS-silica columns. The changes almost suggest that as the proportion of modifier is increased, the mobile phase is in transition from an SFC- to an HPLC-type system in which low retentions would be expected for the amide, alcohols and acids, although the comparison with the retention indices in methanol–water given earlier suggests an even greater contrast.

CONCLUSIONS

The alkyl aryl ketones can be used as retention index standards for SFC on ODS-columns and can compensate for small changes in the pressure. They can also be used as a comparison scale to study the marked changes in selectivities with the proportion of organic modifiers. Basic amines seem to suffer interaction with silanol groups on the stationary phases but aromatic amines are unaffected. The influence of silanols is reduced and the elution of polar compounds is improved by the addition of modifiers to the mobile phase.

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Retention studies in supercritical fluid chromatography

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SUMMARY

The effect of fluid density on solute retention using a mobile phase containing 3% (v/v) propan-2-ol in pentane has been investigated. A linear relationship was established between solute retention times and mobile (fluid) phase density. A new method for the determination of the critical temperature of mobile phase mixtures is described and the chromatographic integrity of gas, supercritical fluid and high-performance liquid chromatography is demonstrated.

INTRODUCTION

Supercritical fluid chromatography (SFC) is a rapidly developing analytical technique for the analysis of non-volatile and thermally labile compounds. SFC was first described by Klesper[1] and further studies by Giddings and Myers[2–4] and by Sie and co-workers[5–9] using carbon dioxide, isopropanol and *n*-pentane as mobile phases have been published. Recently, interest in SFC has increased due to its potential ability to provide faster analysis and higher resolving power when compared to high-performance liquid chromatography (HPLC).

Many of the advantages of gas and liquid chromatography are exploited in SFC. These include: densities ranging between a gaseous and liquid mobile phase, higher solute diffusivities and lower mobile phase viscosities. The solvation power of a supercritical fluid is controllable, because it is principally a function of density (although mobile phase modifiers may also be used to enhance separation). This allows pressure-programming techniques to be used in SFC[10], similar to temperature programming in gas chromatography.

This paper reports the effect of fluid density (attained through temperature and pressure programming) on solute retention and efficiency for a packed-column SFC system using 3% (v/v) propan-2-ol in pentane as the mobile phase. The effect of fluid density using normal paraffins (hexane, heptane, octane, nonane and decane) as the mobile phase is also described. The effect of mobile phase modifiers, *n*-butylamine, and propan-2-ol on solute retention is discussed.

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EXPERIMENTAL

Apparatus

The supercritical fluid chromatograph used has been described previously[11].

Reagents

HPLC-grade propan-2-ol, pentane, hexane, heptane and octane were obtained from Fisons (Loughborough, U.K.). Nonane and decane were obtained from BDH (Poole, U.K.) together with the other chemicals used in this work.

Table I gives a list of solvents and their critical parameters.

TABLE I

CRITICAL PARAMETERS OF MOBILE PHASES

T_c = Critical temperature; P_c = critical pressure; ρ_c = critical density; ρ = density.

Solvent	T_c *(°C)*	P_c *(atm)*	ρ_c *(g cm^{-3})*	ρ *at 200°C and 60 kg cm^{-2} inlet pressure (g cm^{-3})*
Pentane	196.5	33.3	0.237	0.459
Hexane	234.2	29.3	0.234	0.525
Heptane	267.0	27.0	0.232	0.587
Octane	295.6	24.54	0.232	0.613
Nonane	321.4	22.8	—	0.636
Decane	344.3	20.72	—	0.653
Propan-2-ol	235.1	47.0	0.273	
Carbon dioxide	31.3	72.9	0.468	
Methanol	240.0	78.5	0.272	
Propan-2-ol–pentane (3:97)	197.94	33.8	0.238	
Methanol–carbon dioxide (3:97)	37.4	73.03	0.466	

RESULTS AND DISCUSSION

Effect of varying the column temperature of constant inlet pressure

The retention behaviour of aniline and pyridine on a 250 × 4.6 mm I.D. column packed with 7-μm silica particles with a mobile phase composition of 3% (v/v) propan-2-ol in pentane was investigated. The temperature of the column was varied between 160 and 240°C at constant inlet and outlet pressure to observe the behaviour of the test solutes with the fluid below and above its critical temperature. The results obtained are shown in Fig. 1.

The retention times of the solutes decreased approximately linearly with the increase in temperature at a constant inlet pressure of 60 kg cm^{-2}. This observation confirms results obtained by Schoenmakers[12]. The decrease in solute retention observed was principally a result of the increase in the linear mobile phase velocity with temperature through the chromatographic column. Although a slight change in solute partition coefficient does occur with the increase in column temperature at constant inlet and outlet pressure, the effect of this slight change in partition coefficient on

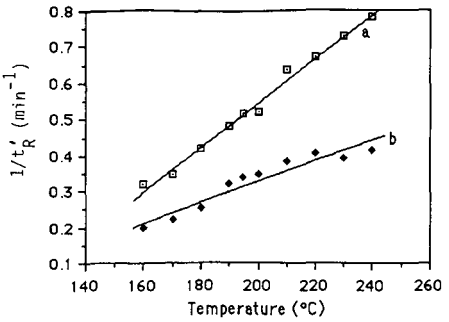

Fig. 1. Effect of column temperature on the reciprocal retention time. Lines: a = aniline (correlation coefficient 1.00); b = pyridine (correlation coefficient 0.96).

solute retention is secondary to the effect of the change in the linear mobile phase velocity through the chromatographic column.

Variation of the column inlet pressure at constant temperature and outlet pressure

The effect of varying the column inlet pressure at constant temperature and outlet pressure on the retention of the test solutes aniline and pyridine was investigated on a 250 × 4.6 mm I.D. column packed with 7-μm silica particles. The mobile phase was propan-2-ol–pentane (3:97).

Fig. 2 illustrates the relationship between the solute retention and the fluid density which is a function of the inlet and outlet pressure of the system. The experiments were performed at three different temperatures: 180, 190 and 200°C. The inlet pressure was varied between 50 and 90 kg cm^{-2} and the outlet restriction was maintained constant concomitant with the inlet pressure. The pressure drop over the chromatographic column for the above experiments was too small (from 1 to *ca.* 1.5 kg cm^{-2}) to be measured accurately and, hence, was considered negligible.

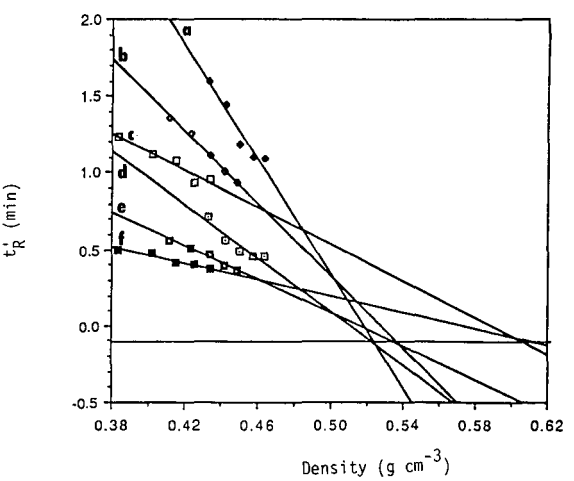

Fig. 2. Plots of adjusted retention time *versus* fluid density at 180, 190 and 200°C. Lines: a = aniline, 200°C; b = aniline, 190°C; c = aniline, 180°C; d = pyridine, 200°C; e = pyridine, 190°C; f = pyridine, 180°C.

A linear decrease in the adjusted solute retention time ($t'_R = t_R - t_0$, where t_R is the retention time and t_0 the dead time) was observed as a result of the increase in density of the mobile phase[11]. From this plot, it is clear that there are differences in the retention behaviour of the test solutes above and below the critical temperature (197.8°C) of the mobile (fluid) phase (see Table I).

From the extrapolation of the relationships in Fig. 2, lines a + d, b + e and c + f intersect at a nearly constant negative adjusted retention time. This may be due to inaccuracy in measuring the dead times of the operational systems and could provide a measure of the dead times of the column in the various operational systems and hence a measure of the dead volume of the column and the volumetric flow-rates. Further work is needed to substantiate this.

The rate of decrease in solute retention with density in the supercritical state (lines c + f) is considerably less than that observed below the critical temperature of the mobile phase (lines a + d and b + e).

In order to explain the general trend of variation in retention time with density observed in these results, the effect of fluid density on solute retention was further studied. The density of the mobile phase was changed using the higher alkanes as the solvents keeping all other operational parameters constant. If the solute retention was observed to decrease linearly with increase in solvent density, it would then be clear that the density of the mobile phase was a principal controlling factor of retention in SFC. This would not take into consideration the effect of changing the polarity of the mobile phase which, under the conditions, would be substantially constant.

Variation in the fluid density by change of solvent

SFC was performed with a series of *n*-alkanes as the mobile (fluid) phase. The first fluid, pentane, was pumped at a constant flow-rate of 1 cm^3 min^{-1} and the temperature of the system was maintained at 200°C, *i.e.* above the critical temperature of pentane. The mobile phase was changed to the higher alkanes (up to *n*-decane) successively while all the other experimental conditions were maintained constant. Under these conditions, the higher *n*-alkanes were below their critical temperatures (see Table I). The test solute used was chrysene on a column packed with 40-μm silica particles (250 × 4.6 mm I.D.). With the same chromatographic column the retention characteristics of this solute were also determined at 200°C using helium as the fluid phase in a gas chromatographic system using a thermal conductivity detector.

Fig. 3 shows that a general decrease in solute retention was obtained with increasing fluid density. These observations confirm that there is a linear relationship between mobile phase density and the solute retention and show that a continuity exists between gas, supercritical, and liquid chromatography and that this is a direct function of the density of the mobile phase.

From the above result it is clear that the use of the higher alkanes which possess higher fluid densities would enable the separation and elution of high-molecular-weight non-polar solutes. This work clearly establishes that there is no real need to operate in the supercritical state with SFC and that the control of the fluid density (achieved through controlling temperature and pressure) is the key factor to attain fast and efficient separations.

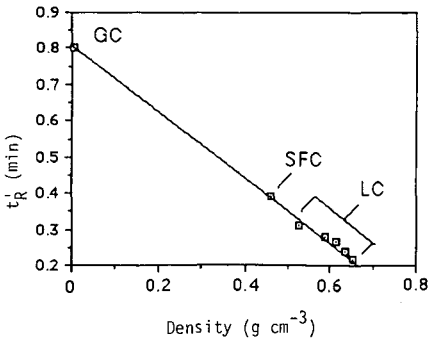

Fig. 3. Plot of adjusted retention time *versus* density of alkanes. Correlation coefficient 0.99.

Effect of column temperature on the dead time in SFC

The effect of the column temperature on the chromatographic dead time was studied using a 250 × 4.6 mm I.D. column packed with 7-μm silica particles.

The SFC system was maintained at a constant inlet pressure of 60 kg cm^{-2}, *i.e.* above the calculated critical pressure for this mobile phase composition (pentane with 3% propan-2-ol). The dead time was obtained using a fixed sample size of potassium nitrate as the unretained solute.

Fig. 4a shows that the dead volume decreases rapidly with temperature until the temperature reaches 196°C, when the rate of decrease in dead volume was significantly reduced up to the maximum temperature employed (240°C). Between 160 and 190°C the mobile phase exists as a liquid phase while above this temperature it exists in the fluid state. This difference in the retention time of the unretained solute is due to the increase in fluid velocity as the critical temperature of the mobile phase is approached. In the supercritical state, the fluid velocity only increases at a rate concomitant with the "expansion" of the fluid with increasing temperature. The critical temperature of the mobile phase mixture was estimated by drawing a vertical line to the x-axis, at the point of interception of the two lines (first line of points obtained below the critical temperature and the second line of points above the critical temperature) (Fig. 4a).

To confirm the above finding, the experiment was repeated for a different mobile phase mixture (3% methanol in carbon dioxide). The temperature of the supercritical fluid chromatograph was varied between 15 and 70°C and a similar behaviour was observed for the rate of change of chromatographic dead time with temperature (Fig. 4b).

This confirms that this approach is indeed useful in obtaining a practical estimate of the critical temperature of a given solvent mixture.

Effect of modifier concentration on solute retention

Organic modifiers may be used to reduce the retention of highly polar solutes in a similar manner as in LC. The effect of including two organic modifiers, *n*-butylamine and isopropanol, in the mobile phase on the retention of aniline using a 250 × 4.6 mm I.D. column packed with 7-μm silica particles was studied. During the experiments the inlet pressure was maintained at 60 kg cm^{-2} and the chromatographic column was maintained at a temperature of 200°C. Fig. 5a shows the effect of the modifier

concentration on solute retention with pentane (above its critical temperature, T_c = 196.5°C) and Fig. 5b with hexane (below its T_c = 234.2°C) as mobile (fluid) phases.

From these plots a decrease in the retention of aniline is observed with an increase in the modifier concentration (*n*-butylamine and isopropanol) in the mobile fluid. It is most unlikely that at this operating temperature (200°C) the modifiers are irreversibly adsorbed onto the surface of the silica, but the observed effect is related to a competition for the adsorption sites on the silica surface between the modifier and the solute molecules[13]. Since the modifier is present in excess with respect to the solute, this will preferentially, but transiently occupy the adsorption sites rendering them unavailable for simultaneous interaction with the solute molecules. This explains why the retention time of the solute molecule decreases with increase in modifier concentration.

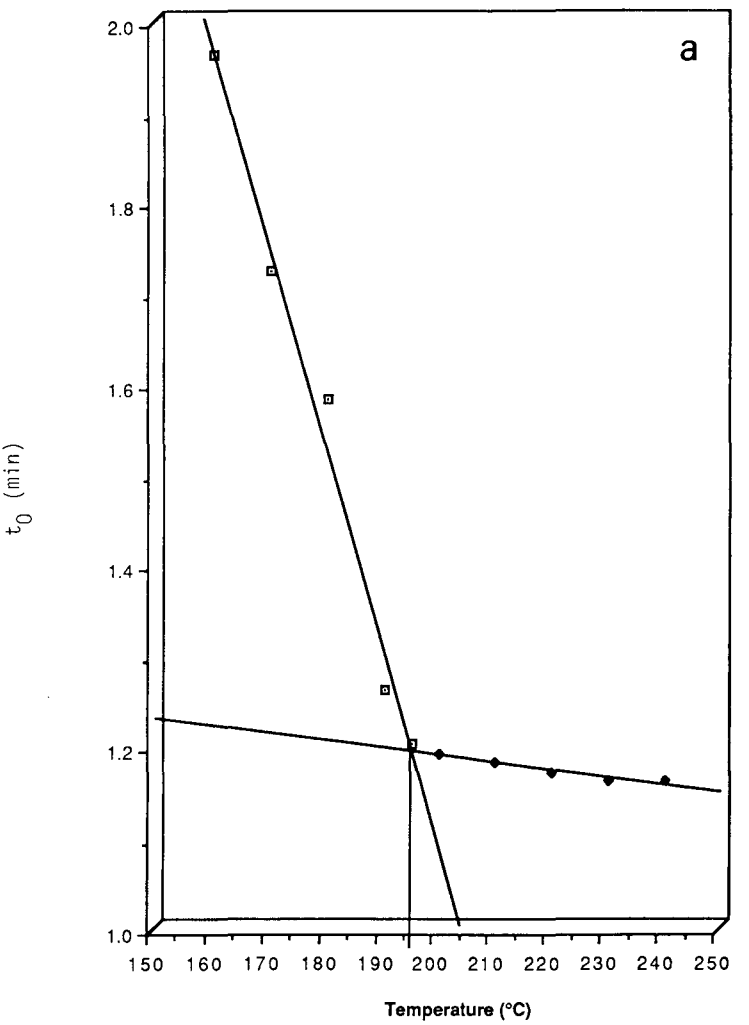

Fig. 4.

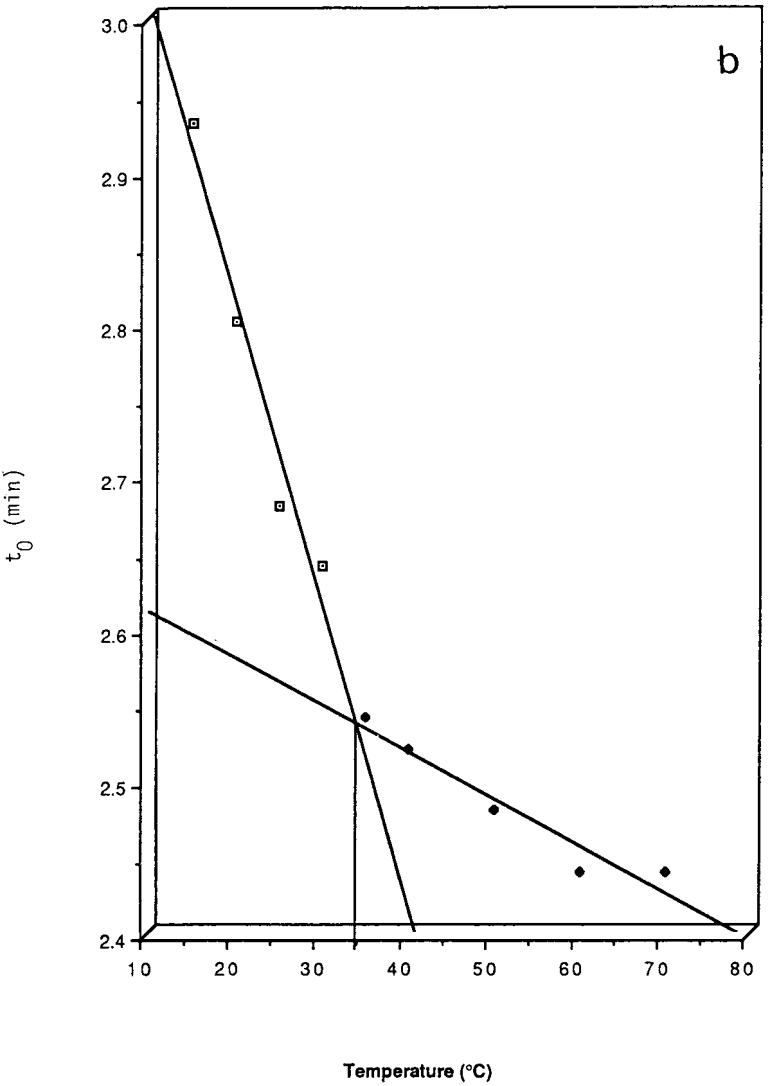

Fig. 4. Effect of column temperature on chromatographic dead time. (a) Mobile phase, propan-2-ol–pentane (3:97); □ = below critical temperature; ◆ = above critical temperature. Lines intersect at 196°C. Critical temperature 197.9°C (calculated). (b) Mobile phase, methanol–carbon dioxide (3:97); □ = below critical temperature; ◆ = above critical temperature. Lines intersect at 35°C. Critical temperature 37.4°C (calculated).

This study was carried out on a silica column. However, other workers have studied modifier effects on cyano, amine and diol stationary phases with different solutes[14–16]. The general trend of their results correlates well with those reported above.

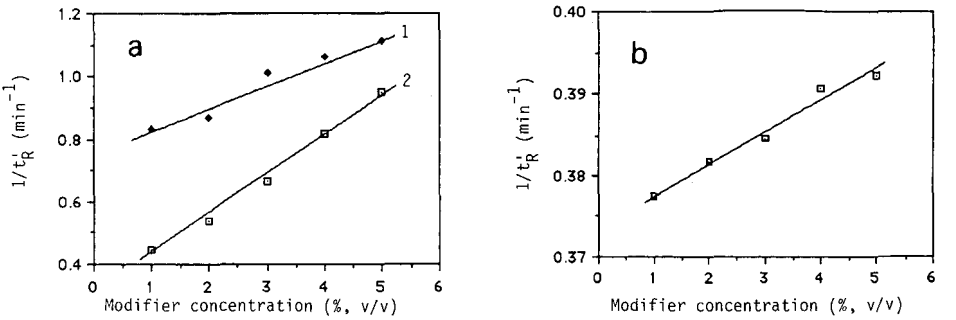

Fig. 5. Effect of propan-2-ol and *n*-butylamine concentration on solute retention. (a) Modifier, propan-2-ol; lines: 1 = pentane (correlation coefficient 0.99); 2 = hexane (correlation coefficient 0.98). (b) Modifier, *n*-butylamine; pentane (correlation coefficient 0.98).

CONCLUSION

A linear relationship has been established between solute retention and mobile (fluid) phase density. From the experiment (Fig. 3), it is clear that the mobile (fluid) phase density and solubility parameter are the factors that control solute retention in SFC and that separations need not be performed above the critical temperature of the mobile phase. Also observed from Fig. 3, is continuity in the three separation techniques, gas chromatography, SFC and HPLC.

Studies on the effect of the mobile (fluid) phase temperature on the dead volume resulted in an interesting observation that varying the mobile phase temperature (at a constant inlet pressure) could be a means of determining the critical temperature of the mobile (fluid) phase mixture.

ACKNOWLEDGEMENTS

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Determination of binary diffusion coefficients of organic compounds in supercritical carbon dioxide by supercritical fluid chromatography

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SUMMARY

Binary diffusion coefficients, D_{12}, in supercritical carbon dioxide were determined in a supercritical fluid chromatographic apparatus by the peak-broadening method. Organic compounds belonging to three different classes, namely ketones, fatty acids and liquid crystals and additionally squalene were investigated as a function of pressure between 9.5 and 18 MPa at about 314 K. The resulting D_{12} values are of the order of 10^{-8} m^2 s^{-1} and ln D_{12} decreases approximately linearly with increasing density of the carbon dioxide.

INTRODUCTION

The determination of gaseous diffusion coefficients by the gas chromatographic peak-broadening method (PBM) was introduced by Giddings[1] in 1960. His results and those of other workers were summarized in a review by Marrero and Mason[2]. Later the method was extended to gas–liquid[3], liquid[4,5] and supercritical fluid systems[6,7]. A critical review of the literature was given by Maynard and Grushka[8].

The theory of diffusion in flowing fluids was first studied by Taylor[9–11] and Aris[12], who solved the problem in a more general form. According to Aris, a sharp band of solute, which is allowed to dissolve in a solvent flowing laminarly in an empty tube, can be described in the limit of a long column as a Gaussian distribution, the variance of which, σ^2, in length units is

$$\sigma(x)^2 = 2D_{\text{eff}}t \tag{1}$$

where t is the time of migration of the peak. D_{eff} is an effective diffusion coefficient, given by

$$D_{\text{eff}} = D_{12} + \frac{r^2u^2}{48D_{12}} \tag{2}$$

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where u is the average solvent velocity, r the inner radius of the tube and D_{12} is the binary diffusion coefficient. The first term describes the longitudinal diffusion in the axial direction. The second term is called the Taylor diffusion coefficient and describes band broadening due to the parabolic flow profile and therefore radial diffusion. The height equivalent to a theoretical plate, H, is a measure of the relative peak broadening and is defined as

$$H = \frac{\sigma(x)^2}{L} \tag{3}$$

where L is the length of the tubing. Substituting eqns. 1 and 2 in eqn. 3 yields

$$H = \frac{2D_{12}}{u} + \frac{r^2 u}{24D_{12}} \tag{4}$$

Eqn. 4 can be rearranged to

$$D_{12} = \frac{u}{4}\left(H \pm \sqrt{H^2 - r^2/3}\right) \tag{5}$$

Of the two possible values of D_{12} only one is meaningful; in this work the negative sign is used.

EXPERIMENTAL

The measurements were carried out with a supercritical fluid chromatographic (SFC) apparatus first designed by Van Wasen *et al.*[7]. Since then, several improvements have been made.

Fig. 1 shows the apparatus used in this work. First the carbon dioxide was

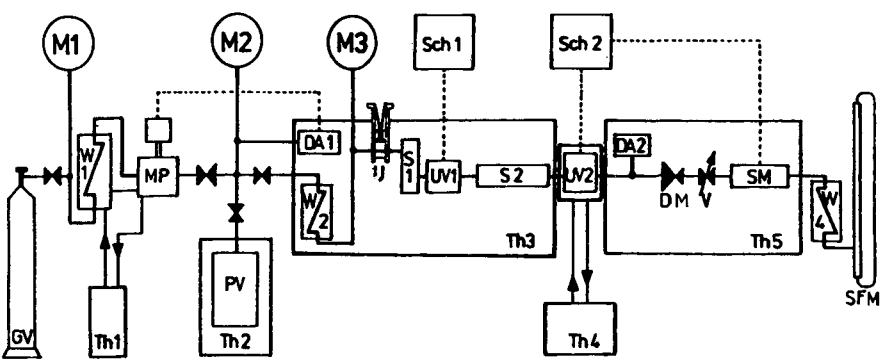

Fig. 1. SFC apparatus for the determination of binary diffusion coefficients. GV = Gas cylinder; W = heat exchanger; MP = diaphragm pump; Th_1 = cryostat; Th_{2-5} = air- and water-baths; PV = buffer volume; M = pressure gauge; DA = strain gauge; IJ = injector; S = column; UV = UV detector; Sch = chart recorder; DM = pressure-reducing valve; V = needle valve; SM = thermal conductivity flow meter; SFM = soap-bubble flow meter.

liquefied and then compressed to the pressure of the experiment by a diaphragm pump (Orlita DMP-AE-10.4), the pulsations of which were eliminated by a volume of buffer. The sample was injected through a high-performance liquid chromatographic valve with a sample volume of *ca.* 0.5 μl. To eliminate the effects of the initial variance of the solute and of the dead volume on peak broadening, the subtraction method of Giddings as adapted by Wasik and McCulloh[13] was used. A short pre-column, 1.26 m long, allowed the development of a symmetrical initial band, which was recorded with a UV detector. Behind the main diffusion column (46.23 m × 0.41 mm I.D.) the end variance was measured by means of a second UV detector. For the usual flow velocity of 0.5 cm s^{-1} a typical experimental run lastened about 2.5 h. The time between two injections was chosen in such a way that overlapping of the peaks was prevented. Finally the fluid was expanded and the flow velocity could be measured by means of a soap-bubble flow meter. The measurements were performed in the range 9–18 MPa at 313.5 and 314.5 K. This corresponds to densities of $0.53 \cdot 10^3$–$0.82 \cdot 10^3$ kg m^{-3}. The initial and the end bands were registered on a chart recorder.

To evaluate D_{12} values from eqn. 4, values of H had to be determined. They were available from the measured chromatograms obtained manually with eqn. 3 and

$$\sigma(x)^2 = \sigma_2(x)^2 - \sigma_1(x)^2 \tag{6}$$

where $\sigma_2(x)^2$ and $\sigma_1(x)^2$ are the variances in length units recorded with the second and first UV detector, respectively. For easier evaluation, the base width w was used instead of the variance:

$$w(x)^2 = 16\,\sigma(x)^2 \tag{7}$$

After transforming $\sigma(x)^2$ into $\sigma(t)^2$ by $\sigma(x)^2 = u^2\sigma(t)^2$, where u is the average flow velocity, the following expression for H was obtained:

$$H = \frac{[w_2(t)^2 - w_1(t)^2]u}{16t_r} \tag{8}$$

t_r being determined from the time difference of the two peaks as shown in Fig. 2.

The main sources of error were the initial peak dispersion, adsorption effects, in particular for low-volatile substances, and the manual determination of the chromatograms. The overall accuracy of the D_{12} values obtained was better than $\pm 6\%$.

Substances

The substances tested belonged to three different chemical classes. In the first group some homologous and branched ketones were measured. In particular these were the symmetric ketones 2-propanone, 3-pentanone, 4-heptanone, 5-nonanone and 6-undecanone, the 2-substituted ketones 2-butanone, 2-pentanone, 2-heptanone and 2-nonanone and the branched isomers 2,4-dimethyl-3-pentanone (DMP), 2,2,4,4-tetramethyl-3-pentanone (TMP) and tricyclo[3.3.1.$1^{3,7}$]decanone (adamantanone). The second group consisted of the fatty acids oleic acid, stearic acid and linolenic acid, all with 18 carbon atoms but a different number of double bonds. Finally, the two

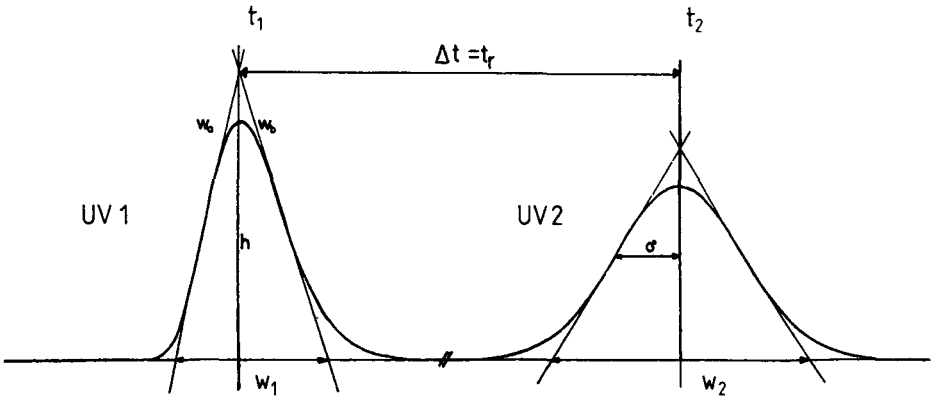

Fig. 2. Determination of D_{12} values from the chromatograms. σ = Variance; W = base width; w_a,w_b = inflection point tangents; h = peak height; t = migration time; $t_r = t_2 - t_1$.

liquid crystals N-(4-methoxybenzylidene)-4-*n*-butylaniline (MBBA) and 4-cyano-4'-*n*-pentoxybiphenyl (5-OCB), both having similar molar masses, and additionally 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene (squalene) were measured.

Most of the substances were from Aldrich (Steinheim, F.R.G.) with purities 98% or better; DMP was from Fluka (Buchs, Switzerland) with a purity better than 98%. MBBA was obtained from Merck (Darmstadt, F.R.G.) and 5-OCB from BDH (Poole, U.K.) with purities of about 98% and better than 99%, respectively.

The mobile phase carbon dioxide was obtained from Linde (Höllriegelskreuth, F.R.G.) with a purity of better than 99.9%.

All low-volatile substances were dissolved before injection. The fatty acids were dissolved in ethanol and the liquid crystals and squalene in heptane and cyclohexane. The solvent had no influence on the value of D_{12}.

RESULTS AND DISCUSSION

In previous papers[14,15], results of measurements mostly on aromatic compounds in different mobile phases were reported. In this paper, new data on some ketones, fatty acids and liquid crystals and additionally squalene in supercritical carbon dioxide are presented. The results are compiled in Tables I, II and III.

In Figs. 3 and 4 the density dependence of the D_{12} values of the symmetric and non-symmetric ketones is shown. The same exponential behaviour is found for all the substances. For comparison, self-diffusion coefficients D_{11} of carbon dioxide[16,17] are also given. They show a density dependence, which is similar to that of the binary diffusion coefficients. As expected the D_{11} values are higher mainly because of the lower molar mass of carbon dioxide. In Fig. 5 reduced diffusion coefficients, $D_k^{red} = D_k/D$(3-pentanone), are plotted against the molar mass of all ketones, k, under test. D_k^{red} are values obtained by dividing all D_{12} values of a substance by the corresponding D_{12} of 3-pentanone measured at the same density and subsequently taking the average. The resulting dependence for the homologous ketones is approximately linear. The

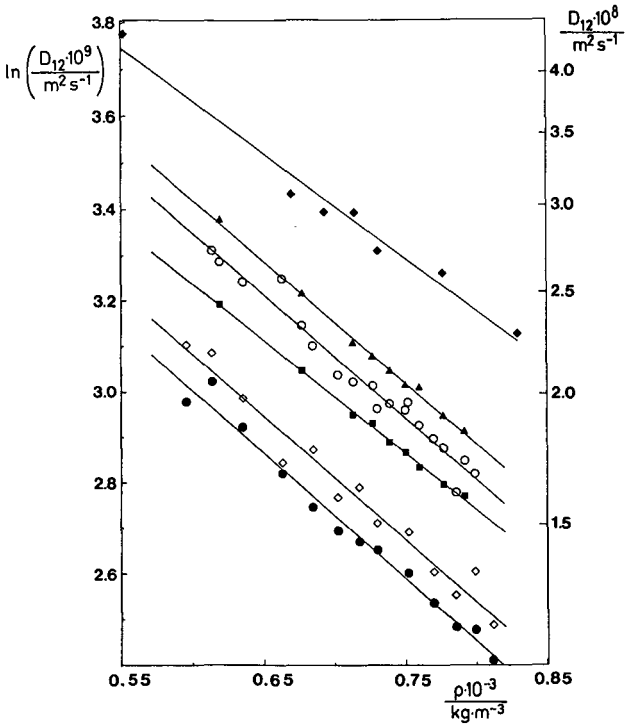

Fig. 3. Logarithm of binary diffusion coefficients, ln D_{12}, of symmetric ketones as a function of density ρ at 314 K. ▲ = 2-Propanone; ○ = 3-pentanone; ■ = 4-heptanone; ◇ = 5-nonanone; ● = 6-undecanone. ◆ = Self-diffusion coefficients, D_{11}, of carbon dioxide according to refs. 16 and 17. Densities of carbon dioxide are taken from ref. 18.

TABLE I

DIFFUSION COEFFICIENTS, D_{12}, OF SOME KETONES IN SUPERCRITICAL CARBON DIOXIDE AT 313.5 K AND DENSITIES, ρ

313.5 K		D_{12} (10^{-8} m^2 s^{-1})					
p (MPa)	*$\rho \cdot 10^{-3}$ ($kg\ m^{-3}$)*	*2-Propanone*	*3-Pentanone*	*4-Heptanone*	*Adamantanone*	*DMP*[a]	*TMP*[b]
16	0.792	1.84	1.72	1.59	–	1.58	1.43
15	0.777	1.90	1.77	1.64	1.44	1.60	1.44
14	0.760	2.03	1.86	1.70	1.50	1.70	1.54
13.5	0.750	2.04	1.93	1.76	1.59	1.73	1.58
13	0.739	2.10	1.96	1.79	1.59	1.77	1.63
12.5	0.727	2.17	2.07	1.87	1.64	1.83	1.67
12	0.713	2.23	2.05	1.91	1.68	1.89	1.78
11	0.677	2.49	2.32	2.11	1.77	2.10	1.91
10	0.619	2.92	2.68	2.43	1.41	2.45	2.22

[a] 2,4-Dimethyl-3-pentanone.
[b] 2,2,4,4-Tetramethyl-3-pentanone.

TABLE II

DIFFUSION COEFFICIENTS, D_{12}, OF SOME KETONES IN SUPERCRITICAL CARBON DIOXIDE AT 314.5 K AND DENSITIES, ρ

314.5 K		D_{12} $(10^{-8}\ m^2\ s^{-1})$						
p (MPa)	$\rho \cdot 10^{-3}$ $(kg\ m^{-3})$	*2-Butanone*	*2-Pentanone*	*2-Heptanone*	*2-Nonanone*	*3-Pentanone*	*5-Nonanone*	*6-Undecanone*
18	0.812	1.75	1.53	1.40	1.31	–	1.20	1.12
17	0.799	1.70	1.69	1.42	1.29	1.68	1.35	1.19
16	0.786	1.79	1.69	1.46	1.19	1.61	1.29	1.20
15	0.770	1.86	1.84	1.49	1.42	1.81	1.35	1.27
14	0.752	1.90	1.81	1.60	1.44	1.97	1.44	1.35
13	0.730	2.13	1.93	1.72	1.48	1.93	1.51	1.38
12.5	0.717	2.18	1.97	1.71	1.64	–	1.63	1.45
12	0.702	2.20	2.12	1.81	1.76	2.09	1.59	1.48
11.5	0.685	2.49	2.24	2.01	1.74	2.22	1.77	1.56
11	0.663	2.55	2.25	2.11	1.92	2.58	1.86	1.68
10.5	0.635	2.59	2.58	2.27	2.00	2.55	1.99	1.96
10.2	0.614	2.94	3.28	–	2.15	2.74	2.19	2.06
10	0.586	2.86	2.79	2.71	2.16	3.17	2.23	1.97
9.7	0.562	3.42	3.28	2.70	–	–	–	–
9.5	0.531	3.46	3.30	2.92	–	–	–	–

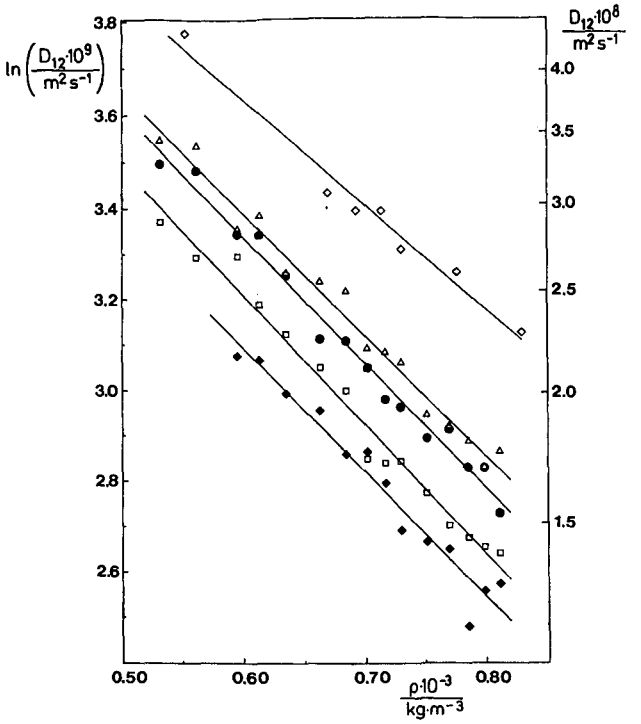

Fig. 4. Ln D_{12} of non-symmetric ketones as a function of density ρ at 314 K. △ = 2-Butanone; ● = 2-pentanone; □ = 2-heptanone; ◆ = 2-nonanone. ◇ = D_{11} values of carbon dioxide.

D_{12} values of the branched C_7 ketone lay in the range of the linear isomers (Fig. 6), whereas for the tetramethylated C_9 isomer there is a trend to higher values (Fig. 7). Tricyclodecanone also shows higher values, as expected because of its nearly spherical shape.

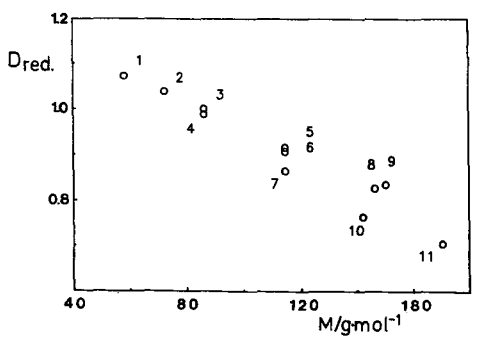

Fig. 5. Reduced diffusion coefficients, $D_k^{red} = D_k/D$(3-pentanone), as a function of molar mass, M_k. 1 = 2-Propanone; 2 = 2-butanone; 3 = 3-pentanone; 4 = 2-pentanone; 5 = 4-heptanone; 6 = 2,4-dimethyl-3-pentanone; 7 = 2-heptanone; 8 = 2,2,4,4-tetramethyl-3-pentanone; 9 = adamantanone; 10 = 2- and 5-nonanone; 11 = undecanone.

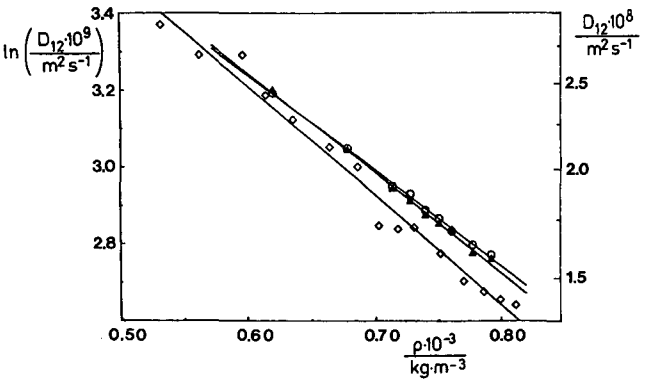

Fig. 6. Density dependence of ln D_{12} of the C_7 isomeric ketones at 314 K. ○ = 4-Heptanone; ▲ = 2,4-dimethyl-3-pentanone; ◇ = 2-heptanone.

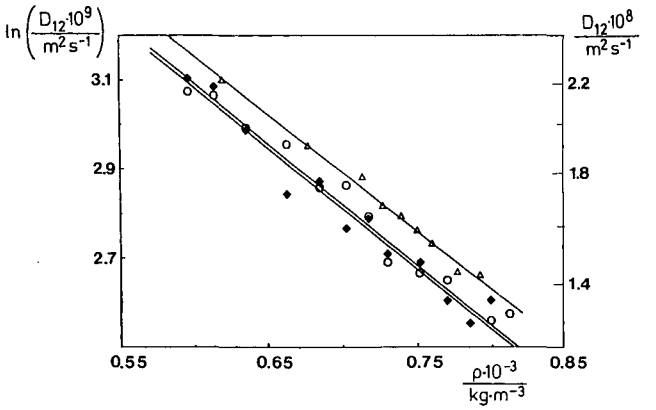

Fig. 7. Density dependence of ln D_{12} of the C_9 isomeric ketones at 314 K. △ = 2,2,4,4-Tetramethyl-3-pentanone; ○ = 2-nonanone; ◆ = 5-nonanone.

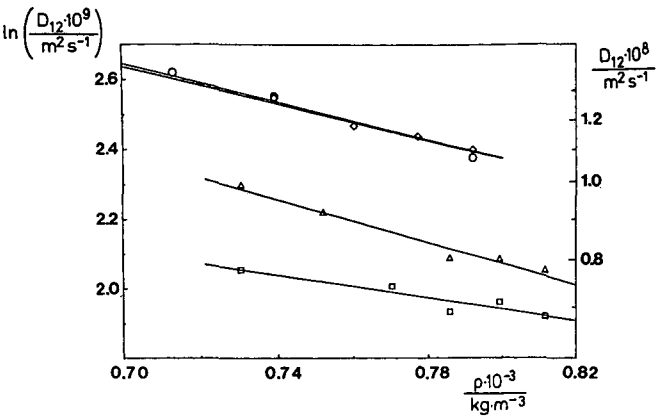

Fig. 8. Comparison of the density dependence of ln D_{12} of some fatty acids and squalene at 314 K. ◇ = Stearic acid; ○ = oleic acid; △ = linolenic acid; □ = squalene.

TABLE III

DIFFUSION COEFFICIENTS, D_{12}, OF SOME LOW-VOLATILE SUBSTANCES IN SUPERCRITICAL CARBON DIOXIDE AT 313.5 AND 314.5 K AND DENSITIES, ρ

313.5 K		D_{12} (10^{-8} m^2 s^{-1})		
p (MPa)	$\rho 10^{-3}$ (kg m^{-3})	*MBBA*[a]	*Stearic acid*	*Oleic acid*
16	0.792	1.00	1.10	1.08
15	0.777	1.04	1.15	–
14	0.760	1.10	1.19	1.19
13.5	0.657	–	–	1.29
13	0.739	1.15	1.28	1.37
12	0.713	1.23	–	1.54
314.5 K		*5-OCB*[b]	*Linolenic acid*	*Squalene*
18	0.812	0.86	0.78	0.68
17	0.799	0.89	0.81	0.71
16	0.785	0.89	0.81	0.66
15	0.770	0.93	–	0.71
14	0.752	0.96	0.92	–
13	0.703	1.06	1.00	0.78

[a] MBBA = N-(4-Methoxybenzylidene)-4-*n*-butylaniline.
[b] 5-OCB = 4-Cyano-4'-*n*-pentoxybiphenyl.

Owing to the strong solvent power of the highly compressed carbon dioxide, it was possible to measure some low-volatile substances such as the fatty acids. Three C_{18} acids were examined which differ only in the number of double bonds. For stearic acid and oleic acid with one double bond no difference exists, as can be seen in Fig. 8. Only linolenic acid with its three double bonds shows distinctly lower D_{12} values. It is evident that these double bonds make the molecule rigid, its effective cross-section increases and the diffusion process will be slower.

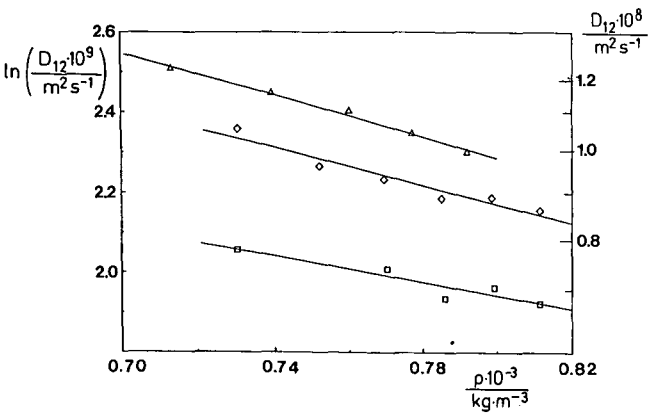

Fig. 9. Comparison of the density dependence of ln D_{12} of the liquid crystals △ = MBBA and ◇ = 5-OCB and also □ = squalene at 314 K.

For the liquid crystals MBBA and 5-OCB a similar effect can be seen. Although both have similar molar masses, their diffusion coefficients differ. The bridged phenyl rings should allow more mobility whereas the biphenyl is rigid. This results in lower diffusion coefficients for 5-OCB (Fig. 9). For squalene small D_{12} values are found as expected because of its high molar mass.

ACKNOWLEDGEMENT

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Analysis of aliphatic and phenolic carboxylic acids by capillary supercritical fluid chromatography–Fourier-transform infrared microspectrometry

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SUMMARY

A mobile phase elimination interface and a microscope accessory are used to combine capillary supercritical fluid chromatography with Fourier-transform infrared spectrometry for analyzing carboxylic acids. Separations of a mixture of oligomers prepared by a self-condensation polymerization of 12-hydroxystearic acid and of a mixture of 16 phenolic carboxylic acids are achieved by using a well-deactivated fused-silica capillary column coated with an oligoethyleneoxide substituted methylpolysiloxane (glyme) stationary phase and carbon dioxide as the mobile phase. Components requiring identification are deposited, with elimination of the carbon dioxide, onto potassium bromide discs as compact spots, from the end of a heated restrictor, which is attached at the end of the column. Unique spectroscopic information is obtained by positioning the spots in the microscope and measuring their infrared spectra. Phenolic acids are unambiguously identified and the degree of polymerization of oligomers in the 12-hydroxystearic acid reaction mixture is indicated from the ratio of ester carbonyl to acid carbonyl absorptions.

INTRODUCTION

Combination of state-of-the-art Fourier-transform infrared spectrometry (FT-IR) and high-resolution capillary chromatography creates a powerful tool for the analytical chemist. Volatile and thermally stable compounds can be analyzed using

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gaseous mobile phases and higher-molecular-weight, thermally labile or polar material can be analyzed using supercritical mobile phases.

Carbon dioxide is the most commonly used supercritical mobile phase, because of its attractive chromatographic and spectroscopic properties. From a chromatographic point of view, the solvating power of carbon dioxide can be increased by programming the mobile phase from gas-like to liquid-like densities. Compounds which are not amenable to analysis by capillary gas chromatography (GC) can therefore be separated at low temperatures and with reasonable efficiency, because of the gas-like viscosity and high diffusivities of solutes in the mobile phase. From a spectroscopic point of view, carbon dioxide absorbs minimally in the mid-IR region and is gaseous under atmospheric conditions. Consequently capillary supercritical-fluid chromatography (SFC) has been coupled to FT-IR using flow-cell and mobile phase-elimination interfaces —a subject which has been reviewed in the literature recently[1,2].

SFC–FT-IR microspectrometry makes use of the mobile phase-elimination approach, where the column effluent is depressurized from a heated restrictor at the end of the capillary column. Involatile analytes, at the low nanogram level, are deposited uncontaminated onto an IR-transparent support as the mobile phase evaporates away. The window is stepped so that the components may be spatially separated for analysis in an FT-IR microscope. An advantage of this interface over the flowcell is that it is compatible with polar or modified mobile phases which may be necessary for chromatography[3]. Further, once a compound is deposited, extensive signal averaging can be performed to improve the quality of the final spectra. The mobile phase elimination method was adapted for SFC–FT-IR by Pentoney and co-workers[4,5], who analyzed polysiloxane oligomers, vegetable oil shortening and a synthetic mixture of ter- and quaterphenyl isomers. Raynor and co-workers have used a similar system for analysing polymer additives[2,6], steroids[7] and polycyclic aromatic hydrocarbons.[8].

The aim of this report is to demonstrate further the capability of SFC–FT-IR microspectrometry for the analysis of aliphatic and phenolic carboxylic acids. These compounds are frequently present in products of plant origin which are important economically. Free fatty acids for example are obtained from vegetable sources such as sunflower seed, and used in large amounts by the food and surface-coatings industries. Methods for analysing these types of materials by SFC[9] and packed-column SFC–FT-IR using a flow cell[10] are documented in the literature. Phenolic acids such as syringic, *p*-hydroxybenzoic and vanillic acids are commonly found in grape and other fruit juices[11], while distilled beverages such as whisky contain other acids such as ferrulic acid, which originate from the cereal used for fermentation[12]. These acids are polar and highly adsorptive solutes, but have appreciable solubility in supercritical carbon dioxide[13]. This indicates that direct analysis by SFC is possible without derivatization, provided that the capillary column is well deactivated and is coated with a stationary phase which has selectivity and capacity for polar solutes and does not interact strongly with acid and hydroxyl groups. For this purpose, Rouse *et al.*[14] have recently developed a 50% oligoethylene oxide-substituted methylpolysiloxane (glyme) stationary phase which has a resolving power similar to that of Carbowax 20M. A 50 μm I.D. fused-silica capillary column coated with an immobilized film of this phase has therefore been investigated for separating the acids.

EXPERIMENTAL

The experimental procedure and instrumentation required for SFC–FT-IR microspectrometry has already been fully described in the literature[6]. However, in order to analyze acidic compounds, various changes have been made to this methodology. These changes are itemized in this section.

Supercritical fluid chromatography

A Lee Scientific (Salt Lake City, UT, U.S.A.) 501 SFC syringe pump was used for density programming the mobile phase. The pump software was run on an IBM XT Model 286 personal computer (IBM, Portsmouth, U.K.). SFC-grade carbon dioxide (Air Products, Rotherham, U.K.) was passed through a basic alumina trap and a 2-μm filter prior to filling the pump. This was carried out to reduce hydrocarbon contamination in the mobile phase. The carbon dioxide was pressurized and delivered by the pump to a Valco C14W microvalve injector fitted with a 200-nl internal sample rotor (Valco Instruments, Houston, TX, U.S.A.). The injector was cooled to approximately 18°C prior to injection with a cooling jacket which was connected to the main water supply. The column was connected to the injector using an inlet splitter (SGE, Austin, TX, U.S.A.) which was adjusted to give a split ratio of approximately 1:4. Separations were performed on a 10 m × 50 μm I.D. fused-silica capillary column, which had been deactivated using a cyanopropyl hydrosiloxane procedure[15] and coated with a 0.25 -μm film of the glyme stationary phase[14,16]. The stationary phase was cross-linked with azo-*tert.*-butane, rinsed with methylene chloride and conditioned prior to use[16]. The capillary column was installed in a Carlo-Erba (Milan, Italy) Fractovap Series 2150 GC oven with a flame ionization detection (FID) system held at 400°C. The column effluent was split between a tapered capillary restrictor[17] in the FID system and a heated transfer line to the mobile phase-elimination interface by using a butt connector and a graphite ferrule (SGE, Milton Keynes, U.K.).

Mobile phase-elimination interface

The transfer line was a 50 cm × 50 μm I.D. piece of deactivated fused-silica tubing (SGE) with a tapered restrictor fabricated at the interface end[17]. The transfer line was inserted into a 50 cm × 1/16 in. O.D. stainless-steel tube maintained at the column temperature. The end 10 cm of the tube was heated to approximately 200°C using a GC injector heating block. The column effluent was split fairly evenly between the FID system and the interface, by cutting the tapered restrictors back until each had a gaseous flow-rate of approximately 1 ml/min (measured at room temperature and at a column pressure of 150 atm). The end of the restrictor was positioned 50–100 μm above the surface of a potassium bromide window which was stepped manually for peak collection. Solutes were deposited with the window at room temperature and held stationary.

FT-IR microspectrometry

Spots on the potassium bromide discs were analyzed using a Spectra-Scope IR microscope accessory (Spectratech, Warrington, U.K.) and a Nicolet 60SX FTIR spectrometer fitted with an MCT detector (Nicolet Instruments, Warwick, U.K.).

The FT-IR sample compartment which housed the microscope was purged with nitrogen to reduce spectral interference from water vapour and carbon dioxide in the atmosphere. An IR spectrum of the background was measured using a clean area of the window. This spectrum was stored and subtracted from the spectra measured from deposited solutes. Spots to be analyzed were positioned in the beam focus of the microscope using the visible transmission viewing mode. After inspection, the IR beam was stopped down to the diameter of the deposit which was typically 100–200 μm for solids and less than 100 μm for liquids (see Results and Discussion section). On average 1000 spectra, with a resolution of 4 cm^{-1} per spot, were measured and co-added in about 5 min to obtain each final spectrum.

Materials

A mixture of oligomers of 12-hydroxystearic acid was obtained from ICI. The aromatic acids used in this study were donated from several sources. Samples were dissolved in analytical-grade methanol or dichloromethane (BDH, Liverpool, U.K.). Spectroscopic grade potassium bromide (BDH) was used for preparing windows for deposition of selected solutes.

RESULTS AND DISCUSSION

The objective of this work was to investigate capillary SFC with carbon dioxide as the mobile phase for separating a range of aliphatic and phenolic carboxylic acids and to assess the suitability of the mobile phase-elimination interface for their collection and subsequent FT-IR analysis.

Mixed oligomers of 12-hydroxystearic acid

The method was first applied to the analysis of a reaction mixture produced by a self-condensation polymerisation of 12-hydroxystearic acid. The reaction schematic in Fig. 1 shows how this material was prepared. At the start of the polymerization, the acid group of one molecule reacts with the hydroxyl group of another with the elimination of water to form an ester. This dimer can then react in the same way to form a trimer and so on. The resultant reaction product therefore consists of a complex mixture of acid oligomers of various chain lengths which are difficult to analyze by GC and high-performance liquid chromatographic (HPLC) methods without derivatization. Capillary SFC is, however, a particularly good technique for direct analysis of this sample as shown by the chromatogram in Fig. 2. For this separation, the mixture was dissolved in dichloromethane and injected into the chromatograph. The carbon dioxide mobile phase (held isothermally at 100°C) was programmed from 0.35 g/cm^3 to 0.76 g/cm^3 at 0.008 g/cm^3 per min after a 10-min isoconfertic period to elute progressively longer oligomer chains. Although oligomers with similar chain lengths have not been fully resolved, an efficient separation has been achieved, which is more than adequate for collecting components at the interface, particularly as little difference was expected between the IR spectra of co-eluting material.

Components associated with each labeled peak in Fig. 2 were collected on the KBr window for subsequent FT-IR analysis. On microscopic examination, each spot was observed to be a collection of small globules which had been spread over an area of 300–500 μm in diameter by the expanding mobile phase leaving the restrictor.

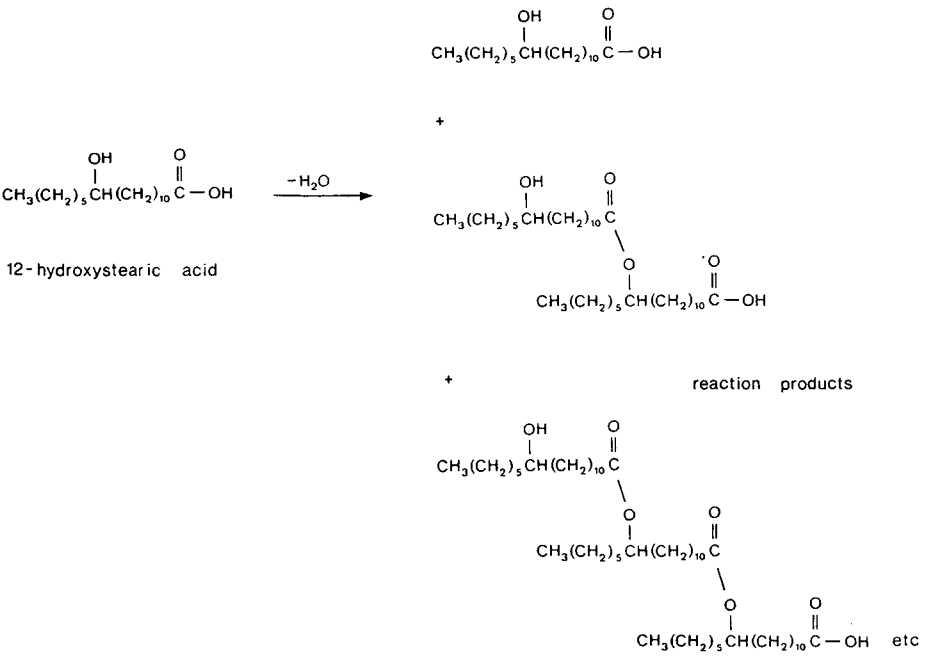

Fig. 1. Self-condensation polymerization of 12-hydroxystearic acid to form oligomers.

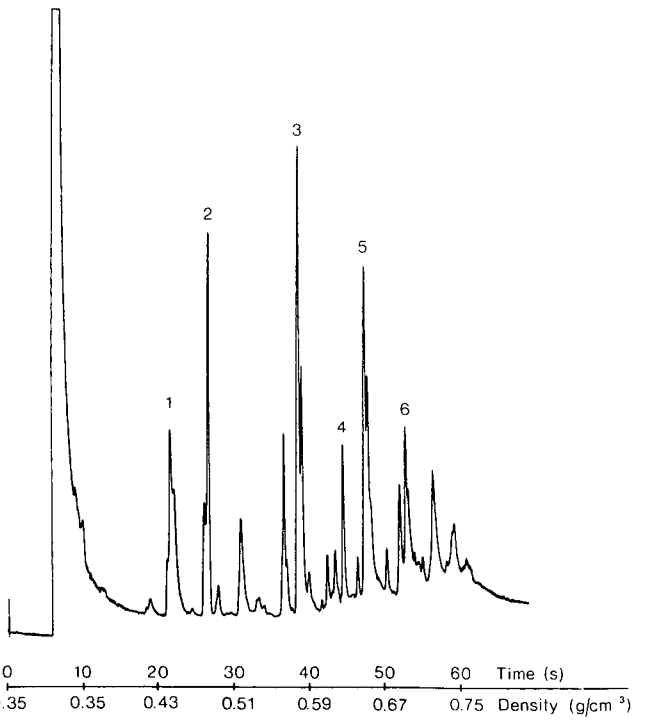

Fig. 2. Separation of 12-hydroxystearic acid oligomers by capillary SFC. Conditions: 10 m × 50 μm I.D. capillary column, glyme stationary phase (0.25 μm film thickness), CO_2 programmed from 0.35 g/cm^3 (10 min) to 0.76 g/cm^3 at 0.008 g/cm^3 per min, 100°C, FID at 400°C.

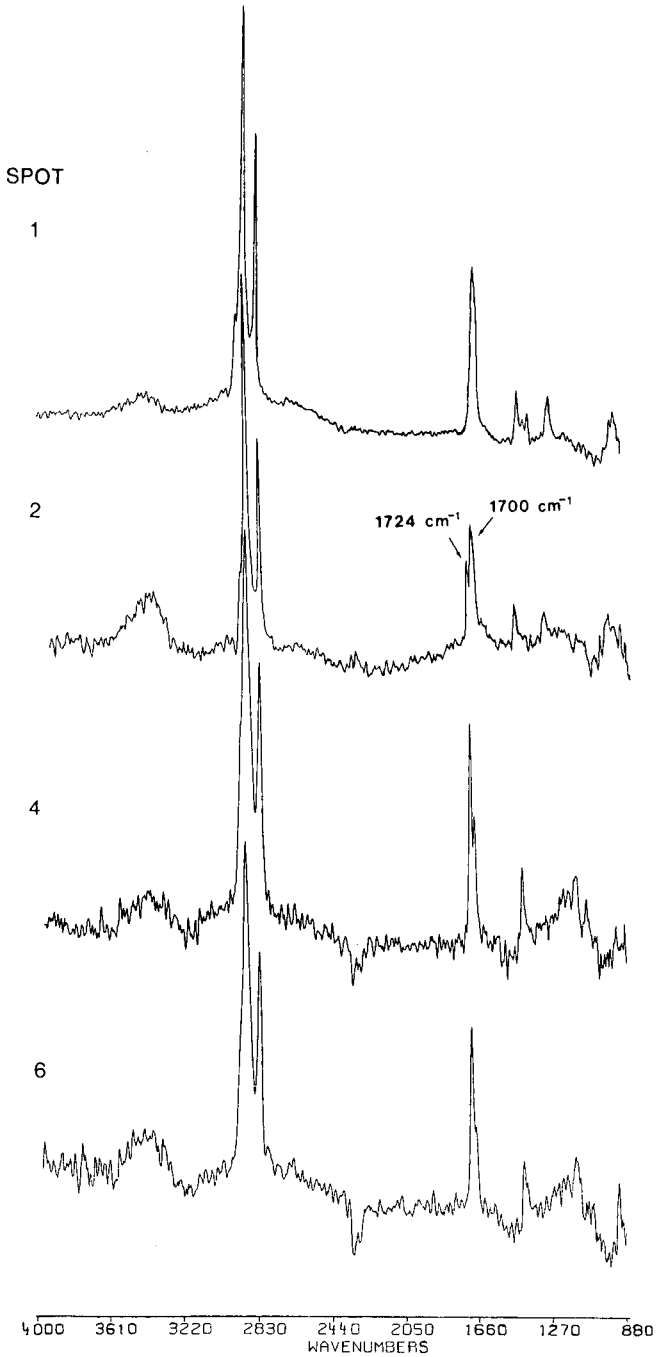

Fig. 3. IR spectra of 12-hydroxystearic acid oligomers separated by capillary SFC in Fig. 2.

Measurement of IR spectra from these samples was more difficult than for solid samples which tend to deposit as compact spots and therefore have greater pathlengths. Pentoney *et al.*[4] have shown that lowering the window temperature improves the deposition characteristics of liquids. However, to achieve this experimentally, the interface becomes significantly more complex and we have found that sufficient spectral information can still be obtained from samples deposited onto the window surface as liquids providing that they are involatile. The IR spectra from spots 1, 2, 4 and 6 shown in Fig. 3, were measured by focusing the microscope beam onto the largest globule of sample in each of the deposits. The beam aperture was stopped down to the approximate size of the globule (typically below 100 μm).

A certain amount of chemical information about the oligomers can be extracted from the 1600–1800 cm^{-1} region of the spectra. The IR spectrum of spot 1 has one absorption at 1700 cm^{-1} in this region. This is assigned to the carbonyl stretching vibration of the 12-hydroxystearic acid which exists (in the deposited state) as a dimer due to hydrogen bonding. The presence of an additional absorption at 1724 cm^{-1} in the IR spectrum of spot 2 indicates that an ester carbonyl is now present in this oligomer. The IR spectrum of spot 4 has a significantly more intense ester carbonyl absorption than acid carbonyl absorption which indicates that this oligomer has a greater chain length (the number of ester groups has increased whereas there is still only one terminal acid group on the end of the oligomer). The effect is further enhanced in the IR spectrum of spot 6, where the acid-carbonyl absorption is only just evident as a shoulder on the ester-carbonyl peak. Accompanying the relative decrease in carbonyl absorption at 1700 cm^{-1}, is the loss of absorption intensity at 1270 cm^{-1}, assigned to the νC–O of the acid group. Conversely, as the relative intensity of the ester νC=O increases this is accompanied by an increase in νC–O–C intensity in the 1210 cm^{-1} region. The difference observed in the intensity of the νO–H near 3400 cm^{-1} between the spectra is due to the difference in the levels of absorbed water in the KBr support in regions of sample deposition compared to regions where background spectra were accumulated.

The above example demonstrates the capability of SFC with FID and FT-IR detection for monitoring reactions. For example, samples could be removed from a mixture at set times during a reaction and analyzed to determine the extent of polymerization. Unfortunately, IR spectroscopy does not give any molecular weight information, which may be important for further identification. However, there is a good possibility that a gold-coated moving-belt type interface could be constructed which would enable SFC to be coupled with both FT-IR and mass spectrometry (MS).

Phenolic acids

Phenolic acids are difficult compounds to analyze, particularly if FT-IR identification of underivatized material is required. As these compounds are solids they pose no potential problem for collection at the mobile phase-elimination interface, provided that they can be efficiently separated. Capillary SFC was investigated using a synthetic mixture containing approximately 500 ng/μl of benzoic acid, cinnamic acid, 4-chlorobenzoic acid, 3,5-dimethoxybenzoic acid, 4-chlorocinnamic acid, vanillic acid, 3,4-dimethoxycinnamic acid, syringic acid, ferrulic acid, sinapic acid, 4-hydroxyphenylpropionic acid, 4-hydroxyphenylacetic acid, 4-hydroxybenzoic acid,

cis-4-hydroxycinnamic acid, *trans*-4-hydroxycinnamic acid and 2,4-dihydroxybenzoic acid in methanol.

The chromatogram in Fig. 4 was obtained after optimizing the conditions for separation. The carbon dioxide mobile was density programmed at 100°C from 0.5 g/cm^3 to 0.74 g/cm^3 at 0.015 g/cm^3 per min after an initial 5-min isoconfertic period. The rapid density ramp was necessary to elute progressively more polar acids and to preserve the peak shapes of the last four eluting acids to some extent. These compounds eluted after the final density (*i.e.*, maximum pressure setting of the pump) had been reached and therefore exhibited band broadening in an analagous fashion to the elution of low-volatile compounds after the final temperature of a GC temperature program has been reached. A more polar or modified mobile phase is necessary if further di- and trihydroxylated phenolic acids are to be chromatographed.

The glyme column showed good selectivity for the phenolic acids, although a certain amount of peak tailing occurred, particularly at temperatures above 130°C. Below 80°C, chromatographic efficiency dropped off considerably, which was probably due to the lower rate of solute diffusion between the mobile and stationary phases at high mobile phase densities. An oven temperature of 100°C was therefore used throughout the study.

The results indicate that the glyme phase has good solubility and diffusion characteristics for organic acids. The medium polarity of the phase ensures only moderate retention of polar solutes which is essential for their elution from the column, when a non-polar mobile phase like carbon dioxide is used. Further, through

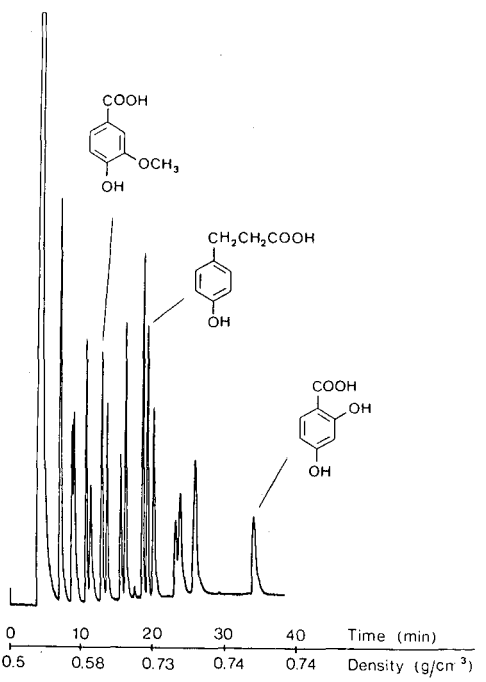

Fig. 4. Capillary SFC separation of phenolic acids. Conditions: 10 m × 50 μm I.D. capillary column, glyme stationary phase (0.25 μm film thickness), CO_2 density programmed from 0.5 g/cm^3 (5 min) to 0.74 g/cm^3 at 0.015 g/cm^3 per min, 100°C, FID at 400°C.

cross-linking and immobilization, the glyme phase is chemically and physically stabilized towards degradation. All of these advantages make the glyme phase an ideal stationary phase for polar isomeric solutes in SFC.

Deposition characteristics of the phenolic acids at the interface were investigated using a five-component mixture containing 4-chlorobenzoic acid, vanillic acid, 3,4-dimethoxycinnamic acid, 4-hydroxyphenylpropionic acid and 2-acetoxynaphth-7-oic acid in methanol. Approximately 100 ng of each component were introduced onto the column so that about 50 ng of each acid was delivered to the interface and also detected by FID (Fig. 5). The acids were all deposited as compact solid spots approximately 200 μm in diameter, from which good-quality IR spectra could be measured. Figs. 6–8 show the IR absorption spectra from spots associated with peaks 2, 4 and 5 in Fig. 5. These spectra are highly characteristic and compare well with those of reference compounds ground into potassium bromide, which indicates that unknown acids deposited at this interface could be positively identified using library-search facilities.

A significant amount of structural information can be obtained from the spectra. For example, phenolic acids have OH stretching absorptions due to both phenolic and carboxylic acid moieties. The phenolic OH stretching absorption is narrow and occurs from 3500–3400 cm^{-1} (3470 cm^{-1} in the case of vanillic acid, Fig. 6), while the OH stretching absorptions of the carboxylic acid are broad due to hydrogen

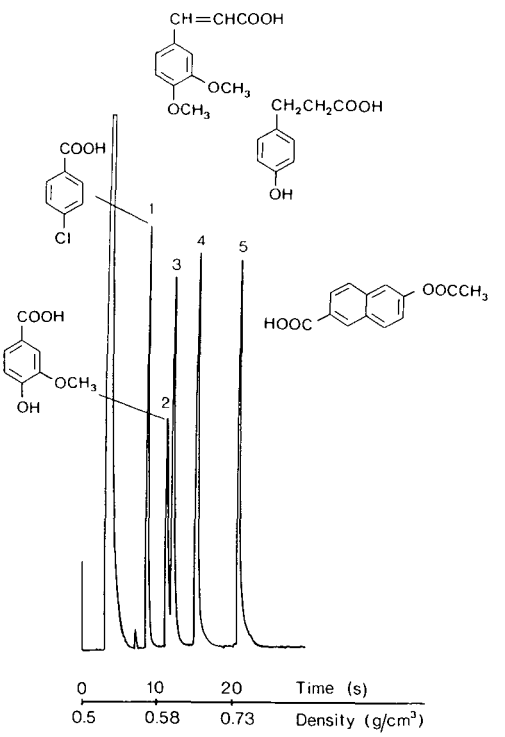

Fig. 5. Separation of 5-component mixture to demonstrate the mobile phase-elimination interface for deposition of phenolic acids. Conditions as in Fig. 4.

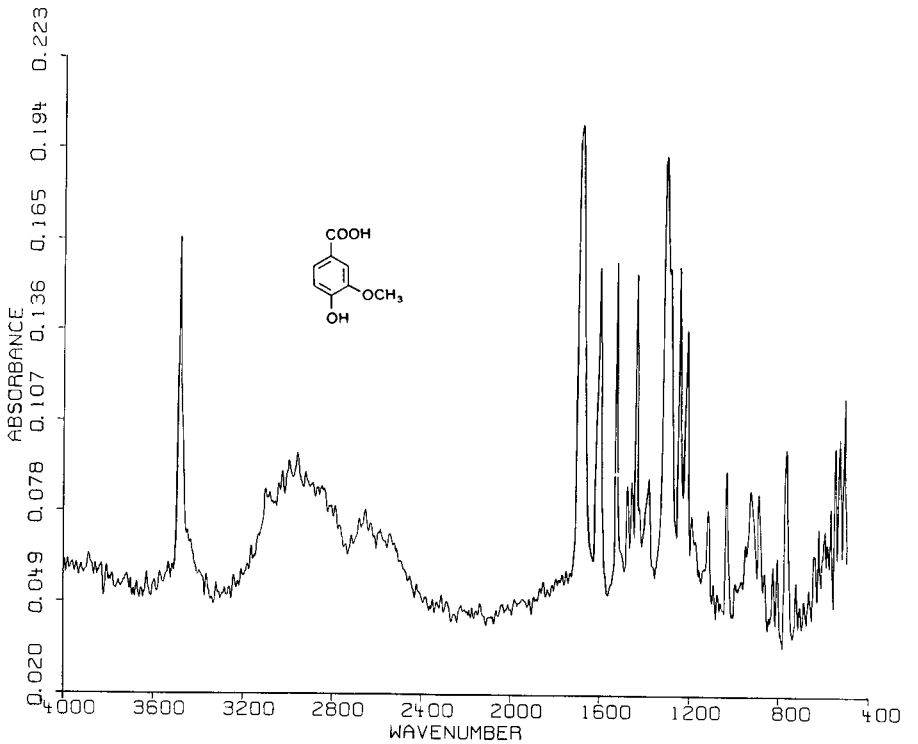

Fig. 6. IR spectrum of vanillic acid (peak 2 in Fig. 5).

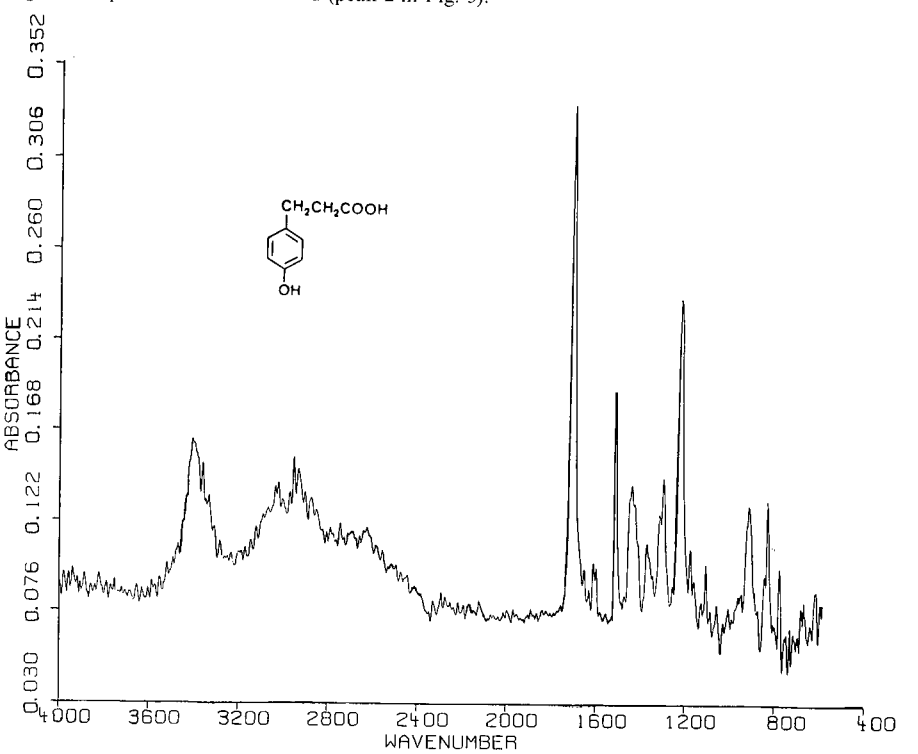

Fig. 7. IR spectrum of 4-hydroxyphenylpropionic acid (peak 4 in Fig. 5).

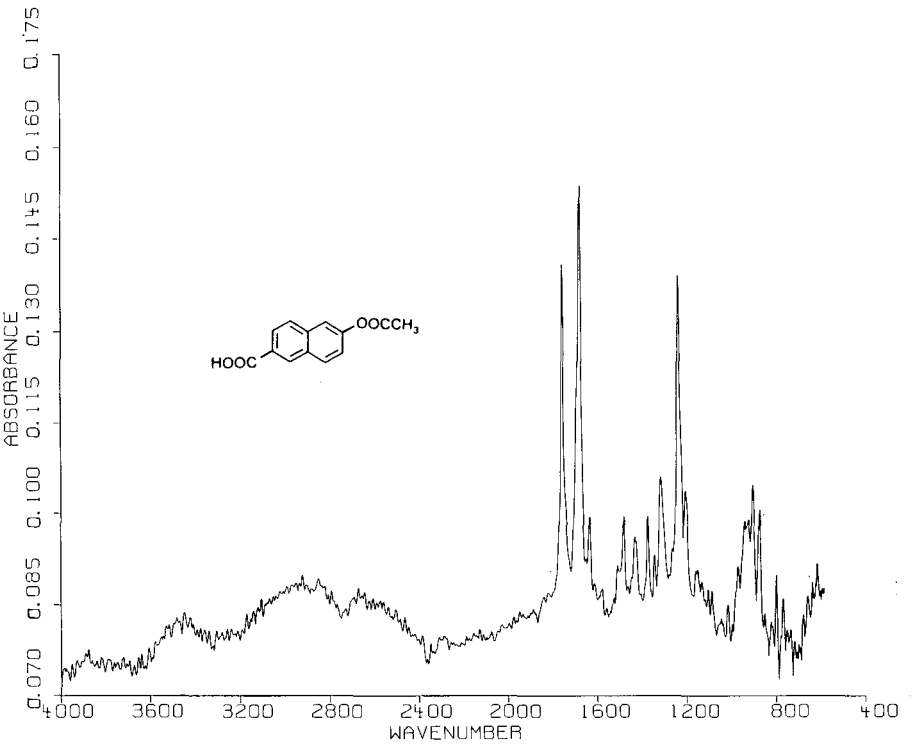

Fig. 8. IR spectrum of 2-acetoxynaphth-7-oic acid (peak 5 in Fig. 5).

bonding and occur from 3400–2500 cm^{-1}. This interaction also results in the acid carbonyl absorption occurring between 1710–1680 cm^{-1} (monomeric acid would be expected to give rise to an absorption near 1750 cm^{-1}). It occurs in the IR spectrum of 4-hydroxyphenylpropionic acid (Fig. 7) at 1701 cm^{-1} and in the IR spectrum of vanillic acid and 2-acetoxynaphth-7-oic acid (Fig. 8) at 1680 cm^{-1}. Acid carbonyl and ester-carbonyl absorptions, which in the case of 2-acetoxynaphth-7-oic acid occur at 1680 cm^{-1} and 1758 cm^{-1}, respectively, are therefore easily distinguished from one another.

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Note

Supercritical fluid chromatography with light-scattering detection

I. Preliminary results of the analysis of polar compounds with packed columns

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Supercritical fluid chromatography (SFC) does not possess a universal detector that compares with the refractive index detector used in high-performance liquid chromatography (HPLC). The flame ionization detector used in capillary SFC precludes the use of polar additives necessary in the analysis of polar products on packed columns.

A new kind of semi-universal detection has recently been developed in HPLC, which has some significant advantages over refractive index detection. This new type of detector is based on scattered light produced by solid or liquid microparticles resulting from the vaporization of effluents. The signal is directly dependent on the analysed solutes when the eluents are totally evaporated. Light-scattering detection (LSD) is compatible with gradient elution and is easily and rapidly operative. We have constructed an LSD instrument based on this principle, and without the limitations inherent in other available apparatus (Applied Chromatography Systems, Luton, U.K.). It is possible to evaporate aqueous–organic[1,2] and aqueous mobile phases[3] with[4] or without addition of volatile salt at low temperatures (40°C) and at high flow-rates (3 ml min^{-1}).

LSD is compatible with SFC as it reveals every solid or liquid solute in suspension in the gas phase resulting from the decompression of the supercritical fluid. It is also compatible with polar additives added to the supercritical fluid. We have recently attempted to develop a specific interface for SFC that can be used directly with the light-scattering HPLC detector[5] and have determined the main factors that influence the detection limit and reproducibility in SFC[6].

This preliminary work emphasizes the advantage of this detection method for non-UV-absorbing or UV-absorbing polar solutes. Three classes of either commercially available or laboratory-made solutes are being studied: O-alkyl glycosides, desulphoglucosinolates and alkylated and non-alkylated polyethylene glycols.

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EXPERIMENTAL

Apparatus

The apparatus used has been described in a previous paper[7]. The following columns were used: 10-μm LiChrospher 100 Diol (125 × 4.6 mm I.D.), 5-μm LiChrosorb CN (150 × 4.6 mm I.D.), both purchased from Merck (Darmstadt, F.R.G.), 7-μm Zorbax CN (150 x 4.6 mm I.D.) purchased from DuPont (Wilmington, DE, U.S.A.), 10-μm μBondapak CN (150 × 3.9 mm I.D.) purchased from Waters Assoc. (Milford, MA, U.S.A.) and 5-μm RSil NO_2 (250 × 4.6 mm I.D.) purchased from RSL (Eke, Belgium).

Chemical and reagents

Carbon dioxide was of B 50 grade (Air Liquide, Paris, France) and methanol of Pestipur grade (SDS, Vitry, France). The solutes (analytical-reagent grade) were dissolved in either methanol–chloroform, pure chloroform or pure methanol, depending of the polarity of the mobile phase. O-Alkyl β-D-glycosides were purchased from Sigma (St. Louis, MO, U.S.A.) and Aldrich (Strasbourg, France), except for octyl β-D-galactopyranoside and octyl β-D-xylopyranoside[8]. Glucosinolates were synthesized in our laboratory[9]. Polyethylene glycols (PEG), ethylene glycol monohexadecyl ether ($C_{16}E_1$), diethylene glycol monohexadecyl ether ($C_{16}E_2$), tetraethylene glycol monohexadecyl ether ($C_{16}E_4$) and hexaethylene glycol monohexadecyl ether ($C_{16}E_6$) were purchased from Fluka (Buchs, Switzerland). 2,16-Di-*n*-octyl-3,6,9,12,15-pentaoxa-1,17-heptadecanediol ($2C_8E_6$) and 2,5,13,16-tetra-*n*-octyl-3,6,9,12,15-pentaoxa-1,17-heptadecanediol ($4C_8E_6$) were synthesized in our laboratory[10].

RESULTS AND DISCUSSION

O-Alkyl glycosides

The analysis of carbohydrates is difficult because of their lack of UV absorbance. However, by means of LSD, an analytical studies of carbohydrates by HPLC or SFC have been carried out with apolar[1,3] and polar[2,7,11,12], stationary phases.

Similar detection difficulties were encountered with O-alkyl glycosides. For these compounds, it is very important to determine the purity or composition of products used such as biological detergents[13], membrane solubilizers or as growth media for RNA and proteins[14]. Different O-alkyl glucopyranosides and maltosides are commercially available. In order to study the hydrophobic and hydrophilic interactions between solutes and stationary phases, we have synthetized other O-alkyl derivatives generated from galactopyranoside and xylopyranoside moieties.

Table I gives the retention times of thirteen compounds on cyano- and diol-bonded silicas. Polar interactions are involved because the retentions are mainly dependent on the sugar moiety. For a given carbohydrate, the corresponding O-alkylated derivatives have lower retentions than the non-derivatized solutes. The solute retention times decrease as the percentage of methanol increases. The percentage of methanol, the pressure of the eluent and the stationary phase directly influence the selectivity. Cyano-bonded silica gave smaller retention times than diol-bonded silica but the selectivity was improved (Table I, octyl β-D-gluco- and -galactopyranosides).

TABLE I

RETENTION TIMES OF GLYCOSIDES AND O-ALKYL GLYCOSIDES ON POLAR PACKED COLUMNS IN SFC AT 40°C

Compound	*Retention time (min)*	
	Column 1[a]	*Column 2*[b]
Octyl-β-D-xylopyranoside	3.6	2.0
Hexyl β-D-glucopyranoside	10.0	3.5
Octyl β-D-glucopyranoside	10.9	3.6
Decyl β-D-glucopyranoside	–	3.8
Dodecyl β-D-glucopyranoside	10.7	3.9
Octyl β-D-galactopyranoside	10.9	4.3
Methyl α,β-D-glucopyranoside	14.8	4.45 and 4.73
Methyl α-D-glucopyranoside	14.8	4.73
Decyl β-D-maltoside	>30	21.2
Dodecyl α,β-D-maltoside	>30	22.5
α,β-D-xylopyranose	–	4.5
α,β-D-galactopyranose	>30	8.0
α-D-glucopyranose	>30	8.7

[a] LiChrospher diol (125 × 4 mm I.D.), CO_2–methanol (93.75:6.25, w/w) 3.9 ml min^{-1}, 145 bar.
[b] Zorbax CN (150 × 4.6 mm I.D.), CO_2–methanol (93.0:7.0, w/w), 3.5 ml min^{-1}, 210 bar.

Fig. 1a confirms the higher selectivity of the Zorbax cyano column compared with the diol column. The chromatogram shows a good separation of α- and β-anomers of methyl D-glucopyranoside and a broad peak with a split top for the α- and β-anomer mixture of glucopyranose.

The number of plates calculated for decyl β-D-maltoside gives a reduced plate height of 6.2 at a linear eluent speed of 3.5 mm s^{-1}. This value if lower than that obtained with non-derivatized glycosides using SFC[7] or HPLC[15].

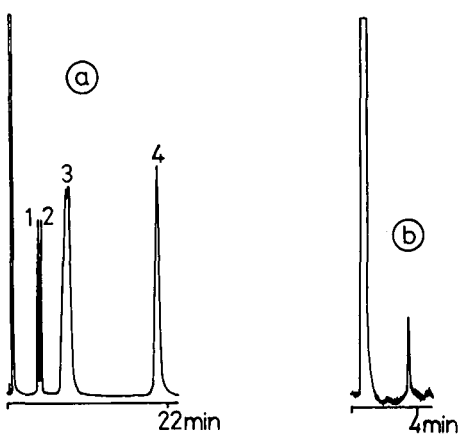

Fig. 1. Chromatograms of glycosides and O-alkyl glycosides on a polar packed column. Column: Zorbax CN (150 × 4.6 mm I.D.). Eluant: CO_2–methanol (93.75:6.25, w/w), 3.9 ml min^{-1}, 145 bar. Solutes: (a) 1 = methyl β-D-glucopyranoside, 2 = methyl α-D-glucopyranoside, 3 = α,β-D-glucopyranose, 4 = decyl β-D-maltoside; (b) octyl β-D-glucopyranoside (amount, 100 ng).

The chromatogram in Fig. 1b illustrates the detection limit, giving a signal-to-noise ratio >10 for an injection of 100 ng (20 μl of a 5 ppm solution of octyl β-D-glucopyranose). Larger sample injection volumes permit the determination of O-alkyl glycosides at levels as low as 1 ppm.

Desulphoglucosinolates

Studies on glucosinolates or desulphoglucosinolates are extremely important as these compounds are found in various plant materials (rape, lupin, etc.) and also cause physiological effects in animals such as inappetence and goitrogenic effects[16]. Stuctures of desulphoglucosinolates are shown in Fig. 2.

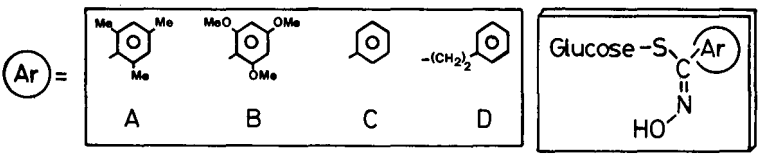

Fig. 2. Structures of desulphoglucosinolates. Me = CH_3.

We have been studying the synthesis, extraction from raw materials and analysis of natural and artificial glucosinolates and desulphoglucosinolates. Preliminary results for desulphoglucosinolates obtained using SFC are reported in Table II. Three desulphoglucosinolates, A, B and C, not yet found in plants and one natural desulphoglusinolate, D (trivial name gluconasturtin), were synthesized[9] (see Fig. 2). UV detection or LSD can be used for the analysis of these compounds.

The retentions of desulphoglucosinolates are generally higher than that of glucopyranose. The selectivities are dependent on the nature of the stationary phase and on the composition of the mobile phase. Nitro-bonded silica is more selective for desulphoglucosinolates, especially B and D. This result corroborates the results obtained with non-derivatized glycosides[7].

The retention times of desulphoglucosinolates A, B and C were lower (A and C) or higher (B) than that of the natural desulphoglucosinolate D. Consequently, A, B

TABLE II

RETENTION TIMES OF DESULPHOGLUCOSINOLATES ON POLAR PACKED COLUMNS IN SFC AT 40°C

Compounds	*Retention time (min)*	
	Column 1[a]	*Column 2*[b]
A	10.7	6.2
B	15.9	12.3
C	14.6	7.4
D	15.7	7.6
D-Glucose	8.1	7.1

[a] LiChrospher diol (125 × 4 mm I.D.), CO_2–methanol (90:10, w/w), 4.1 ml min^{-1}, 180 bar.
[b] RSil NO_2 (250 × 4.6 mm I.D.), CO_2–methanol (89.3:10.7 w/w), 4.6 ml min^{-1}, 245 bar.

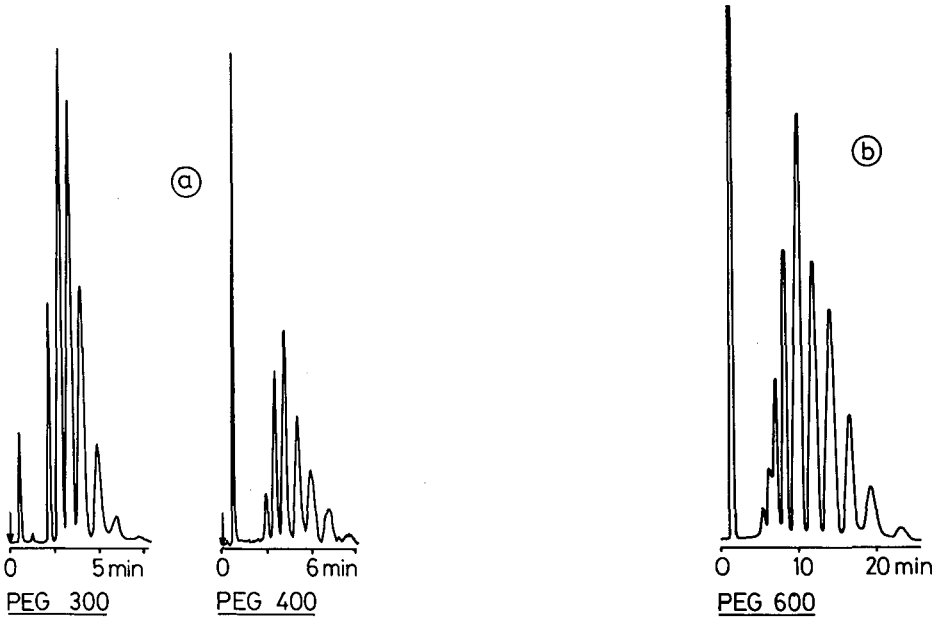

Fig. 3. Chromatograms of polyethylene glycol (PEG) 300, 400 and 600 on polar packed columns. (a) Column: LiChrosper Diol (125 × 4 mm I.D.). Eluent: CO_2–methanol (95.2:4.8, w/w), 3.4 ml min^{-1}, 4000 p.s.i. (b) Column: LiChrospher Diol (125 × 4 mm I.D.). Eluent: CO_2–methanol (90.9:9.1, w/w), 1.8 ml min^{-1}, 2500 p.s.i.

and C can be used as internal standards in glucosinolate analysis as they have never been identified in natural glucosinolate mixtures and therefore could not lead to incorrect quantitative analyses.

One of the major interests in LSD is the possibility of obtaining a mass response within a simple solute class[1,3,17]. This is advantageous for the quantitative analysis of complex glucosinolate mixtures compared with UV detection, which largely depends of alkyl or aryl substituents.

It has been shown that UV detection in SFC, when compared with UV detection in HPLC, provides quantitative results which are less precise[18]. We have observed the same loss of precision in the SFC–LSD[12] analysis of sugars.

TABLE III

RETENTION TIMES (min) OF ALKYLATED POLYETHYLENE GLYCOLS ON A μBONDAPAK CN COLUMN

Column: μBondapak CN (150 × 3.9 mm I.D.), CO_2–methanol (97.1:2.9, w/w), 4.15 ml min^{-1}, 224 bar.

Compound	*Retention time (min)*	*Compound*	*Retention time (min)*
$C_{16}E_1$	0.7	$C_{16}E_6$	7.2
$C_{16}E_2$	0.8	$4C_8E_6$	3.8
$C_{16}E_4$	1.4	$2C_8E_6$	7.1

Straight and derivative polyethylene glycols

Non-ionic detergent such as PEG or alkylated PEG are very used in various fields. Our laboratory is interested in the specific synthesis of non-UV-absorbing derivatives of PEG in order to produce reference compounds usable for the characterization and identification of complex commercially available PEG mixtures. Commerical PEG 300, 400 and 600 have been analysed both by HPLC[19,20] and SFC using capillary columns and with parameter programming (density–pressure[21] and temperature[22]).

The chromatograms in Fig. 3 illustrate some preliminary results obtained on packed columns. They show an isocratic SFC elution using a diol column and a carbon dioxide–methanol mobile phase. From the shape of the chromatogram, it is easy to recognize the oligomer distribution.

Table III illustrates the important role of the polar ethoxy group in determining the retention time. With a constant alkyl chain length (C_{16}) the retention times increase with increase in the ethylene oxide number: $C_{16}E_6 > C_{16}E_4 > C_{16}E_2 > C_{16}E_1$. With a constant number of ethylene oxide groups, the retention times decrease with increase in the number of alkyl chains: $2C_8E_6 > 4C_8E_6$. A more detailed study will attempt to elucidate the different factors (*e.g.*, alkyl chain length and alkyl chain number) involved in the retention of these compounds.

CONCLUSION

SFC of polar compounds using polar packed columns needs a polar modifier to the carbon dioxide supercritical fluid. Examples of separations of three classes of compounds have been shown. LSD afford a universal detection method that is compatible with SFC using a polar modifier, and opens up a new field of potential applications of such analyses.

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Supercritical fluid chromatography–mass spectrometry of non-ionic surfactant materials using chloride-attachment negative ion chemical ionization[a]

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SUMMARY

Capillary supercritical fluid chromatography interfaced with chemical ionization mass spectrometry (SFC–CI-MS) provides high-efficiency separations and identification for characterization of alcohol ethoxylate and other non-ionic surfactant mixtures. When using the capillary direct interface for SFC–CI-MS, however, the role of the chromatographic mobile phase during ionization must be considered. The presence of carbon dioxide or nitrous oxide in the chemical ionization source of a Hewlett-Packard 5985 gas chromatography–mass spectrometry system configured for SFC–CI-MS significantly influences the appearance of positive and negative ion chemical ionization mass spectra. Positive and negative ion mass spectra produced using methane, isobutane or ammonia all display extensive fragmentation and adduct ion formation in the presence of carbon dioxide or nitrous oxide. Surfactant sample fragmentation can be limited or eliminated through the use of difluorodichloromethane (Freon-12) as the reagent gas for chloride-attachment negative ion chemical ionization. Only the chloride-attachment adduct anion is produced for alkoxylated surfactants and free fatty acids, even with a two-fold excess of carbon dioxide present in the ion source.

With CI conditions that permit the production of only molecular species, effects due to restrictor-heater temperature and interface design and operation could be evaluated. A comparison of direct supercritical fluid injection *versus* heated probe inlet introduction for surfactant samples was also possible. Chloride-attachment negative ion CI-MS following capillary SFC may be the preferred system for obtaining molecular weight and telomer distribution information on commercial alcohol alkoxylates and other non-ionic surfactant mixtures.

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INTRODUCTION

Non-ionic surfactants are consumed at a rate of 800 million pounds annually in the U.S.A., 75% of this in household detergent products. Supercritical fluid chromatography (SFC) provides for high-efficiency separations for the analysis of these and related materials [1–3]. The interface of SFC with chemical ionization mass spectrometry (CI-MS) extends the utility of the technique by providing identification information to complement the efficient separation. Information required for non-ionic surfactant characterization includes identification of hydrophobe type, usually an alkyl or alkyl-substituted aryl group, hydrophobe distribution and the type, length and distribution of the hydrophile, usually repeating ethylene or propylene ethers. Characterization using MS is often enhanced by limiting fragmentation of higher-molecular-weight species.

A probe-mounted capillary direct interface for SFC–CI-MS was developed and an initial evaluation of its performance conducted[4]. When the system was further evaluated for applicability to characterization of alkoxylated surfactants, the positive and negative ion CI mass spectra produced were found to be strongly influenced by the presence of the chromatographic mobile phase in the ion source. These results were contrary to results reported for other sample types and SFC–CI-MS systems[5,6]. The influence of the mobile phase on ionization complicated evaluation of the SFC–CI-MS interface and limited application of the system for surfactant characterization.

To determine whether observed effects were sample specific and to understand the role of mobile phase on CI following capillary SFC, conditions in the ion source of the SFC–CI-MS system were studied with selected fatty acid and alkoxylated surfactant mixtures. Various sample introduction methods were employed with positive and negative ion CI performed with and without supercritical fluid solvents present. The influence of carbon dioxide and nitrous oxide on CI of non-ionic surface-active materials was evaluated and conditions defined which limited such influence. Parameters were established which allowed evaluation of interface conditions, sample transfer and system transmission independent of mobile phase. The system was finally employed to obtain average molecular weight and hydrophile distribution information on alkoxylated mixtures that had been extensively studied by other MS methods[7].

EXPERIMENTAL

All work was conducted on a Model 602 SFC system (Lee Scientific, Salt Lake City, UT, U.S.A.) consisting of a computer-controlled syringe pump for delivery of pressurized carbon dioxide or nitrous oxide (AGL, Clifton, NJ, U.S.A.), a Model 7526 helium-actuated HPLC-type injector (Rheodyne, Cotati, CA, U.S.A.) with a 0.5-μl volume internal loop, an oven capable of isothermal or temperature-programmed operation, gas chromatography (GC)-type flame ionization detection (FID) system or a probe-mounted interface for MS[4]. Operation in either pressure or density programming modes was possible. Sample injections were made at ambient temperature and split using the manufacturer-supplied "T". Volumetric split ratio (usually 50:1) was controlled by flow through a length of 10-μm I.D. fused-silica tubing or a similar length of 50-μm I.D. tubing with a polished restriction. A 10 m ×

50 μm I.D. fused-silica open tubular column with a biphenyl–methylpolysiloxane (30:70) stationary phase (SB-Biphenyl-30) was used for chromatography. Direct fluid injection experiments were conducted at 125°C using various lengths of deactivated fused-silica tubing, both uncoated and with stationary phase. Injections were made at 100 bar followed by a 140 bar/min increase to 400 bar, sufficient to separate the analyte from the injection solvent. Lee Scientific porous "frit" restrictors were used for pressure (flow) restriction. Restrictors were connected to the column terminus using a zero-dead-volume union. Restrictor-heater temperatures for the FID system were from 350 to 400°C. For SFC–CI-MS the restrictor terminus was kept flush with the interface probe exit. Mobile phase linear velocity was *ca.* 2 cm/s, volumetric flow-rate was *ca.* 4 μl/s, measured at a carbon dioxide density of 0.2 g/ml. The design and operation of the SFC–CI-MS interface probe has been fully described previously[4]. The interface probe was operated at SFC oven temperature and the restrictor-heater temperature was varied between 200 and 450°C.

MS was performed on a Hewlett-Packard 5985B GC–MS system operated in both positive and negative CI modes. The interface probe was inserted through the interlock used for the tuning or direct insertion probe (DIP). As with the DIP, the SFC–CI-MS interface probe mates with a tapered port on the ion source, ensuring a well confined volume, relative to the GC–electron impact (EI) ionization-MS configuration. The restrictor terminus was within 5 mm of the source volume entrance. Ion source temperature was 200°C, with a starting source pressure (column flow plus reagent gas) of $1.2 \cdot 10^{-4}$ Torr. Final ion source chamber pressure at completion of density-programmed SFC was *ca.* $2.0 \cdot 10^{-4}$ Torr. No effort was made to maintain constant mobile phase–reagent gas ratio. Methane, isobutane, ammonia and Freon-12 of the highest available purity (AGL) were used as CI reagent gases. The mass spectrometer was controlled and data were collected on a Teknivent (St. Louis, MO, U.S.A.) Vector/One MS data system, requiring manual tuning and calibration. The system was always operated at unit mass resolution to *m/z* 614 (perfluorotributylamine).

All samples were used in solution in dichloromethane (Optima grade, Fisher Scientific, Fair Lawn, NJ, U.S.A.) at concentrations of 1–5 mg/ml for single compounds or simple mixtures or 2% (w/w) for complex mixtures. Non-ionic alcohol ethoxylate samples were obtained from numerous sources at Unilever Research. "OXYPRUF" alkoxylated pyrazoles (Olin Chemical) were obtained from Dr. Kenneth L. Busch. Homogeneous dodecanol ethoxylate samples were purchased from Nikko Chemical (Tokyo, Japan). Fatty acid mixtures were obtained from commercial sources or from the Chem Services Surfactant Chemicals Kit (Chem Services, West Chester, PA, U.S.A.).

RESULTS AND DISCUSSION

The applicability of the SFC–CI-MS interface for characterization of surfactant materials was first tested by comparing the results produced using FID with those produced using CI-MS detection. Fig. 1 shows the SFC–FID chromatogram and the SFC–CI-MS reconstructed total ion chromatogram (ammonia CI from *m/z* 150 to 1000) obtained for a poly(ethylene glycol) methyl ether sample. Comparisons between FID and MS detection for these and related poly(ethylene glycol) (PEG) and

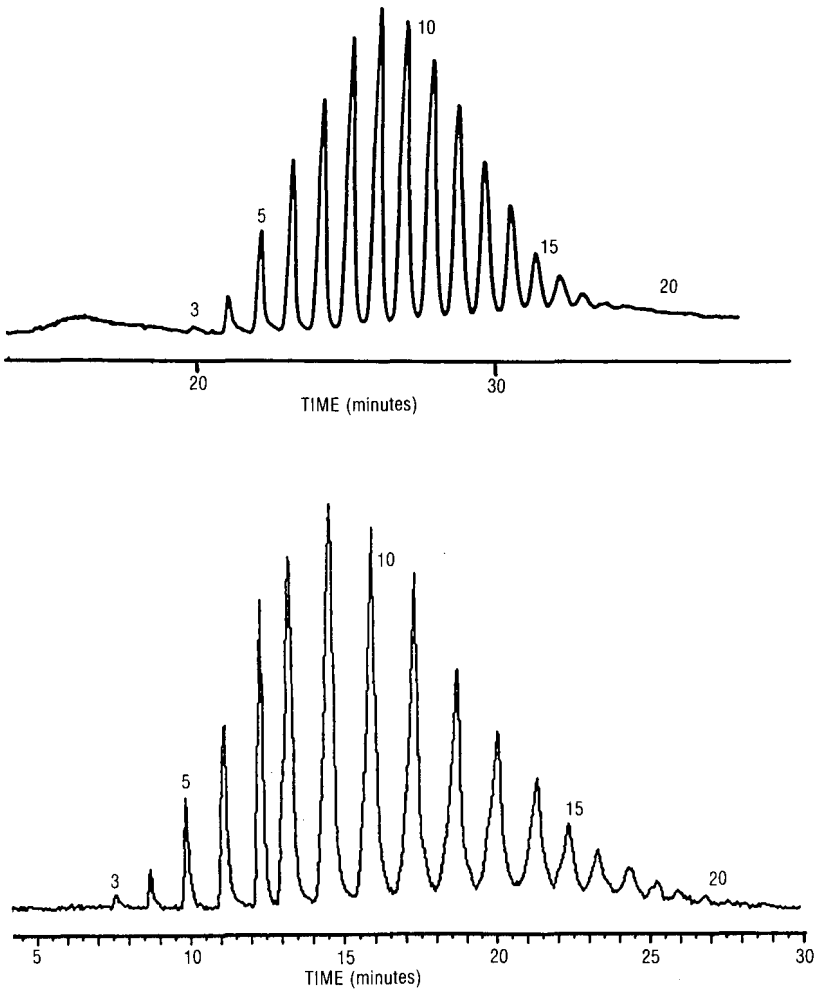


Fig. 1. Capillary supercritical fluid chromatographic separations of a poly(ethylene glycol) methyl ether sample (average molecular weight 550) using flame ionization (top) and ammonia positive ion CI-MS (bottom) for detection. Separations were conducted at 125°C, isothermal, using carbon dioxide as the mobile phase. A two-step density program, from 0.2 g/ml to 0.52 g/ml at 0.033 g/ml/min followed by a 0.009 g/ml/min increase to 0.69 g/ml was used to obtain separation. Numbers indicate moles of ethylene oxide per molecule.

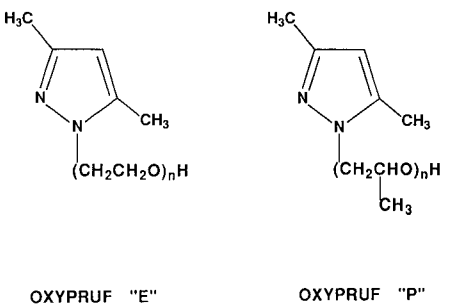
poly(propylene glycol) (PPG) samples all displayed comparable separations with baseline resolution and narrow peak widths and efficient transfer and detection of higher molecular weight species. Samples were chosen to fall within the mass range of the HP5985 quadrupole mass spectrometer, 1000 dalton. In all cases, conditions developed for separation with FID could be directly transferred to SFC–CI-MS[4]. One difference found in the use of SFC–CI-MS was shorter dead times and shorter retention times for all analytes when the same restrictor was used in both SFC–FID and SFC–CI-MS. This increase in mobile phase velocity, due in part to expansion

into the CI ion source vacuum, was not found to adversely affect the separations of PEG, PPG and PEG methyl ether samples.

Ammonia positive ion mass spectra of PEG methyl ether telomers were characterized by ammonium adduct ions $(M + NH_4)^+$ as the base peaks and minor contributions from fragment ions. Isobutane positive ion mass spectra displayed protonated molecules $(M + H)^+$ as the base peaks and limited amounts of fragment ions. These spectra correspond to the type of CI spectra for alcohol ethoxylates reported by Rudewicz and Munson[8]. Fragmentation, to aid in structural characterization of unknowns, could be increased by using methane or argon as the reagent gas[4].

Mobile phase effects

Alkoxylated pyrazoles, manufactured by Olin Chemical under the OXYPRUF tradename, provided a more rigorous test of the SFC–CI-MS interface. Ethoxylated (OXYPRUF-E) and propoxylated (OXYPRUF-P) dimethyl pyrazoles are highly viscous liquids with vapor pressure low enough to allow for direct introduction into the source of a mass spectrometer. They are stable and of relatively low mass, amenable to many forms of sample introduction and mass analysis. For these reasons the OXYPRUFS were used to develop a comprehensive comparison of various desorption ionization techniques[7,9].



OXYPRUF "E" OXYPRUF "P"

Fig. 2 shows the carbon dioxide capillary SFC separation of OXYPRUF E and P mixtures using FID. These alkoxylated antioxidants were found to be readily amenable to analysis using capillary SFC and of sufficiently low volatility to test the SFC–CI-MS interface. The ability to compare SFC–CI-MS results with mass spectral results using desorption ionization techniques permitted evaluation of possible mass discrimination effects. The number-(M_n) and weight-(M_w) average molecular weights for these samples obtained from SFC–FID and desorption MS data are summarized in Table I. The results from SFC–FID characterization agreed quite well with data from gel permeation chromatography (GPC) (within about 5%), used in the study of desorption ionization as the non-MS "standard"[7].

Unlike results with PEG and PPG, SFC–CI-MS of alkoxylated pyrazole samples did not correspond to SFC–FID results. Fig. 3 shows averaged mass spectra, positive ion ammonia CI, obtained for OXYPRUF P and E following direct supercritical fluid injection (no chromatography). Direct supercritical fluid injection was employed in an effort to minimize differences in source conditions over the course of a chromatographic separation[10]. All of the OXYPRUF mixture components were ionized under the same interface and CI source conditions (temperatures, pressures,

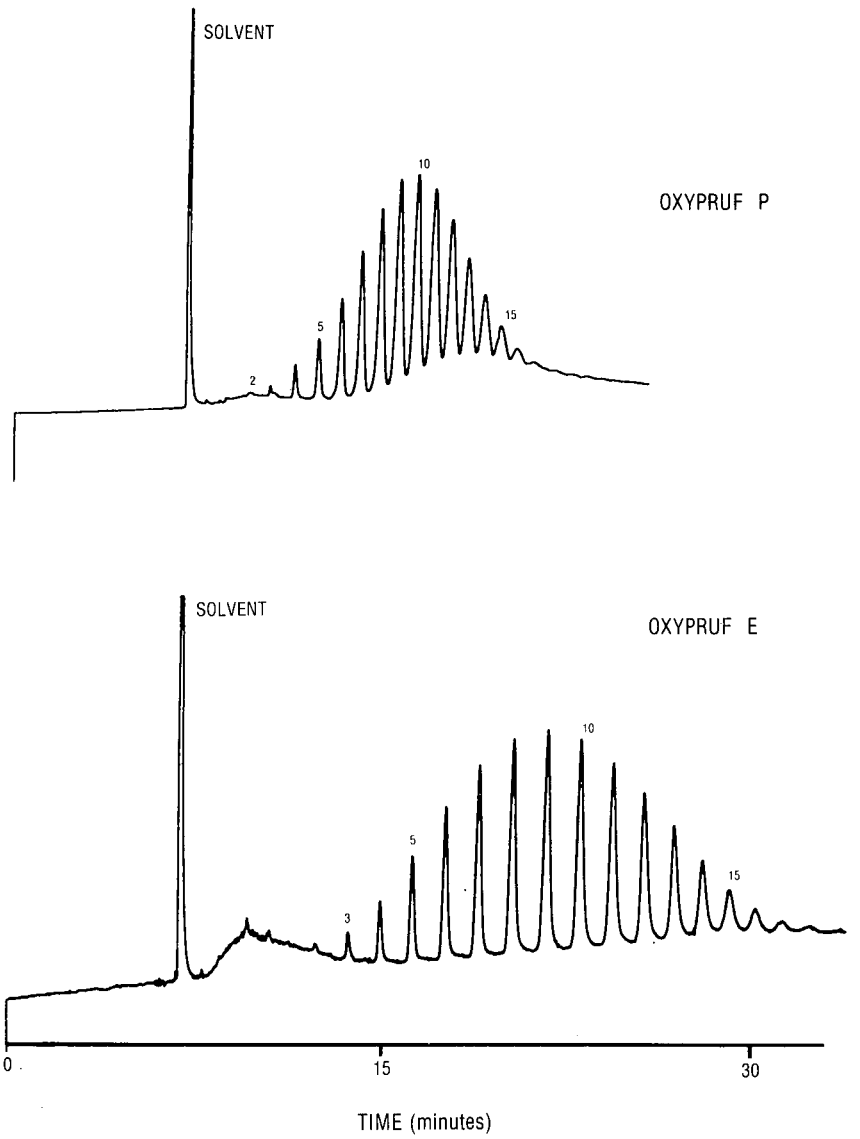


Fig. 2. Capillary supercritical fluid chromatographic separations of propoxylated (OXYPRUF "P", top) and ethoxylated (OXYPRUF "E", bottom) dimethylpyrazole obtained using FID. Carbon dioxide was used as the mobile phase with the same chromatographic conditions as described for Fig. 1 used for both samples. Restrictor-heater temperature was 375°C. Numbers indicate moles of ethylene or propylene oxide per molecule (n).

mobile phase flow-rate and reagent gas composition). Although protonated molecules are the most abundant ions in the spectra, abundance of fragment ions is greater than was seen for PEG or PPG samples and the average molecular weights and telomer distributions do not correspond with the SFC–FID data (Table I). The greater abundance of unresolved material present in OXYPRUF "E" (Fig. 2, bottom) may

TABLE I

NUMBER-AVERAGE (M_n) AND WEIGHT-AVERAGE (M_w) MOLECULAR WEIGHTS OF ALKOXYLATED PYRAZOLE MIXTURES OBTAINED FROM CHROMATOGRAPHIC AND MASS SPECTROMETRIC DATA

GPC and desorption ionization mass spectral data from ref. 7. FAB = fast atom bombardment; NICI = negative ion chemical ionization; PICI = positive ion chemical ionization; SF-DFI = direct supercritical fluid introduction; SIMS = secondary-ion mass spectrometry. For other abbreviations, see text.

Method	*OXYPRUF "E"*		*OXYPRUF "P"*	
	M_w	M_n	M_w	M_n
SFC–FID	582	556	747	710
SF-DFI				
NH_3 PICI-MS	420	390	506	473
Cl^- NICI-MS	545	535	617	585
FAB	512	471	603	549
SIMS	538	501	642	601
Electrospray	587	562	665	630
EI	375	360	423	390
GPC	617	570	735	681

account for part of the apparent difference in the amount of fragmentation in the mass spectra in Fig. 3. Some physical changes have been noted in the source stocks of this sample and further MS studies of this material are currently in progress[11]. These results were also found when the samples were analyzed using complete chromatographic separations[12]. These spectra correspond to the type described by Pinkston *et al.*[13] obtained in SFC–CI-MS. Attempts to minimize production of fragment ions through control of restrictor-heater temperature or use of another mobile phase (nitrous oxide) were unsuccessful. Influence of ion source temperature and role of mobile phase modifiers are currently under study.

One drawback of the OXYPRUF samples was the inability to analyze isolated telomers under a variety of conditions. Mass spectra obtained as single telomers were eluting from the SFC column indicated lower-molecular-weight components were transferred and ionized intact, as evidenced by production of only protonated molecules. Higher-molecular-weight species were found to produce less abundant protonated molecules and extensive fragmentation[12]. The same behavior was found for ethoxylated *n*-alcohol surfactants, although not to the same extent. Homogeneous alcohol ethoxylates were available and used to study the effect of various source and introduction conditions and reagent gases and ion source gas mixtures.

Ethoxylated dodecanols, containing an ethoxylated chain from one to ten units long, were analyzed using both direct surpercritical fluid injection and heated DIP introduction. DIP introduction allowed evaluation of the effect of thermal decomposition on the samples. Positive and negative ion CI mass spectra were obtained using a variety of reagent gases and source gas pressure combinations. Complete details of the study of alcohol ethoxylate surfactants by SFC–CI-MS are described elsewhere[14]. Briefly, results of the study indicated samples up to the dodecanol decaethoxylate species ($C_{12}H_{25}[OCH_2CH_2]_{10}OH$) produced predominantly protonated molecules or ammonium adduct ions when introduced from the heated DIP (*ca.* 280°C) using

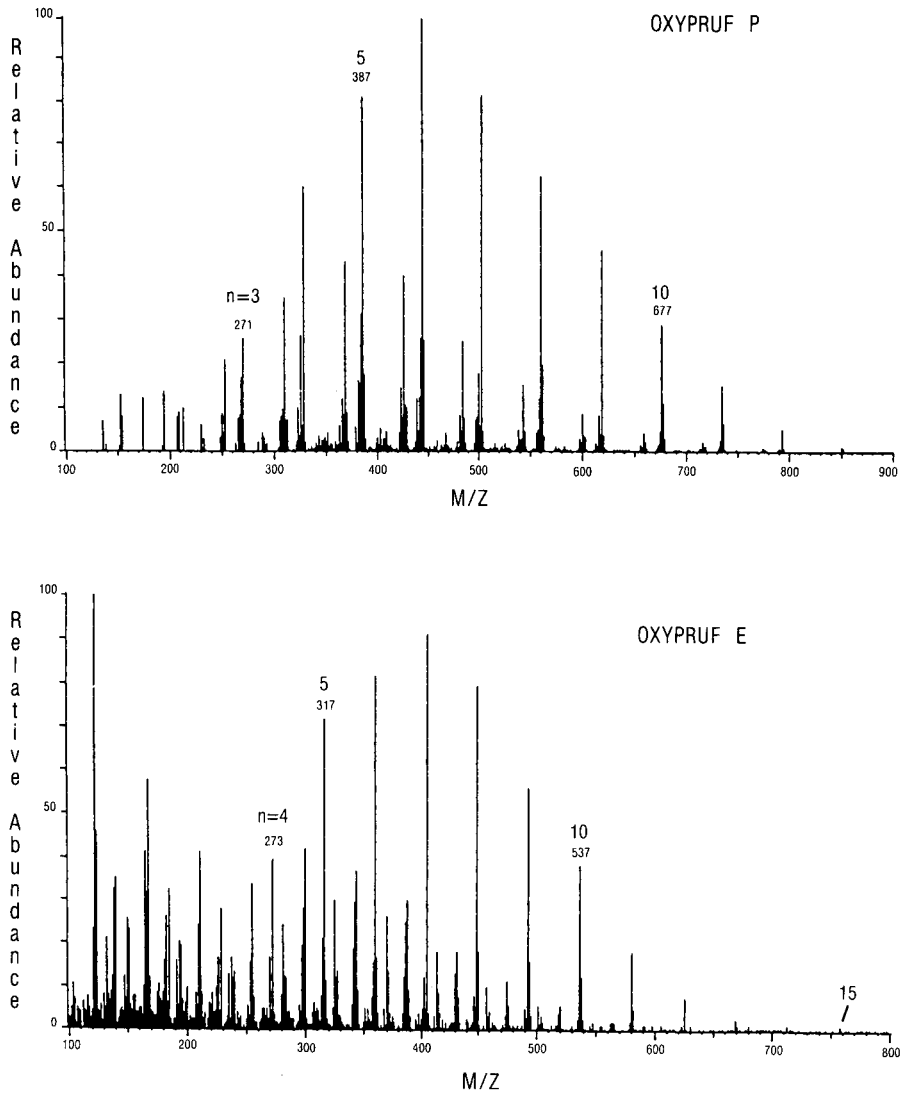


Fig. 3. Positive ion ammonia chemical ionization mass spectra of OXYPRUF "P" and OXYPRUF "E" mixtures obtained following direct supercritical carbon dioxide injection. Injection and interface conditions, described in the Experimental section, were similar for both samples. Restrictor-heater temperature was 385°C.

isobutane or ammonia as the reagent gas, up to a source pressure of $2 \cdot 10^{-4}$ Torr. Thermal decomposition did not contribute to these mass spectra. When carbon dioxide or nitrous oxide was introduced along with CI reagent gas, the relative abundance of fragment ions increased. The results for dodecanol–pentaethoxylate ionized using isobutane in the presence of carbon dioxide are summarized in Table II. An increase in the relative concentration of carbon dioxide or nitrous oxide resulted in an increase

TABLE II

RELATIVE ABUNDANCE OF THE *m/z* 133 FRAGMENT ION FROM THE PROTONATED MOLECULE OF PENTAETHYLENEGLYCOL DODECYL ETHER (*m/z* 407) OBTAINED AT VARIOUS ION SOURCE GAS COMPOSITIONS

Isobutane pressure (Torr)	*Carbon dioxide pressure (Torr)*	*% Relative abundance m/z 133 ion*[a]
$1.0 \cdot 10^{-4}$	0	17.5
$1.0 \cdot 10^{-4}$	$0.5 \cdot 10^{-4}$	19.2
$1.0 \cdot 10^{-4}$	$1.0 \cdot 10^{-4}$	27.1
$0.5 \cdot 10^{-4}$	$0.5 \cdot 10^{-4}$	39.8
$0.5 \cdot 10^{-4}$	$1.0 \cdot 10^{-4}$	34.1

[a] Protonated molecule, *m/z* 407, was the base peak in all cases.

in the abundance of fragment ions. Only one fragment ion, *m/z* 133, is listed but similar changes were found for many fragment ions. The relative abundance of the molecular ion (M^+) did not appear to change but the role of charge exchange with carbon dioxide could not be discounted from these data.

Chlorinated hydrocarbons and chlorofluorocarbons as reagent gases for CI have been employed in the analysis of polysaccharides[15]. The mild chloride-attachment negative ionization was tried with ethoxylated alcohols and found to yield only chlorine adduct ions $(M + Cl)^-$ even with a two-fold excess of carbon dioxide present[12,14]. The negative ion CI mass spectrum (averaged) of a mixture of OXYPRUF "P" and PPG produced using dichlorodifluoromethane following supercritical carbon dioxide direct fluid injection is shown in Fig. 4. The mass spectrum is composed primarily of $(M + Cl)^-$ adduct ions for the OXYPRUF and PPG samples. The expected chlorine isotope abundance is found in all cases for the OXYPRUF species. The chlorine isotope ratios for the PPG species are shifted due to water loss ions from OXYPRUF materials. This effect is more noticeable for the higher *m/z* value ions. The calculated average molecular weights (Table I) compare quite favorably with the FAB, SIMS and electrospray ionization data. Production of water loss ions and possible discrimination against higher molecular weight materials might account for the discrepancy between SFC–CI-MS (chloride attachment) and SFC–FID data. Results with OXYPRUF "E" were similar (Table I) and results from propoxylated hydrazines (OXYPRUF "6") also agreed well with other methods[7,9].

Interface parameter effects

With conditions that produce solely molecular species, the role of various interface and source parameters could be studied. One variable studied was the direction of CI reagent gas introduction. The present design of the SFC–CI-MS interface permits CI reagent gas introduction coaxially along the SFC pressure restrictor. Alternatively, reagent gas is introduced through the GC–MS interface, the conditions used for capillary GC–CI-MS on this system. Coaxial CI reagent gas introduction was found to permit more stable temperature control of the SFC–CI-MS restrictor heater but no measurable change in the mass spectra produced. Positive and negative ion CI mass spectra produced were of slightly greater intensity, but no statistically

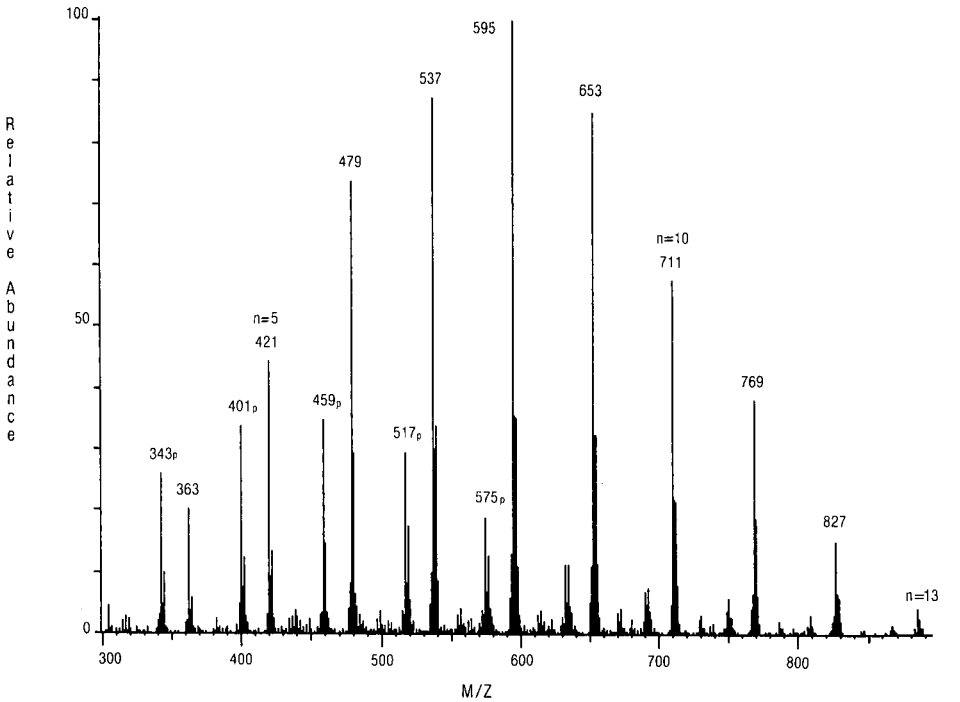


Fig. 4. Negative ion dichlorodifluoromethane (Freon-12) chemical ionization mass spectrum of a mixture of OXYPRUF "P" and poly(propylene glycol) obtained following supercritical carbon dioxide direct injection. Poly(propylene glycol) chloride adduct ions are marked with a "P". Injection and interface conditions were similar to those used for positive ion ammonia chemical ionization.

significant change has been found. There was no change in the ions produced in the mass spectra.

The effects of restrictor-heater temperature were probed using underivatized ("free") fatty acids. In preliminary evaluation[4] and analysis of OXYPRUF materials, restrictor-heater temperature was found to have little effect. Temperatures determined as applicable for SFC–FID were directly transferred to SFC–CI-MS. It was not apparent whether restrictor-heater temperature strongly influenced production of water loss ions for the OXYPRUF samples. Results with free fatty acids were noticeably different.

Fig. 5 shows the reconstructed total ion chromatogram for the SFC–NICI-MS analysis of a mixture of fatty acids from a marine fish oil (521, Chem Services). Separation was achieved using a linear 140-bar/min pressure program on the 10 m × 50 μm biphenyl phase column. Advantages of fast pressure program rates for rapid separations on small bore fused-silica columns have been clearly described[16,17]. The separation shown in Fig. 5 was conducted at a restrictor-heater temperature of 250°C. The chloride attachment negative ion mass spectra of the C_{18} and C_{22} acids from the mixture are shown in Fig. 6. The mass spectra are characterized by the production of the chloride adduct ion, $(M + Cl)^-$. The ion at m/z 291 in the C_{18} mass spectrum is from the "tail" of the C_{16} acid chromatographic peak about 12 s earlier, and the m/z

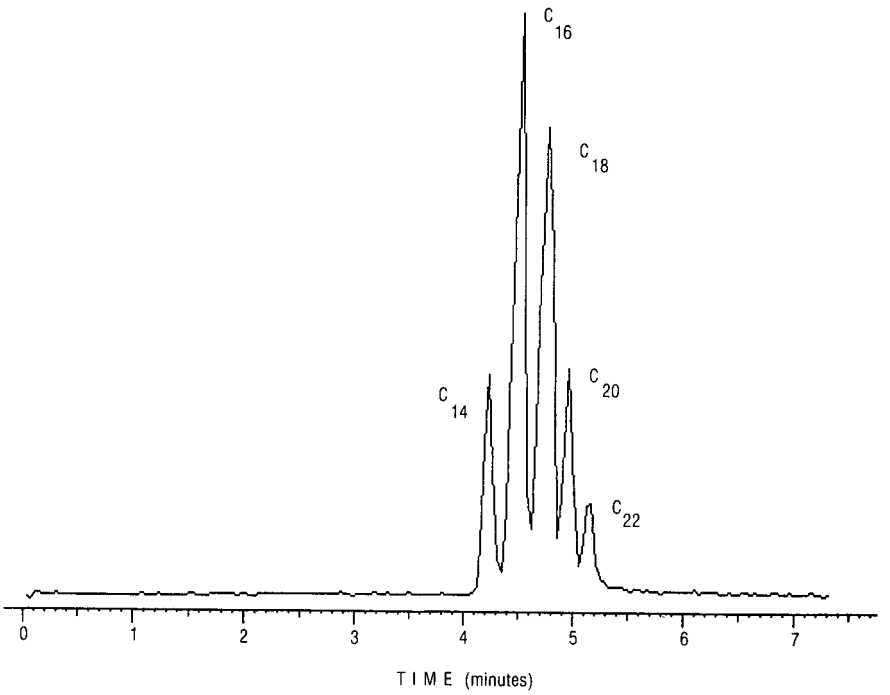


Fig. 5. Reconstructed total ion chromatogram of the rapid capillary carbon dioxide supercritical fluid separation of a hydrogenated marine fish oil fatty acid mixture produced using dichlorodifluoromethane negative chemical ionization mass spectrometry detection. A linear 140-bar/min pressure program was used to produce this separation at 125°C, isothermal, on a 10 m × 50 μm SB-Biphenyl-30 open tubular column. Full scan (100–400 dalton) mass spectra were obtained at 0.7 s/scan.

353 ion corresponds to an $(M - H + Cl_2)^-$ species. The m/z 155 ion in the C_{22} spectrum is a cluster ion due to the reagent gas. When produced at the restrictor-heater temperature used for SFC–FID, 333°C, the chloride-attachment mass spectra in Fig. 7 are obtained. These spectra show extensive fragmentation and the formation of ions which correspond in mass to unusual adduct ions, $(M + CH_2Cl)^-$ and $(M + C_2H_4Cl)^-$. As analytes are chromatographically separated from higher homologues, these ions could not be produced from coeluting species (Figs. 5 and 6). Further, there are no C_{19} fatty acids present in the fish oil sample that could yield the m/z 333 ion. The adduct ions may be formed from reaction of the thermally degraded materials with intact molecules. What is not clear is where in the system thermal decomposition may be occurring. It may be that analyte molecules contact hot surfaces in the interface restrictor heater and decompose (pyrolyze) prior to introduction to the CI ion source[18]. This type of pyrolysis–SFC–CI-MS interface has been proposed as a means of extending SFC–CI-MS to very involatile materials[5]. It is also possible that reactions are occurring on or near ion source surfaces in close proximity to the interface probe[19].

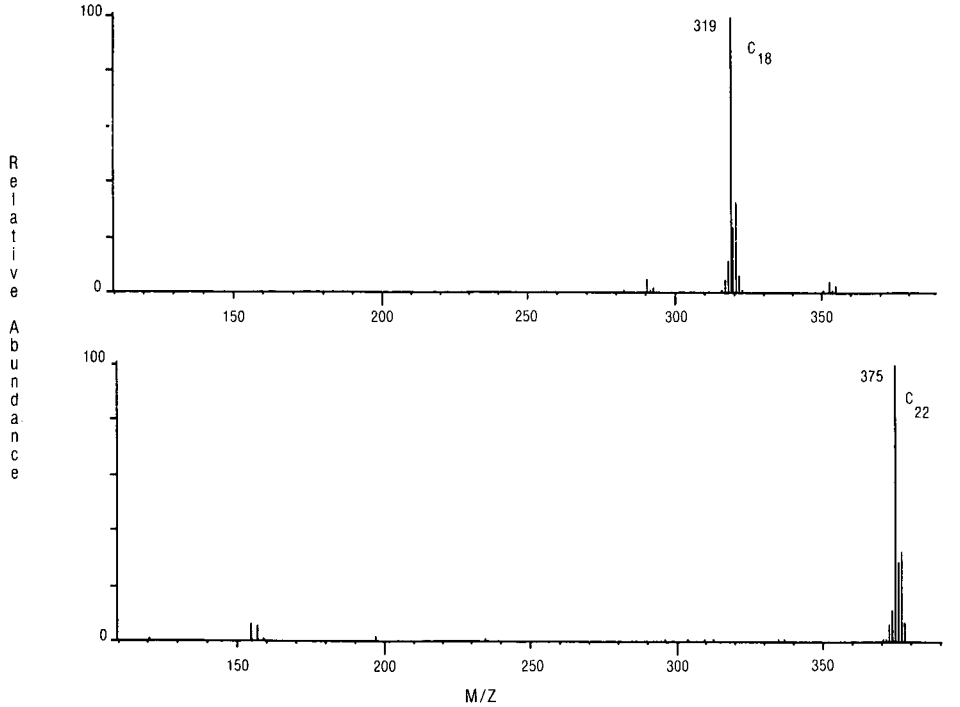


Fig. 6. Dichlorodifluoromethane negative ion chemical ionization mass spectra of the C_{18} and C_{22} saturated fatty acids from the marine fish oil mixture separated in Fig. 5. These mass spectra were obtained at an ion source temperature of 200°C and a restrictor-heater temperature of 250°C.

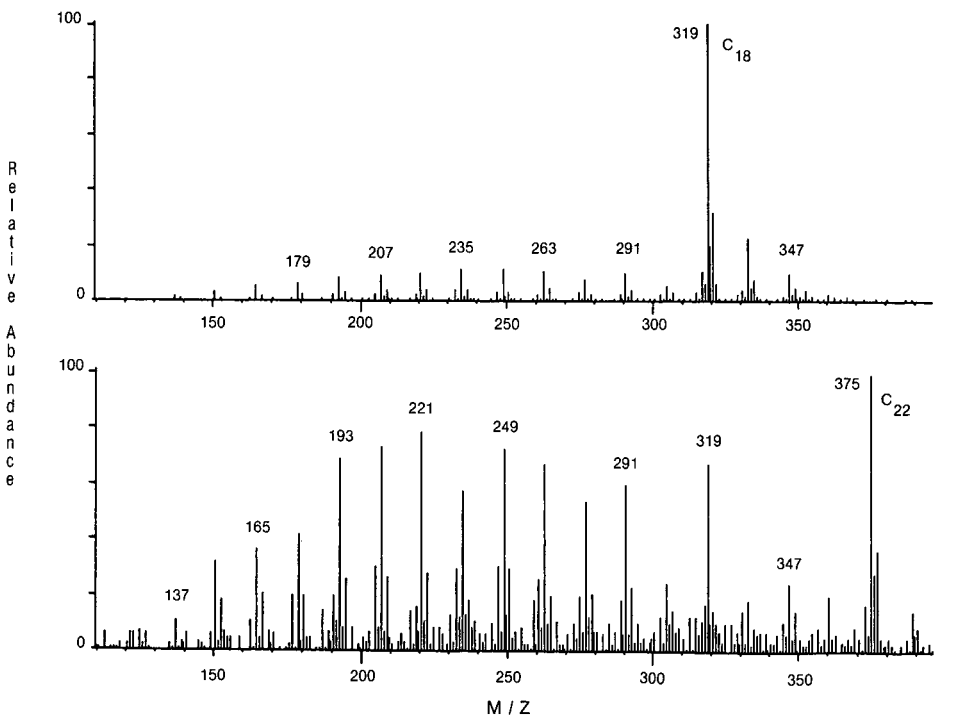


Fig. 7. Dichlorodifluoromethane negative ion chemical ionization mass spectra of the C_{18} and C_{22} fatty acids from the fish oil mixture. Supercritical fluid chromatographic conditions were similar to those used for Fig. 6, with the restrictor heater operated at 333°C.

Application to surfactant characterization

The free fatty composition of a soap bar will influence the physical properties and performance of the product. The analysis of free fatty acids from soap bars is currently performed by GC of methyl esters (FAMEs) derived from the soap bar acids. This widely accepted technique provides a suitable standard against which SFC–NICI-MS methods can be measured. Fig. 8 shows the averaged chloride-attachment negative ion CI mass spectrum of a fatty acid mixture from a soap bar separated using fast capillary SFC. The average mass spectrum presents data on all fatty acids present in the most concise form. Unlike FAME analysis by GC, there was no need to derivatize the acids prior to SFC–CI-MS analysis. Chloride adduct ions were found for the C_6 through C_{18} even carbon number normal saturated fatty acids, the C_{15} and C_{17} saturated fatty acids and C_{16} and C_{18} fatty acids with one and two unsaturation sites ($C_{16:1}$, $C_{18:1}$ and $C_{18:2}$). The composition of this mixture, as determined from SFC–NICI-MS data, is given in Table III. The data indicate that this acid composition was prepared from a mixture of coconut oil and tallow fatty acids, a common combination in commercial soap bars. Also given Table III are weight percentages for this same sample as determined by the GC–FID method (FAMEs). The results from SFC–NICI-MS correspond quite well with GC data, confirming the applicability of the SFC method for analysis of surfactant materials. Some of the discrepancies between data sets may result from the need to deconvolute isotope signals for unsat-

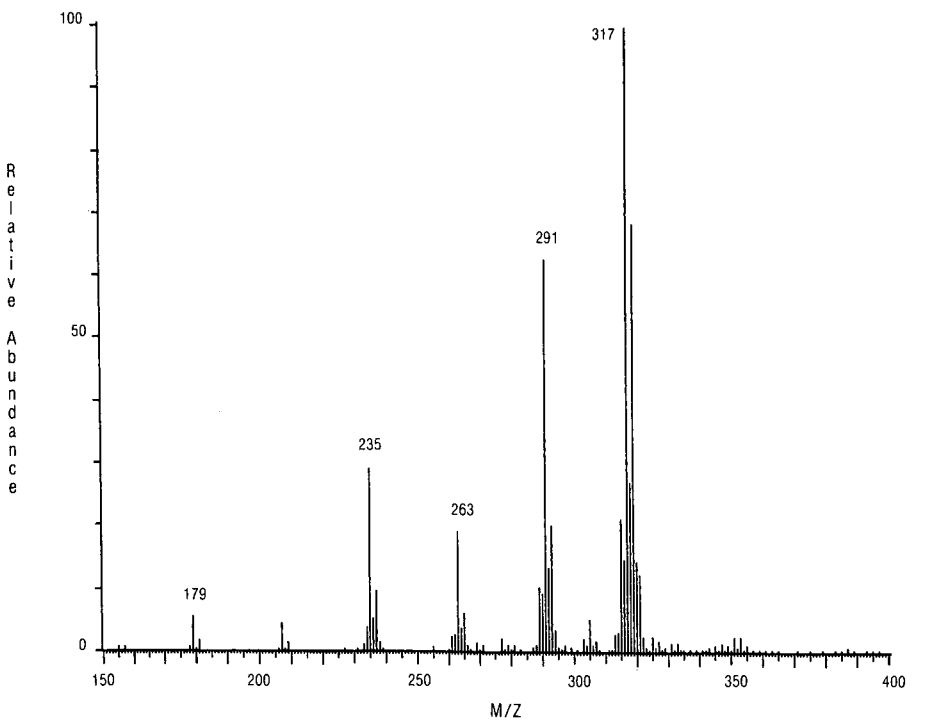


Fig. 8. Averaged dichlorodifluoromethane negative ion chemical ionization mass spectrum of a fatty acid mixture, derived from a soap bar, obtained following capillary supercritical fluid chromatography using a carbon dioxide mobile phase and the conditions described for Fig. 5.

TABLE III

COMPOSITION OF FATTY ACID MIXTURE FROM SOAP BAR OBTAINED FROM GC AND SFC–NICI-MS DATA

Fatty acid, carbon number:degree of unsaturation	*Composition (%, w/w)*	
	GC–FID(FAMEs)	*SFC–NICI-MS*
8:0	0.6	1.1
10:0	0.9	1.1
12:0	7.8	7.5
14:0	5.9	5.6
15:0	0.4	0.8
16:1	3.6	3.5
16:0	21.6	21.2
17:0	1.2	2.0
18:2	3.6	8.0
18:1	35.6	35.0
18:0	16.4	14.4

urated fatty acids ($C_{18:0}$, $C_{18:1}$ and $C_{18:2}$) where the ^{37}Cl isotope of the unsaturated species and the ^{35}Cl isotope of the saturate species are isobaric. There may also be some loss of the more volatile C_6 and C_8 acids during sample pretreatment and derivatization for GC(FAMEs) analysis. Efforts are in progress to evaluate other fluorocarbon reagent gases for negative CI to produce monoisotopic adduct ions, $(M + F)^-$, for analysis of mixtures of saturated and unsaturated materials.

CONCLUSIONS

Presence of the mobile phase gas in the CI source of a system configured for SFC–CI-MS can strongly influence the appearance of the positive and negative ion CI mass spectra obtained. This condition can be used to produce desirable results, such as library-searchable "EI-like" carbon dioxide charge exchange mass spectra[4] or using the mobile phase as a buffer gas for electron-capture negative CI[20,21]. The materials used as mobile phase modifiers can also be used to such advantages[5,14]. For evaluation of an SFC–CI-MS interface and for analyses that require optimum production of parent species, the ion source and reagent gas mixture conditions must be properly chosen. The use of chlorofluorocarbons as negative ion CI reagent gases was found to yield solely chloride adduct ions for surfactant materials containing at least one hydroxyl function. This type of ionization was not influenced by the presence of mobile phase gases, even when the mobile phase was present in large excess. Production of only parent species following capillary SFC allowed the study of alkoxylated surfactants, free fatty acids and SFC–CI-MS interface conditions affecting analyses of such materials. The SFC–NICI-MS method compares well with other MS techniques for analysis of surfactant materials and GC techniques for fatty acid analyses.

Still to be studied is the effect of ion source temperature on the production of CI mass spectra of materials transported using supercritical fluids. In progress is an evaluation of the effect of mobile phase modifiers on CI mass spectra following

capillary SFC. Concurrently, the role of restrictor type, frit, integral or tapered, on the effects noted here is under investigation. Finally, the ability to fully utilize the deliberate pyrolysis of very low volatility analytes in SFC–CI-MS requires further study.

ACKNOWLEDGEMENTS

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Ion mobility detection of polydimethylsilicone oligomers following supercritical fluid chromatographic separation

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SUMMARY

A mixture of polydimethylsilicones (Dow Corning 200), average molecular weight 2000 a.m.u., was separated by simultaneous density and temperature-programmed supercritical fluid chromatography and detected by ion mobility detection. Ion mobility spectra were captured by Fourier transform ion mobility spectrometry. Using information from these spectra it was possible to selectively detect a single compound in the complex mixture. A detector temperature investigation demonstrated that, for the efficient transfer of high-molecular-weight compounds from the column to the detector, the interface to the detector must be heated. Using a 50 μm I.D. column, a Guthrie-type restrictor and a detection temperature of 250°C, as many as 70 oligomers were separated and detected.

INTRODUCTION

The potential for developing a detector for supercritical fluid chromatography (SFC) based on the principles of ion mobility spectrometry (IMS) has been previously demonstrated[1–4]. Advantages of the ion mobility detection (IMD) system for SFC include: (1) sensitivity to a wide variety of compounds, such as those that do not contain a large molar absorptivity, (2) selectivity based on size and shape rather than hetero-atom content, (3) compatability with many flame-ionizable supercritical mobile phases and (4) ruggedness of detector due to ambient pressure operation.

In one study[1], the SFC separation of Triton X-100 surfactant demonstrated successful detection of the polymer in several of the IMD system's operational modes. More importantly, drift spectra were captured for individual oligomers, which provided qualitative information about the size of each oligomer. Selective detection was demonstrated for several single oligomers in the mixture using drift time data. The highest-molecular-weight compound detected was a Triton X oligomer of *ca.* 1000 a.m.u.

In early attempts to interface IMD to SFC, a decrease in SFC resolution was encountered. This was most likely due to inefficient transfer of the analytes from the chromatograph to the detector resulting from operation of the IMD system at a

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relatively low temperature and a long viscous restrictor serving as the interface between SFC and IMD. The importance of temperature in the transfer of solutes from a supercritical fluid has been well documented[5,6]. It has also been found that a long viscous restrictor does not transfer solutes out of the supercritical fluid as well as either a tapered restrictor or a frit restrictor[7].

In this work the effect of the IMD temperature on the separation of high-molecular-weight polymers was studied. Ion mobility spectra were collected for individual oligomers of the polymer mixture and, using the information from the ion mobility spectra, selective detection of a contaminant in the polymer was demonstrated.

EXPERIMENTAL

The SFC system used in this work was constructed using a high-pressure syringe pump (Alpine West Labs, Provo, UT, U.S.A.) and a GC oven (Model 5830; Hewlett-Packard, Avondale, PA. U.S.A.). The components of the chromatograph have been described in more detail in an earlier publication[1]. The SFC system was operated in two different configurations. The first configuration had a 100 μm I.D. column with a splitless injection system and a viscous restrictor. The second configuration had a 50 μm I.D. column, a split injection and a Guthrie-type restrictor.

The 100 μm I.D. column was 20 m long and coated with a DB-1 stationary phase, 0.4 μm film thickness (J & W Scientific, Folsom, CA, U.S.A.). The flow restrictor was a 10 cm × 10 μm I.D. uncoated fused-silica capillary attached to the column with a butt connector (Supelco, Bellefonte, PA, U.S.A.). A double-ended ferrule, which had been cut to *ca.* 3/4 of its original length, was used to connect the column directly to the injector. The O.D. of the column was large enough so that the column could not pass through the injector body and damage the sample rotor. Compression of the ferrule kept the column centered and eliminated any dead volume in the injector. A 60-nl sample volume was injected directly onto the column.

The 50 μm I.D. column was 20 m long and was coated with an SB-Methyl-100 stationary phase, 0.25 μm film (Lee Scientific, Salt Lake City, UT, U.S.A.). The injection volume was 60 nl, split *ca.* 20:1. The flow restrictor was 12 cm × 50 μm I.D. fused-silica which had been tapered at one end in the manner described by Guthrie and Schwartz[8]. The restrictor was calibrated to provide a linear velocity of about 3 cm/s at a constant temperature of 100°C and constant pressure of 100 atm and was attached to the end of the column with a butt connector, 0.4 mm I.D. (Scientific Glass Engineering, Houston, TX, U.S.A.). Inside the butt connector the end of the column and the non-tapered end of the restrictor were inserted into a length of 200 μm I.D. fused silica. This ensured end-to-end alignment of the column and restrictor.

The IMD system used for this work was similar to earlier designs[9,10]. A schematic of the drift tube is shown in Fig. 1. One modification in this design was that the collector was completely surrounded by a grounded drift ring to reduce background noise. Also, a new method of ion-gate construction produced gates which were less prone to failure at high temperatures. Although there are several methods of gathering drift spectra[10], Fourier transfrom ion mobility spectrometry (FT-IMS) was used in this study[11]. Chromatographic detection was accomplished by continuous mobility monitoring (CMM)[12].

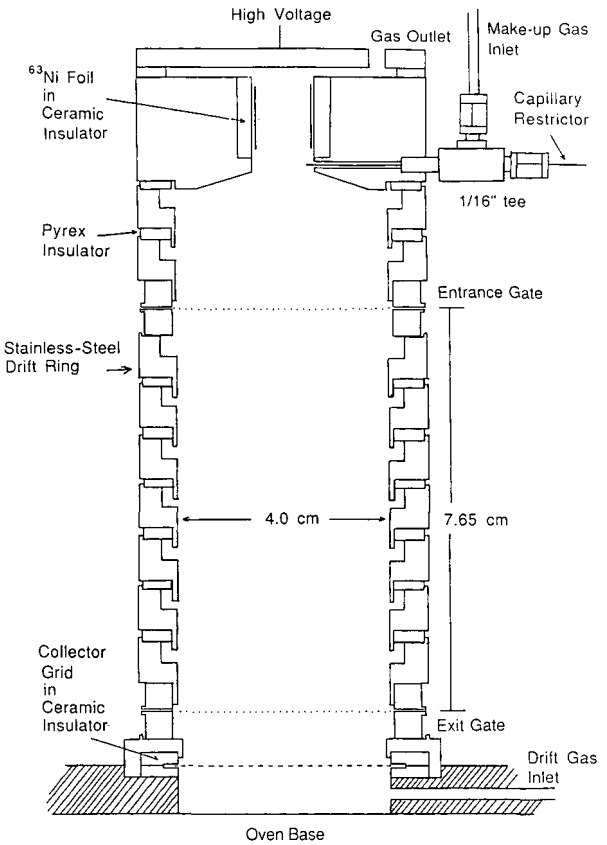

Fig. 1. Schematic of the ion mobility detector drift tube.

Electronics

Schematics of control electronics are shown in Fig. 2. The components of the system were an IBM microcomputer, a high-voltage source and gate controller (WSU Technical Services), a scanning square wave generator (SSQW) (WSU Technical Services), a Keithley 417 picoammeter (Keithley Electronics, Cleveland, OH, U.S.A.), an interface box and a chart recorder and integrator. The signal source for the CMM mode of operation and the data collection system for the FT-IMS mode were both built into the IBM microcomputer equipped with a multi-input/output expansion card, a memory expansion card, a math coprocessor and a PCI-2000 intelligent instrumentation system (Burr-Brown, Tucson, AZ, U.S.A.).

The timer module generated the logic for the opening and closing of the gates in the CMM mode of operation. Two timer element outputs were used as the pulses for the opening and closing of the entrance and exit gates. A combination of compiled basic and assembly language programs were used to control the timing of the gates.

In the FT-IMS mode both gates were opened and closed simultaneously while the opening and closing of the gates were scanned through a frequency range. The SSQW acted as the signal source for gate control in the FT-IMS mode of operation.

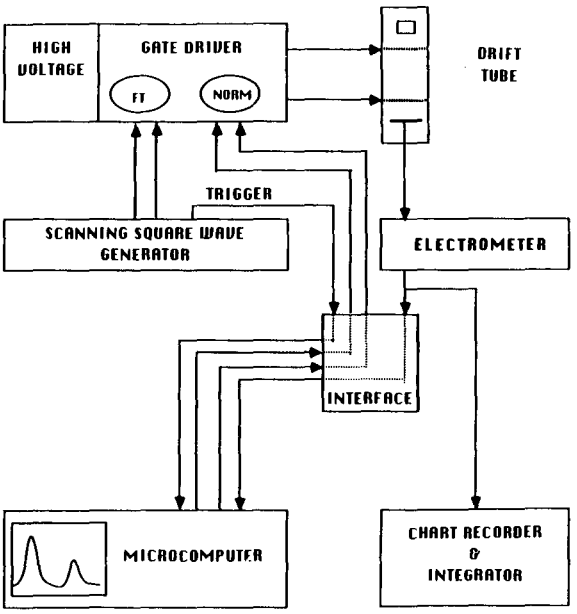

Fig. 2. Schematic of the electronics for the ion mobility detector.

As ions drifting through the drift region came in and out of phase with the frequency of the gates, they created an interference pattern at the collector. This interference pattern was collected by the computer as a mobility interferogram and converted to a time-domain drift spectrum via a fast-FT algorithm.

The IMD controller converted the logic signals from the signal sources to the proper voltages to open and close the ion gates. The devices for the gate control were contained on two custom PC cards (Redmond's Circuits, Redmond, WA, U.S.A.), one for each gate.

The chart recorder and integrator was a Hewlett-Packard 18817A GC terminal. The output of the picoammeter was routed through a voltage divider to an auxiliary analog/digital (A/D) board of the chromatograph.

The interface box was designed and built in-house. It performed several functions. In the CMM mode of operation the interface box configured the timer elements of the timer module so that the drift window was created, configured the system to an internal trigger signal set in the computer software and inverted the logic signals from the timer module so that they were compatible with the transistor-transistor logic (TTL) of the IMD controller. In the FT-IMS mode the interface box routed the output of the picoammeter to the A/D module of the computer, configured the system to an external trigger signal from the SSQW, and set the full-scale input voltage of the A/D module. This last function was used as an additional gain switch.

Operating conditions

Pure carbon dioxide (instrument grade, Liquid Air, Tacoma, WA, U.S.A.) was used as the mobile phase for all separations. Later *et al.*[13] showed that much higher

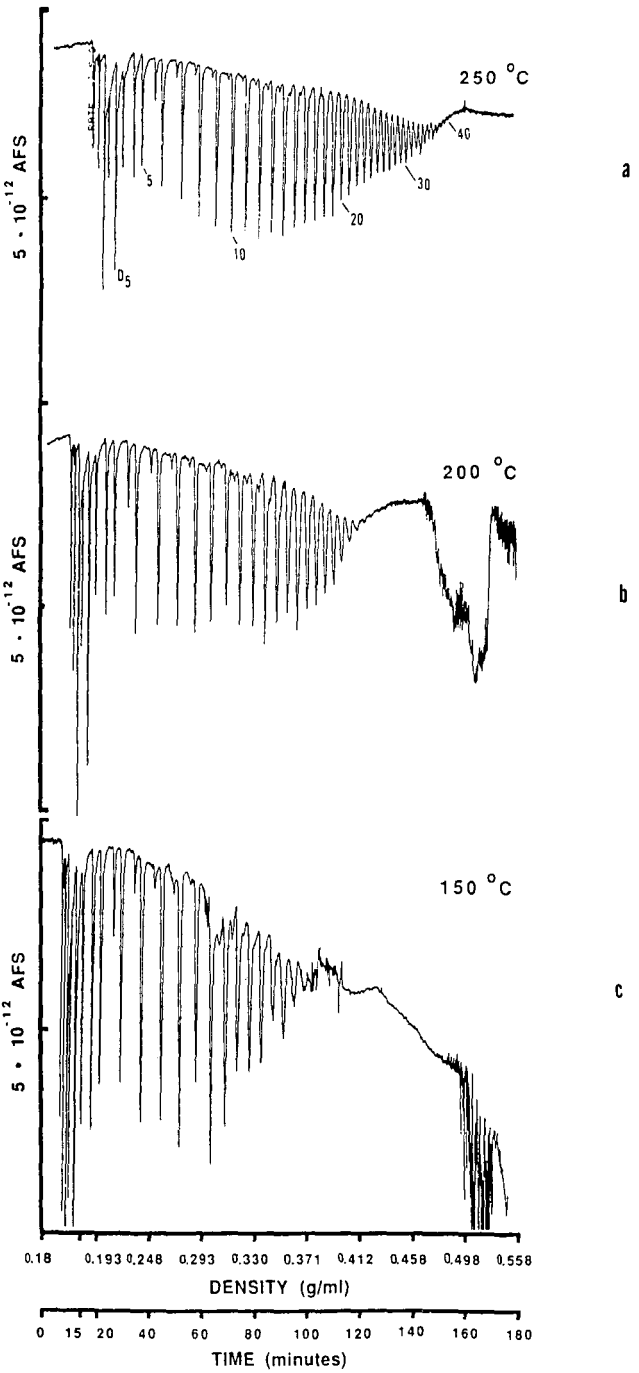

Fig. 3. SFC–IMD separation of Dow Corning 200. J & W Scientific column, 20 m × 100 μm I.D., DB-1 stationary phase, 0.4 μm film. Detector temperature: (a) 250°C, (b) 200°C, (c) 150°C.

efficiency separation of polydimethylsilicones could be achieved by simultaneous density and temperature programming than by either density or temperature programming alone. Unfortunately, the SFC system used for this work was capable of linear density programming at a constant temperature, but was incapable of compensating for the change in temperature as the chromatographic run progressed. Therefore, temperature and density were programmed independently, *i.e.*, the temperature was programmed from 100°C to 140°C, but density was programmed at an *assumed* constant temperature of 100°C. The density line shown along the bottom of the chromatograms has been corrected for temperature programming.

RESULTS AND DISCUSSION

Temperature effect

Fig. 3 shows the separation of Dow Corning 200 at IMD temperatures of 150, 200 and 250°C. In each case the detector had been tuned to monitor the depletion of the reactant ions (those ions present in the spectrometer when the sample is not in the spectrometer). This reactant ion monitoring mode is a non-selective method of detection[1–8]. Other pertinent detector conditions are given in Table I. All three separations were carried out on the 100 μm I.D. column with splitless injection and a viscous restrictor. In each case the sample concentration was *ca.* 1 mg/ml of the polydimethylsilicone polymer, Dow Corning 200. Temperature and density programs are given in the figure.

There are several important features in Fig. 3 that should be noted. First, in Fig. 3c the IMD operating temperature of 150°C is the same operating temperature used in the earlier Triton X-100 separation[1]. Only, *ca.* eighteen peaks can be seen in this chromatogram, far short of the number of oligomers expected in the sample. Also, at the end of the chromatogram was a large unresolved peak. This was most likely due

TABLE I

DETECTOR CONDITIONS

IMD	
Nitrogen drift gas	400 ml/min
Nitrogen make-up gas	100 ml/min
Temperature	250°C[a]
Electric field	+ 316 V/cm
Ion drift length	7.6 cm
Ambient pressure	698 mmHg
FT-IMS	
Scan range	20–10 020 Hz
Scan time	10.24 s
Gate voltage	+/− 20 V
Continuous mobility monitoring	
Drift time base	24 ms
Gate voltage	+/− 40 V
Drift times monitored	noted in figures

[a] Except where indicated.

to samples which had precipitated in the restrictor and then, as the mobile phase density increased, was eventually eluted, but poorly separated.

The effect of increasing the detector temperature to 200°C can be seen in Fig. 3b where the number of peaks increased to around 25. Although this was an improvement, fewer peaks were eluted than expected and unresolved peaks were found between 160 and 180 min.

Fig. 3a shows the same separation at an IMD temperature of 250°C. In this chromatogram the number of peaks suggests the separation of 50 compounds in the polymer mixture. The numbers over individual peaks represent the number of monomers believed to be in the peak. The identification relied on the comparison of this separation to previous separations with assigned monomer numbers[13]. Note that this chromatogram lacked the poorly resolved group of peaks near the end that were prominent in the two chromatograms at lower detector temperatures.

Ion mobility spectra

Using the FT capabilities of the detector, spectra were obtained for individual

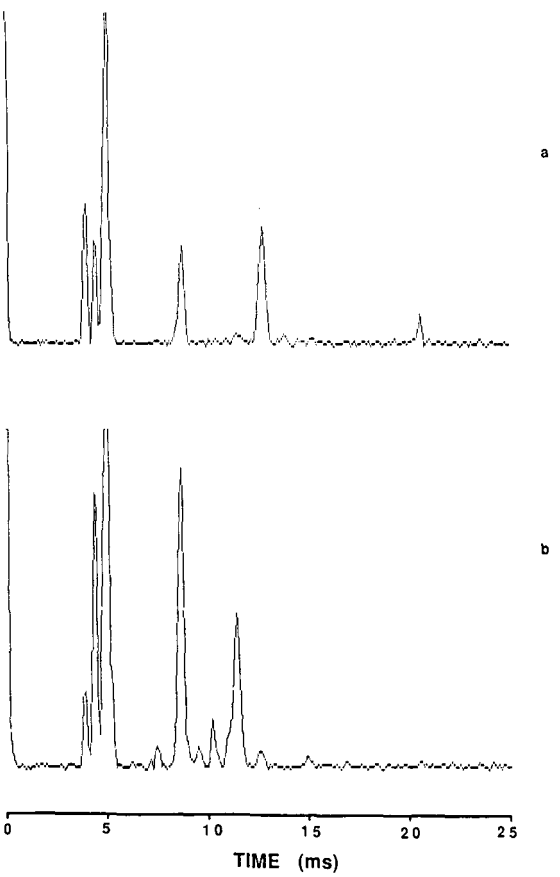

Fig. 4. Drift spectra of Dow Corning 200 oligomers. (a) Drift spectrum captured at 27 min, (b) drift spectrum captured at 64 min.

peaks of the Dow Corning 200. Twenty-eight spectra were collected during a single chromatographic run over a time period of 120 min. Fig. 4 shows the spectra collected for peaks which eluted at 27 and 64 min. (Chromatographic conditions are given in Fig. 3, detector conditions given in Table I). The spectrum shown in Fig. 4b for the peak which eluted at 64 min is representative of most of the spectra collected. All of the spectra contained more than one product ion and the majority showed two major product ion peaks at drift times of 8.8 and 11.4 ms.

Spectra were collected during a single chromatographic run. During the run it was possible to observe the major product ions increasing and decreasing in intensity as the chromatographic peaks passed through the detector. Early in the chromatogram it was possible to observe the absence of any product ion peaks, which would correspond to the valleys in the chromatogram. Later in the chromatography, after *ca.* 70 min, the presence of some of the compounds in the detector was almost continuous and it was not possible to capture a spectrum without the major product ions.

Minor peaks varied in number and drift time. The drift times of the minor peaks shown in Fig. 4b are 7.5, 9.5, 10.2 and 12.5 ms. The peak at *ca.* 14 ms is a noise artifact.

Of the twenty-eight spectra collected during chromatography only the peak at 27 min showed a unique drift spectrum. The spectrum for this compound can be seen in Fig. 4a. The drift spectrum had two major product ion peaks at 8.8 and 12.7 ms. The product ion at 12.7 ms was the largest product ion of major intensity in the Dow Corning 200. The unique features of this peak suggest that this compound is a contaminant in the polymer mixture. The drift times of the reactant ions and product ions in Fig. 4 are summarized in Table II.

Chromatography

Using the information from the drift spectra it was possible to selectively detect the peak at 27 min. The IMD system was tuned to monitor only ions with drift times between 12.2 and 13.2 ms. Selective detection can be seen in Fig. 5a. From the reten-

TABLE II

DRIFT TIMES AND REDUCED MOBILITIES

Compound	*Drift time (ms)*	*Reduced mobility* ($cm^2/V \cdot s$)
Reactant ions	4.0	2.88
	4.5	2.56
	5.0	2.31
Peak at 27 min	8.8	1.31
	12.7	0.91
Peak at 64 min	7.5	1.54
	8.7	1.33
	9.5	1.21
	10.2	1.13
	11.4	1.01
	12.7	0.92

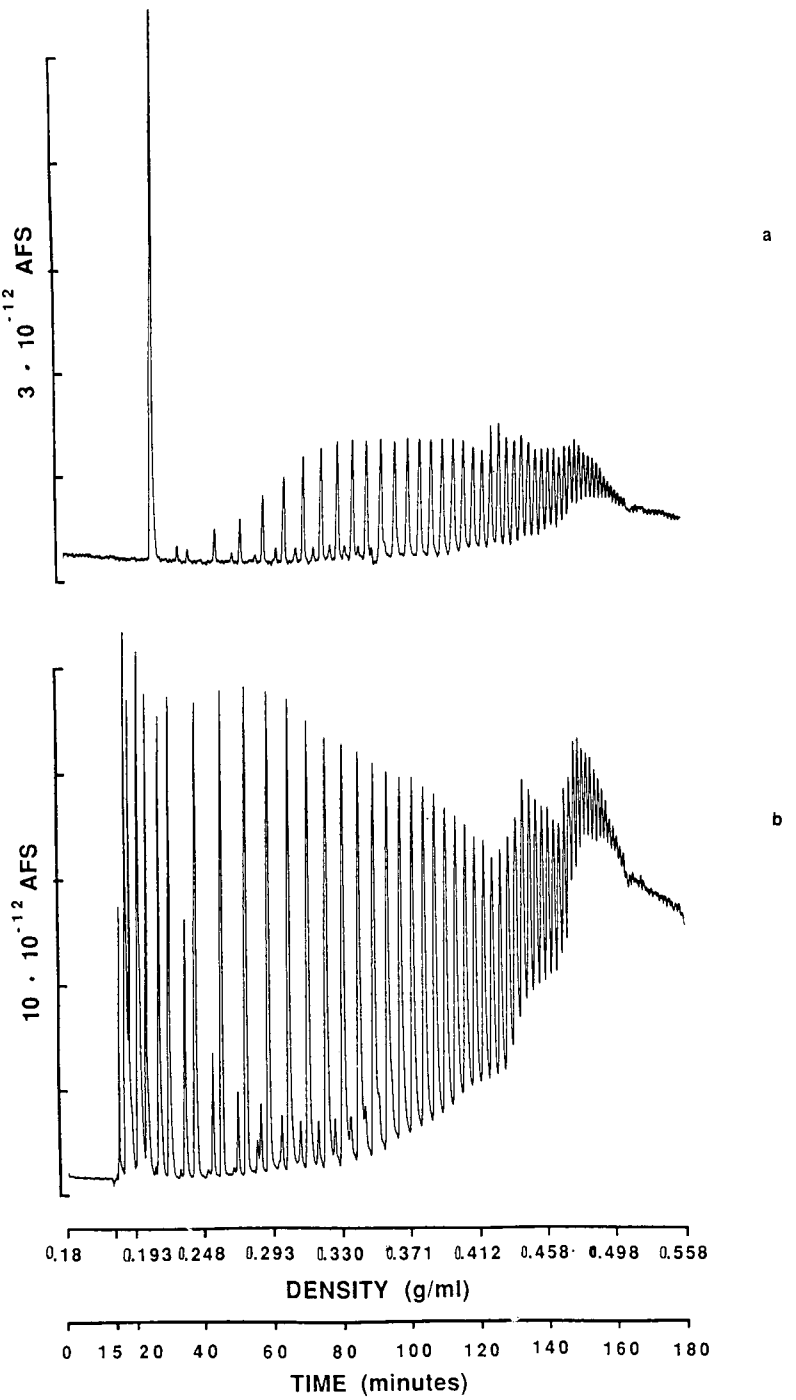

Fig. 5. Selective detection of polydimethylsilicone oligomers. (a) Drift window 12.2 to 13.2 ms, (b) drift window 7 to 15 ms.

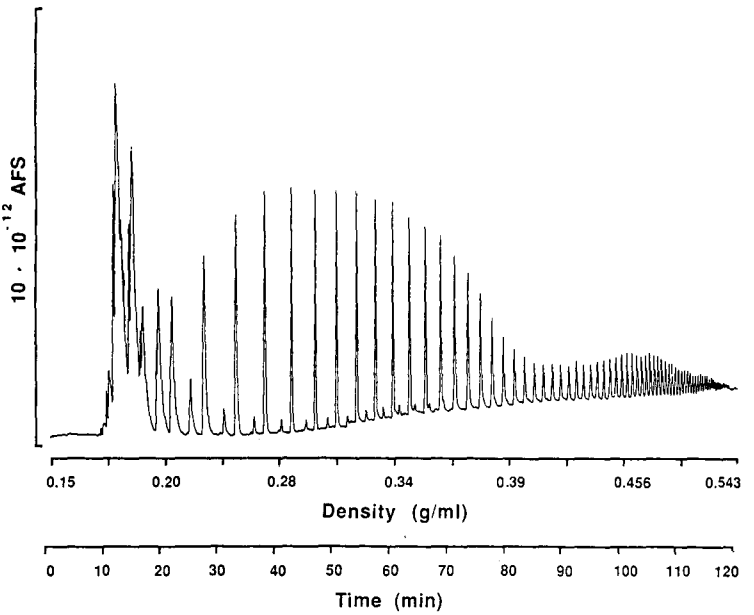

Fig. 6. SFC–IMD separation of Dow Corning 200. Lee Scientific column, 20 m × 50 μm I.D. column, SB-Methyl-100 stationary phase, 0.25 μm film. IMD temperature 250°C.

tion time data in Fig. 5 and matching the chromatographic profiles of the figure to the chromatograms obtained by Later *et al.*[13], it appears that the selectively detected peak is a compound which elutes before the fifth oligomer. For comparison, drift times between 7 and 15 ms were monitored to provide non-selective detection of the Dow Corning 200, shown in Fig. 5b. From Fig. 5b it appears that the Dow Corning 200 mixture was composed of two series of peaks: a major series and a minor series. Without mass spectral data for the product ions produced by these peaks it is difficult to determine the identity of the ions.

Finally, Fig. 6 shows the separation of the Dow Corning 200 using the 50 μm I.D. column with a split injection and Guthrie restrictor. The Dow Corning 200 sample was prepared at a concentration of 5% in hexane. The detector was programmed to monitor drift times between 7 and 15 ms. As can be seen in the figure, the efficiency of the separation and the number of peaks observed have been increased. It is estimated that compounds corresponding to oligomers of 70 monomer units were detected. Furthermore, the use of a higher-efficiency column has shortened the analysis time by an hour. With higher-efficiency columns and superior restrictors the performance of the IMD was roughly equivalent to separations using flame ionization detection[13]. The unique advantages of selective detection and qualitative information from the drift spectra are not affected by the improvement in the chromatography. It is likely that if the temperature of the IMD could be raised to match normal operating temperatures of the flame ionization detection, the performance of the IMD could be improved beyond what is shown here.

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Integrated analysis of solid samples by on-line supercritical fluid extraction–gas chromatography

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SUMMARY

A system for direct coupling of supercritical fluid extraction with carbon dioxide and capillary gas chromatography (GC) has been developed. Use of a double-oven gas chromatograph allows precise thermostating of all parts used for the extraction and sample transfer process. The valve position and the temperature are controlled by the GC panel. The system allows extraction chambers with dimensions from the micro to the semi-preparative scale. The modifications necessary for changing the vessel size and operation mode have been minimized. For small sample sizes, the whole extract is transferred to the analytical column and cryofocused there. The handling of larger amounts of sample or the performance of equilibration studies can be effected by using a "time-split" injection mode. The performance of the system was evaluated by the analysis of soil, plant material and smoke particles trapped on Tenax.

INTRODUCTION

The isolation of organic compounds from complex matrices is a limiting step in analysis because complete decomposition as in elemental analysis is not possible. Solvent extraction is time consuming and often requires large amounts of solvent. In addition, concentration and purification of the extracts are necessary in most instances. Thermal methods are very sensitive, but limited either by the thermal stability of the analyte or by the sorbents used for preconcentration.

A new approach to the solution of these problems is provided by supercritical fluid extraction (SFE). Although the basic principles of the solvation power of supercritical fluids had been known for a long time[1], it took almost a decade until Zosel *et al.*[2] introduced this technique for industrial-scale applications. Owing to the physicochemical properties of supercritical fluids, *i.e.*, a lower viscosity and higher diffusion coefficients than liquids, combined with higher solubility than in the vapour phase, they offer a number of advantages, *e.g.*, their use as a mobile phases in chromatography, as was first described in 1962[3]. A number of extraction processes on an analytical scale have been reported in recent years[4–23]. Extraction of environmental pollu-

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tants and pesticides from sorbent traps such as Tenax has been demonstrated[4–6]. Extraction of soil with supercritical methanol has been applied successfully to determine the amount of pesticide which is not extractable by liquids[7].

The liquids corresponding to supercritical fluids often have very low boiling points. Therefore, sample concentration, which is essential in trace analysis, can easily be achieved by reducing the pressure. The first application of a "solvent-free" micro-extraction and sample transfer to chromatographic analysis was demonstrated by Stahl and co-workers[8–10], who coupled SFE with thin-layer chromatography and studied the solubility behaviour of various compound classes in supercritical carbon dioxide. On-line coupling of SFE with high-performance liquid chromatography (HPLC)[11,12], packed[13,14] and capillary supercritical fluid chromatography (SFC)[15,16] has also been reported.

Although it seems that SFE–SFC is the most favourable system, because the dissolved extracts and the chromatographic carrier are in the same physical state, interfacing is difficult. Flow-rates and inner diameters of capillary SFC columns are small and only small amounts of fluid can be injected directly, because peak focusing, which is done by raising the column temperature, is successful only for high-boiling compounds. The use of external traps is possible, but their size and void volume have to be very small, thus also limiting this method to high-boiling compounds. Interfacing with packed-column SFC is similar to interfacing with HPLC, but in many instances the separation cannot be carried out by using pure carbon dioxide. With modifiers such as methanol, however, only HPLC detectors can be used, so that, compared with HPLC, SFC offers only the advantage of a higher resolving power.

Although gas chromatography (GC) is limited to thermally stable compounds, interfacing SFE with GC is a favourable approach that has also been reported[17–22]. The resolving power and detection sensitivity of GC are high. Also, extracts can be powerfully focused at the column head, so that there is no need for concentration loops, even if large amounts are transferred directly to the column. The solvation power of pure carbon dioxide is limited to apolar or slightly polar compounds. For the direct interfacing of SFE with GC this is not a general disadvantage. Compounds dissolved in supercritical carbon dioxide may be suitable for GC analysis. However, this is not expected for strongly polar compounds, which often tend to decompose at the temperatures required for GC analysis.

The aim of this investigation was to establish a multi-purpose SFE–GC system for research studies. Coupling of a continuous extraction chamber with the GC column provides the highest possible sensitivity. Quantification is easy to accomplish, because the recoveries of extracted compounds are usually very high. This is favourable for the ultra-trace analysis of aerosol particles or of airborne trace compounds collected on sorbent tubes in remote areas.

The volume of the extraction cell is limited to 200–300 μl in this method. Larger cell dimensions require either extremely long extraction times or high flow-rates of carbon dioxide. Both result in a poor trapping efficiency and, therefore, low resolution and bad peak shapes.

On the other hand, the high sensitivity of this method can easily cause overloading of the analytical column. Reduction of the sample size creates problems with regard to the homogeneity of the material. No representative quantification can be achieved by using sample sizes of 1 mg. For these reasons, a versatile SFE–GC system must be carefully designed for coping with these different requirements.

EXPERIMENTAL

Supercritical fluid supply

The supercritical fluid was supplied either by a programmable, computer-controlled high-pressure syringe pump (Lee Scientific, Salt Lake City, UT, U.S.A.; Model 600) or a constant-pressure HPLC pump with slow speed drive equipped with commercially available cooling jackets (Milton Roy, Riviera Beach, FL, U.S.A.; CP 3000). The syringe pump was operated at 8°C and the HPLC pump heads were cooled to 0°C with an external cryostat.

Carbon dioxide was of SFC grade (Scott Gases, Plumsteadville, PA, U.S.A.) and used without further purification, except for passing it through a 2-μm inlet filter.

SFE–GC instrumentation

The separation and extraction were performed with a double-oven gas chromatograph (Siemens, Karlsruhe, F.R.G.; Sichromat II), equipped with flame ionization and electron-capture detectors and suitable for cryogenic operation. Data analysis was performed by using an integrator (Shimadzu, Kyoto, Japan; CR 2-A). A schematic diagram of the system is shown in Fig. 1.

The columns used were 50 m × 0.32 m I.D. coated with either SE-30 (film thickness 0.5 μm) or SE-52 (0.25 μm) (Macherey, Nagel & Co., Düren, F.R.G.). The columns were connected with a retention gap (1–2 m × 0.32 mm I.D.) to promote cryogenic concentration and to protect the column. Direct connection of the column to the outlet restrictor of the extraction cell resulted in visible damage to the stationary phase at the column entrance after a few extraction cycles. Moreover, the fused

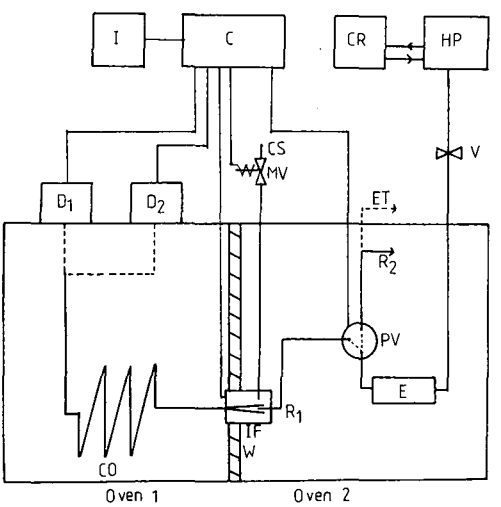

Fig. 1. Schematic diagram of the SFE–GC system. HP = high-pressure pump; CR = cryostat; V = high-pressure shut-off valve; E = extraction cell; PV = air-actuated three-way valve; IF = thermostated SFE–GC coupling unit (for details, see Fig. 2); W = oven separation wall; CO = capillary column; D_1, D_2 = electron-capture and flame ionization detectors, respectively; R_1 = restrictor for on-column deposition; R_2 = waste restrictor; ET = external trap; C = GC control unit; CS = carrier gas supply; MV = magnetic carrier shut-off valve; I = integrator; thin lines, control circuits; thick lines, transfer lines.

silica became fragile. For this reason part of the retention gap (*ca.* 15 cm) was removed after 10–20 extraction cycles. Supercritical conditions were maintained in the extraction cell with straight restrictors made of fused silica (10–20 cm × 15 μm I.D.; Lee Scientific), resulting in flow-rates of gaseous carbon dioxide of about 30–80 ml/min.

The carrier gas supply (helium at about 40 cm/s) and the restrictor were connected to the retention gap with a custom-made T-piece (Fig. 2). Heating of this interface reduced clogging of the restrictor and was performed by a heating block operated at 150°C, which was fixed into the separating wall of the gas chromatograph. During the extraction, the carrier gas was shut off, because the high back-pressure of the GC column caused transfer of carbon dioxide to the carrier gas supply, resulting in long equilibration times after the extraction. An additional magnetic valve was used to flush the carrier gas supply, if necessary. The flow-rates of gaseous carbon dioxide caused problems with the flame of the flame ionization detector and therefore ignition before starting the analysis was necessary and was performed by using the GC time programme. The operation of the electron-capture detector was unaffected by the large amounts of carbon dioxide and the baseline was stable after flushing the column with carrier gas. Moreover, this detector responsed linearly to carbon dioxide, so that the extraction could easily be monitored.

Extraction cells could easily be constructed of empty HPLC columns equipped with sintered-steel frits of 2 mm × 2 μm pore size and standard reducing fittings. For small amounts of solid samples, an extraction cell made from modified standard fittings was used (see Fig. 3).

Sample transfer from the extraction cell to the column or waste was accomplished by using an air-actuated three-way valve (Valco C3W; VICI, Schenkon, Switzerland). The valve position was switched using the time programme of the GC controller. The transfer lines were made out of 1/16 in. × 0.007 in. or 1/16 in. × 0.25 mm I.D. stainless-steel tubing.

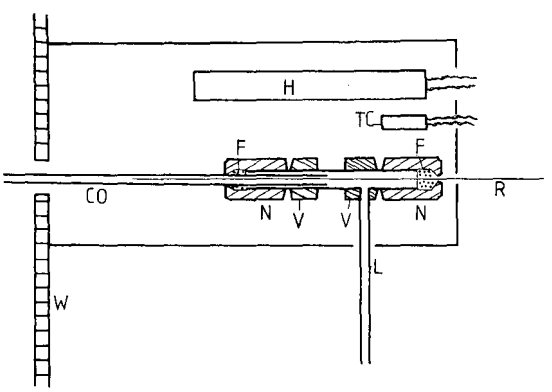

Fig. 2. Interface for on-line SFE–GC coupling. CO = retention gap, 0.32 m I.D.; R = Restrictor; N = 1/16 in. SGE nuts; F = Vespel ferrules; V = 1/16 in. Valco fittings; L = 1/16 in. × 0.5 m I.D. stainless-steel tube from carrier supply; H = heating unit; TC = thermocouple; W = separation wall of gas chromatograph.

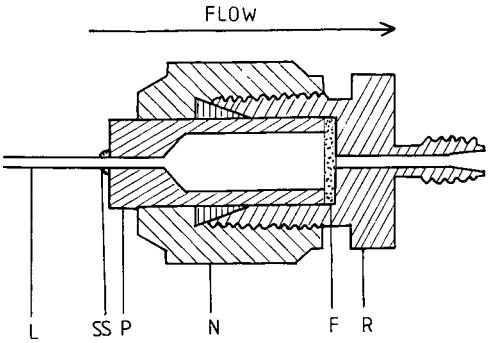

Fig. 3. Micro extraction cell for solid samples. P = 1/4 in. plug; L = 1/16 in. × 0.007 in. I.D. stainless steel line; R = 1/4 in. × 1/16 in. zero volume reducer; F = 2 mm x 2 μm porous stainless steel frit; SS = silver soldering.

Operation modes

The waste outlet of the valve was either equipped with an additional restrictor (R_2) or closed with a Vespel ferrule, depending on the operation mode used.

Sorbent cartridges were analysed with an additional 5 cm × 15 μm restrictor placed inside oven II (see Fig. 1). After transferring the extract to the column and switching the valve to the waste position, the sorbent traps were cleaned for the next use by raising the pressure to 40 MPa and venting the solutes to R_2 during the analysis. Additionally, the waste restrictor could be used to flush all lines with supercritical carbon dioxide before analysis.

Fractionation was carried out in a similar way except that the waste restrictor was replaced with a vespel ferrule. To avoid possible losses of extracts, the carbon dioxide supply was closed during the analysis.

Time-split injections were performed with a closed waste outlet and with the valve switched to the waste position during the equilibration period (10–20 min). Sample injection was carried out by switching the valve to the column restrictor. The amount sample deposited on the column could easily be varied by changing the time of the sample transfer. Transfer of the extracts to an external trap is also possible (ET, dashed line in Fig. 1).

RESULTS AND DISCUSSION

Different kinds of samples were chosen for evaluation of the performance of the described SFE–GC system. Fir needles (*Abies alba*) were analysed using the 'time-split' injection mode after a 12-min equilibration period. The chromatogram obtained by this method is shown in Fig. 4. Most compounds detected in the samples are terpenes and sesquiterpene hydrocarbons, *i.e.*, C_{10}, C_{15} and oxygenated C_{10} hydrocarbons.

The volume of the extraction cell was 1 ml. There is no limitation to the size of the extraction cell in this method. Amounts of sample large enough to represent true bulk properties can be analysed. Owing to the short injection time, the peak shape was good even for volatile compounds and using thin-film columns. Exact quantifica-

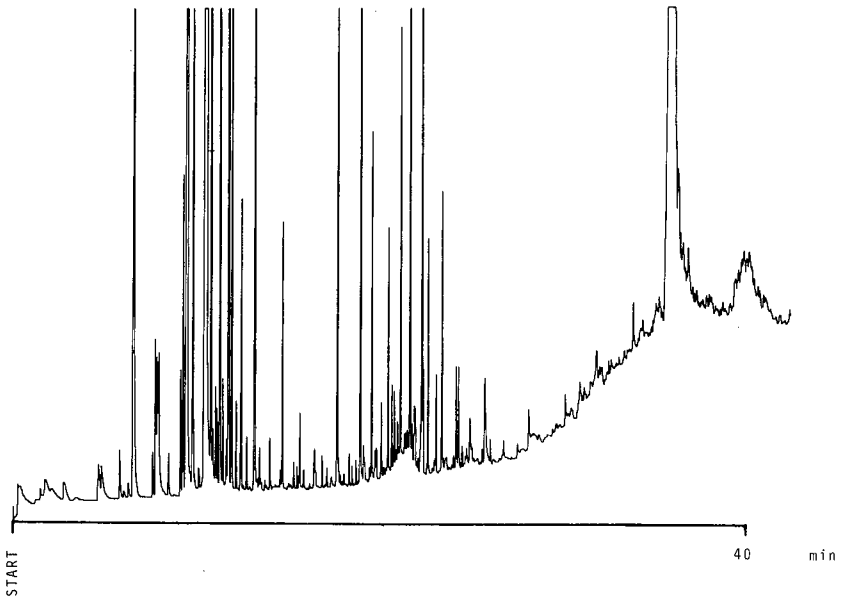

Fig. 4. SFE–GC in the time-split injection mode. Sample: *Abies alba* needles, 680 mg; extraction cell, 1 ml; 12-min equilibration at 30 MPa, injection for 3 s at 0°C; column, 50 m × 0.32 mm I.D.; stationary phase, SE-52, d_f 0.25 μm; flame ionization detection; temperature programme, 2 min at 0°C, 15°C/min to 80°C, 8°C/min to 280°C.

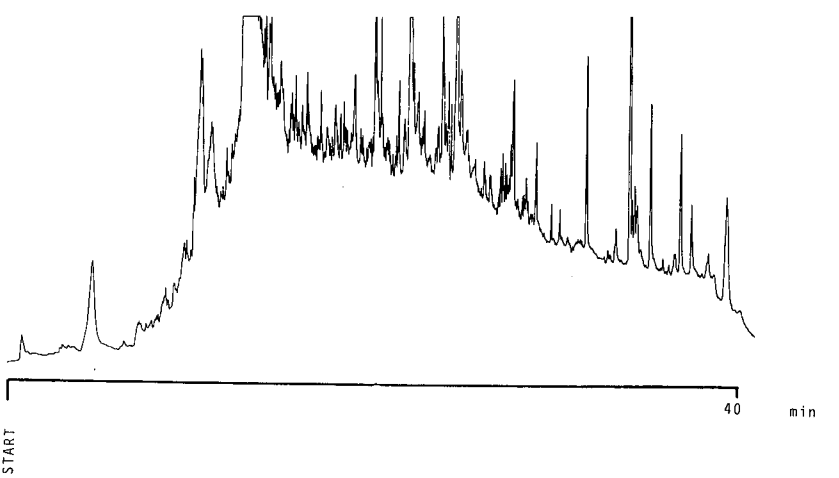

Fig. 5. SFE–GC of cigarette-smoke particles trapped on Tenax. Extraction at 25 MPa and 50°C for 12 min, trapping at 0°C; extraction cell, 200 μl; column 50 m × 0.32 mm I.D.; stationary phase, SE-52, d_f 0.25 μm; flame ionization detection; temperature programme, 1 min at 0°C, 15°C/min to 100°C, 10°C/min to 300°C.

tion can be achieved in a similar way as for headspace analysis, if the equilibration concentrations are known. Measurement of these concentrations can easily be performed by this system, but complications are expected owing to matrix effects, especially for biological samples with variable water content.

Sorbents used for preconcentration of airborne pollutants can easily be ana-

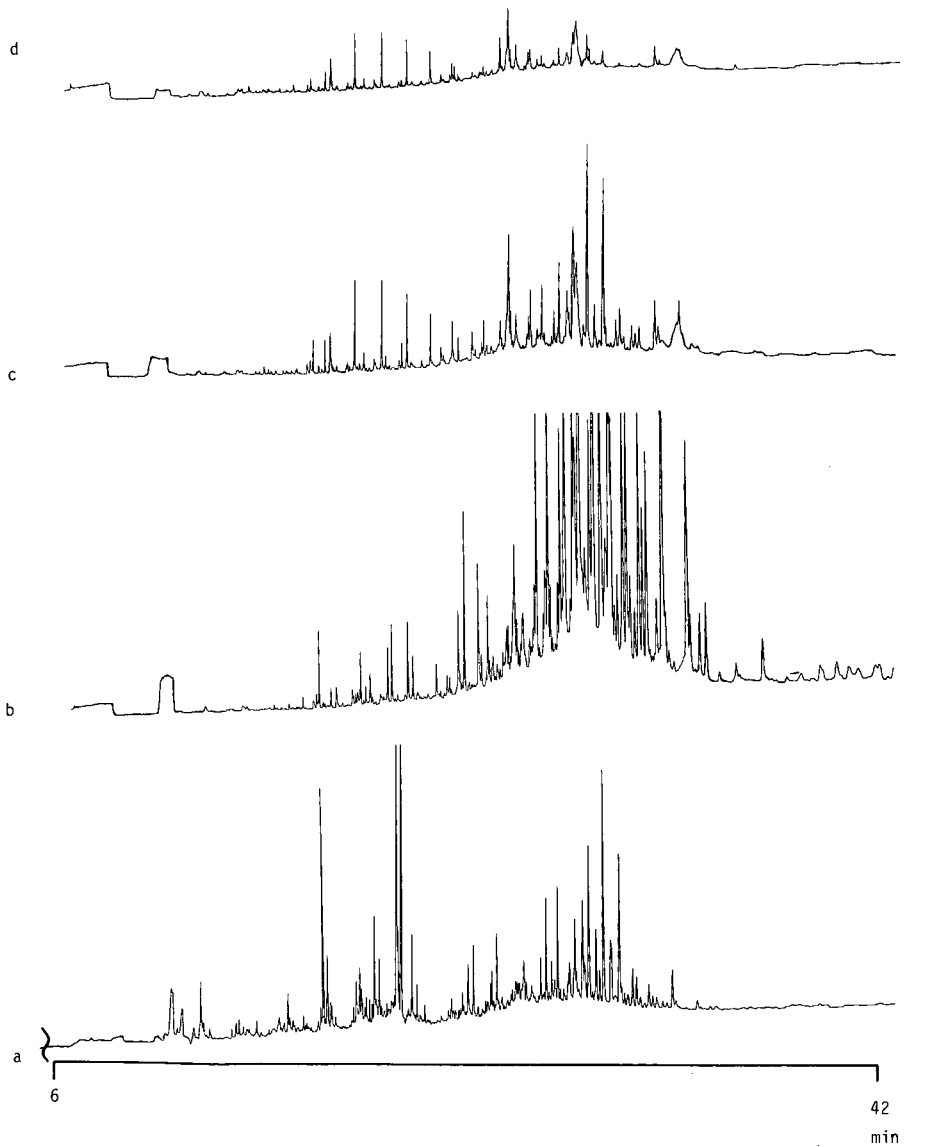

Fig. 6. Fractionation by SFE. Sample 20 mg of soil; extraction cell as in Fig. 3. (a) Extraction at 8 MPa for 12 min; (b) extraction at 16 MPa for 12 min; (c) extraction at 26 MPa for 12 min; (d)) system blank at 16 MPa for 12 min. Trapping at 0°C; column 50 m × 0.32 mm I.D.; stationary phase, SE-30, d_f 0.52 μm; electron-capture detection; temperature programme, 3 min at 0°C, 10°C/min to 280°C.

lysed by this system. Fig. 5 shows the SFE–GC of cigarette smoke particles (phenolic and N-heterocyclic compounds) trapped on Tenax.

Extraction and fractionation of compounds sensitive to the electron-capture detector from a soil sample that had been exposed to laboratory air for several years is shown in Fig. 6a–c. Volatile compounds are quantitatively recovered in the first fraction, in which only trace amounts of less volatile solutes are detectable. Extraction at higher pressures yielded these less volatile solutes and additional amounts of intermediate volatile compounds. A third fraction extracted at 26 MPa yielded only traces of additional material and indicated that the recoveries of the compounds were almost quantitative. A number of peaks that are present in all fractions are due to the blank. A chromatogram obtained under similar conditions without sample is shown in Fig. 6d.

Blank values obtained with the SFC-grade carbon dioxide were acceptable for trace analysis using electron-capture detection, but for flame ionization detection, however, the blank values are higher and can interfere with analytes of low concentration. This fact has not been mentioned by other workers studying upper ppm levels[17–21,23] or using lower flow-rates of carbon dioxide[22]. It seems that additional contamination is emitted by new seals of the pump and valves. The syringe pump used for delivery of carbon dioxide for more than 1 year resulted in a lower blank value than an HPLC pump that had not been used very long.

No problems concerning the stability of the restrictor mentioned by others[17–20] were observed. Therefore, the interface described seems to be more favourable than inserting and removing the restrictor through an on-column injector.

Although, as reported[18], low temperatures resulted in better peak shapes for the early eluting compounds, problems caused by clogging of the column (not the restrictor) were observed below 0°C. This problem appears to be related to the expansion of the carbon dioxide and the small amounts of water contained in the samples. No clogging of the column was observed after trapping of the extracts of double the amount of similar samples (soil) at −196°C obtained by the thermal desorption method.

CONCLUSION

Supercritical fluid extraction is a powerful technique for isolating organic compounds from complex matrices. On-line coupling with GC minimizes the time consumption and avoids contamination or sample losses. The present SFE–GC system combines the advantages of different operation modes with precise and simple control of the extraction and analysis parameters. The potential of the system has been successfully demonstrated with different kinds of samples. Quantification aspects, especially for the time-split injection mode, and reduction of blank values will be the aim of further research.

ACKNOWLEDGEMENTS

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Fractionation of lemon-peel oil by semi-preparative supercritical fluid chromatography[a]

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SUMMARY

Fractionation of cold-pressed lemon-peel oil into several compound types, namely hydrocarbons, alcohols and aldehydes, esters and others, was demonstrated using a semi-preparative supercritical fluid chromatographic system. By utilizing stepwise pressure and modifier programming, it was possible to load 0.5 ml (about 0.5 g) of lemon-peel oil onto 50 mm × 7.2 mm I.D. column packed with silica gel (10–20 μm). The technique can be used to produce new flavours by remixing fractions in different proportions, and for simple removal of terpenes from the oil.

INTRODUCTION

Problems with supercritical fluid chromatography as a preparative separation method

Although there have been useful reports on preparative supercritial fluid chromatography (SFC) by Jentoft and Gouw[1], Hartmann and Klesper[2] and Perrut and Jusforgues[3], the technique has not yet been widely accepted, because fractions are collected in high-pressure vessels when a fluid such as carbon dioxide is used. Therefore, in order to obtain the fractions, the operator needs to wait until the last fraction has been collected, and then the pressure in the vessel must be reduced to atmospheric pressure. Hence the fractions cannot be dealt with as easily as those obtained in preparative liquid chromatography.

For easy operation of a preparative SFC system, it was necessary to develop a back-pressure regulating and fractionating system, which allowed operation under normal atmospheric pressure. For this purpose we developed a back-pressure regulator having an internal volume of less than 10 μl[4]. This back-pressure regulator is suitable for collecting solutes, in addition to applying a back-pressure, even for semi-preparative SFC, owing to the extremely low internal volume which prevents the remixing of solutes, as reported previously[5].

Lemon-peel oil

Citrus essential oil is a fairly expensive material used in the perfume and flavour

[a] Presented in part at the *International Conference on Supercritical Fluids, Nice, October, 1988.*

industries. In general, the oil is isolated from citrus-fruit peel with a cold-press machine. However, fresh cold-pressed oil deteriorates easily and develops an off-flavour (deteriorated odour), owing to the instability of terpene hydrocarbons, including limonene. Although these hydrocarbons are major components of the oil, they contribute little to the characteristic citrus aroma and flavour of the oil and are readily oxidized to off-flavour-producing compounds. Therefore, in order to increase the stability of the oil as a commercial product, the terpenes are removed by distillation. On the other hand, oxygenated compounds, such as aldehydes, alcohols and esters, are enriched because these are responsible for the flavour of the oil[6,7].

Recently, the extraction and enrichment of lemon peel oil with supercritical carbon dioxide have been examined by Calame and Steiner[8], Stahl and Gerard[9], Coppella and Barton[10] and Sugiyama and Saito[11]. Fractionation of components by supercritical-fluid extraction (SFE) was also investigated Stahl and Gerard[9] but was not very successful because a pressure near the critical pressure gave a higher selectivity for the terpenes relative to the oxygenated compounds, but a low extraction yield, whereas a higher pressure gave a higher yield but a lower selectivity.

This paper describes the fractionation of lemon-peel oil into several compound types, namely hydrocarbons, alcohols and aldehydes, esters and others by a semi-preparative SFC method.

EXPERIMENTAL

Materials and column

Cold-pressed lemon oil was donated by Mr. Sugiyama of Morinaga (Yokohama, Japan).

Standard-grade carbon dioxide was purchased from Toyoko Kagaku (Kawasaki, Japan) and was used as the mobile phase. High-performance liquid chromatographic (HPLC)-grade ethanol was purchased from Wako (Osaka, Japan) and used as a modifier for chromatographic elution. A JASCO (Tokyo, Japan) SuperPak SIL column (50 mm × 7.2 mm I.D.) packed with silica gel (10–20 μm) was used for the separation of the oil.

Apparatus

Fig. 1 shows a schematic diagram of the system. A JASCO Model 880-PU HPLC pump (1) with a cooling jacket kept at -5°C was used for the delivery of liquified carbon dioxide. Two 880-PU pumps (2 and 3) were used for solvent delivery. As the amounts of the lemon-peel oil fractions obtainable were estimated to be of the order of 10^{-3}–10^{-2} g based on the previous investigations[11], and as the fractions consist of volatile compounds, a third pump (3) was added in addition to the modifier pump. This pump delivered ethanol at a low flow-rate (0.05 ml/min), which was mixed with the column effluent at a tee-joint[12] placed downstream of the detector[11] to collect small amounts of volatile fractions efficiently. By this arrangement, the fractions could be obtained as ethanol solutions.

A six-way switching valve (7) (Model 7000; Rheodyne, Cotati, CA, U.S.A.) and a needle value (8) (Model 02-0120; SSI, State College, PA, U.S.A.) were used for by-passing the column and for releasing the fluid compressed and stored in the column when the column was removed from the line. This configuration is important for

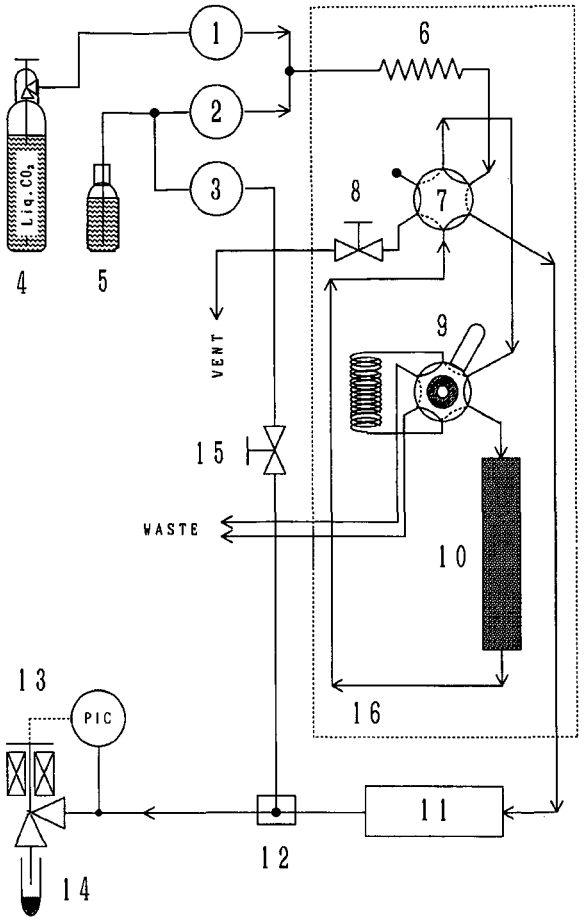

Fig. 1. Schematic diagram of coupled SFE–semi-prepararative SFC system. Components: 1 = carbon-dioxide pump; 2 = modifier pump; 3 = solvent pump; 4 = carbondioxide cylinder; 5 = modifier solvent; 6 = pre-heating coil; 7 = six-way switching valve; 8 = needle valve; 9 = injection valve; 10 = column; 11 = photodiode-array UV detector; 12 = tee-joint; 13 = back-pressure regulator; 14 = collection tube; 15 = shut-off valve; 16 = oven.

safety when a column larger than 4.6 mm I.D. is used, because such a column accumulates a large amount of energy in the form of compressed gas. For example, several litres of carbon dioxide are compressed to a few millilitres, and this energy can be explosively released if a column end-fitting is accidentally loosened. In the above arrangement, the column can be by-passed from the main line by means of the six-way valve (7), and the pressure in the column can be released slowly to atmospheric pressure by opening the needle valve (8). The column can then be disconnected from the line.

A Rheodyne Model 7125 injector (9) with a 0.5-ml loop was used for injecting cold-pressed lemon-peel oil.

A JASCO Model Multi-330 photodiode-array multi-wavelength UV detector (11) was used for monitoring the column effluent. A JASCO Model 880-81 back-pressure regulator (13) with a spring-loaded collection tube (14) was used for application of a back-pressure and for collecting components. The pre-heating coil (6), six-way valve (7), injector (9) and column (10) were placed in an oven (16) (JASCO Model 865-CO).

An HP 5890A capillary gas chromatograph (Hewlett-Packard, Avondale, PA, U.S.A.) was used for gas–liquid chromatographic (GLC) analysis of SFC fractions of the oil.

RESULTS AND DISCUSSION

SFC fractionation of cold-pressed oil

SFC fractionation of the cold-pressed oil was performed by injecting 0.5 ml of the oil directly onto the separation column using the ordinary valve injector. The column outlet pressure was kept at 10 MPa for the first 9.0 min, then increased to 20 MPa and held for 4.0 min, and finally ethanol was added at a flow-rate of 0.05 ml/min. The carbon dioxide flow-rate was kept constant at 2.2 g/min throughout the elution. The column temperature was constant at 40°C. The above conditions were determined bearing in mind the real-time monitoring of the spectrum using the multi-wavelength UV detector. The collection tube was changed manually every time the pressure was changed or modifier was added.

Fig. 2 shows a three-dimensional chromatogram of the oil obtained by the above procedure. The numbers shown under the time axis are time frames, which correspond to fractions collected.

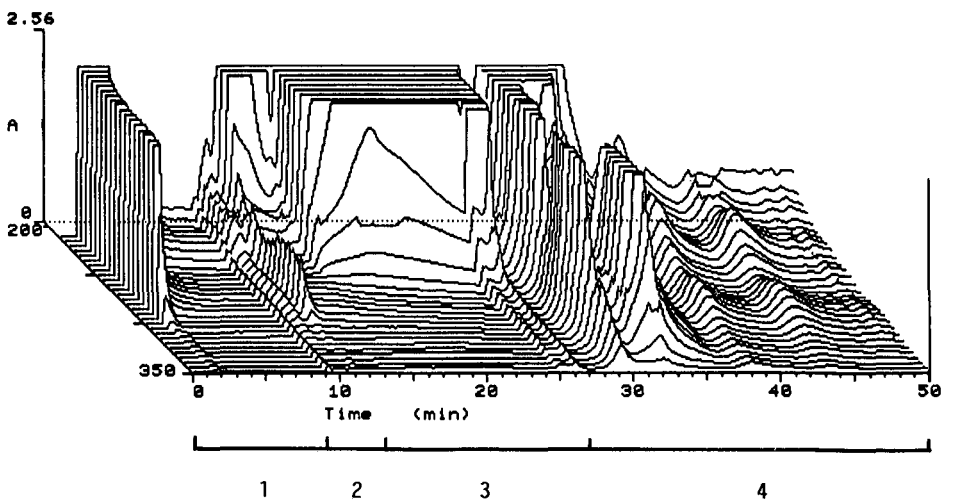

Fig. 2. Three-dimensional cromatogram of cold-pressed lemon peel oil obtained by semi-preparative SFC. The numbers under the time axis indicate fraction numbers, which are refered to as fractions 1–4 in the text and Table I. (From ref. 12).

GLC analyses of cold-pressed oil and its SFC fractions

Cold-pressed oil. Fig. 3 shows the GLC of the original cold-pressed oil without SFC fractionation, which we used as the reference. Peak assignment was performed by comparing the retention times of the components with the chromatogram of the same oil obtained in previous experiments[11] and by reference to the chromatograms of cold-pressed oil in the literature[6,10]. In these experiments, component peaks were identified by gas chromatography-mass spectrometry. Table I lists the compound names and the percentage peak area for each peak. As shown, the most abundant component is limonene, the peak area of which occupies more than 65% of the total area for all assigned peaks.

Fraction 1. This fraction was a colourless solution, which first smelt like a fresh lemon when it was collected. However, this aroma deteriorated to an off-flavour in a

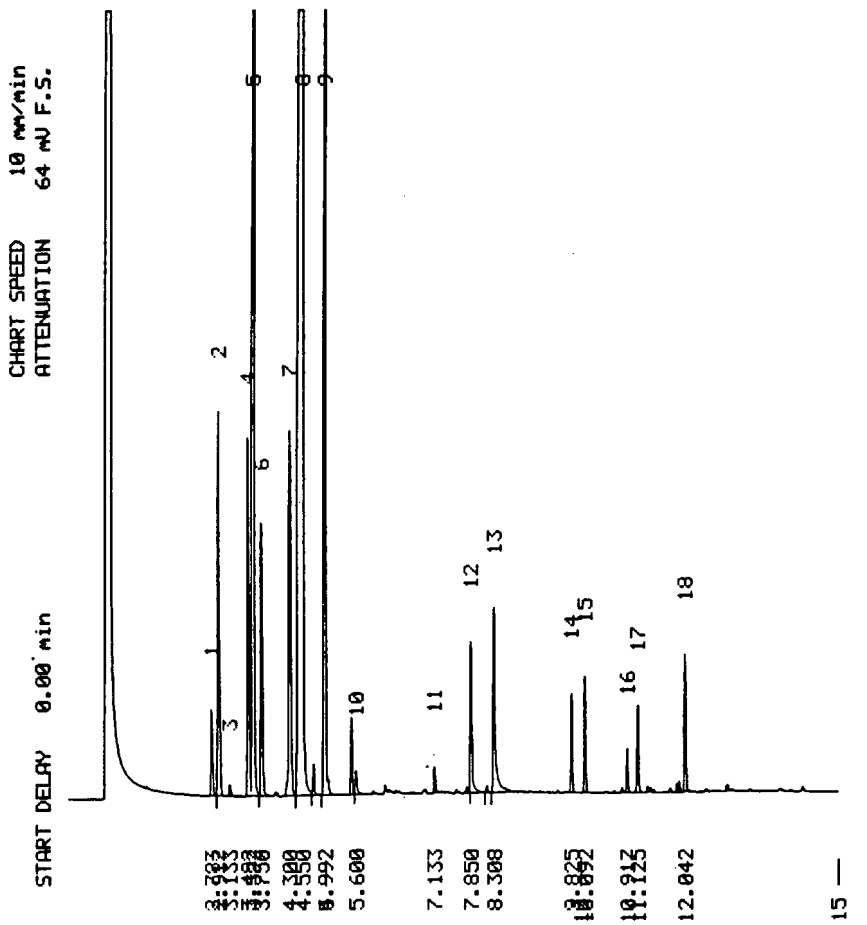

Fig. 3. GLC of cold-pressed oil. Peak numbers correspond to those in Table I. GLC conditions: column, Ultra-1 (20 m × 0.2 mm I.D.; Hewlett-Packard); detector, flame ionization; column temperature, 80°C, held for 3 min, then increased at 10°C/min to 180°C; injection volume, 2 μl (splitting ratio = 1:100); carrier gas, helium at 180; kPa. Numbers at the horizontal axis are retention times in min. (From ref. 12).

few days. Fig. 4 shows the GLC of fraction 1 obtained from the semi-preparative SFC of the oil, the chromatogram of which is shown in Fig. 3. Components are also listed in Table I. It is clear that this fraction contains only terpenes and no oxygenated compound except for peak 17 (α-bergamotene).

Fraction 2. This fraction was also a colourless solution. Its smell was slightly acidic like the aroma arising from a whole lemon being juiced in a kitchenmixer. Fig. 5 shows the GLC of this fraction. As shown in Table I, the major components are neryl acetate and geranyl acetate.

Fraction 3. This fraction was also colourless. Its aroma was similar to that of fraction 2, but less acidic. Fig. 6 shows the GLC of this fraction. It contained only aldehydes and alcohols as listed in Table I.

Fraction 4. This fraction was slightly yellow. its smell was completely off flavour, *i.e.*, like old shellaced wooden furniture. As shown in Fig. 2, this fraction included strongly UV- absorbing substances, which were suggested to be compounds containing benzene rings. There was no significant peak in the chromatogram, which is not shown here. This fraction contained slight precipitation, indicating that the substances in the fraction have higher molecular weights than those in fractions 1–3, and that the substances are non-volatile.

TABLE I

AMOUNTS (%) OF COMPONENTS OF SFC FRACTIONS OF COLD-PRESSED OIL

Peak No.	*Component*	*SFC fraction*			*Cold-pressed*[a]
		1	*2*	*3*	
1	α-Thujene	0.44			0.44
2	α-Pinene	2.01			1.92
3	Camphene	0.19			0.06
4	Sabinene	0.69	0.31		1.98
5	β-Pinene	11.96	0.22		12.37
6	Myrcene	1.47	1.09		1.44
7	α-Terpinene	3.00	0.95	0.18	2.53
8	Limonene	69.89	4.85	0.53	66.80
9	γ-Terpinene	9.12	0.34		8.02
10	Citronellal		2.07	2.52	0.17
11	Terpineol		0.44	11.67	0.12
12	Neral			24.52	0.80
13	Geranial			59.52	1.26
14	Neryl acetate		39.12	0.18	0.41
15	Geranyl acetate		50.06	0.41	0.49
16	β-Caryophyllene	0.21			0.20
17	α-Bergamotene	0.42	0.56		0.40
18	β-Bisabolene	0.61			0.59

[a] Cold-pressed oil without fractionation, given as a reference.

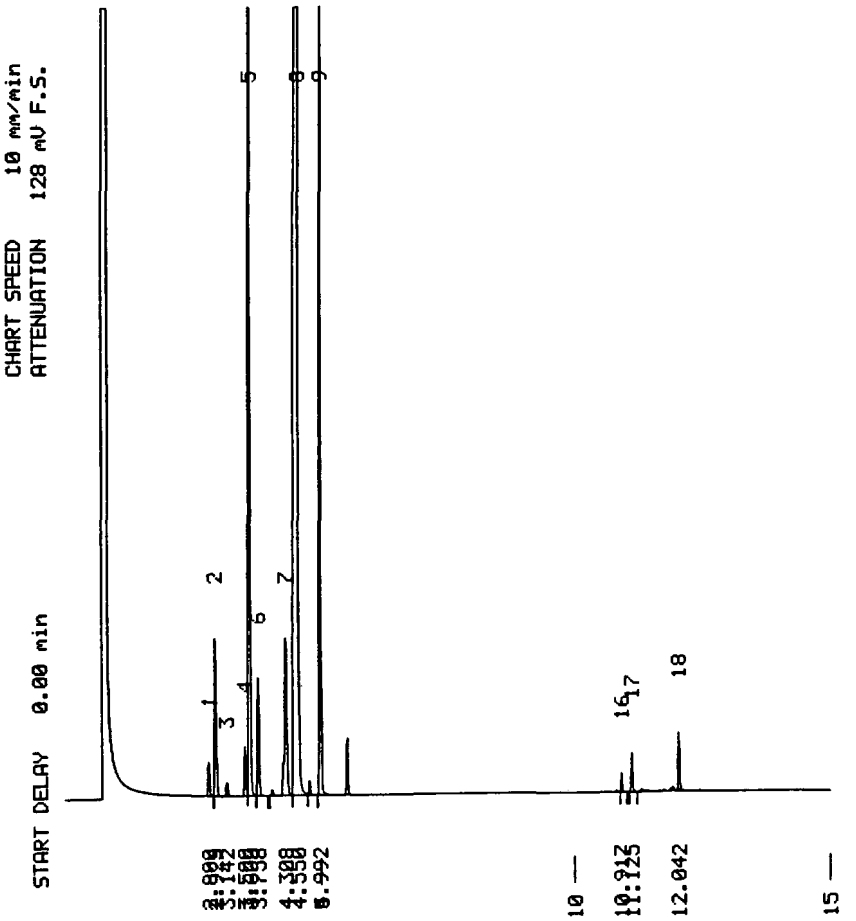

Fig. 4. GLC of fraction 1. Conditions as in Fig. 3. (From ref. 12).

CONCLUSION

Fractionation of lemon-peel oil was successfully performed by semi-preparative SFC. It is remarkable that 0.5 ml (about 0.5 g) of the oil could be fractionated into several compound types by using a relatively small column of 50 mm × 7.2 mm I.D. This means that the amount injected was about 20% of the weight of stationary phase in the column. This is higher than would be expected from the solubility of a hydrocarbon in supercritical carbon dioxide (only a few percent by weight). This high loading capacity is due to the stepwise pressure and modifier programming, which result in step-by-step injection by the stepwise change in the mobile phase strength.

The results suggest that the solute migration mechanism in our experiment was not based on an equilibrium distribution of the solute between the stationary phase

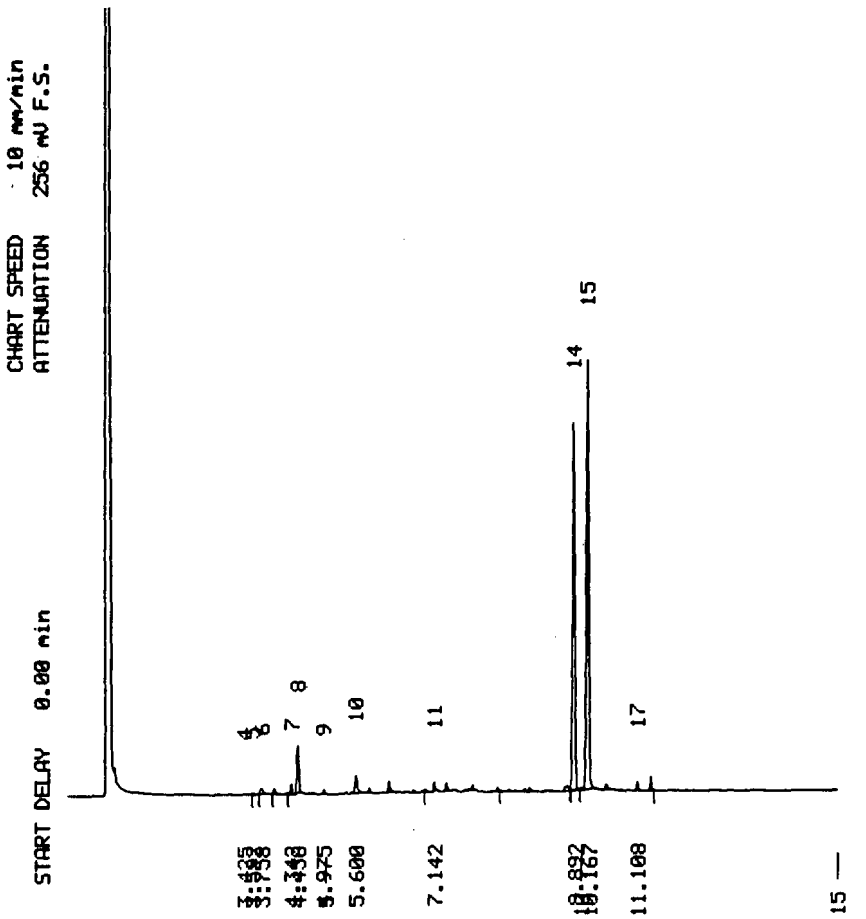

Fig. 5. GLC of fraction 2. Conditions as in Fig. 3. (From ref. 12).

and the mobile phase. Rather, it is based on absorption–desorption by the stepwise change in the mobile phase strength. Therefore, the injection is also made step-by-step, which can be regarded as programmed extraction on the column head. This may be the reason why such a high loading was possible.

This technique is more suitable for compound-type fractionation than separation into single compounds. The high loading capacity makes the preparative separation of SFE extracts of natural products feasible. The present technique can be used to produce new flavours by remixing fractions in different proportions and for the removal of terpenes from the oil.

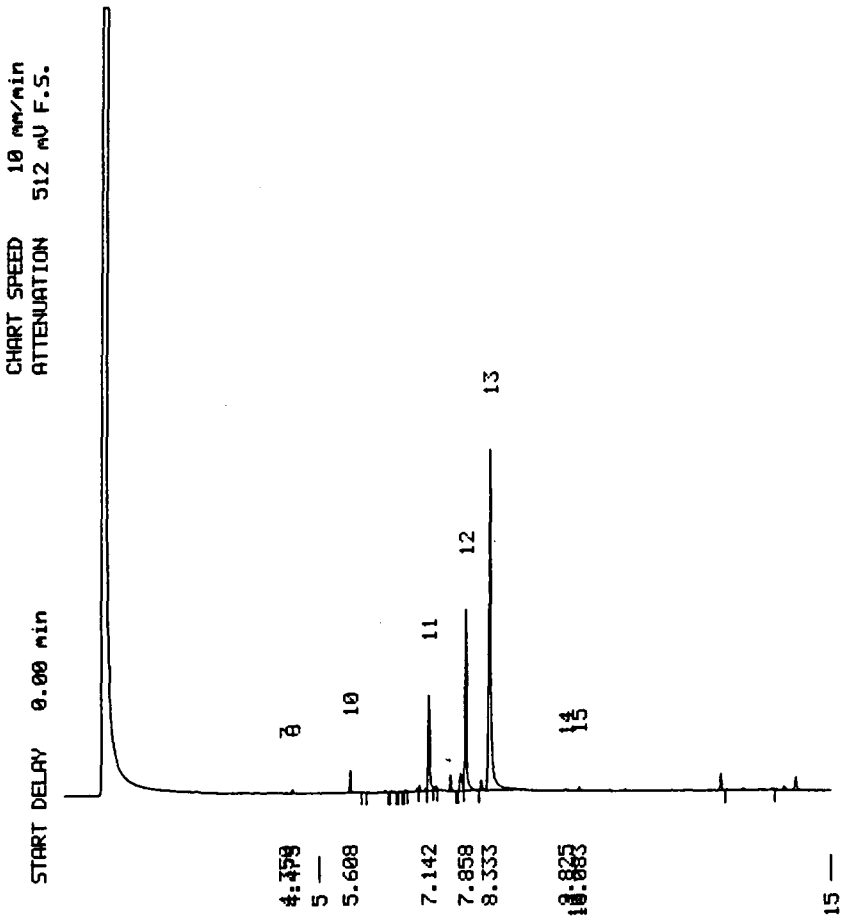

Fig. 6. GLC of fraction 3. Conditions as in Fig. 3. (From ref. 12).

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CHROM. 22 253

Extraction of major alkaloids from poppy straw with near-critical mixtures of carbon dioxide and polar modifiers

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SUMMARY

Near-critical extraction of major alkaloids from poppy straw was performed successfully with a simple device consisting mainly of two chromatographic pumps and a pressure regulator. The optimum extractant, consisting of carbon dioxide, methanol and water, gave a quantitative extraction of thebaine, codeine and morphine in 20 min. The method was compared with a classical liquid–solid extraction procedure and carbon dioxide was shown to act as a transporting agent of the extraction solvent (methanol–water) into the vegetable matrix.

INTRODUCTION

The use of supercritical fluids for analytical extraction has recently attracted increasing attention and several new applications have been reported[1–16]. In comparison with classical liquid–solid extraction methods, supercritical fluid extraction (SFE) offers many potential advantages[17,18]. *e.g.*, faster and more efficient extractions, increased selectivity, easier analyte fractionation and coupling with on-line analytical methods[19].

These potential advantages of SFE are due to the properties of fluids above their critical pressure and temperature. Such fluids have densities 100–1000 times greater than those of gases and solvating properties comparable to those of liquids. Their viscosities and diffusion coefficients are intermediate between those of liquids and gases. Solvent mixtures can also be used to achieve higher solvent strength and increased selectivity. Further, by using a fluid such as carbon dioxide with a relatively low critical temperature (31°C), extractions can be performed under mild thermal conditions. Finally, supercritical fluids have a solvent power close to that of liquids and offer better mass transfer. This clearly provides the potential for more rapid and more efficient extractions than classical liquid phases owing to a more rapid and more complete penetration into a solid matrix.

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Many experimental data are available on the solubility and extractibility of natural products such as steroids, alkaloids, anti-cancer agents, flavours and aromas, oils from seeds, caffeine from coffee beans and fatty acids from fish oils[17,20]. To determine the solubilities of compounds in supercritical fluids, Stahl and co-workers[21,22] developed a microextraction procedure directly coupled with thin-layer chromatography. Using this apparatus, they compared the capacities of carbon dioxide and nitrous oxide for extracting opium alkaloids from plant material. Nitrous oxide appeared to be a better extractant than carbon dioxide, but its extracting power remained weak. It was concluded that it was worth investigating the influence of polar modifiers which, when added to a supercritical fluid, could increase its solvating power.

In this paper, the near-critical extraction of alkaloids from poppy straw is described. As classical liquid–solid extraction procedures are often time consuming and tedious[23–25], it was of interest to develop a rapid, near-critical fluid extraction procedure that is readily applicable in routine analysis. The study resulted in a quantitative extraction of the three major alkaloids in 20 min. Special efforts were made to define the respective roles of carbon dioxide and polar modifiers in the extraction process.

EXPERIMENTAL

Chemicals and reagents

Carbon dioxide was of technical grade (Air Liquide, Lyon, France), methanol of high-performance liquid chromatographic grade (Prolabo, Paris, France) and methylamine was used as a 40% aqueous solution (Merck, Darmstadt, F.R.G.). Thebaine, codeine and morphine standards were obtained from Francopia (Paris, France).

Extraction procedure

Extraction was effected by percolation of a near-critical mixture (carbon dioxide–polar modifier) through a column containing poppy straw (obtained from Sanofi-Chimie, Aramon, France) previously ground and sieved. After decompression, the extract was collected by inserting the outlet tubing in a three-necked flask which contained *ca.* 5 ml of methanol. The methanolic solution was then analysed off-line by subcritical fluid chromatography.

The extraction cells were stainless-steel columns with stainless-steel frits maintained at both ends by Swagelock fittings. Two columns were used, 25 × 0.75 cm I.D. and 25 × 0.46 cm I.D., containing 4 and 2 g of poppy straw, respectively. For all experiments, the particle size of the poppy straw was between 250 and 500 μm.

To maintain near-critical pressures inside the extraction cells, we first used fused-silica capillaries and tapered stainless-steel tubes as the outlet. With carbon dioxide–polar modifier mixtures, these two kinds of restrictors did not work properly as they became plugged and did not ensure a constant pressure in the system. Finally, we used the same pressure regulator as in packed-column supercritical fluid chromatography (Model 26-3220-24004 valve; Tescom, Minneapolis, MN, U.S.A.). The whole extraction apparatus is shown in Fig. 1. The two pumps were the same as those used in our chromatographic studies[26].

It must be noted that this pressure-regulating system can only be used with

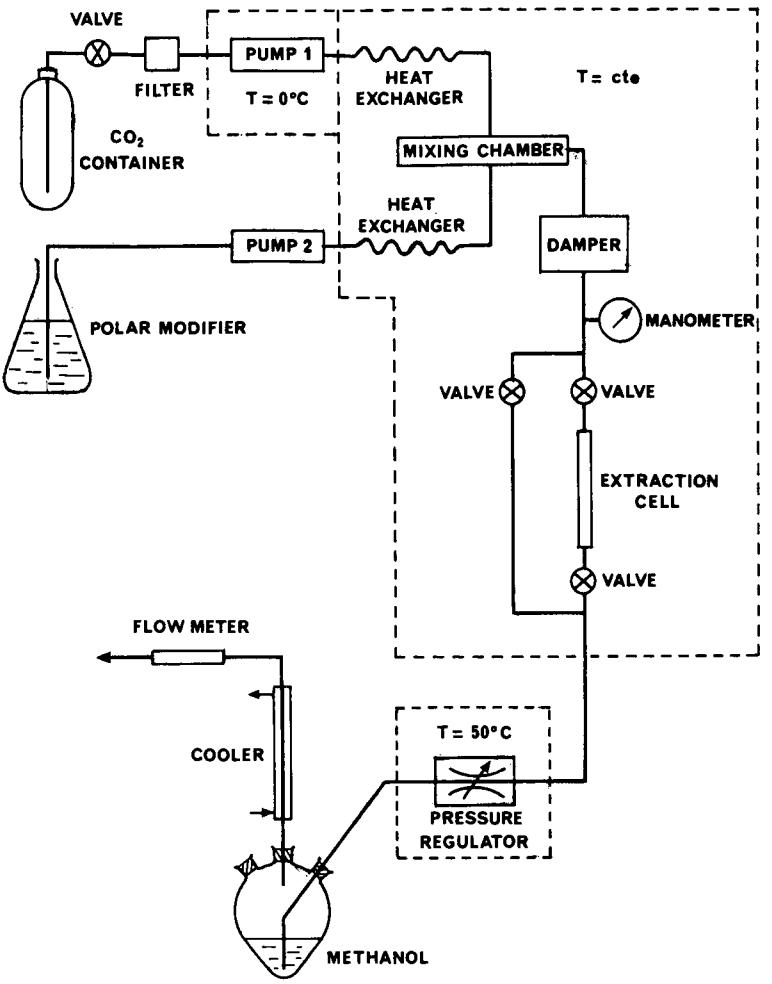


Fig. 1. Experimental set-up.

extraction phases containing a polar modifier. After decompression, gaseous carbon-dioxide and liquid modifier are obtained; the transfer of the extract is then due only to the liquid phase.

Chromatographic analysis of extracts

The chromatographic system used has been described elsewhere[26]. All chromatographic analyses were effected with a stainless-steel column (23 × 0.46 cm I.D.) packed with 5-μm LiChrosorb Si 60 silica (Merck). The mobile phase was a subcritical mixture of carbon dioxide–methanol–methylamine–water (83.00:15.90:0.14:0.96, w/w). A typical chromatogram of the extract aliquot is shown in Fig. 2, revealing three major alkaloids (thebaine, codeine and morphine) and two unidentified constituents.

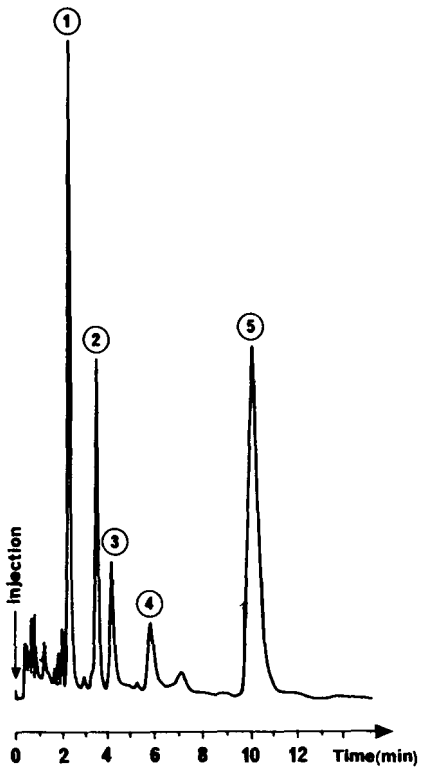


Fig. 2. Subcritical-fluid chromatography of a poppy straw extract. Column, 23 × 0.46 cm I.D.; stationary phase, bare silica LiChrosorb Si 60, 5 μm; mobile phase, carbon dioxide–methanol–methylamine–water (83.00:15.90:0.14:0.96, w/w); flow-rate, 8 ml min^{-1} CO_2 at 0°C; mean pressure, 220 bar; temperature, 41°C; detection, UV at 220 nm. Solutes: 1 = thebaine; 2, 4 = unidentified; 3 = codeine; 5 = morphine.

RESULTS AND DISCUSSION

The first experiments were performed with pure carbon dioxide, fused-silica capillaries and tapered 1/16-in. stainless-steel tubes maintaining the pressure above 200 bar in the extraction cell. No significant extraction was observed, in agreement with results of Stahl *et al.*[21]. Therefore, we studied the influence of various polar modifiers which could increase the dissolving power of the extractant for alkaloids. Owing to the high polar modifier contents, it must be noted that the extraction phase was in a near-critical but subcritical state rather than in a supercritical state[27].

Influence of polar modifiers

Three modifiers were considered: methanol, methylamine and water. All experiments were performed with a constant mass flow-rate through the extraction cell.

Influence of methanol. Mobile phases consisting of mixtures of carbon dioxide and methanol from 90:10 to 50:50 (w/w) were percolated through the vegetable

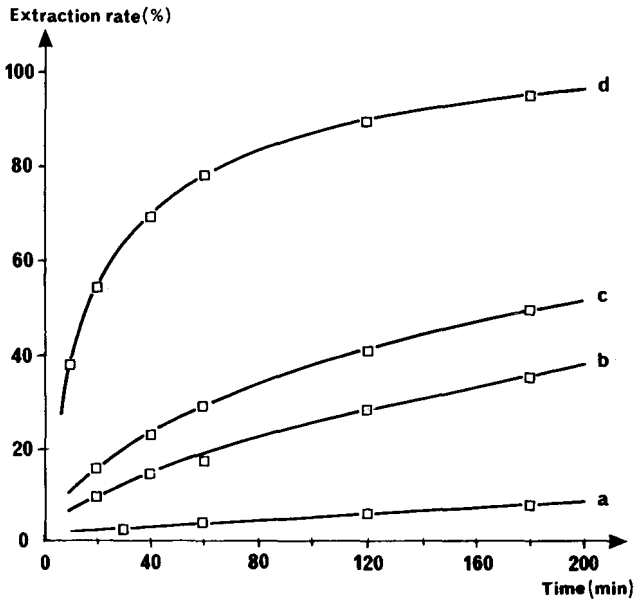


Fig. 3. Influence of methanol on the extraction curves of morphine at constant mass flow-rate (the extraction rate is the ratio of the amount extracted to the maximum extractable amount). Poppy straw granulometry, 250–500 μm; extraction pressure, 200 bar; temperature, 40.5°C; mass flow-rate, 2.11 g min^{-1}. Extractant, carbon dioxide–methanol: (a) 90:10; (b) 82:18; (c) 75:25; (d) 50:50 (w/w).

material. Kinetic extraction curves for morphine are given in Fig. 3. They show that very high percentages of methanol are necessary to ensure quantitative extraction in less than 20 min. The extraction phase would then be nearly a conventional liquid and all the potential gain in mass transfer would be lost. It was therefore necessary to find a polar modifier efficient at lower concentrations.

Influence of methylamine. In the subcritical fluid chromatography of opium-alkaloids, methylamine has been proved to be a very efficient polar modifier even at low concentrations[26], so we added methylamine to the extraction mixture. As shown in Fig. 4, the addition of a small amount of methylamine and water to the mobile phase considerably increased the extraction power: a mixture of 25% methanol, 0.22% methylamine and 0.34% water having the same effect as 50% methanol.

In spite of its strong extraction power, the methylamine–water mixture had the severe drawback that morphine was particularly sensitive to light in the presence of the amine (90% degradation after exposure to light for 3 h). Therefore, the methylamine–water mixture was not chosen as the final polar modifier.

Influence of water. Increasing the water content in the extraction fluid increased the extraction rate at a given time for alkaloids, as shown in Fig. 5 for thebaine. A similar behaviour was observed for all alkaloids. With 10% (w/w) water in the mobile phase, the time necessary to obtain a quantitative extraction is less than 30 min. Without water, even after 180 min quantitative extraction is not achieved.

However, even with methanol in the mobile phase, 10% water appeared to be the maximum value, higher concentrations resulting in degradation of the vegetable

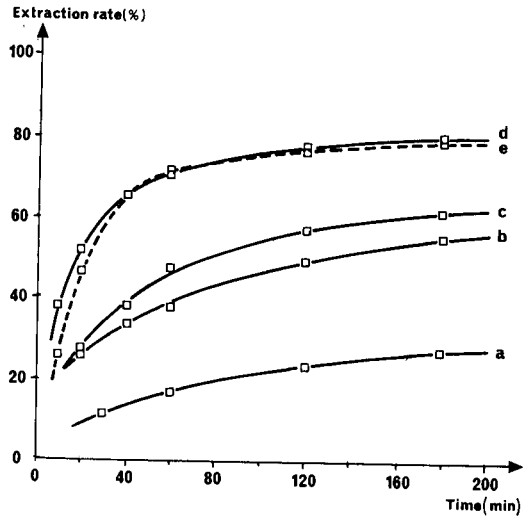


Fig. 4. Influence of methanol and methylamine on the extraction curves of codeine at constant mass flow-rate. Conditions as in Fig. 3. Extractant for curves (a)–(d) as in Fig. 3; (e) carbon dioxide–methanol–methylamine–water (75.00:24.44:0.22:0.34, w/w).

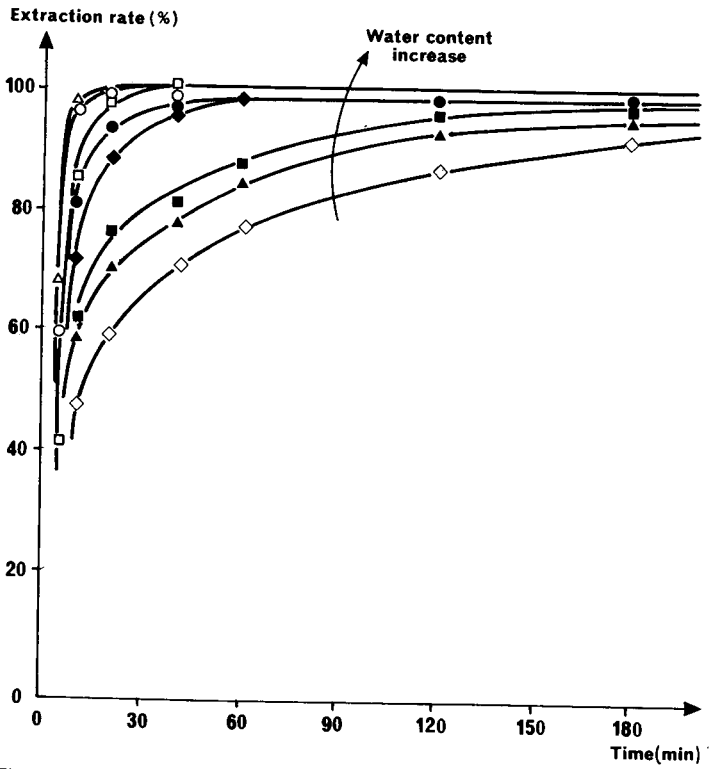


Fig. 5. Influence of water on the extraction curves of thebaine at constant mass flow-rate. Conditions as in Fig. 3 except extractants, carbon dioxide–methanol–water (◇) 50:50:0; (▲) 50:49.5:0.5; (■) 50:49:1; (◆) 50:46:4; (●) 50:44:6; (□) 50:40:10; (○) 50:36:14; (△) 50:32:18 (w/w/w).

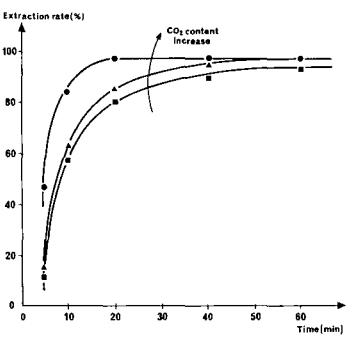
material with solid particles being collected in the three-necked flask. In addition, with more than 10% water in the extraction phase, the pressure regulator did not work properly.

Role of carbon dioxide

As morphine alkaloids are polar, it was clear that the higher the polar modifier flow-rate the faster would be their quantitative extraction. However, it was of interest to determine the extent to which the addition of carbon dioxide to the extraction phase could improve the transport phenomena. Fig. 6 shows the evolution of the kinetic extraction curves for morphine when the modifier flow-rate was maintained constant at 1.28 ml min^{-1} and the carbon dioxide flow-rate was increased from 0 to 5.2 ml min^{-1}. From 0 to 2.6 ml min^{-1}, the addition of carbon dioxide gave a reduction of a factor of 3 in the time required for quantitative extraction (from 60 to 20 min). Above 2.6 ml min^{-1}, carbon dioxide appeared to have no significant influence.

We also studied the influence of carbon dioxide during the extraction. The methanol flow-rate was maintained constant, but its concentration varied from 100 to 8% owing to the increase in the carbon dioxide flow-rate. Experiments were performed for three extraction times and the results are presented for thebaine in Fig. 7. It can be seen that the shorter the extraction time, the greater is the possible gain achieved by the addition of carbon dioxide to pure methanol. For an extraction time of 15 min, 72% carbon dioxide in the extraction phase tripled the extraction rate obtained with pure methanol; at a time of 60 min, the maximum gain was obtained with only 35% carbon dioxide in the extractant and the extraction rate increased by a factor of 1.2.

From this study, it can be deduced that the contributon of carbon dioxide to the extraction is probably only a volume effect; at the beginning of the extraction, carbon dioxide improves the transport of the modifier into the vegetable matrix and hence is favourable for the extraction. In contrast, at the end of the extraction, the solvating



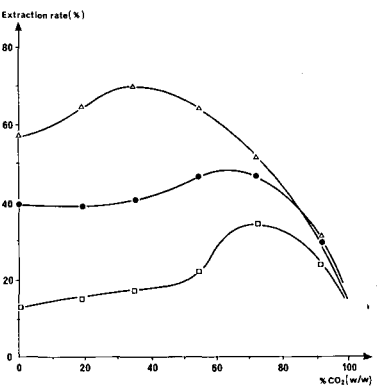


Fig. 6. Influence of carbon dioxide on the extraction curves of morphine at constant flow-rate of polar modifier, methanol–water (84:16, v/v) at 1.28 ml min^{-1}. Carbon dioxide flow-rate: (■) 0; (▲) 0.6; (●) 2.6, 3.9 and 5.2 ml min^{-1} at 0°C. Other condition as in Fig. 3.

Fig. 7. Influence of carbon dioxide content on the extraction rate of thebaine at constant flow-rate of methanol for three extraction times. Operating conditions as in Fig. 3 except flow rates: methanol, 0.35 ml min^{-1}; carbon dioxide, 0.07–3.30 ml min^{-1} at 0°C. Extraction times: (□) 15; (●) 30; (△) 60 min.

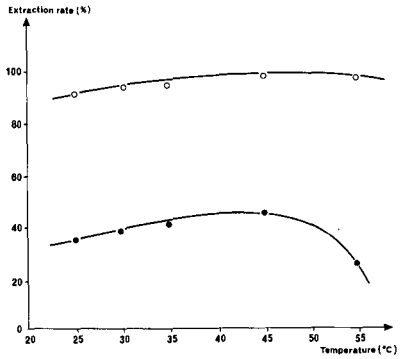


Fig. 8. Influence of temperature on morphine extraction rates. Extraction pressure, 200 bar; extractant, carbon dioxide–methanol–water (70:24:6, w/w/w); flow-rates, carbon dioxide, 2.6 ml min^{-1} at 0°C and polar modifier 1.28 ml min^{-1}. Extraction times: (●) 5; (○) 40 min.

power of the extractant is the major parameter and carbon dioxide which has a low polarity, is less beneficial for the extraction process.

These hypotheses were confirmed by thermodynamic results. Pressure (from 130 to 290 bar) was found to have no real influence on the curves shown in Fig. 7 (slightly higher extraction rates being obtained, however, near 200 bar). The influence of temperature was studied from 25 to 65°C with a carbon dioxide–methanol–water (70:24:6, w/w/w) extraction phase at 200 bar. The results are shown in Fig. 8. For a 5-min extraction, a maximum appears at *ca.* 45°C; for a 40-min extraction, in contrast, the temperature seems to have no real influence. These results confirm that the physical state of carbon dioxide only plays a role during the first few minutes of the extraction.

Optimized extractant

From the preceeding results two main conclusions can be drawn: the solvating power of the extractant is due only to the polar modifier, and the greater the modifier flow-rate the greater is the extraction rate at a given time; carbon dioxide at 45°C and 200 bar can increase the extraction rate at the beginning of the extraction by improving the transport of the polar modifier into the vegetable matrix.

These considerations are illustrated in Fig. 9. It can be seen that quantitative extraction can be achieved with 1.28 ml min^{-1} of pure modifier in about 60 min. This time can be reduced to 20 min by tripling the volume flow-rate, either with pure modifier or with carbon dioxide. It must be noted that, although carbon dioxide has a low polarity, it does not affect the extraction power at the end of the extraction.

Finally, with 1.28 ml min^{-1} of polar modifier [methanol–water (84:16, v/v)] quantitative extraction of the three major alkaloids in the poppy straw sample was achieved under optimum conditions in 20 min with an extraction phase composed of carbon dioxide–methanol–water (70:24:6, w/w/w). An increase in the carbon dioxide concentration did not improve the efficiency of the extraction. Quantitative results obtained for thebaine, codeine and morphine with this procedure were in very good agreement with those given by the classical liquid–solid extraction method (less than a 3% difference).

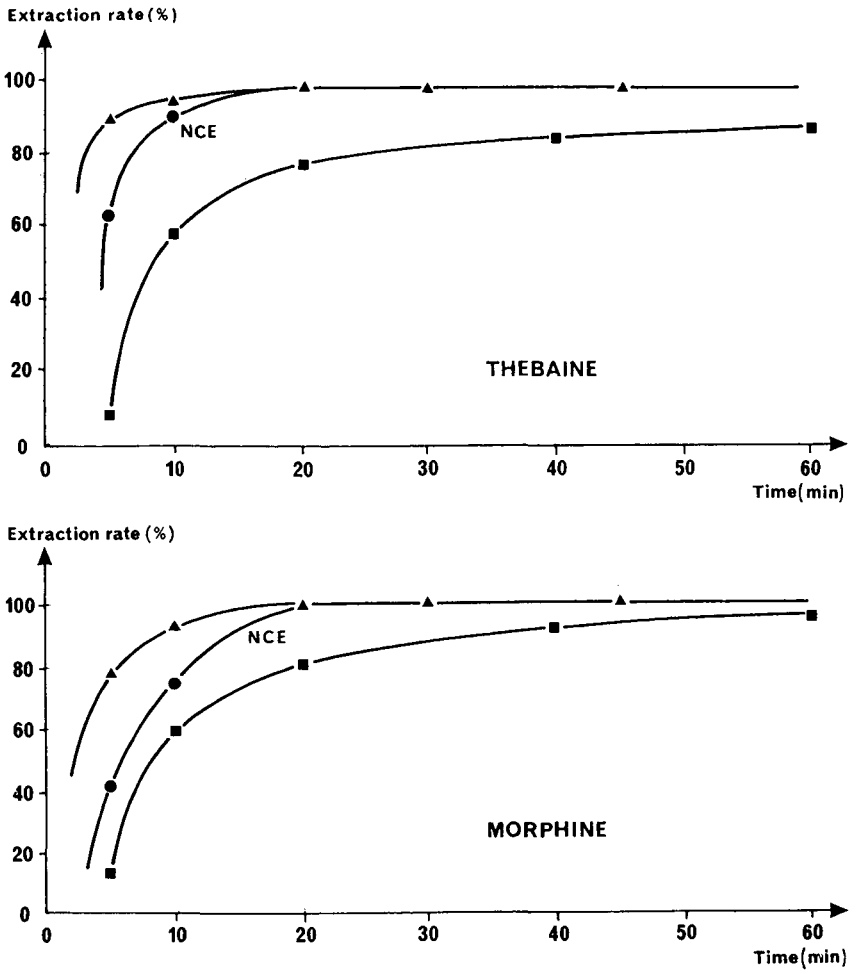


Fig. 9. Comparison of liquid and near-critical (NCE) extractions of thebaine and morphine. Pressure, 200 bar; temperature, 45°C; polar modifier, methanol–water (84:16, v/v). Flow-rates: liquid extraction (pure polar modifier), (■) 1.28 and (▲) 3.88 ml min^{-1}; near-critical extraction (●), polar modifier 1.28 ml min^{-1} and carbon dioxide 2.6 ml min^{-1} at 0°C.

CONCLUSION

It has been demonstrated that carbon dioxide cannot be considered as an extractant of morphine alkaloids from vegetable material but as a cheap and efficient transporting agent for the extraction solvent. Because of its high diffusivity, carbon dioxide improves the penetration of the extractant into the vegetable matrix and then, by decompression, the gas is very easily removed from the extracts. In 20 min, thebaine, codeine, and morphine can be extracted quantitatively by an extractant consisting of carbon dioxide–methanol–water (70:24:6, w/w/w) at 45°C and 200 bar.

As a similar extraction can be achieved with the pure liquid polar modifier at the same total volume flow-rate, near-critical fluid extraction may appear to be more difficult to perform. However, the entire extraction process was performed using a current chromatographic device and there are two main advantages of a near-critical extractant: from an economical point of view, it is of interset to replace a.significant fraction of methanol in the extraction phase (up to two thirds) by carbon dioxide; and the evaporation of carbon dioxide after decompression leads to an extract three times more concentrated than that with the liquid extraction procedure.

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CHROM. 22 249

Isolation of tocopherols from wheat germ oil by recycle semi-preparative supercritical fluid chromatography[a]

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SUMMARY

Isolation of tocopherols from wheat-germ oil was achieved by recycle semi-preparative supercritical fluid chromatography using two 250 mm × 10 mm I.D. columns packed with 5-μm silica gel. β-Tocopherol was fractionated after recycling twice and α-tocopherol after two additional recycles. Peak assignment was performed by comparing the UV spectra of the fractionated substances, which were obtained during the chromatographic run using a photodiode-array UV detector, with those of standard tocopherols. The identification of the fractionated substances was confirmed from the IR spectra. The purities of α- and β-tocopherols were found to be about 85 and 70%, respectively, by capillary gas chromatography.

INTRODUCTION

We have previously reported[1,2] a method for the extraction and fractionation of substances from complex mixtures such as natural products by using supercritical carbon dioxide both as an extraction medium and as a mobile phase in preparative chromatography, and we have demonstrated the enrichment of tocopherols from wheat germ. The method was based on coupled supercritical fluid extraction (SFE)–preparative supercritical fluid chromatography (SFC). It was reported[2] that tocopherols were enriched from 0.05 to about 5%, *i.e.*, by a factor of 100, in a single run of coupled SFE–preparative SFC. However, even after repeated chromatography of the α-tocopherol fraction, the final concentration was only about 20%, owing to the low initial concentration (0.05%) of the compounds in the complex matrix of wheat germ.

The major components of wheat-germ oil are triglycerides with various lengths of alkyl chains. These are co-eluted as a broad peak with small peaks on it, resembling a gel-permeation chromatogram of an oligomer mixture. This broad peak interferes with the isolation of tocopherol peaks in preparative separation. In order to separate tocopherol peaks from the triglyceride peak in preparative SFC, a highly efficient

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column with a high loading capacity is required. However, the use of a long column packed with microparticulate (less than 5 μm) material results in a decrease in the column efficiency owing to too high a column pressure drop (ΔP), as reported by Schoenmakers[3] and Schoenmakers and Uunk[4].

Recycle operation is very suitable for preparative SFC, because the method offers a high plate number without increasing the column pressure drop (ΔP), as was reported previously[5]. In addition, the consumption of mobile phase fluid can be drastically reduced. In this paper we describe the isolation of tocopherols from wheat-germ oil by recycle semi-preparative SFC.

EXPERIMENTAL

Materials

Wheat-germ oil was extracted and tocopherols were enriched by the method described previously[2]. The tocopherol concentration of the oil was determined to be about 4% by high-performance liquid chromatography (HPLC).

Chemicals

Standard-grade carbon dioxide was purchased from Toyoko Kagaku (Kawasaki, Japan) and was used as the mobile phase. HPLC-grade ethanol (Wako, Osaka, Japan) was mixed with carbon dioxide as a modifier solvent. HPLC quantitation-grade α- and β-tocopherol standards (purity 98% by HPLC) (Eizai, Tokyo, Japan) were used as the standard samples for the UV and IR spectrometric analyses.

Potassium bromide powder used in the diffuse-reflectance infrared (IR) spectral measurements was from Jasco (Hachioji, Tokyo). Chloroform of special reagent grade (Wako) was used for dissolving samples for the IR measurements.

Apparatus

Two Jasco SuperMegaPak SIL columns, 250 mm × 10 mm I.D., were used for the SFC separations. The packing material was 5-μm silica gel.

Recycle SFC system. A flow diagram of the recycle semi-preparative SFC system is shown in Fig. 1. It is similar to that used in our previous study[5], but an additional column (10′) is placed between the switching valve (13) and in-line pump (8) (Jasco 880-PU HPLC pump with modification for alternative dual-piston operation). This modification is required in order to have the pump serve as an in-line pump, because such a pump is needed to suck and discharge fluid simultaneously in order to circulate the fluid in a closed loop line without causing pressure fluctuations.

The column serves to retain components that have only weak retentions on it. This is very important for the practical application of recycle SFC. Capacity factors (k') of peak components may be in the range from 0 to 10 when natural products are involved. This means that the migration velocities of the components in a column vary by the same factors. As a result, components with smaller capacity factors often pass through the column several times before components with greater capacity factors are eluted from the column. This means that some components which have already been separated can be re-mixed as the number of recycles increases. Therefore, some means of retaining the components with smaller k' is required in order to operate recycle SFC successfully.

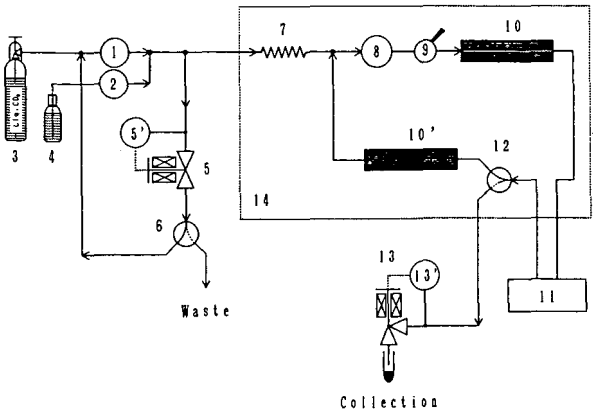

Fig. 1. Flow diagram of recycle preparative SFC. Components: 1 = CO_2 pump; 2 = modifier pump; 3 = liquid CO_2; 4 = modifier solvent; 5 = back-pressure regulator; 5′ = pressure transducer for controlling 5; 6 = switching valve; 7 = pre-heating coil; 8 = in-line pump; 9 = injector; 10 = first separation column; 10′ = second separation column; 11 = photodiode-array UV detector; 12 = switching valve; 13 = back-pressure regulator for collection; 13′ = pressure transducer for controlling 13; 14 = air circulating oven.

Liquid carbon dioxide from a cylinder (3) and modifier solvent (4) are delivered respectively by pumps 1 (Jasco 880-PU with head cooled to −5°C) and 2 (standard 880-PU) to the recycling flow line in the constant-flow mode. The fluid pressure is controlled by a back-pressure regulator (5) (Jasco 880-81), whose operating principle is based on flow switching[6], feeding back the excess amount of mobile phase to the inlet of pump 1 or passing it to waste from the outlet of the regulator via switching valve 6 when a modifier solvent is used.

In the recycling flow line, the in-line pump (8), injector (9), separation column (10), photodiode-array UV detector (11), switching valve (12) and second column (10′) are placed in series and connected in a closed loop. A back-pressure regulator (13) (Jasco 880-81) is connected to switching valve 12 for collection (fractionation) and/or passage to waste of peak components.

By means of this arrangement, the components having smaller k' value can be retained in column 10′ whereas the target or unwanted peak components are passed to the back-pressure regulator 13, preventing the components with smaller k' from being re-mixed and from leaving column 10 together with the target components. The significance of this arrangement is that the fluid flow is completely stopped although the pressure is maintained by the fluid delivery system even while the target is being collected.

Infrared spectrophotometer. A Jasco Model IR-700 grating infrared spectrophotometer was used with a diffuse reflectance accessory (Model DR-81) for the measurement of IR spectra of the fractionated substances and standard tocopherols.

Gas chromatograph. A Hewlett-Packard (Avondale, PA, U.S.A.) Model 5890A gas chromatograph was used with flame ionization detection (FID). A J&W (Folsom, CA, U.S.A.) DB-5 capillary column (15 m × 0.25 mm I.D.) with 5% diphenyl–95% dimethylpolysiloxane phase (0.1-μm thickness) was used for the examination of the purities of fractionated tocopherols.

RESULTS AND DISCUSSION

About 20 g of wheat-germ powder were subjected to SFE–preparative SFC in order to prepare wheat-germ oil, and the tocopherol-rich fraction was collected. Table I shows the mass balance for the process.

Fractionation of α- and β-tocopherols -

About 200 mg of the tocopherol-rich portion of the oil were collected as reported previously[1,2] and 98 mg of the fraction were subjected to recycle SFC. Fig. 2 shows the chromatogram and the contour plot obtained in the recycle operation. The carbon dioxide flow-rate was 5.0 ml/min, the modifier flow-rate 0.20 ml/min and the in-line pump flow-rate 5.0 ml/min. The first back-pressure was set at 25 MPa and the second at 20 MPa.

As shown in the chromatogram monitored at 220 nm, there is a broad peak with small peaks superimposed on it. The broad peak consists of triglycerides, which are the main constituents of the oil, and the first and second peaks on the tailing part of the peak are assumed to represent α- and β-tocopherols, because they have UV absorption maxima at *ca.* 290 nm and have similar retention times to those obtained in preliminary experiments under the same conditions without recycling. The procedure for recycling and fractionation was as follows:

(1) The first portion, triglycerides, eluting in the period between 0 and 6.5 min, shown as A, was passed to waste.

(2) The second portion, shown as B, containing α- and β-tocopherols, was passed to the second column to be retained.

(3) While the tocopherols were retained in the second column, the third portion shown as C was passed to waste.

(4) Then, only portion B was recycled.

A peak appeared at 42 min due to triglycerides, which were now completely separated from the tocopherol peaks at 62 and 71 min. The peak of triglycerides at 42 min was passed to waste. The contour plot showed that the β-tocopherol peak at 71 min contains no co-eluted compounds, and consequently it was fractionated.

The α-tocopherol peak at 84 min still contained some co-eluted compounds and was therefore recycled again. The peak at 109 min showed that the concomitants were

TABLE I

MASS BALANCE OF THE SFE–PREPARATIVE SFC PROCESS

Component	*Amount (g)*
Wheat germ before extraction	21.72
Wheat germ after extraction	17.65
Total extracts	4.07
Collected oil	1.78
Tocopherol-rich fraction	0.20
Water content in wheat germ[a] (10%)	2.17
Recovered extract	4.15 (102%)

[a] The water amount was obtained by weighing the material before and after the separate freeze-dry process. The value agreed with the supplier's value.

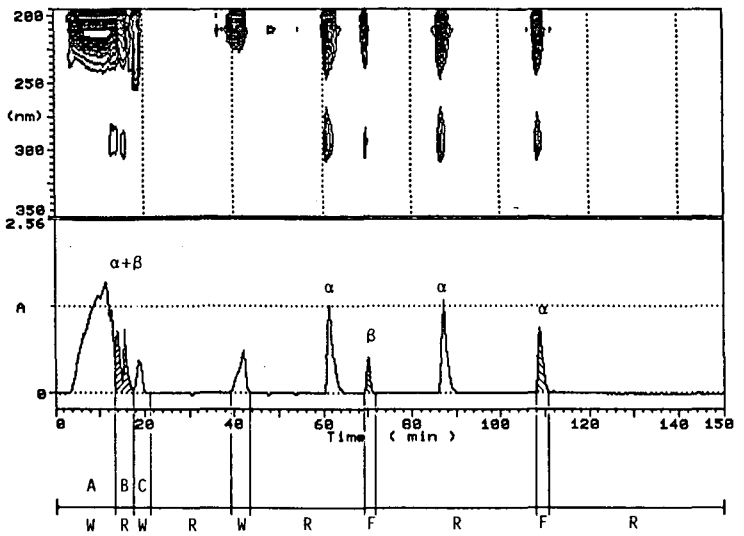

Fig. 2. Recycle chromatogram and contour plot of wheat-germ oil containing tocopherols. The axis and characters below the time axis represent the time frames for wasting, shown as W, recycling as R and fractionation as F. A represents the wasted portion of the peak of triglycerides, B contains tocopherols and C an unknown lipid.

separated from the main component, *i.e.*, α-tocopherol, and consequently it was fractionated.

The collected amounts were measured to be 1.0 mg for both tocopherols.

Peak assignment and determination of purity from UV spectral data

Peak assignment. Figs. 3A and 4A show the UV spectra of α- and β-tocopherols in the first cycle, *i.e.*, without recycling, taken at 13.51 and 15.35 min, respectively. Figs. 3B and 4B show the spectra after recycling taken at 108.9 and 69.51 min. Fig. 3C and 4C are spectra of the standard tocopherols obtained in a separate experiment. As can be seen, the spectra A are severely distorted in the region below 230 nm by co-eluted compounds, and steep rises toward lower wavelengths are seen in both spectra. β-Tocopherol was well purified after one additional cycle and the spectrum at 69.51 min shows a clear inflection point at 220 nm, which is also seen in the standard spectrum in Fig. 4C. On the other hand, α-tocopherol required two further cycles to be separated from co-eluted compounds which have relatively strong UV absorption at 210 nm. The spectrum at 108.29 min also exhibits a clear shoulder, as shown in Fig. 3B.

Purity check. In order to check the purities of the fractions, the peaks monitored just before fractionation were examined closely by using the data processor of the photodiode-array UV detector. Fig. 5A and B shows normal chromatograms at 230 and 295 nm, and ratio chromatograms between the above wavelengths over the time range 107–111 min for α-tocopherol and 68–71 min for β-tocopherol. The ratio chromatogram in Fig. 5A has a very rectangular shape, which means that the ratio of the UV absorptions at 230 and 295 nm remains constant. The absorption at 230 nm potentially represents tocopherols, fatty acids and their esters and the absorption at

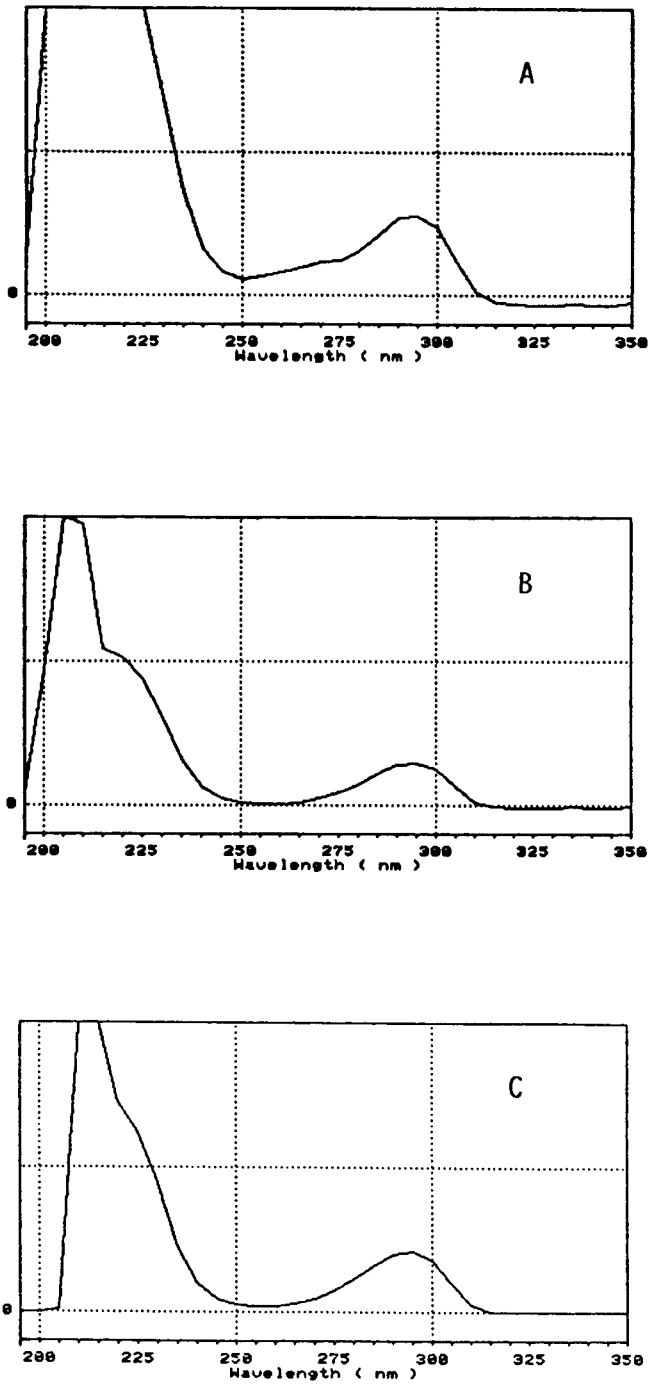

Fig. 3. UV spectra of the α-tocopherol peak in (A) the first cycle, (B) the last cycle (fractionated) and (C) the standard.

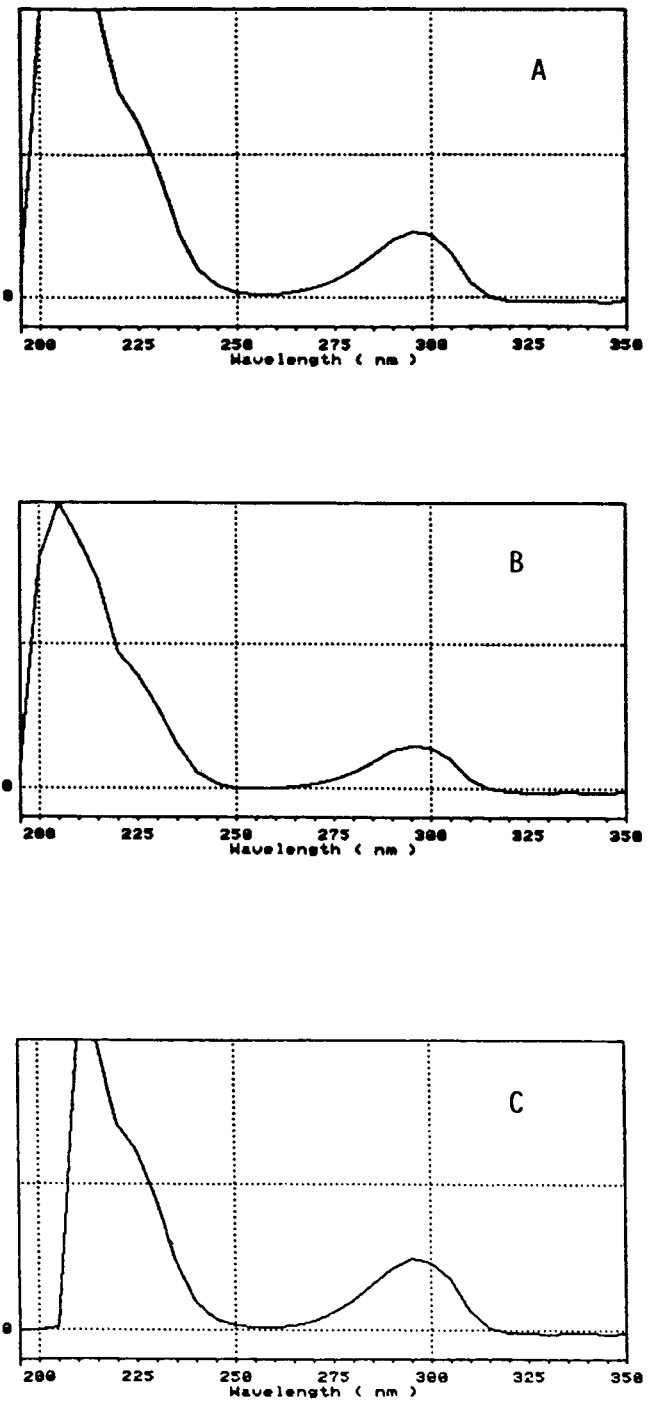

Fig. 4. UV spectra of the β-tocopherol peak in (A) the first cycle, (B) the last cycle (fractionated) and (C) the standard.

295 nm represents only tocopherols. Therefore, spectrometrically, this means that the UV spectrum along the time axis over the peak does not change its shape but only the intensity changes, indicating the absence of components with different UV spectra. The ratio chromatogram in Fig. 5B also shows a rectangular shape. However, it is slightly convex at the top, indicating that there is a small amount of other compounds which give rise to a higher absorption at 230 nm at the middle part of the peak. This peak may include β-tocopherol as the main component and a minor impurity, possibly free fatty acids and/or their esters.

For reference, a ratio chromatogram obtained in the first cycle obtained under the same data processing conditions is shown in Fig. 6. Tocopherols are considered to

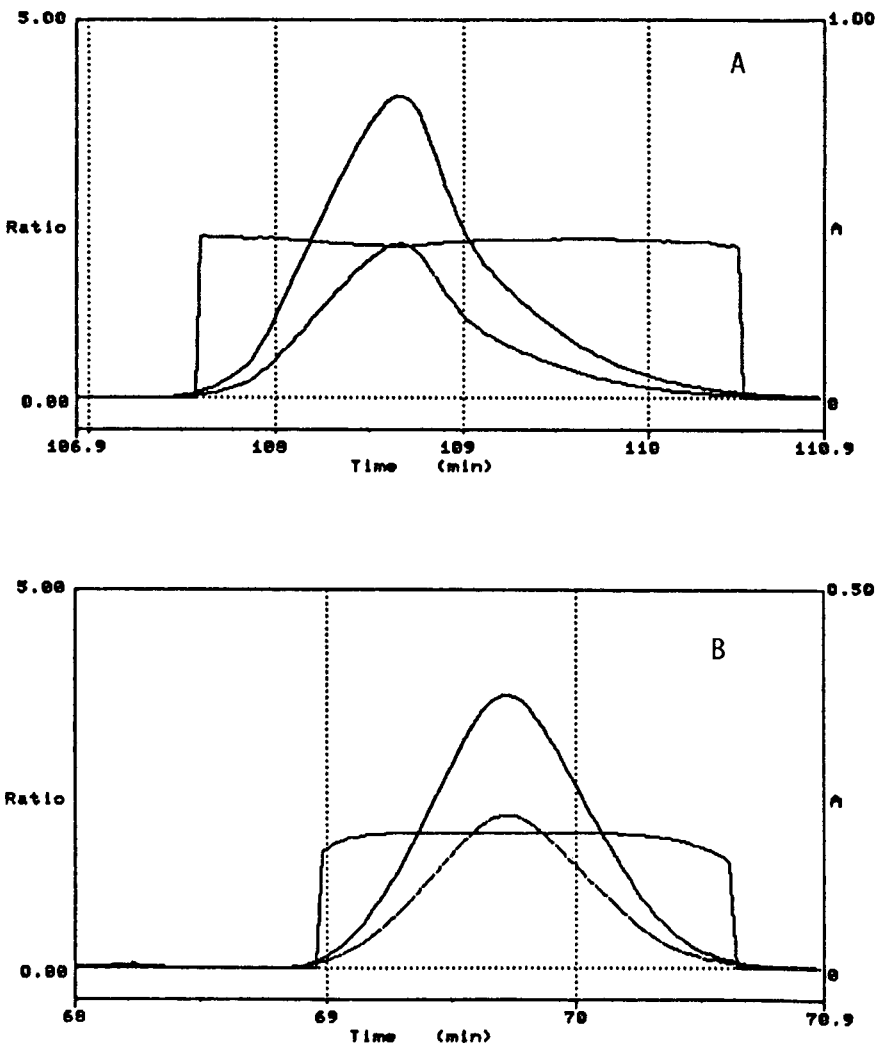

Fig. 5. Ratio chromatograms (230/295 nm) of (A) α-tocopherol peak and (B) β-tocopherol peak.

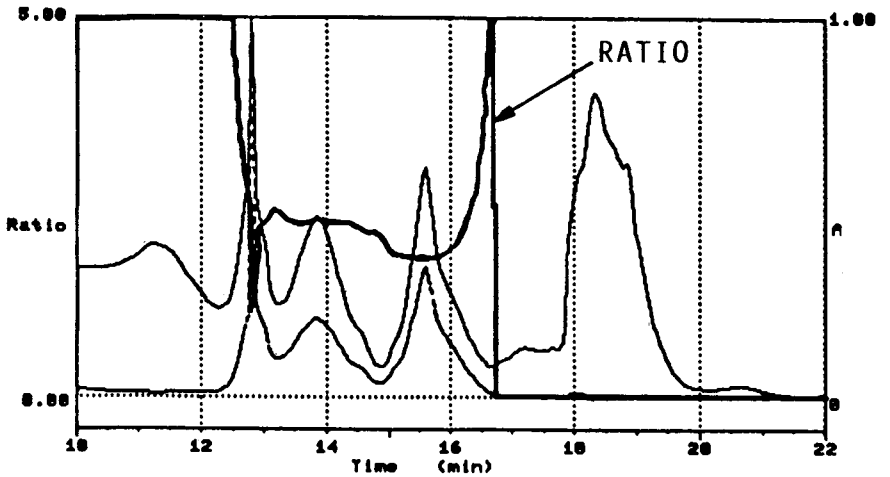

Fig. 6. Ratio chromatogram of the tocopherol-rich portion of the first cycle.

be eluted in the time range 12–17 min. However, the ratio chromatogram in this range is very unstable and wavy, resulting from co-eluted compounds, as a result of co-eluting compounds.

Determination of tocopherol contents in peaks. The amounts of the tocopherols included in the above peaks were calculated by the following procedure. Necessary values obtained from the literature[3] are listed in Table II.

The amounts of α- and β-tocopherols included in the peaks were calculated by using the above peak areas, peak heights, molar absorption coefficients, optical path length (0.5 cm) and the flow-rate (5.0 ml/min) set for the in-line pump, assuming that the concentration distributions of the peaks are Gaussian. The values obtained are 4.9 mg for α-tocopherol and 1.3 mg for β-tocopherol. The above calculation was based on the assumption that the molar absorption coefficients in the liquid solvent (ethanol) were similar to these in supercritical carbon dioxide with a similar density. The total amount of tocopherols, 6.2 mg, is in fair agreement with the amounts of tocopherols in the injected oil, which is about 4 mg (4% in 98 mg).

The amounts of fractionated tocopherols were weighed to be about 1 mg for both α- and β-tocopherols after eliminating ethanol, used as a modifier, by

TABLE II

VALUES USED IN THE CALCULATION OF THE AMOUNTS OF TOCOPHEROLS

Molecular weights and molar absorption coefficients (ε) are taken from ref. 7. Other values were obtained from the experimental results.

Compound	*Molecular weight*	*ε (l mol^{-1} cm^{-1})*	*Peak area*[a]	*Peak height*[a]
α-Tocopherol	430.7	$32.6 \cdot 10^3$ (292 nm)	24.8 A s	0.430 A
β-Tocopherol	416.3	$37.2 \cdot 10^3$ (296 nm)	9.33 A s	0.202 A

[a] A = absorbance.

evaporation. These values do not agree with the above values, and only about one third to half of the tocopherols in the peaks was recovered. The reason of this disagreement is considered to be that part of the tocopherols was removed by ethanol mist in the carbon dioxide vented to the waste line.

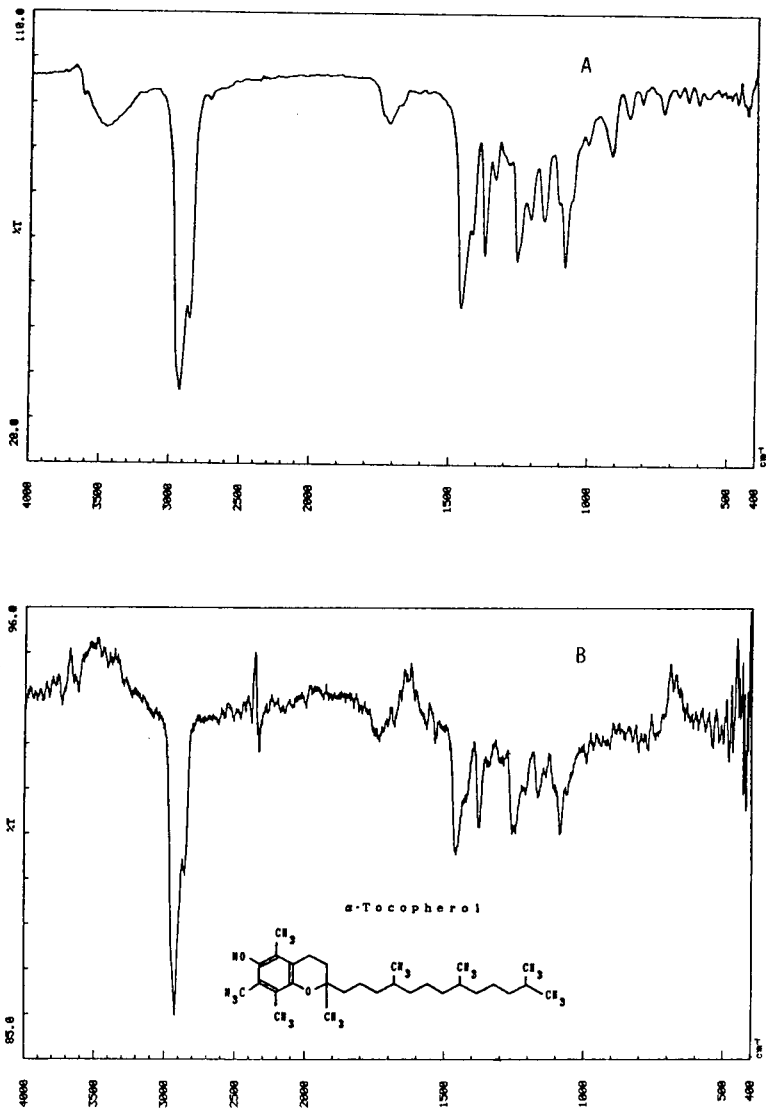

Fig. 7. IR spectra of (A) the fractionated α-tocopherol and (B) the standard. The sample was prepared by evaporating ethanol, which was added as a modifier, from the α-tocopherol fraction and dissolved in chloroform. The chloroform solution of the fraction was deposited on KBr powder. After drying at ambient temperature, the KBr powder containing the compound was subjected to IR measurement using a diffuse reflectance attachment.

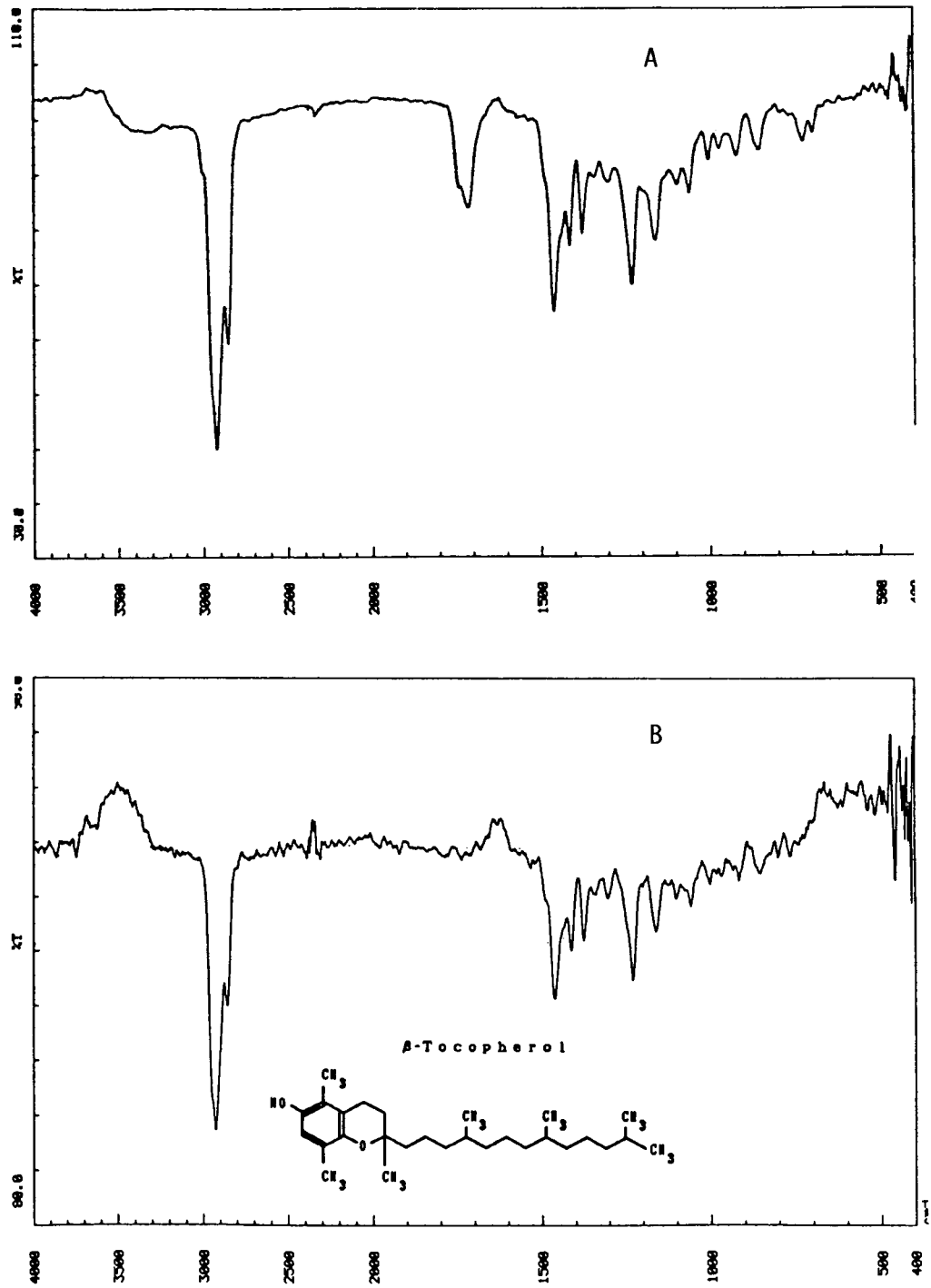

Fig. 8. IR spectra of (A) the fractionated β-tocopherol and (B) the standard. Conditions as in Fig. 7.

Identification from IR spectra

In order to confirm the peak assignment in the UV spectra, IR spectra of the fractionated and the standard tocopherols were measured by employing the diffuse reflectance method. Figs. 7A and 8A show the measured IR spectra of fractionated α- and β-tocopherols, respectively. Figs. 7B and 8B are the spectra of the standard compounds. These spectra are background compensated by subtracting the blank spectrum from the measured spectra. As shown, the measured spectra are almost identical with those of the standards. The characteristic IR absorption bands found in the literature[8] are listed in Table III. The relatively poor signal-to-noise ratios of the spectra of the standards are due to the low concentrations of the standard solutions which were originally meant for HPLC quantification.

All the absorption bands appeared in the standard and measured spectra except for those due to the hydroxyl group. This may be due to over-compensation of the absorption caused by water in potassium bromide and in the environment. Accordingly, the fractionated substances are confirmed to be α–β-tocopherols.

However, in addition to the characteristic bands, weak absorptions at about 1720 cm^{-1} are observed in all the spectra. This may be due to carbonyl groups, which give rise to a strong band at 1900–1550 cm^{-1} due to stretching of the C=O bond. This suggests either that very small amounts of free fatty acids and/or their esters exist as impurities, or that a small portion of tocopherols was already oxidized.

Gas chromatographic analysis of fractionated tocopherols

So far, the fractionated tocopherols have been investigated by spectrometric methods, with the following results: (i) identification was achieved by IR spectrometry; (ii) tocopherol purities are very high; and (iii) the IR spectra suggest the presence of compounds containing carbonyl groups as minor impurities.

Prior to the GC analysis of the fractionated tocopherols, a standard mixture containing various triglycerides and the SFE-extracted wheat-germ oil were separately injected to establish GC conditions that could elute these compounds. The following conditions were established: initial temperature = 250°C; temperature ramp rate = 10°C/min; initial time = 0 min; final temperature = 350°C; final time = 8 min; injector temperature = 300°C; detector temperature = 350°C; flame ionization detection (FID); carrier gas = helium at 150 kPa.

Fig. 9 shows the gas chromatogram of the fractionated α-tocopherol. As can be seen, there is only a single main peak and a series of very small peaks. The main peak is α-tocopherol and the small peaks may be fatty acid esters, including mono- and diglycerides. The IR spectrum shown in Fig. 7A supports this assumption. The purity of α-tocopherol is determined to be about 85% based on the area percentage, *i.e.*,

TABLE III

CHARACTERISTIC IR ABSORPTION BANDS (cm^{-1}) OF α- AND β-TOCOPHEROLS[8]

Compound	*OH*	*CH*	*C=C (arom.)*	*CH_3*	*CH_2*	*C–O (aryl)*	*C–O (alkyl)*	*CH*
α-Tocopherol	3310	2930	1620	1460	1375	1270	1160	850
β-Tocopherol	3320	3940	1595	1458	1373	1229	1158	860

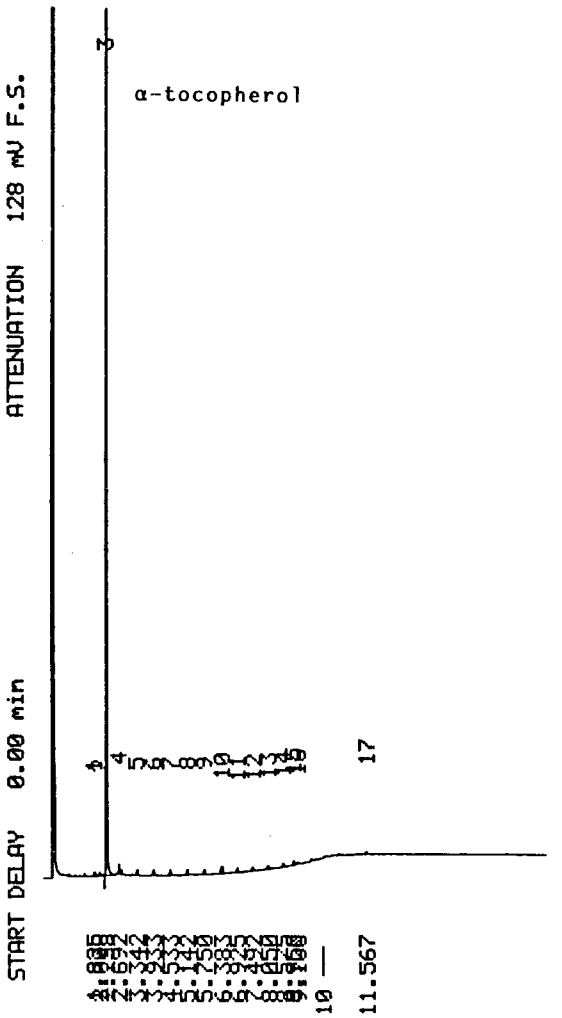

Fig. 9. Gas chromatogram of the fractionated α-tocopherol. A 1-μl volume of the fractionated α-tocopherol (obtained as an ethanol solution) was injected with a splitting ratio of 1:100. For other GC conditions, see text. Numbers at horizontal axis are retention times in min.

assuming that the response factors for FID and the injection splitting ratios of α-tocopherol and the impurities mentioned above are identical.

Fig. 10 shows a comparable chromatogram of β-tocopherol. There is also only a single main peak and a series of very small peaks. However, the retention times of the impurity peaks are very different from those for α-tocopherol. These may be fatty acid esters, possibly mono- and diglycerides with higher molecular weights. The purity of β-tocopherol is determined to be 70% based on the area percentage, assuming the same as above.

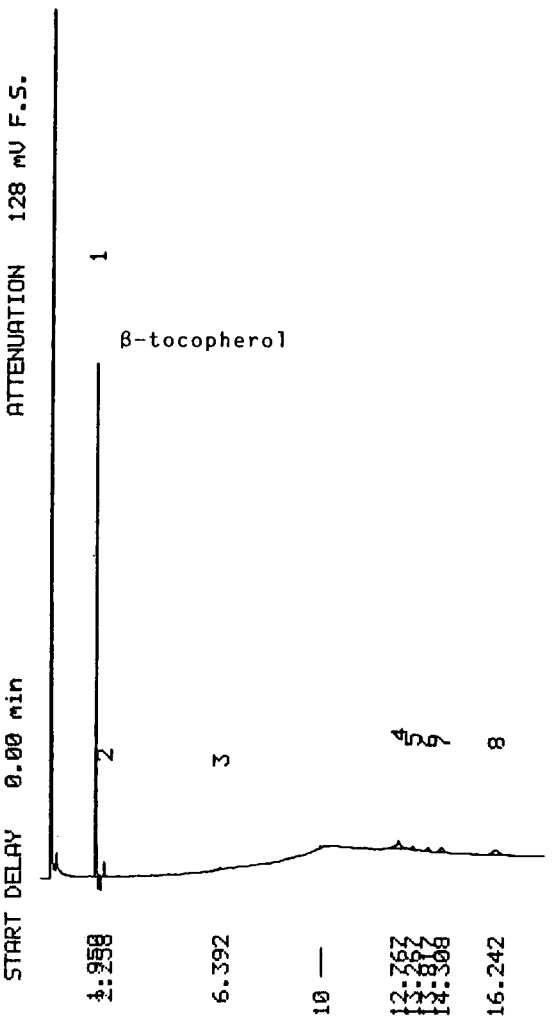

Fig. 10. Gas chromatogram of the fractionated β-tocopherol. Conditions as in Fig. 9.

CONCLUSION

The isolation of tocopherol from wheat-germ oil has been achieved successfully by recycle preparative SFC. The purities of α- and β-tocopherols are calculated to be about 85 and 70%, respectively. These values can be improved by performing a few additional cycles.

The recoveries during the fractionation of peaks are only 30–50%, which is unsatisfactory. This is probably because the compounds were removed by the ethanol mist in the vent line. In order to improve the recoveries, a cold trap may help.

Recycle preparative SFC is very suitable for the isolation of fat-soluble compounds from complex matrices, as demonstrated. Also, it is suitable for the

separation and fractionation of compounds of which the α-values are small and cannot be improved by varying the mobile-phase conditions, *i.e.*, by a change in density or temperature or the addition of a modifier solvent. In such a case, the column length has a major effect on the resolution. Recycle preparative SFC offers a high efficiency, because the pressure drop ΔP is minimized, regardless of the number of recycles, which is virtually equivalent to using a long column with a minimum pressure drop. Typical examples of such applications include the separation of optical isomers.

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Quantitative analysis of additives in polymers using coupled supercritical fluid extraction–supercritical fluid chromatography

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SUMMARY

A procedure is described for the quantitative analysis of additives in polymers by a coupled supercritical fluid extraction (SFE)–supercritical fluid chromatography (SFC) system. Various polyethylene and polypropylene samples from several manufacturers were extracted by SFE and the extracts analyzed by SFC. Successful extractions and analyses were performed on ten different additives ranging from butylhydroxytoluene (218 a.m.u.) to Irganox 1010 {pentaerythritol tetrakis[3-(3,5-di-*tert.*-butyl-4-hydroxyphenyl)propionate], 1178 a.m.u.}. Extraction efficiencies are generally greater then 92%. This technique provides a rapid and accurate alternative for investigators who may normally use a traditional solvent extraction method followed by chromatography or spectroscopy.

INTRODUCTION

Analysis of polymer additives is important in both research and quality control for manufacturers and users of various polymers including polyolefins, synthetic rubbers, polystyrene, etc. Raw materials and finished products are analyzed for these additives. The compounds have a wide variety of physical (*i.e.* volatility and molecular weight) and chemical (*i.e.* amides, esters) characteristics. Consequently, a number of different chromatographic methods have been used for analysis of polymer additives. Gas chromatography (GC) is limited to the separation of low-molecular-weight, volatile, thermally-stable compounds[1,2], although some compounds with a molecular weight greater than 1000 daltons have been analyzed using high-temperature GC[3]. The most widely used method of analysis for these compounds, high-performance liquid chromatography (HPLC), lacks a simple sensitive universal detector that is compatible with all liquid mobile phases[4–7].

More recently, supercritical fluid chromatography (SFC) using flame-ionization detection (FID) and Fourier-transform infrared (FT-IR) detection has been applied to the analysis of polymer additives[8,9]. Its was shown in these reports and it has been our experience that many of the more common polymer additives can be analyzed by SFC using a single mobile phase (CO_2), column type and chromatographic parameters.

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Off-line liquid phase extraction before analysis of these compounds in polymer samples generally involves many time consuming steps, including Soxhlet extraction, concentration, clean-up, reconcentration and reconstitution of the sample in an appropriate solvent for analysis by GC, LC or SFC. Hirata and Okamoto[10] have successfully used supercritical fluid extraction (SFE) as a single rapid method to collect additives for subsequent analysis by micro-LC. There have been a number of publications demonstrating the utility of SFE directly coupled with GC[11–15], LC[16,17] ad SFC[18–27] for facile analysis of a wide variety of analytes in complex matrixes.

In this work, we describe a method for the direct SFE–SFC analysis of additives in polymers. Analysis of polymer additives using a coupled SFE–SFC system is an effective alternative method. Additives are typically extracted under relatively mild conditions. Oligomers are also extracted, but at much higher pressures. This enables the investigator to selectively extract either oligomers or additives. When necessary, ultraviolet and flame ionization can be used in series as detectors for SFC, a single high-pressure extraction analysis can be employed to provide the concentration of a chromophoric additive against an oligomer fingerprint. Extraction–analysis times are generally under 1 h.

The coupled system was used because of the high degree of automation. When the SFE–SFC system is automated, sample handling is reduced to loading the extraction vessel with the sample. This aids in eliminating variations in quantitative results.

Using the coupled SFE–SFC system, the objective was to extract various additives from several commercial polymer samples and to quantitate the level of additives in the sample. In defining the analytical methods, the key questions addressed were: (1) Is the analysis reproducible? (2) Is the analysis quantitative? (3) Is the quantitation linear over the range of concentrations expected for the analysis? (4) What is the recovery level of the analytes of interest? (5) Will chromatographic efficiency degrade for the SFE–SFC analysis of a sample relative to direct injection SFC analysis of the standard?

EXPERIMENTAL

Materials

The equipment used (Fig. 1) was a Model 311B extractor/accumulator, a Model 10000 SFC–GC system, and a Model 747DS data system (all by Computer Chemical

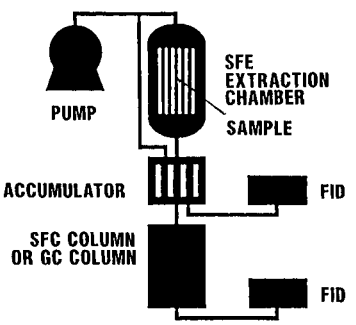


Fig. 1. Schematic of a coupled SFE–SFC system.

Systems, Avondale, PA, U.S.A.). The CCS Model 311B was equipped with a 0.5-ml volume extraction chamber and a 100 × 2 mm accumulation column containing 5-μm Nucleosil Cyano packing. The Model 10000 SFC–GC system was equipped with a post-column crimped stainless-steel restrictor calibrated to an expanded gas flow-rate of 20 ml/min at 2000 p.s.i. to 100 ml/min at 6000 p.s.i. (column oven temperature 150°C). The FID system was maintained at 350°C. UV detection was via a Linear™ UVIS 204 fitted with a high-pressure detector cell (Linear Instruments, Reno, NV, U.S.A.). Separations were achieved using a 250 × 1 mm Deltabond™ 300 Octyl column (Keystone Scientific, State College, PA, U.S.A.). Baker-analyzed HPLC-grade dichloromethane used to dissolve standards of the additives was purchased from VWR Scientific (Philadelphia, PA, U.S.A.). The supercritical fluid used for extraction and analysis was SFC grade from Scott Specialty Gases (Plumsteadville, PA, U.S.A.).

Creation of calibration curves for additives

Standards of the additives were prepared in dichloromethane. Concentrations varied according to additive concentrations in the polymer samples to be analyzed. A solution volume containing a known amount of the additive standard(s) was then applied via a microsyringe to a bed of quartz wool in the extraction vessel. The additives were extracted using supercritical CO_2 for 10 min at 50°C and 6000 p.s.i. The extract was accumulated by cryofocussing on the accumulator column at 10°C. When extraction was complete, the sample was desorbed at 50°C from the accumulator onto the analytical column for analysis. A second extraction–analysis was performed to determine whether all the additive standard(s) had been extracted from the quartz wool bed. In no case was residual additive detected.

Several differing amounts of the additives were extracted. These data points were plotted to provide a curve, the slope of which was an area response factor (μg additive/area counts) and could be compared directly to area counts observed in

TABLE I

RESULTS OF DUPLICATE SUPERCRITICAL FLUID EXTRACTIONS AND CHROMATOGRAPHY ON ERUCAMIDE STANDARDS OVER THE RANGE 12.5–100 μg

Linear regression: standard deviation = $2.6 \cdot 10^2$; slope = $1.4 \cdot 10^4 \pm 2.8 \cdot 10^2$ counts/μg; y-intercept = $4.2 \pm 1.6 \cdot 10^4$; correlation coefficient = 0.9

Aliquot (μ)	*Concentration (μg/μl)*	*μg*	*Area counts (10^5)*	*Percent difference*
2.5	5	12.5	1.9	0
2.5	5	12.5	1.9	
5.0	5	25.0	4.4	1.4
5.0	5	25.0	4.3	
5.0	10	50.0	7.6	0.9
5.0	10	50.0	7.6	
10.0	10	100.0	14.6	1.0
10.0	10	100.0	14.8	

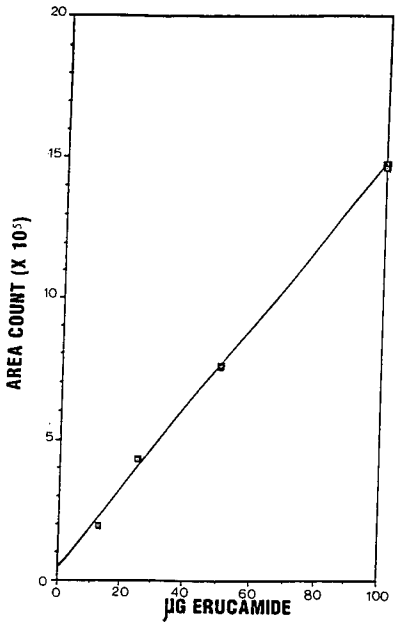


Fig. 2. Calibration curve of the data for the erucamide standards obtained from Table I. $y = 0.4 + 0.1\,x$; correlation coefficient = 0.9.

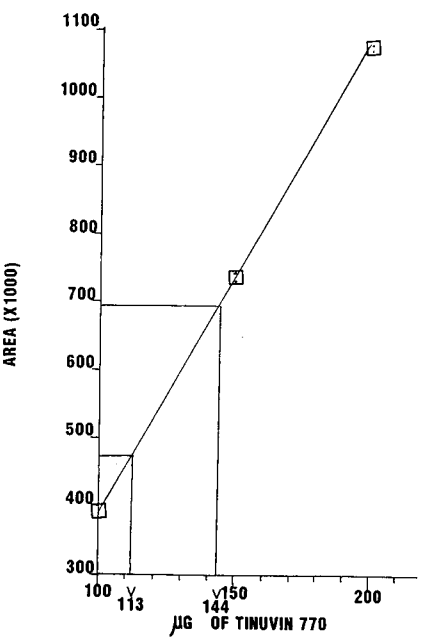


Fig. 3. Calibration curve for Tinuvin 770 over the range of 100–200 μg.

actual polymer samples. Table I shows the results of duplicate supercritical fluid extractions and chromatography on erucamide standards over the range of 12.5 μg to 100.0 μg. Fig. 2 is the calibration curve of the data for the erucamide standards obtained from Table I. Fig. 3 is a calibration curve for Tinuvin 770 over the range of 100–200 μg. Fig. 4 shows the calibration curve for Irgafos 168 and Irganox 1010 over the range of 10–60 μg.

SFE of polymer samples

When calibration curves were complete, ground samples of polymer (40–80 mesh) were placed in the extraction vessel. Extraction conditions varied from sample to sample, but generally fell into two categories: low-pressure and high-pressure extractions. Low-pressure extractions were carried our at 2000 p.s.i. for a duration of 30 min (expanded gas flow at the extractor restrictor was 80–100 ml/min). High-pressure extractions were performed at 6000 p.s.i. for 15 min (expanded gas flow at the extractor restrictor was 300–400 ml/min). In both cases, extraction temperature (50°C), accumulation temperature (10°C) and desorption temperature (50°C) remained constant.

RESULTS AND DISCUSSION

The first concern was reproducibility of the analysis. Fig. 5 shows the comparison of two extraction–analyses performed on the same polyethylene sample. Because

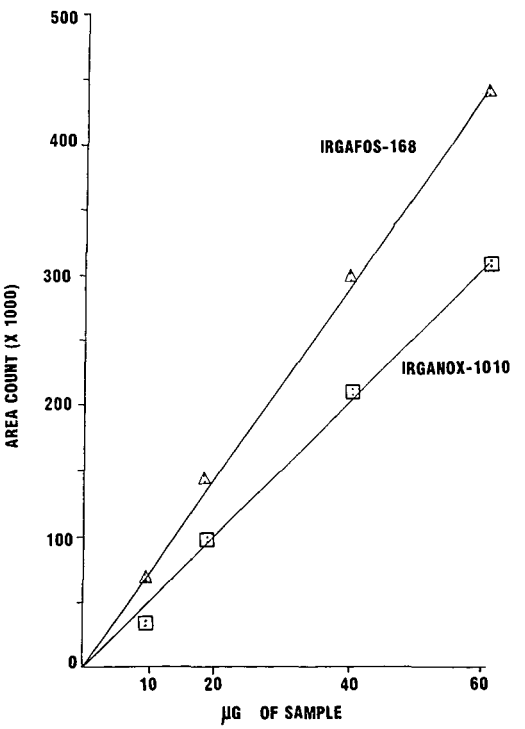


Fig. 4. Calibration curve for Irgafos 168 and Irganox 1010 over the range of 10–60μg.

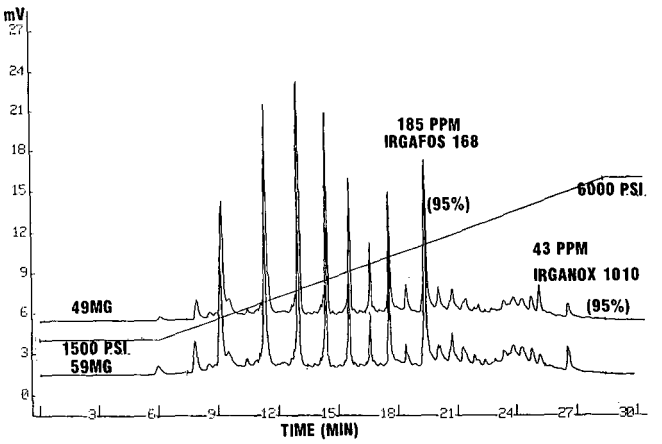


Fig. 5. Comparison of two extraction–analyses performed on the same polyethylene sample. Extraction parameters: 6000 p.s.i. for 15 min. at 50°C, desorption temperature 50°C. SFC parameters: 1500 p.s.i. starting pressure held for 6 min, then 200 p.s.i./min to 6000 p.s.i. Column: 250 × 1 mm Deltabond 300 Octyl, FID 350°C, oven temperature 150°C.

an area response factor is being used for quantitation and the response is linear over a wide range, it is not necessary to keep sample weight constant. In this case a 49 mg sample and a 59 mg sample were extracted. Using the area response factor, the concentrations of Irgafos 168 and Irganox 1010 determined experimentally were within 5% of concentration supplied by the manufacturer.

The next concern was the ability to achieve quantitative results. In addition to the results obtained in Fig. 5, Fig. 6 is a comparison of one 50-μg standard of erucamide to an extraction of a commercial polyethylene sample also containing 50 μg of

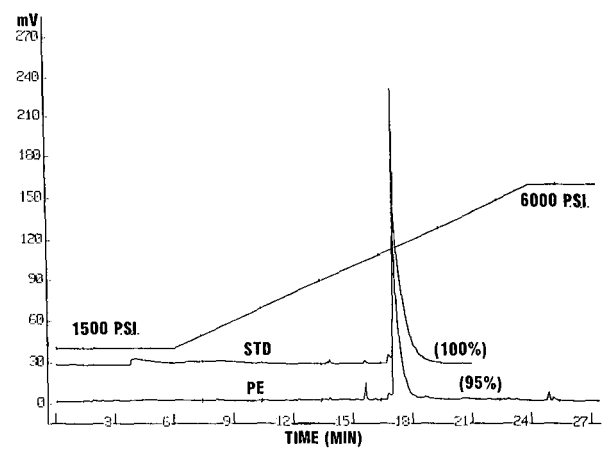


Fig. 6. Comparison of one 50-μg standard (STD) of erucamide to an extraction of a commercial polyethylene (PE) sample also containing 50 μg of erucamide. Extraction duration 10 min, other conditions as in Fig. 5.

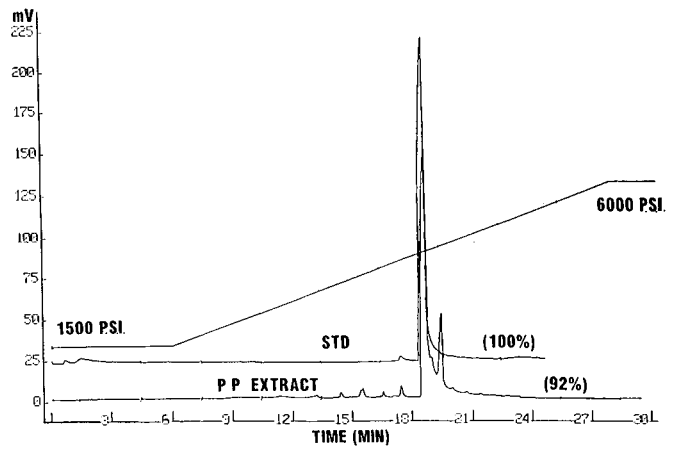
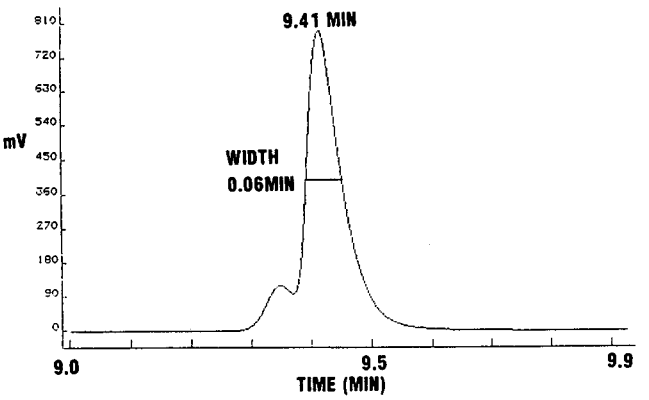


Fig. 7. Comparison of one 150-μg standard of Tinuvin 770 to an extraction of a commercial polypropylene (PP) sample also containing 150 μg Tinuvin 770. Conditions as in Fig. 6.



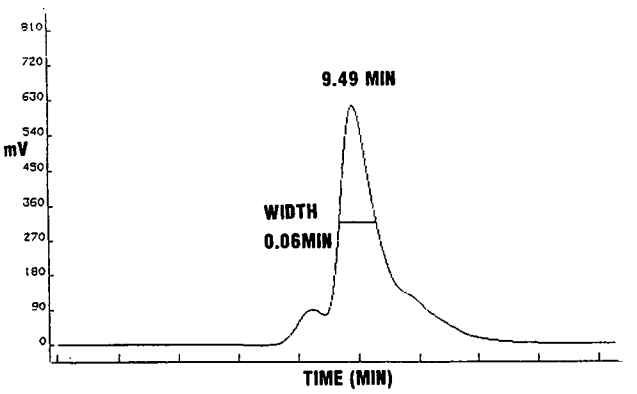


Fig. 8. Comparison of a direct injection SFC analysis (top) to a coupled SFE–SFC analysis (bottom) of Irgacure 651. SFC parameters as in Fig. 5, SFE parameters as in Fig. 6. Theoretical column plate count: SFC: 136 389; SFE–SFC: 134 207.

erucamide. The amount of erucamide in the polyethylene sample was 95% of that in the erucamide standard. Fig. 7 shows the analysis of Tinuvin 770 in polypropylene employing the same SFE–SFC method. In this case, recovery was 92% of the expected result, although no additional Tinuvin 770 was noticed in a subsequent extraction–analysis.

Linearity of the calibration is very important. When the calibration curve is linear and passes through the origin, an area response factor can be calculated using a single-point calibration. The examples in Figs. 2, 3 and 4 indicate that the quantitation is linear.

Figs. 5, 6 and 7 also provide data on the recovery level of the analytes of interest. The lowest recovery level was 92%, with higher values more common. All analyses were followed by second extractions in order to determine if an incomplete extraction had occurred. In the polymer samples, an additional 5–8% of the additives were observed in the second extraction. This is in agreement with an extraction efficiency of 92% or greater for the initial extraction.

Chromatographic efficiency of the coupled SFE–SFC system is comparable to that achieved by direct injection SFC. Fig. 8 is a comparison of an SFC injection to an SFE–SFC analysis of Irgacure 651. In both cases, the peak width at half-height remained 0.06 min, while the apparent theoretical column plate count of the SFE–SFC analysis was 98.4% of that achieved with the direct injection analysis. The difference observed in the retention time of 0.08 minutes between the SFC and SFE–SFC analyses is due to a slightly longer sample path when using the extractor. To compensate for this difference standards are generally run by spiking the extraction vessel as was done in this study.

Fig. 9 is a typical example an extraction–analysis of polyethylene using the coupled SFE–SFC system. In this case, three additives were succesfully extracted from the polymer matrix using a low-pressure extraction. Quantitation of these re-

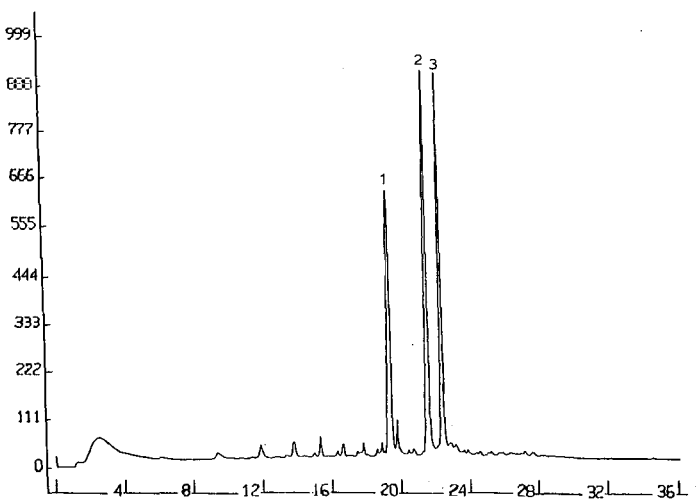


Fig. 9. Typical extraction analysis of three additives from a polyethylene sample. Extraction pressure: 2000 p.s.i., duration 30 min. All other parameters as in Fig. 5. Peaks: 1 = Tinuvin 326 (601 ppm); 2 = Irgafos 168 (737 ppm); 3 = Irganox 1076 (543 ppm).

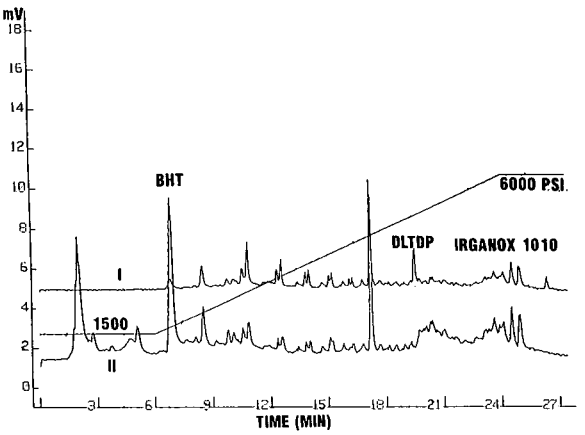


Fig. 10. Analysis of two polyethylene samples from the same manufacturer each containing different additives. Sample I contains dilaurylthiodiproprionate (DLTDP) and Irganox 1010, sample II contains butylhydroxytoluene (BHT). Conditions as in Fig. 6.

sults indicated that the concentration of the additives in the polyethylene were within 8% of the manufacturers formulation.

Fig. 10 is an analysis of two polyethylene samples from the same manufacturer, but containing different additives. Sample I contained dilaurylthiodipropionate and Irganox 1010 while Sample II contained butylhydroxytoluene. Note the similarities in the oligomer fingerprint, and also the presence of the large peak at *ca.* 18 min which could be an unidentified additive.

Fig. 11 is an example of the utility of a UV detector when the oligomers interfere with additive identification/quantitation. This is an atypical polyethylene sample containing an oxidative colorant which causes rapid degradation of the polymer. Usually the oligomer fingerprint is not as pronounced *versus* the additive peaks.

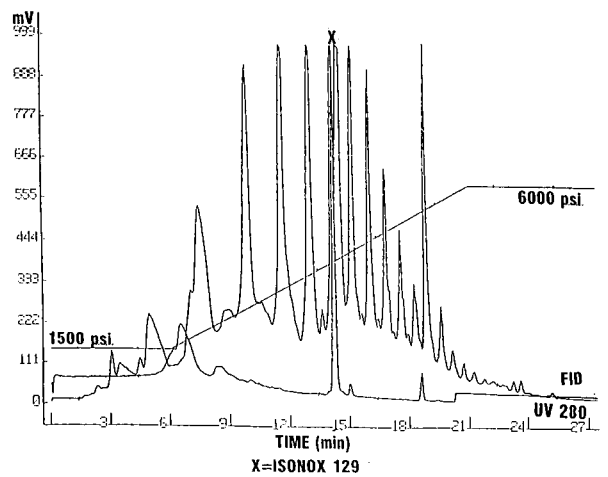


Fig. 11. The utility of a UV detector when oligomers interfere with additive identification/quantitation. Partially oxidized polyethylene. Conditions as in Fig. 9.

CONCLUSION

Successful on-line extraction and chromatographic analysis of additives in polymers was performed using coupled SFE–SFC. Accurate quantitation was achieved along with high extraction efficiency. This technique should prove a viable alternative to traditional off-line liquid-phase extraction and analysis methods. Continuing efforts are being focused on optimization of the procedure in order to allow investigators to further reduce method development time while maintaining high efficiency.

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Characterization of polymer additives by supercritical fluid chromatography and by liquid chromatography

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SUMMARY

Various polymer additives were characterized by chromatographic methods. As many of the additives lack chromophores for UV absorption, the detection has to be based mainly on refraction or light scattering in liquid chromatography (LC) and on flame ionization or light scattering in supercritical fluid chromatography (SFC). More than fifteen additives were analysed essentially by the same SFC method, except with varying density gradients, whereas two different columns and several different eluents were required in LC. This illustrates that SFC has an obvious advantage over LC for the characterization of this large group of lipophilic compounds.

INTRODUCTION

In order to improve the physical properties of synthetic polymers, most polymeric materials contain antioxidants, UV stabilizers, metal deactivators, slip agents, antiblock agents and antistatic agents. The need for such additives in polyolefins has been well studied[1]. Even if many additives have multiple functions, the number of additives routinely used in the manufacture of polyolefins exceeds 20, although not all added together, as some additives are better suited than others for a particular product or purpose. As the purity and amount of additive affect the properties of the polymer, there is a need for analytical methods to characterize the additive and also to determine the amount present. Further, many commercial additives are not single components, but may contain homologues or byproducts in significant amounts. For products used in food processing and packaging there is also a need to determine the amount of additives released to the foodstuff.

Owing to the relatively high molecular weights involved, liquid chromatography has been the most commonly used chromatographic technique for additives. Unfortunately, many additives show little UV absorption. Previously this led to the use of refractive index (RI) detectors, without gradient capability and with low sensitivity. Supercritical fluid chromatography (SFC) has recently been demonstrated to be valuable for this type of compound[2–5], particularly owing to the ability to use the mass-sensitive flame ionization detector.

In an industrial production control laboratory, there is always a need for several

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analytical techniques operating simultaneously, as many operators may use the systems around the clock. Thus, if one chromatographic system could be used for most of the additives, this would represent a major advantage over a system with dedicated instruments and methods for each additive. Based on earlier experience in SFC with some additives[2,5], the main purpose of this study was to examine if essentially one technique could be utilized for the purity control of most of the additives used in the manufacture of polyolefins. After having developed liquid chromatographic (LC) and SFC methods for most of the additives, the results for each additive were also compared to check whether one method had clear advantages for a particular additive.

The LC methods mainly involved the use of light-scattering detection (LSD) for compounds without strong chromophores, permitting the use of gradient elution where that was required. The combination of SFC and LSD, first reported by Carraud *et al.*[6], was included on some occasions in order to compare the results on open capillaries with packed columns or because other methods did not give a satisfactory result.

EXPERIMENTAL

SFC–FID

The instruments were either a Model 3000 supercritical fluid chromatograph (Carlo Erba, Milan, Italy) or a laboratory-built system based on a Model μLC-500 pump (ISCO, Lincoln, NE, U.S.A.)[7–9]. The latter was equipped with solvent venting injection[7–9], whereby 0.5-μl volumes were injected with venting times of approximately 20 s. The columns were 10 m × 50 μm I.D. SB-Phenyl-5 or SB-Biphenyl-30 with 0.5- and 0.25-μm films (Lee Scientific, Salt Lake City, UT, U.S.A.). The restrictors were ceramic frit restrictors from Lee Scientific or laboratory-made integral restrictors. The linear flow-rate in the columns was 2–4 cm/s and that in the precolumn (2 m × 50 μm I.D. coated with a 0.2-μm film of DB-1) was 15–20 cm/s. Carbon dioxide, grade 4.8, was obtained from AGA Norgas (Oslo, Norway).

SFC–LSD

The instrument consisted of a Carlo Erba Phoenix 20 syringe pump connected to packed fused-silica columns and a modified Varex VLSD-101 laser light-scattering detector. The modification, which has been published[5], consisted mainly in removal of the ordinary nebulizer and use of the drift tube as a column oven. The restrictor, which could be heated, functioned as the nebulizer. The detector time constant was set at 0.2 s. The injector could be heated by a jacket and a cartridge heater[5]. The laboratory-made integral restrictors had a thin (1 mm) ceramic frit at the inlet to prevent plugging by foreign particles[5]. *n*-Propanol was added to the carbon dioxide as a modifier, with another pump[5]. The fused-silica columns were packed[10] with different reversed-phase materials.

LC–LSD

The LC results were partly obtained on 4.6 mm I.D. columns connected to the unmodified Varex detector, and partly on packed 0.32-mm fused-silica columns connected to the detector which was equipped with a modified nebulizer to accommodate the low LC flow-rates. The modifications have been published[10]. The

LC pumps were Waters Assoc. Model 6000 A (Millipore–Waters, Milford, MA, U.S.A.). For the packed capillaries the pump was equipped with an open split to maintain a constant flow at the low flow-rates[10].

LC–UV detection

For the few UV-absorbing additives, the samples were injected on a packed fused-silica column which was connected to an SPD-2AM variable-wavelength absorbance detector (Shimadzu, Kyoto, Japan). The detector was modified for packed capillaries with a fused-silica capillary flow cell. The time constant was set at 0.2 s. The mobile phase was delivered by a Waters Assoc. Model 590 pump in the constant pressure mode, with an open split.

RESULTS AND DISCUSSION

Owing to their low solubility in aqueous solutions, very few of the additives can be chromatographed by ordinary reversed-phase methods in LC. In order to be able to run gradients, where this might be needed, non-aqueous reversed-phase and normal-phase elution on amino-modified silica was chosen for the LC experiments.

Irgafos P-EPQ (MW 979) is an organic phosphonite with aromatic substituents, allowing the use of UV detection. With non-aqueous reversed-phase chromatography, the choice of the C_{18} material had a considerable effect on the peak profiles (Fig. 1). With a packing material containing a relatively high density of residual silanol groups, most of the sample was adsorbed on the column (Fig. 1A). With more deactivated packing materials, the chromatograms mainly contained three peaks, in approximately the same ratio based on UV and LSD (Fig. 1B and C). Detection at 220 nm was chosen to reduce the influence of variations in molar absorptivities. With capillary SFC the resolution was improved, resulting in four major peaks and several minor peaks (Fig. 1D). One impurity is tris(2′,4′-di-*tert.*-butylphenyl) phosphite, another additive.

Irganox PS 802 (MW 683) is the distearyl ester of thiodipropionic acid, which has little UV absorbance. The purity tests showed that LC with LSD and SFC with flame ionization detection (FID) gave approximately the same results (Fig. 2).

Armostat 400, N,N′-bis(2-hydroxyethyl)-C_{12}–C_{16}-diamine, also lacks strong chromophores. Owing to the basic functions the mixture was best separated on the amino column in LC. The resolution was slightly better in LC than in SFC (Fig. 3). The retention on the Biphenyl-30 column was higher than that on the Phenyl-5 column, but the resolution of the mixture was not actually improved. Whether the secondary amino groups reacted with carbon dioxide, is not known, but so far there is nothing to indicate that this had happened.

Hostanox SE 10, dioctadecyl disulphide (MW 571), is a non-polar additive with few apparent impurities in LC. According to the chromatogram obtained by LSD, the purity was better than 90%. Better resolution was obtained with SFC, however, showing an actual purity of only 70% by using FID (Fig. 4).

In a routine industrial purity test with reversed-phase LC and RI detection, the three additives oleamide, stearamide and erucamide appeared to be essentially pure, with one peak each (Fig. 5A). This is the only example within the compounds tested where a small amount of water improved the peak shape in a reversed-phase system.

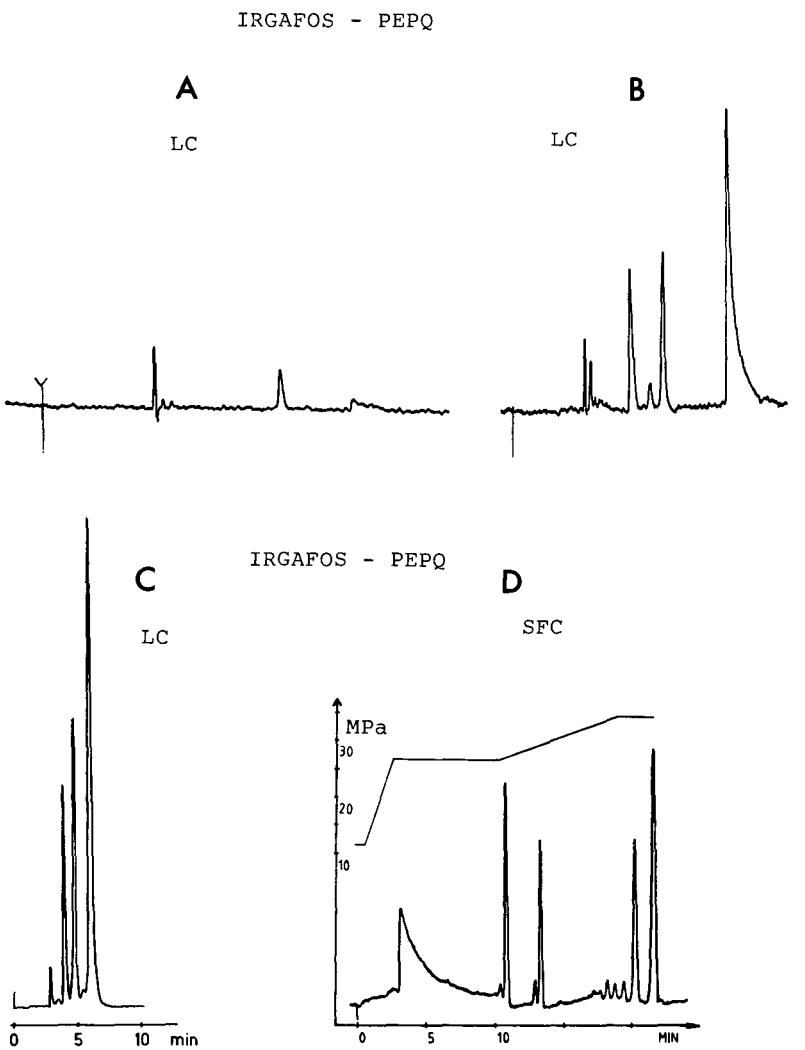

Fig. 1. Separation of Irgafos P-EPQ (0.1 μg) by LC on (A) 3-μm Spherisorb ODS, (B) 4-μm Novapak C_{18} in 20 cm × 0.32 mm I.D. packed fused-silica columns and (C) 5-μm Supelco LC-18-DB (25 cm × 4.6 mm I.D.) with acetonitrile–chloroform (65:35), with (A and B) UV detection (220 nm) and (C) LSD. Chromatogram D shows the SFC separation with carbon dioxide on the 50-μm Phenyl-5 column with FID.

The amino column could not be used owing to the strong retention of byproducts with acidic functions. With a standard SFC method the purities of the three fatty amides were determined to 90, 60 and 90%, respectively (Fig. 5B–D). The main impurity in stearamide was stearic acid.

Glyceryl monostearate is another additive without strong chromophores, making UV detection difficult. In LC with LSD the best resolution was obtained on an amino column with a small amount of methanol in dichloromethane. A gradient was required to elute the components in a reasonable time (Fig. 6A). The shortest elution

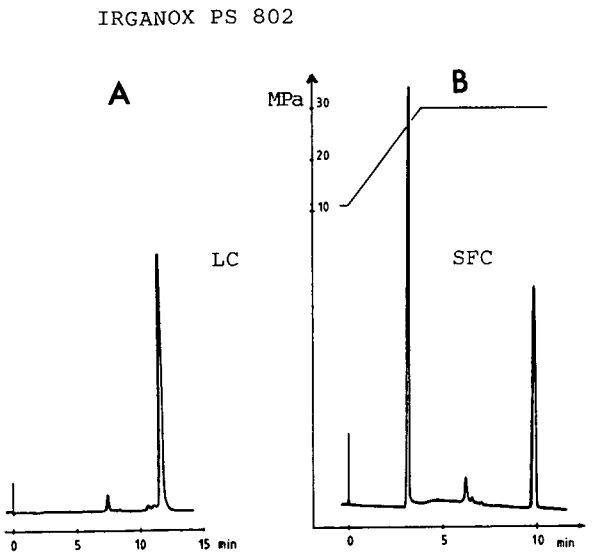

Fig. 2. Separation of Irganox PS 802 by (A) LC on a Supelco LC-18-DB column (25 cm × 4.6 mm I.D.) with acetone and LSD and (B) SFC with carbon dioxide on the Phenyl-5 column with FID. The first peak in B is a solvent peak.

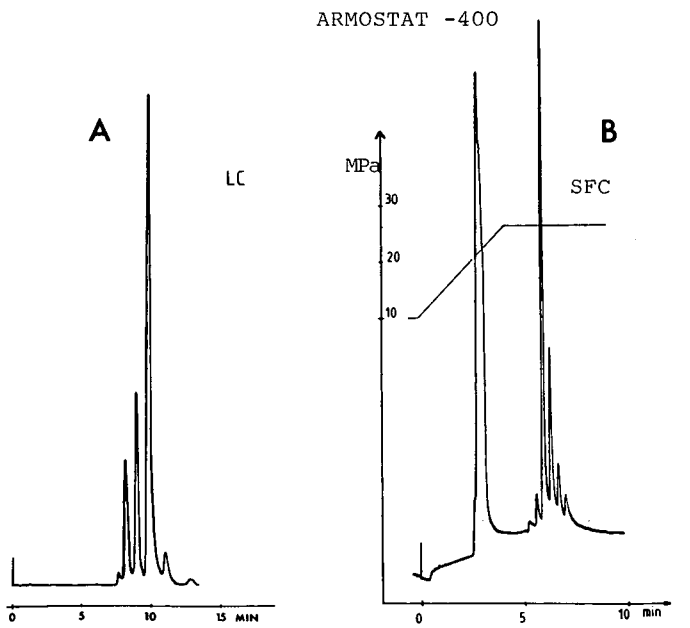

Fig. 3. Separation of Armostat-400 by (A) LC on a 5-μm Supelcosil LC-NH_2 column (25 cm × 4.6 mm I.D.) with hexane–methanol (95:5) and LSD and (B) SFC with carbon dioxide on the Biphenyl-30 column with FID.

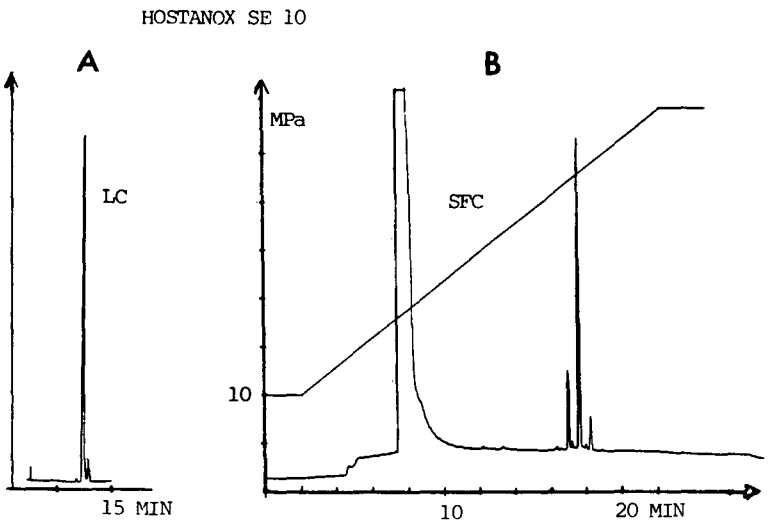

Fig. 4. Separation of Hostanox SE-10 by (A) LC on a Supelco LC-18-DB column (25 cm × 4.6 mm I.D.) with acetone and LSD and (B) SFC with carbon dioxide on the Phenyl-5 column with FID.

time was obtained on a packed column in SFC, with *n*-propanol-modified carbon dioxide and LSD (Fig. 6B). The best quantitative analysis, however, was achieved by SFC on an open-tubular column with FID (Fig. 6C). Owing to the non-linearity of the light-scattering detector, the amounts of triglycerides and other small peaks are misrepresented compared with the FID trace. The actual content of glyceryl monostearate in this technical-grade product was determined by FID to be only 30%. In SFC the elution pattern was similar on the packed and on the open-tubular column, according to the molecular weight, in contrast to the LC normal-phase pattern where the triglyceride peak eluted first.

N,N′-Ethylenebisstearamide is a fatty amide for which we could find no satisfactory LC separation method. The solubility in pure carbon dioxide was low, but the addition of *n*-propanol to the carbon dioxide, heated injection and LSD finally led to the separation of three components by SFC[5].

The following commercial additives were purity tested by SFC with pure carbon dioxide on a Biphenyl-30 or a Phenyl-5 column, with variations in the density gradient: Irgafos P-EPQ, Irgafos 168, Atmer 129, Hostanox SE-10, Sumilizer BHT, Armostat 400, Irganox 1010, Irganox 1076, Irganox MD 1024, Tinuvin 120, Tinuvin 327, Tinuvin 770, Irganox PS 802, Radiamuls 142, Crodamide OR, Crodamide ER and Crodamide SR. The Phenyl-5 column could be used for all the additives. The Biphenyl-30 column was tried only where stronger interactions with the stationary phase was expected to improve the resolution. Chromatograms with essentially one peak in both LC and SFC and some of the previously published SFC separations have not been included in this paper.

In order to test the purity of all the additives properly by LC, several different systems were needed (Table I), requiring much more instrumentation in product control compared with SFC. This illustrates that SFC had a definite advantage over LC for this group of lipophilic compounds.

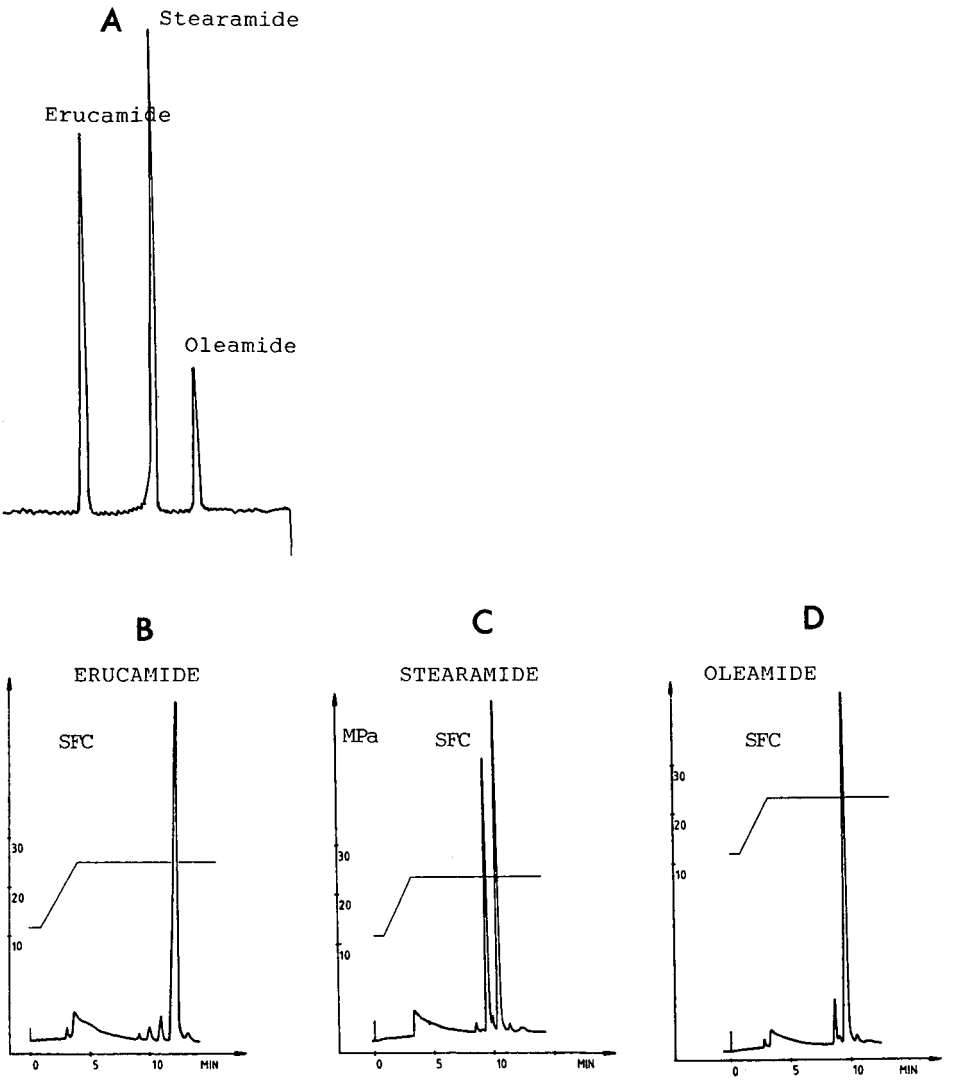

Fig. 5. Purity tests of erucamide, stearamide and oleamide by (A) LC on a 5-μm Spherisorb ODS column with water–methanol (4:95) and RI and (B, C, D) SFC with carbon dioxide on the Phenyl-5 column with FID.

Polyolefin pellets or films are routinely analysed for their additives content, often after Soxhlet extraction of the product. Examples of SFC analyses of the extracts of two different polyolefin products are shown in Fig. 7. By use of the solvent venting injection technique[7–9], volumes of up to 1 μl could be injected.

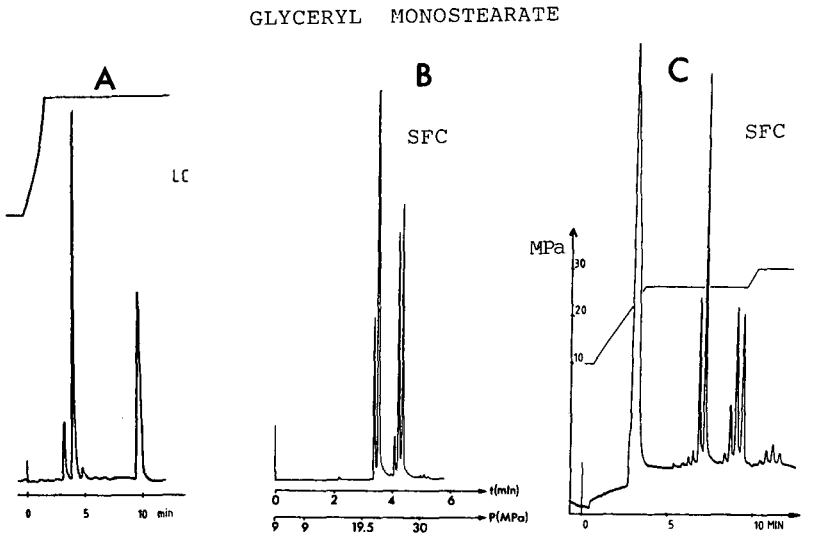

Fig. 6. Separation of technical-grade glyceryl monostearate by (A) LC on a Supelcosil-LC-NH_2 column (25 cm × 4.6 mm I.D.) with 0.6–3% methanol in dichloromethane and LSD, (B) SFC on a 4-μm Novapak-C_{18} column (10 cm × 0.32 mm I.D.) with 2.9 mol-% *n*-propanol in carbon dioxide and LSD and (C) SFC on the Biphenyl-30 column with carbon dioxide and FID.

TABLE I

LC CONDITIONS FOR CHARACTERIZATION OF ADDITIVES

Additive	*Column*	*Mobile phase*
Irgafos P-EPQ	C_{18}	Acetonitrile–chloroform (65:35)
Irgafos 168	C_{18}	Acetonitrile–chloroform (65:35)
Atmer 129	Amino	0.6–3% methanol in chloroform
Radiamuls 142	Amino	0.6–3% methanol in chloroform
Hostanox SE-10	C_{18}	Acetone
DSTDP	C_{18}	Acetone
Armostat 400	Amino	Methanol–hexane (5:95)
Sumilizer BHT	C_{18}	Acetonitrile–acetone (65:35)
Irganox 1010	C_{18}	Acetonitrile–acetone (65:35)
Irganox 1076	C_{18}	Acetonitrile–acetone (65:35)
Irganox MD 1024	C_{18}	Methanol–water (75:25)
Tinuvin 327	Amino	Methanol–dichloromethane (10:90)
Tinuvin 770	Amino	Methanol–dichloromethane (10:90)
Crodamide OR, ER, CR	C_{18}	Methanol–water (96:4)

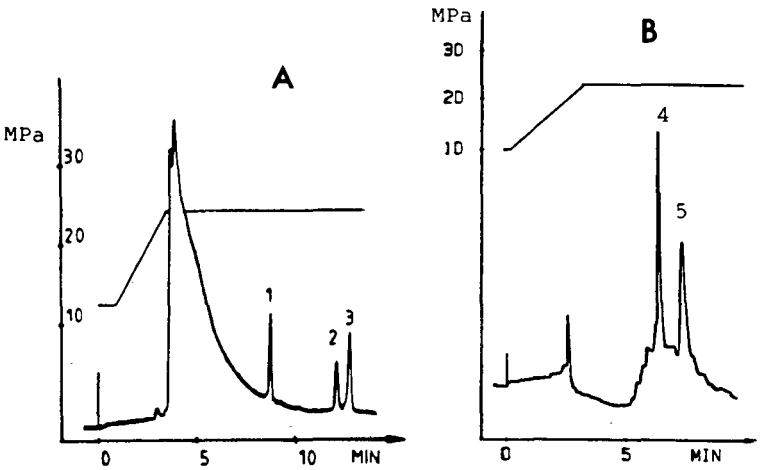

Fig. 7. Determination of the additives (1) Tinuvin 120, (2) Tinuvin 770, (3) Tinuvin 327, (4) stearamide and (5) erucamide in two extracts (A, B) from polyolefin pellets by SFC with carbon dioxide on the Phenyl-5 column with FID.

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CHROM. 22 247

Analysis of triazine and triazole herbicides by gradient-elution supercritical fluid chromatography

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SUMMARY

A mixture of substituted triazine and triazole herbicides is separated on an analytical-scale column by gradient elution using supercritical fluid CO_2 with increasing methanol content and flow-rate. High efficiency and fast analysis times are achieved with complete resolution of all the compounds. The effect of CO_2 flow-rate on the separation has been studied and it is shown that an increase in the overall mobile phase flow-rate by a factor of two reduces the analysis time drastically with no loss in resolution. Also, the effects of the oven temperature and the column outlet pressure on the separation have been studied.

INTRODUCTION

Triazines and triazoles are widely used as herbicides and fungicides. Several methods have been developed for the analysis of triazines by both gas chromatography (GC) and high-performance liquid chromatography (HPLC)[1–3]. GC with flame based detectors and GC coupled with spectroscopic detection have been employed for the analysis of volatile components[4–11]. HPLC methods, on the other hand, have been applied to the analysis of high molecular weight and thermally labile herbicides and metabolites[12–17]. Ultraviolet and mass spectrometric (MS) detection in both the positive and negative ion modes have been used for triazine detection. Positive-ion thermospray MS detection appears to be the most common MS method for LC effluent containing triazines.

Supercritical fluid chromatography (SFC) has gained popularity for the analysis of various classes of compounds of industrial interest. Specifically, SFC–MS has been employed to analyze pesticides with a basic triazine structure. In this regard, six triazine derivatives were separated on an analytical-scale packed column with methanol-modified CO_2 and the mass spectrum of each solute was achieved with a thermospray interface[18]. In another study, a triazole fungicide metabolite was separated by SFC on a capillary column with electron-capture detection[19].

None of the above mixtures of herbicides contained both triazine- and triazole-based compounds. The non-volatile nature of triazoles and triazines restrict

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their analysis by GC. Since SFC offers certain advantages, such as faster analysis and higher resolution per unit time over HPLC, SFC has been applied to the analysis of a model mixture (Fig. 1). Since several highly polar components of our synthetic mixture did not elute with 100% CO_2 from either packed or capillary columns, a modified graded mobile phase has been employed for their elution from an analytical scale packed column.

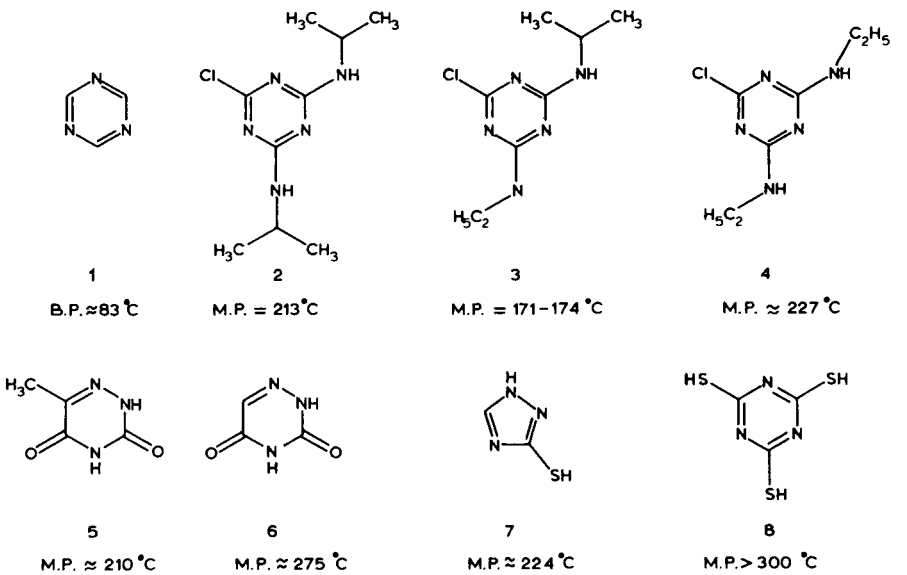

Fig. 1. Structures of triazine and triazole derivatives.

Composition mobile phase gradients were first introduced in SFC by Klesper and Schmitz[20] for effecting the separation of polymeric materials. Its application, however, to SFC has not been widespread . The effect of increasing percent of polar modifier in SCF on the elution and retention of fat-soluble vitamins has previously been reported by Board *et al.*[21]. A mixture of 24 derivatized amino acids has also been separated using a gradient mobile phase of CO_2 and methanol containing tetramethylammonium hydroxide. Nearly complete resolution of 22 derivatives was achieved in 15 min[22]. In this paper we describe the separation of our mixture of herbicides with a CO_2–methanol gradient.

EXPERIMENTAL

A Hewlett-Packard (Avondale, PA, U.S.A.) 1082B liquid chromatograph modified for supercritical fluids was used to deliver CO_2 (Scott Specialty Gases, Plumsteadville, PA, U.S.A.) to the system. This instrument was equipped with an Hewlett-Packard 78795 variable-wavelength ultraviolet detector. A Suprex (Pittsburgh, PA, U.S.A.) Model 200A micro-LC syringe pump was used to deliver methanol

(Fisher Scientific, Fairlawn, NJ, U.S.A.). Liquid methanol and supercritical CO_2 were introduced in a T-mixing chamber (Lee Co., West Brook, CT, U.S.A.) and the resulting mixed mobile phase was passed on to the column. A back-pressure regulator was used to maintain system pressure. A Deltabond® (Keystone Scientific, Bellefonte, PA, U.S.A.) cross-linked cyanopropyl bonded silica column of 25 cm × 4.6 mm I.D., 5 μm particle size, was used to develop the separation. All triazine- and triazole-based compounds were purchased from Aldrich (Milwaukee, WI, U.S.A.). The injected solution had a concentration of 200 ng/μl of each component prepared in HPLC-grade methanol. An injection volume of 1.0 μl was employed.

RESULTS AND DISCUSSION

The dual-pump system employed in this study is similar to that described previously[22]. A separation of the eight component mixture was unsuccessfully tried on several conventional analytical-scale columns packed with different stationary phases such as amino, cyano and octadecyl modified silicas under isocratic conditions. Various concentrations of methanol and different temperatures were attempted to develop the separation. The complete separation of all the components in the mixture was, however, achieved only using gradient elution. An oven temperature of 60°C and a flow-rate of 2 ml/min of CO_2 on a highly deactivated (Deltabond) cyanopropyl column with an outlet pressure of 4000 p.s.i. was employed. The percent methanol was increased to about 33% by the end of the chromatographic run as shown in Fig. 2 with an analysis time of less than six minutes. Throughout the separation, the flow of CO_2 was maintained constant while the flow of methanol was gradually increased. Unsubstituted *sym*-triazine (1,3,5-triazine) elutes first followed by the chloro-substituted *sym*-triazines. Among these substituted triazines, the one with two propyl groups elutes before the analyte with one propyl and one ethyl followed by the analyte with two ethyl groups. The two *asym*-(1,2,4-)triazines elute next followed by the two thiol containing compounds. The baseline shifted slightly when the amount of methanol in the mobile phase reached approximately 30%.

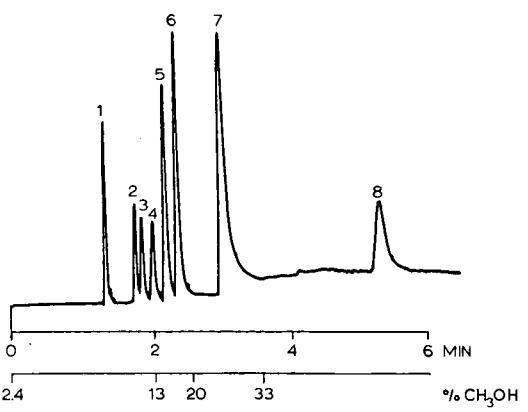

Fig. 2. Separation of triazine mixture at a flow-rate of 2 ml/min of CO_2, 250 × 4.6 mm Deltabond cyanopropyl (5 μm), 60°C, outlet pressure 4000 p.s.i., UV (440 nm). Peak Nos. correspond to the structures in Fig. 1.

The same separation was then carried out under exactly identical conditions but at a CO_2 flow-rate of 4 ml/min (Fig. 3). The flow of methanol in this case was also increased in order to achieve a gradient that was similar to the previous case. Both separations are quite comparable. The resolution between the closely related compounds, namely propazine, atrazine and simazine, is slightly less at the higher flow-rate but the total analysis time is reduced by a factor of approximately two. The peak area for component **8** has decreased somewhat at the higher flow-rate.

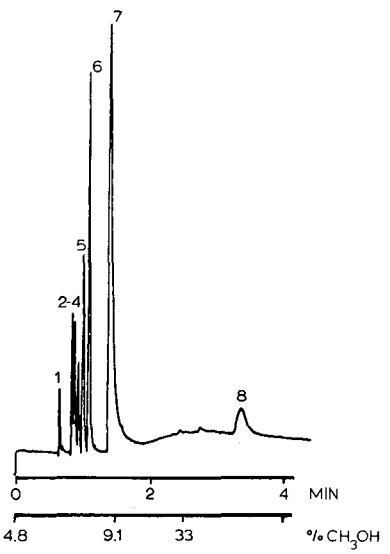

Fig. 3. Separation of triazine mixture at a CO_2 flow-rate of 4 ml/min: Peaks and conditions as in Fig. 2.

The next goal was to develop the same separation at a different temperature and/or a different pressure and study the effect of the changed parameters on resolution. Fig. 4 showns a separation which was performed at the same conditions as for Fig. 2, but at a temperature of 100°C. This separation at the elevated temperature (at 2 ml/min) was very similar to that achieved at the lower temperature. Peak shapes and retention times were almost identical in both cases. The increase in temperature probably does not change the density and the solvating power of the methanol-modified mobile phase significantly thereby having little effect on the separation.

This observation, of course, precludes any effect of changing linear velocity on the separation. Next, the outlet pressure was reduced to 2000 p.s.i. with the same gradient and flow-rate as shown in Fig. 2, and the separation was carried out at 60°C (Fig. 5). All of the components are retained longer due to the reduced density (2000 *vs.* 4000 p.s.i.) of the mobile phase. Yet, the resolution between peaks is comparable to the previously described separations. It is important to note that the mobile phase is in the subcritical state with reference to both temperature and pressure in the initial stages of the separation even with 2.4% methanol. The *sym-* and *asym*-triazines required a higher percentage (20%) of methanol for elution at the lower pressure. The most

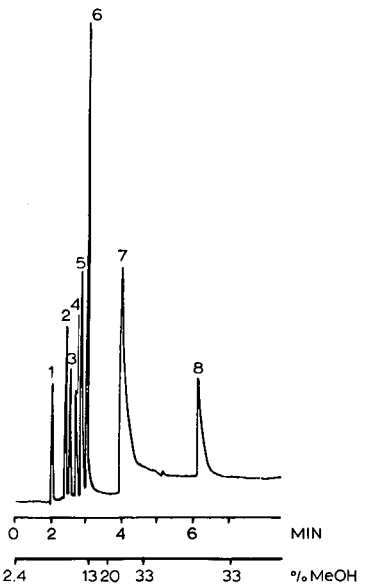

Fig. 4. Separation of triazine mixture at 4000 p.s.i. outlet pressure and 100°C. Peaks and other conditions as in Fig. 2. MeOH = Methanol.

polar component of the mixture, trithiocyanuric acid, is little affected by changes in both temperature and pressure.

The addition of tetramethylammonium hydroxide to methanol was found to

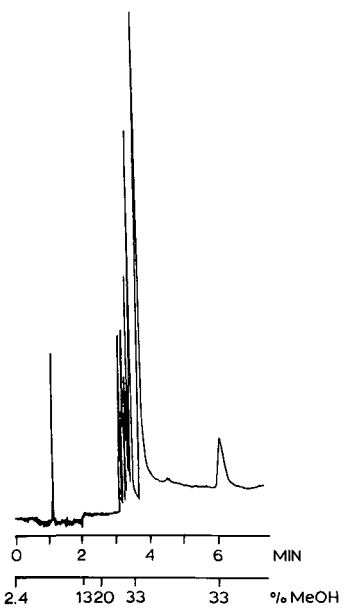

Fig. 5. Separation of triazine mixture at 2000 p.s.i. outlet pressure and 60°C. Peaks and other conditions as in Fig. 2.

have a great effect on the gradient mobile phase elution of acidic and basic phenylthiohydantoin-amino acids as reported previously by Berger *et al.*[22] The same base was added to methanol (0.001 *M*) and the separation of triazines was attempted. The addition of base in this case was found to have a slightly negative effect on the elution. Hexanol instead of methanol was also tried for the triazine. The kinetics of mixing of CO_2 and hexanol were, however, found to be very slow with our instrumental set-up. The failure to achieve a homogeneously mixed mobile phase manifested itself in a very unstable and noisy baseline probably due to air bubbles introduced into the flow cell.

In summary, it can be concluded from these experiments that little apparent loss in resolution occurred with a 100% increase in flow-rate. Further, if pressure is relatively high, the separation under subcritical conditions is quite comparable to the separation achieved under supercritical conditions. Gradient mobile phase elution SFC using a high percentage of modifier appears to bridge SFC and HPLC with the added advantage that faster analysis times can be achieved relative to HPLC. Furthermore, higher mobile phase flow-rates in gradient SFC with packed columns reduce the analysis time drastically with little change in resolution, thereby making this a viable technique for routine analysis.

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CHROM. 22 254

Analysis of sugars by supercritical fluid chromatography using polar packed columns and light-scattering detection

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SUMMARY

The application of supercritical fluid chromatography (SFC) with polar packed columns and light-scattering detection for the analysis of sugars is reported. Cyano-, diol- and nitro-bonded silicas were used with carbon dioxide–methanol mobile phases and a comparison of sugar retention was carried out. These SFC systems showed different selectivities from that found in high-performance liquid chromatography. The association of a constant flow-rate of carbon dioxide and a variable flow-rate of methanol affords the elution of mono-, di- and trisaccharides in the same analysis without baseline drift.

INTRODUCTION

Many papers have been published on the analysis of sugars by high-performance liquid chromatography (HPLC)[1–4]. This interest is due to the difficulties of these analyses, resulting from the instability and relatively short life of the systems[5] and the need for a simple and sensitive detection method[6].

Amino-bonded silicas are often used, but a loss of sugars is observed with reducing sugars that form Schiff bases[7], necessitating accurate calibration for quantitative analysis. In contrast, diol-bonded silicas do not show this problem and afford the advantage of a very low hydrolysis rate with aqueous eluents[5]; consequently their use in gradient elution with light-scattering detection (LSD) is to be preferred. On the other hand, diol-bonded silicas offer a lower selectivity for sugars than do amino-bonded silicas. Moreover, peak broadening occurs with these aqueous eluents due to the anomeric forms of reducing sugars at the time of mutarotation[2,4].

To avoid these difficulties and to obtain better selectivity, we used certain polar bonded silicas in combination with supercritical fluid chromatography (SFC). Sugar analysis was previously carried out by SFC with capillary columns and carbon dioxide after derivatization in order to render the solutes less polar[8,9].

With packed columns, the addition of a polar modifier to the eluent increases the solubility of polar solutes, which is necessary for an acceptable elution time. However, the use of flame ionization detection, often used with capillary columns and carbon

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dioxide, becomes impossible with packed columns and a polar modifier. For non-UV-absorbing compounds we achieved the first coupling of SFC on packed columns using carbon dioxide with LSD[10,11]. This constitutes a universal detection method using SFC with packed columns and polar modifiers. Recently we have described an SFC method with apolar packed columns for the determination of sugars in tobacco[12].

In this paper we show how columns packed with polar bonded silicas can be used to analyse sugars by SFC with greater selectivity than HPLC.

EXPERIMENTAL

Apparatus

Carbon dioxide, kept in a cylinder with an eductor tube connected to a Waters Assoc. (Milford, MA, U.S.A.) Model M 45 pump, was passed through an ethanol cooling bath. The pump head was cooled to improve efficiency. The flow-rates and the weight percentages of carbon dioxide and methanol given in tables and figures have been corrected to take into account the pumping yield and the pump head temperature. The polar modifier (methanol) was added using a Jasco (Tokyo, Japan) Model 2510 pump. The two solvents were mixed in a Knauer (Berlin, F.R.G.) mixer. The column temperature (40°C) was controlled by a water-bath. The loop of a Rheodyne (Cotati, CA, U.S.A.) Model 7125 valve was immersed in the same water-bath.

A Model Sedex 45 evaporative light-scattering detector (Sedere, Vitry sur Seine, France) was used. A fused-silica capillary tube (160 × 0.075 mm I.D.) was chosen as a restrictor instead of the conventional nebulizer for HPLC[10,11]. Consequently, with such a restrictor, variations in flow-rate provide a pressure gradient. The polarity of the mobile phase increases with increase in the flow-rate of methanol. Some experiments with large amounts of methanol in the mobile phase were performed under sub-critical conditions.

Columns

The following columns were used: 10-μm Lichrospher Diol (250 × 4.6 mm I.D.), 5-μm LiChrosorb CN (150 × 4.6 mm I.D.), both from Merck (Darmstadt, F.R.G.), 7-μm Zorbax CN (150 × 4.6 mm I.D.) from DuPont (Wilmington, DE, U.S.A.), 5-μm RSil NO_2 (250 × 4.6 mm I.D.) from RSL (Eke, Belgium) and 10-μm μBondapak CN (150 × 3.9 mm I.D.) from Waters Assoc.

Chemicals and reagents

Carbon dioxide (Air Liquide, Paris, France) was of B 50 grade and was flushed through molecular sieves before the pump. Pestipur-grade methanol was purchased from SDS (Vitry, France). Only a few grades of methanol are suitable as polar modifiers. With others a high baseline noise is observed; HPLC- and spectroscopic-grade methanol must be tested before use. The noise may be partly caused by the amount of dry residue, but this is not the only cause. Studies on the quality of solvents for HPLC and SFC suitable for this detector are currently in progress.

The solutes (analytical-reagent grade) were dissolved in methanol–water, chloroform–methanol or pure methanol. Chloroform–methanol was preferred in order to avoid problems such as band broadening and peak splitting resulting from injection of solvents of high elution strength.

RESULTS AND DISCUSSION

In order to compare the capabilities of the HPLC and SFC separation systems for the analysis of sugars and polyols on polar stationary phases, it was necessary to explore a large number of solvent mixtures as mobile phases.

In this SFC study, we only investigated carbon dioxide–methanol mixtures in order to determine the influence of the methanol concentration on the retention of carbohydrates and corresponding polyols on some polar bonded silicas. The results should be compared with those obtained using organic HPLC mobile phases (*e.g.*, chloroform–methanol) having a polarity similar to that of carbon dioxide–methanol mixtures in SFC. Such a study is currently in progress; so far only the acetonitrile–water mobile phase has been investigated and consequently useful comparisons cannot be given in this paper.

With the carbon dioxide–methanol systems, peak broadening occurs only for certain sugars and as all solutes were injected separately we do not show the chromatograms of mixtures but report for discussion the retention times in two tables.

For the different stationary phases the mobile phase compositions and the pressures were chosen in order to maintain a similar retention for all the compounds.

TABLE I

RETENTION TIMES (min) OF MONOSACCHARIDES AND POLYOLS ON POLAR BONDED SILICAS IN SFC AT 40°C AND WITH CO_2–METHANOL MIXTURES

Compound	*Column No.*[a]				
	1	*2*	*3*	*4*	*5*
2-Deoxy-D-ribose	2.4	2.1	1.1	3.9	3.3
L-Rhamnose	3.6	3.2	4.5	5.8	4.1
D-Ribose	3.8	3.4	4.8	5.6	4.7
meso-Erythritol	3.9	3.5	3.6	5.0	4.6
L-Arabinose	4.4	3.7	3.5	6.2	5.0
D-Xylose	4.5	4.0	4.5	6.4	4.6
D-Fructose	6.0	5.2	3.7	7.6	6.5
L-Sorbose	6.2	5.2	3.7	9.0	6.2
Xylitol	7.0	5.1	8.7	7.3	7.6
D-Galactose	8.0	7.5	7.0	–	8.0
D-Mannose	8.0	4.5	6.7	9.4	7.0
D-Glucose	8.5	7.8	6.5	10.6	8.0
meso-Inositol	13.0	12.0	9.8	19.0	–
D-Mannitol	14.0	8.0	15.0	10.8	10.2
D-Sorbitol	–	–	–	11.0	11.7

[a] 1 = Zorbax CN (150 × 4.6 mm I.D.), CO_2–methanol (93.5:6.5, w/w), 4.35 ml min^{-1}, 3700 p.s.i.;
2 = μBondapak CN (150 × 3.9 mm I.D.), CO_2–methanol (95.9:4.1, w/w), 3.37 ml min^{-1}, 3900 p.s.i.;
3 = LiChrosorb CN (150 × 4.6 mm I.D.), CO_2–methanol (96.4:3.6, w/w), 3.35 ml min^{-1}, 3900 p.s.i.;
4 = Lichrospher Diol (250 × 4.6 mm I.D.), CO_2–methanol (83.7:16.3, w/w), 1.79 ml min^{-1}, 3900 p.s.i.;
5 = RSil NO_2 (250 × 4.6 mm I.D.), CO_2–methanol (87.0:13.0, w/w), 3.8 ml min^{-1}, 3500 p.s.i.

Influence of methanol content

As seen in Table I, the mobile phase for Zorbax CN (column 1) requires a higher methanol content than that for μBondapak (column 2) and LiChrosorb CN (column 3) to elute sugars with similar retentions. Using Lichrospher Diol (column 4) and RSil NO_2 (column 5), the methanol content is much higher, indicating strong interactions of sugars with these packings.

The data in Table I may be compared with those in Table II. The retention times decrease with increase in the amount of methanol in the mobile phase and consequently the disaccharides are more easily eluted (sucrose, trehalose, lactose and maltose; see Table II). As shown in Fig. 1, by increasing the flow-rate of methanol while maintaining a constant flow-rate of carbon dioxide, a composition and flow-rate gradient can be realized, allowing the elution of mono-, di- and trisaccharides in the same analysis. This promising technique, which is easily compatible with LSD, will subsequently be applied to other samples.

Comparison of selectivities on cyano-bonded silicas

Sugars are not retained in HPLC on cyano-bonded silicas using an acetonitrile–water eluent. As seen in Table I, the elution sequence in SFC follows the order of molecular size (*i.e.*, the number of carbon atoms) on Zorbax and μBondapak CN. This sequence is changed on LiChrosorb CN: fructose and sorbose show lower retentions than molecules having fewer carbon atoms (xylose, ribose).

Different selectivities of the three cyano-bonded silicas are noted. Galactose and mannose are not separated on Zorbax and LiChrosorb CN, but are separated on μBondapak CN. In contrast, galactose and mannitol have similar retention times on μBondapak CN, whereas Zorbax and LiChrosorb CN provide a good selectivity. Only LiChrosorb CN easily separates fructose and xylitol; poor results were obtained using

TABLE II

RETENTION TIMES (min) OF MONO- AND DISACCHARIDES ON POLAR BONDED SILICAS IN SFC AT 40°C AND WITH CO_2–METHANOL MIXTURES

Compound	*Column No.*[a]		
	1	*2*	*3*
2-Deoxyribose	1.5	–	–
L-Rhamnose	1.9	–	–
D-Ribose	–	–	3.8
D-Fructose	2.7	2.5	4.0
D-Glucose	3.3	3.2	–
meso-Inositol	6.0	4.9	9.1
D-Sorbitol	–	–	5.9
Sucrose	8.3	7.6	9.7
Trehalose	14.1	15.1	–
Lactose	16.6	17.2	–
Maltose	–	14.1	13.3

[a] 1 = Zorbax CN (150 × 4.6 mm I.D.), CO_2–methanol (90.2:9.8, w/w), 4.09 ml min^{-1};
2 = μBondapak CN (150 × 3.9 mm I.D.), CO_2–methanol (94.7:5.3, w/w), 3.85 ml min^{-1};
3 = Lichrospher Diol (250 × 4.6 mm I.D.), CO_2–methanol (78.3:21.7, w/w), 1.94 ml min^{-1}.

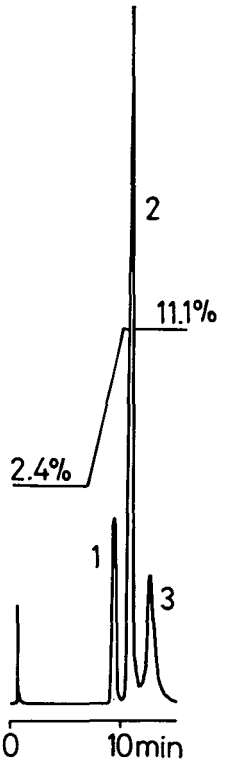

Fig. 1. Gradient elution chromatogram of sugars. Column, μBondapak CN. Mobile phase, CO_2–methanol. Gradient conditions: t = 0 min, 97.6:2.4 (w/w), 3.3 ml min^{-1}; t = 7 min, 97.6:2.4 (w/w), 3.3 ml min^{-1}; t = 10 min, 88.9:11.1 (w/w), 3.7 ml min^{-1}; flow-rate of CO_2 constant at 3.2 ml min^{-1}. Solutes: 1 = fructose; 2 = sucrose; 3 = raffinose.

Zorbax CN for the separation of *meso*-inositol and mannitol whereas μBondapak CN and LiChrosorb CN lead to a good selectivity but to reverse elution orders.

Different selectivity of polar bonded silicas

Table I illustrates the different selectivities of polar bonded silicas in SFC and the larger retention of polyols with regard to the corresponding sugars.

Mannose and glucose are poorly resolved in HPLC on aminopropylsilica. In SFC with a similar selectivity a better resolution is obtained owing to a good efficiency on Lichrospher Diol (Fig. 2) and on RSil NO_2, whereas μBondapak CN provides a large selectivity (Table I). Glucose, mannitol and sorbitol are not separated on aminopropylsilica in HPLC and on Lichrospher Diol in SFC. Although it is not useful for the HPLC of sugars, as it gives very short retention times, RSil NO_2 permits a good separation of these three compounds in SFC (Fig. 2).

The change in the elution sequence of sorbose and xylitol on Lichrospher Diol, RSil NO_2 and LiChrosorb CN columns may be noted from Table I. *meso*-Erythritol, xylose and rhamnose are not separated in HPLC on a Lichrospher Diol column. In

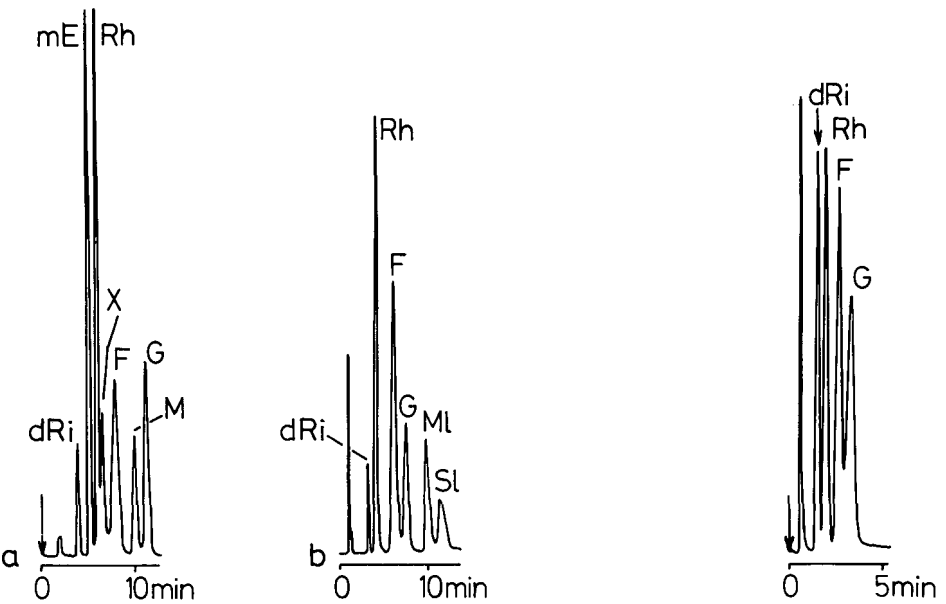

Fig. 2. Separation of sugars in SFC packed columns. (a) Lichrospher Diol column. Eluent: CO_2–methanol (84.5:15.5, w/w), 1.77 ml min^{-1}, 3900 p.s.i. (b) RSil NO_2 column. Eluent: CO_2–methanol (87.0:13.0, w/w), 3.8 ml min^{-1}, 3500 p.s.i. dRi = 2-Deoxy-D-ribose; mE = *meso*-erythritol; Rh = rhamnose; X = xylose; F = fructose; M = mannose; G = glucose; Ml = mannitol; Sl = sorbitol.

Fig. 3. Separation of sugars in SFC on Zorbax CN column. Eluent: CO_2–methanol (91.1:8.9, w/w), 4.49 ml min^{-1}, 3500 p.s.i.

contrast, this column is the only one that permits the separation of these solutes in SFC (Table I and Fig. 2).

Figs. 2 and 3 illustrate good separations of sugars with polar bonded silicas in SFC. In HPLC polyols and corresponding sugars generally show similar retentions on aminopropylsilica[1]. In SFC, and more especially on RSil NO_2, polyols show greater retention than do sugars (*e.g.*, mannitol and mannose, sorbitol and glucose, xylitol and xylose). Finally, *meso*-inositol, a cyclic polyol, yields a high retention as in HPLC.

CONCLUSION

The analysis of polar compunds such as sugars can easily be carried out by SFC using polar bonded phases and with carbon dioxide–methanol as the mobile phase. Light-scattering detection is presented as a universal method using SFC with packed columns and polar modifiers. This system provides a greater range of selectivity than in HPLC.

The possibility of changing the modifier flow-rate permits the elution of mono-, di- and trisaccharides in the same analysis without baseline drift. The chromatographic behaviour of sugars will be investigated in SFC with other apolar and polar bonded silicas. A comparison of selectivities will be presented later.

Research is in progress on other cyano-bonded silicas. Factor analysis of the chromatographic results[13] will emphasize the factors that affect SFC selectivity.

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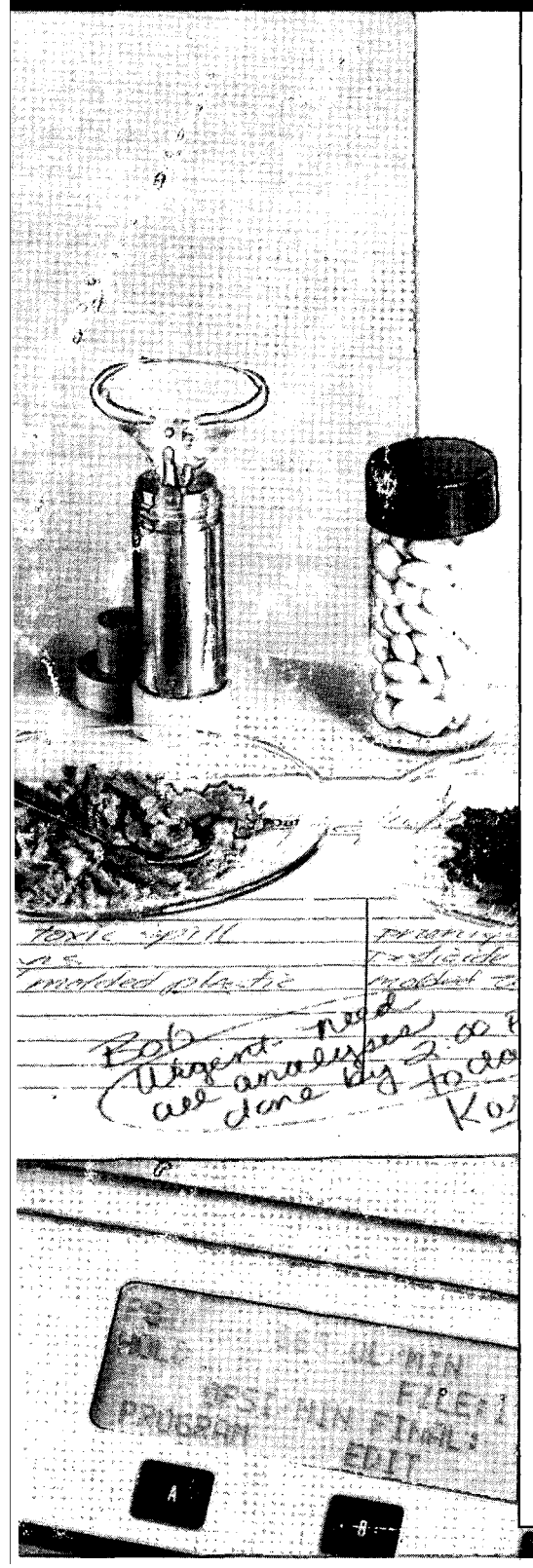

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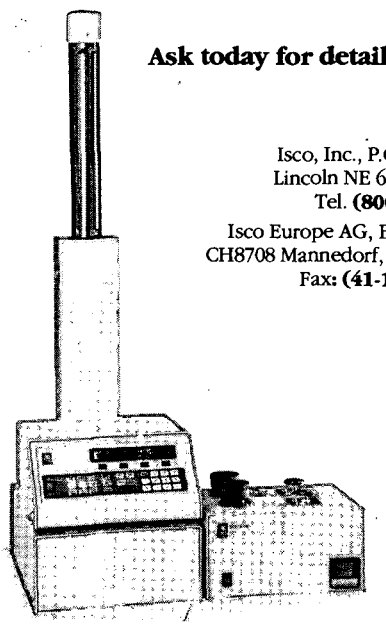

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