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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 dis 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.

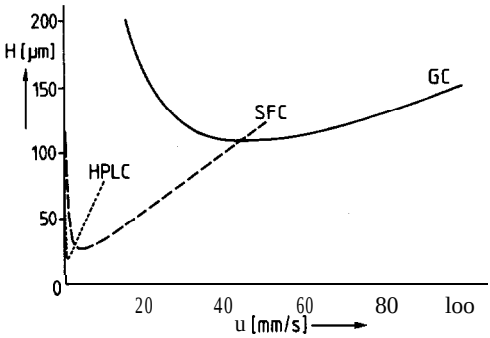

Fig. 1. ***H vs. u curves*** for packed columns used in HPLC, SFC and GC. d_p = 10 µm. Mobile phase: HPLC, methanol; SFC, CO_2; GC, N_2. Sample: HPLC, benzene; SFC and GC, methane.

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*. 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 CC-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', 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

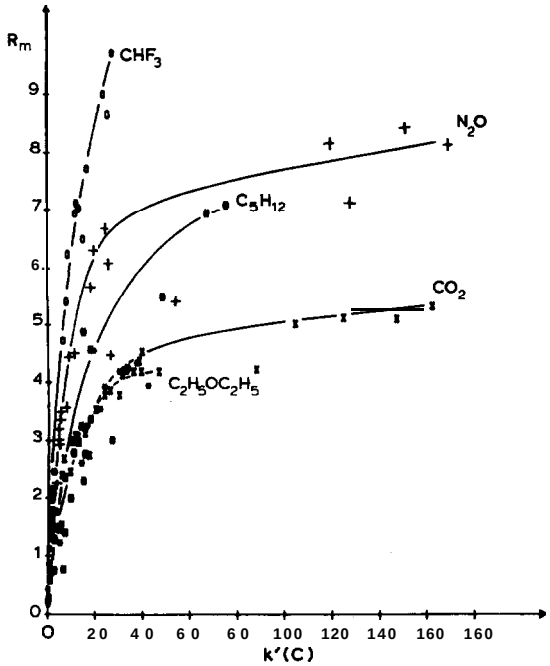

Fig. 2. Dependence of the resolution, R_m, between naphthalene, anthracene, pyrene and chrysene on k' (C) in different mobile phases at different temperatures and pressures. Column: 250 x 4.6 mm I.D., unmodified silica, 10 μm. (Redrawn with permission from ref. 18.)

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	u v	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	u v	26
CO_2 modified with C_1OH, C_3OH, THF, DMSO, SF_6, acetonitrile, methoxyethanol, Freon 11	Diol Octyl Octyl, end-capped	Modifier effects	u v	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 pm Cyan0 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-S 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	Cyan0 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.

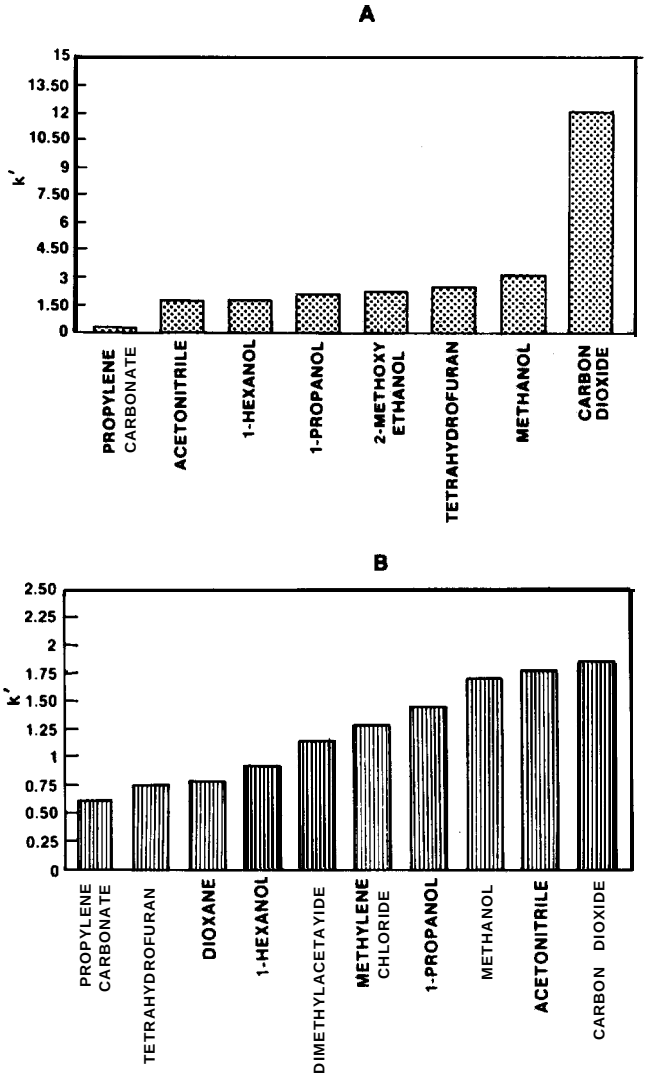

Fig. 3. Effects of 6 mol-% modifier in CO_2 on the capacity factor of (A) 4-nitroaniline and (B) hexachlorobenzene. Conditions: (A) column, 250 × 4.6 mm I.D., cyano, 5 μm, 4500 p.s.i., 40°C; (B) column, 200 × 4.6 mm I.D., ODS, 5 μm, 2800 p.s.i., 40°C. (Redrawn with permission of Preston Publications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

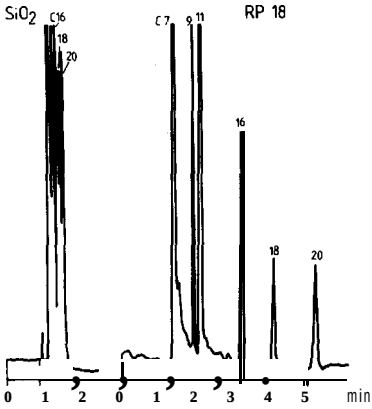

Fig. 4. Separation of *n*-alkanes on a silica and a reversed phase. Conditions: columns, 250 x 4. 1 mm I.D., silica, 10 μm and 250 x 4.1 mm, RP-18, 10 μm; CO_2, 115 bar, 40°C. (Redrawn with permission from ref. 2.)

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.

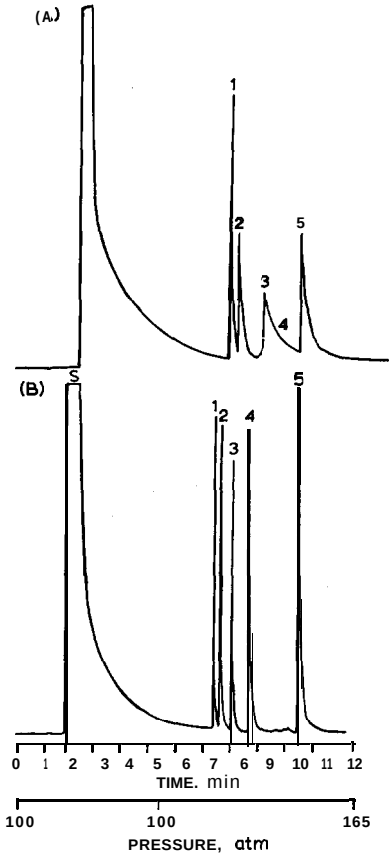

Fig. 5. Separation of a polarity test mixture on (A) a conventional cyanopropyl and (B) cyanopropyl Deltabond column. Conditions: CO_2, 60°C. 1 = n-Pentadecane; 2 = phenyl acetate; 3 = acetophenone; 4 = 2,6-dimethylaniline; 5 = phenol. (Redrawn with permission from ref. 39.)

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.

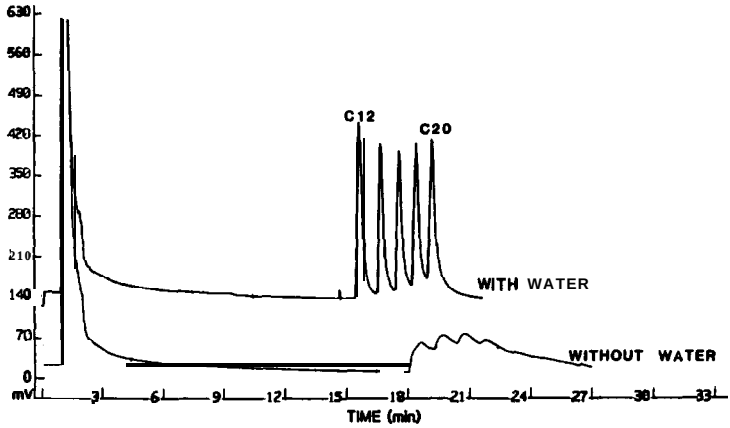

Fig. 6. Influence of water as a modifier on an SFC separation of $C_{12}, C_{14}, C_{16}, C_{18}$ saturated free fatty acids. Conditions: column, 100 × 1 mm I.D., Nucleosil cyano, 5 μm; 1800 p.s.i. for 2 min, 1800–6000 p.s.i. at 100 p.s.i./min; 70°C. (Redrawn with permission from ref. 28.)

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	u v	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	u v	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 pm	C_5 modified with dioxane	UV	64
Liquid crystal mixtures	CP-Spher C_{18}, 8 μm	CO2	u v	65
Styrene oligomers	LiChrosorb Si 100, 10 μm	C_5	u v	66
Optimization strategy for oligomer separations	LiChrosorb Si 60, 10 μm	Various gradients	u v	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 moditied with 10% CO_2	FID	69
Ecdysteroids	Spherisorb cyanopropyl Spherisorb ODS-2, 5 μm	CO_2	u v	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
a-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.

REFERENCES

1 G. Guiochon and C. L. Guillemin, ***Quantitative Gas Chromatography (Journal of Chromatography Library,*** Vol. 42), Elsevier, Amsterdam, 1988.
2 H. Engelhardt and A. Gross, ***J. High Resolut. Chromatogr. Chromatogr. Commun.,*** 11 (1988) 38.
3 M. Ashraf-Khorassani and L. T. Taylor, ***J. High Resolut. Chromatogr. Chromatogr. Commun., 12 (1989) 40.***
4 M. Raynor, J. Kithinji, I. Barker, K. Bartle and I. Wilson, ***J. Chromatogr., 436 (1988) 497.***
5 B. Gemmel, F. P. Schmitz and E. Klesper, ***J. Chromatogr., 455 (1988) 17.***
6 J. L. Janicot, M. Caude and R. Rosset, ***J. Chromatogr., 437 (1988) 351.***
7 K. R. Jahn and B. W. Wenclaviak, ***Anal.*** Chem., 59 (1987) 382.
8 Y. Hirata and F. Nakata, ***Chromatographia, 21 (1986) 627.***
9 P. Carraud, D. Thiebaut, M. Caude, R. Rosset, M. Lafosse and M. Dreux, ***J. Chromatogr. Sci., 25 (1987) 395.***
10 W. Gmiir, J. 0. Bosset and E. Plattner, ***Chromatographia, 23 (1987) 199.***
11 P. G. Sim, C. M. Elson and M. A. Quilliam, ***J. Chromatogr., 445 (1988) 239.***
12 A. J. Berry, D. E. Games, I. C. Mylchreest, J. R. Perkins and S. Pleasance, ***J. High Resolut. Chromatogr. Chromatogr. Commun.,*** 11 (1988) 61.
13 R. D. Smith and H. R. Udseth, ***Anal. Chem., 59 (1987) 13.***
14 J. B. Crawther and J. D. Henion, ***Anal.*** Chem., 57 (1985) 2711.
15 C. C. Johnson, J. W. Jordan, L. T. Taylor and D. W. Vidrine, ***Chromatographia, 20 (1985) 717.***
16 K. H. Shafer, S. L. Pentoney and P. R. Griffith, ***Anal. Chem., 58 (1986) 58.***
17 D. Leyendecker, D. Leyendecker, F. P. Schmitz and E. Klesper, ***J. Chromatogr., 371 (1986) 93.***
18 D. Leyendecker, D. Leyendecker, F. P. Schmitz and E. Klesper, ***J. Liq. Chromatogr.,*** 10 (1987) 1917.
19 D. Leyendecker, F. P. Schmitz and E. Klesper, ***J. Chromatogr., 315 (1984) 19.***
20 S. B. French and M. Novotny, ***Anal.*** Chem., 58 (1986) 213.
21 J. W. Hellegeth, M. G. Fessehaie and L. T. Taylor, ***Chromatographia, 25 (1988) 172.***
22 T. Takeuchi, T. Niwa and D. Ishii, ***Chromatagraphia, 23 (1987) 929.***
23 M. W. Raynor, G. F. Shilstone, K. D. Bartle, A. A. Cleary and B. W. Cook, paper presented at the Tenth International Symposium on Capillary Chromatography, 1989.
24 E. Stahl, K. W. Quirin and D. Gerard, ***Verdichtete Gasezur Extraktion und Raffination***, Springer, Berlin, 1987.
25 F. P. Schmitz, D. Leyendecker and D. Leyendecker, ***J. Chromatogr., 389 (1987) 245.***
26 A. L. Blilie and T. Greibrokk, ***Anal. Chem., 57 (1985) 2239.***
27 J. M. Levy and W. M. Ritchey, ***J. High Resolut. Chromatogr. Chromatogr. Commun., 8 (1985) 503.***
28 F. 0. Geiser, S. G. Yocklovich, S. M. Lurcott, J. W. Luthrie and E. J. Levy, ***J. Chromatogr., 459 (1988) 173.***
29 J. M. Levy and W. M. Ritchey, ***J. High Resolut. Chromatogr. Chromatogr. Commun.,*** 10 (1987) 493.

30 W. Steuer, M. Schindler, G. Schill and F. Erni, *J.* Chromarogr., 447 (1988) 287.
31 T. A. Berger, J. F. Deye, M. Ashraf-Khorassani and L. T. Taylor, *J. Chromatogr. Sci.*, 27 (1989) 105.
32 P. J. Schoenmakers and L. G. M. Uunk, Eur. Chromarogr. News, 1 (1987) 3.
33 P. J. Schoenmakers, ***J. High Resolut. Chromatogr. Chromatogr. Commun.,*** 11 (1988) 278.
34 H. E. Schwartz, P. J. Barthel, S. E. Moring and H. H. Latter, *LC* CC, ***Mag. Liq. Gas Chromarogr., 5 (1987) 490.***
35 D. R. Gere, R. Board and D. McManigill, ***Anal.*** Chem., 54 (1982) 736.
36 H. Engelhardt, A. Gross, R. Mertens and M. Petersen, ***J. Chromarogr., 477 (1989) 169.***
37 G. Schomburg, A. Deege, J. Köhler and V. Bien-Vogelsang, ***J. Chromarogr., 282 (1983) 27.***
38 H. Engelhardt and H. Löw, ***Fresenius Z. Anal.*** Chem., 330 (1988) 396.
39 M. Ashraf-Khorassani, L. T. Taylor and R. A. Henry, ***Anal. Chem., 60 (1988) 1529.***
40 F. Nevejans and M. Verzele, ***J. Chromatogr., 406 (1987) 325.***
41 P. Morin, M. Caude and R. Rosset, ***J. Chromatogr., 407 (1987) 87.***
42 T. Takeuchi, T. Niwa and D. Ishii, ***Chromatogruphia, 25 (1988) 332.***
43 P. Macaudiere, A. Tambute, M. Caude, R. Rosset, M. A. Alembik and I. W. Wainer, ***J. Chromatogr., 371 (1986) 177.***
44 D. Ishii and T. Takeuchi, ***J. Chromafogr. Sci., 27 (1989) 71.***
45 B. Gemmel, F. P. Schmitz and E. Klesper, ***J. High Resolut. Chromatogr. Chromatogr. Commun.,*** 11 ***(1988)*** 901.
46 B. Gemmel, F. P. Schmitz and E. Klesper, ***J. Chromatogr., 455 (1988) 17.***
47 K. Anton, N. Pericles, S. M. Fields and H. M. Widmer, ***Chromatographia, 26 (1988) 224.***
48 P. Mourier, P. Sassiat, M. Caude and R. Rosset, ***J. Chromatogr., 353 (1986) 61.***
49 A. Nomura, Y. Yamada and K.-I. Tsonuda, ***J. Chromatogr., 448 (1988) 87.***
50 J. M. Levy and W. M. Ritchey, ***J. Chromafogr., 24 (1986) 242.***
51 E. Klesper, D. Leyendecker and F. P. Schmitz, ***J. Chromatogr., 366 (1986) 235.***
52 D. Leyendecker, F. P. Schmitz and E. Klesper, ***Chromatographiu, 23 (1987) 171.***
53 D. Leyendecker, D. Leyendecker, F. P. Schmitz, B. Lorenschat and E. Klesper, ***J. Chromatogr., 398 (1987) 105.***
54 M. E. McNally, J. R. Wheeler and W. R. Melander, *LC* CC, Mug. ***Liq. Gas Chromatogr., 6 (1988) 816.***
55 D. R. Gere, ***Application Note, No. 800-1,*** Hewlett-Packard, Avondale, PA.
56 D. R. Gere, ***Application Note, No. 800-2,*** Hewlett-Packard, Avondale, PA.
57 D. R. Gere, ***Application Note, No. 800-3,*** Hewlett-Packard, Avondale, PA.
58 D. R. Gere, T. J. Stark and T. N. Tweenten, ***Application Note, No. 800-4,*** Hewlett-Packard, Avondale, PA.
59 D. R. Gere, ***Application Note, No. 800-5,*** Hewlett-Packard, Avondale, PA.
60 D. R. Gere, ***Application Note, No. 800-6,*** Hewlett-Packard, Avondale, PA.
61 E. Lundanes and T. Greibrokk, ***J. Chromatogr., 349 (1985) 439.***
62 R. M. Campbell and M. L. Lee, ***Anal.*** Chem., 58 (1986) 2247.
63 H. E. Schwartz and R. G. Brownlee, ***J.*** Chromatogr., 353 (1986) 77.
64 F. P. Schmitz, H. Hilgers and B. Gemmel, ***J.*** Chromatogr., 371 (1986) 135.
65 P. J. Schoenmakers, F. C. Verhoeven and H. M. van den Bogaert, ***J. Chromatogr., 371 (1986) 121.***
66 D. Leyendecker, D. Leyendecker, F. P. Schmitz and E. Klesper, ***Chromatogruphia, 23 (1987) 38.***
67 F. P. Schmitz, D. Leyendecker, D. Lcyendecker and B. Gemmel, ***J. Chromatogr., 395 (1987)*** 111.
68 M. Ashraf-Khorassani, J. W. Hellegeth and L. T. Taylor, ***Anal. Chem., 59 (1987) 2077.***
69 R. M. Campbell, N. M. Djordjevic, K. E. Markides and M. L. Lee, ***Anal.*** Chem., 60 (1988) 356.
70 J. L. Janicot, M. Caude and R. Rosset, ***J. Chromatogr., 437 (1988) 351.***
71 J. L. Veuthey, M. Caude and R. Rosset, ***Chromatographia, 27 (1989) 105.***
72 P. A. Mourier, E. Eliot, M. H. Caude, R. Rosset and A. Tambute, ***Anal.*** Chem., 57 (1985) 2819.
73 S. Hara, A. Dobashi, K. Kinoshita, T. Hondo, M. A. Alembik and I. W. Wainer, ***J. Chromatogr., 371 (1986) 153.***
74 S. Hara, A. Dobashi, T. Hondo, M. Saito and M. Senda, ***J. High Resolut. Chromatogr. Chromatogr. Commun., 9 (1986) 249.***
75 P. Macaudiere, M. Caude, R. Rosset and A. Tambute, ***J. Chromatogr., 405 (1987) 135.***
76 A. Dobashi, Y. Dobashi, T. Ono, S. Hara, M. Saito, S. Higashidate and Y. Yamauchi, ***J.*** Chromatogr., 461 (1989) 121.
77 R. E. Jentoft and T. H. Gouw, ***J. Chromatogr. Sci., 8 (1970) 138.***
78 J. A. Niemann and L. B. Rogers, ***Sep. Sci.***, 10 (1975) 517.
79 J. A. Graham and L. B. Rogers, ***J. Chromatogr. Sci., 18 (1980) 75.***

80 M. Novotny, W. Bertsch and A. Zlatkis, *J.* Chromatogr., 61 (1971) 17.
81 Y. Hirata, F. Nakata and S. Murata, ***Chromatographia, 23 (1987) 663.***
82 T. Takeuchi, Y. Hashimoto and D. Ishii, J. ***Chromatogr., 402 (1987) 328.***
83 Q. L. Xie, K. E. Markides and M. L. Lee, presented at the *10th* ***International Symposium on Capillary Chromatography, Riva del Garda, 1989.***