Journal of Chromatography A, 658 (1994) 293–313
Elsevier Science B.V., Amsterdam

CHROM. 25 586

Review

Advances in supercritical fluid chromatographic processes

Michel Perrut

Separex, B.P. 9, F-54250 Champigneulles (France)

ABSTRACT

Analytical supercritical fluid chromatography (SFC) is now coming to age with widespread use in many laboratories, and chromatographic processes using supercritical eluents are now considered as potential solutions for large-scale fractionations. The hydrodynamics of supercritical fluids through porous media and retention phenomena are analysed prior to describing both adsorption–desorption processes and "classical" preparative elution chromatography, which appears to be a competitor to preparative HPLC especially when non-polar molecules are to be handled. The combination of the high selectivity of chromatographic interactions and the unique properties of supercritical fluids (mainly CO_2) will probably lead to promising applications mainly in the pharmaceutical and fine chemical industries and possibly also in pollution abatement in the next decade.

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1. BRIEF HISTORICAL REVIEW

In 1822, Cagniard de la Tour [1] discovered critical phenomena and in 1879 Hannay and Hogarth [2,3] demonstrated the solvent power of supercritical fluids ($P > P_c$, $T > T_c$) or subcritical tions leading to both a detailed knowledge of their physico-chemical properties and industrial applications really started in the second half of this century, mainly in Germany, where most of the pioneering work was undertaken in the late 1960s and throughout the 1970s. Except for

0021-9673/94/$26.00
SSDI 0021-9673(93)E1019-V

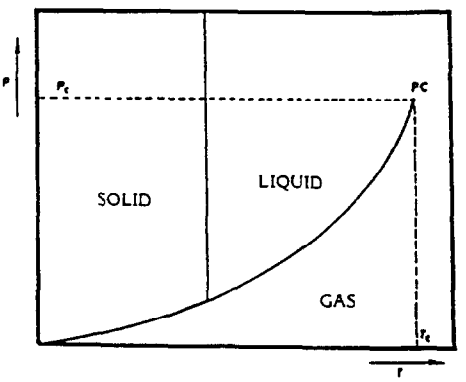

Fig. 1. Diagram of a pure compound: P = pressure; T = temperature; PC = critical point.

large-scale applications in oil industry (residuum deasphalting processes), most developments were made in the food industry in the 1980s; however, numerous applications are now either on-stream or under investigation in various areas, with particular attention in the pharmaceutical industry [4–7].

Among these applications, analytical supercritical fluid chromatography (SFC) is now coming to age with a wide availability of equipment from several suppliers worldwide. Similarly, chromatographic processes are being developed as production techniques with gaseous and mainly liquid eluents [8,9]. In addition to the first small-scale preparative operations [10], two chromatographic modes with supercritical eluents have been applied to large-scale production as described in several patents: the frontal mode [11–13] (adsorption–desorption) in 1977–79 and the elution mode [14] in 1982, leading to industrial developments in the 1990s.

2. SUPERCRITICAL FLUIDS

For any pure compound, there is a transition state called the "critical" state: for temperatures below the critical temperature T_c (or pressures below the critical pressure P_c), two phases, liquid and vapour, co-exist; for temperatures above T_c (or pressures above P_c), there is only one phase. The critical point (PC) on the phase diagram of a pure compound can be considered as the end-point of the vaporization curve, the point at which no liquid exists (Fig. 1). In fact, supercritical fluids ($P > P_c$, $T > T_c$) or subcritical liquids ($P > P_c$, $T < T_c$) are currently used at temperatures near the critical temperature for which their solvent power is important for "reasonable" pressures ($P_c < P < 4P_c$).

Compared with liquid solvents, supercritical fluids possess a greater diffusivity, D, and equivalent density, ρ, and a much lower viscosity, μ. Table 1 shows the ranges of variation of these parameters for gases, liquids and supercritical fluids.

The thermodynamic properties of mixtures are very complex and many phase diagrams for binary systems have been described [4,5,15,16]. However, the evaluation of equilibria has not yet

TABLE 1

PHYSICO-CHEMICAL CHARACTERISTICS OF CLASSICAL AND SUPERCRITICAL FLUIDS (from ref. 18).

Fluid	ρ (kg m^{-3})	μ (cP) (10^{-3} Pa s)	D (cm^2 s^{-1}) (10^{-4} m^2 s^{-1})
Gas (1 atm, 15–30°C)	0.6	0.01	0.1
	2	0.03	0.4
Supercritical fluid:			
T_c, P_c	200	0.01	$0.7 \cdot 10^{-3}$
T_c, $4P_c$	500	0.03	
	400	0.03	$0.2 \cdot 10^{-3}$
	900	0.09	
Liquid (organic solvents, water), 1 atm, 15–30°C	600	0.2	$0.2 \cdot 10^{-5}$
	1600	3	$2 \cdot 10^{-5}$

been fully solved. An empirical correlation, proposed by Chrastil [17], is relatively simple and reliable:

$$C = \rho^{\alpha} \exp(a/T + b) \quad (1)$$

where C is the solubility of a solute in a supercritical fluid and a, b and α are empirical constants. This correlation correctly predicts the variation of solubility: solubility increases with increasing density ρ (or pressure) at constant temperature; and solubility may increase or decrease when the temperature rises at constant pressure.

Numerous recent studies allow a correct description to be obtained and in this respect a paper by Brennecke and Eckert [16] is useful; the use of the Peng–Robinson equation of state provides improved results, as confirmed by many workers. These properties make supercritical fluids "adjustable" solvents with a continuous transition between "excellent" solvents under supercritical conditions and very "poor" solvents in the state of a compressed gas, and most processes are based on these solvent power variations. For ternary or more complex systems, phase diagrams are relatively similar to those of liquid phases; in general, they are very complex with zones where one, two or three phases coexist. As for liquid–liquid or liquid–solid equilibria, for ternary or more complex systems, supercritical fluids can be considered as selective solvents allowing selective extraction or fractionation.

Among supercritical solvents, special attention has been paid to carbon dioxide (CO_2). Its convenient critical parameters ($T_c = 31°C$, $P_c = 7.38$ MPa) permit processing near ambient temperature and under "acceptable" pressures (8–20 MPa). Another advantage of CO_2 is its non-toxic, non-combustible and "natural" character. Consequently, CO_2 seems to be an excellent solvent for the food, pharmaceutical and cosmetic industries where extraction and fractionation have to be carried out at temperatures as close as possible to ambient temperature to avoid thermal degradation and without the use of hazardous chemicals.

However, for many applications, the solvent power of pure CO_2 is limited and only low-polarity compounds are soluble at an acceptable level. Moreover, in many instances, the selectivity of the supercritical fluid is not sufficient for the separation of compounds similar in chemical structure or molecular mass. Much work has been done on the modification of the properties of CO_2 by the addition of one or more co-solvent(s) to increase the solvent power or selectivity of the fluid [16].

Polar co-solvents, such as frequently used alcohols (*e.g.*, methanol and ethanol), lead to increased selectivity and solvent power *vis-à-vis* molecules interacting with the co-solvent (dipole–dipole interactions, hydrogen bonding, proton exchange–donor properties). Non-polar co-solvents (*e.g.*, light hydrocarbons) are applied to increase the solvent power for the extraction of heavy compounds such as triglycerides.

Even if co-solvent addition complicates the separation of products and solvent, it is required in many instances for both analytical or preparative SFC in order to modify retention times and peak shapes [18,19]. Moreover, it should be noticed that water, despite its very low solubility in CO_2, plays an important role and may drastically modify the selectivity of supercritical CO_2 extraction and chromatography.

3. CHROMATOGRAPHIC PROCESSES

Chromatographic processes are mainly used for fractionation purposes, although some reaction–fractionation combinations have been investigated, but seldom used. The concept of chromatography implies interactions of mixtures with a stationary phase, essentially a fixed bed of a porous medium, in the presence or not of a fluid phase, called the eluent, in a gaseous, liquid or supercritical state. Among the numerous other chromatographic modes that are classically used with liquid or gaseous eluents [20], few can be adapted to supercritical fluids owing to technological difficulties and we shall restrict our analysis to the two main modes: elution and frontal.

As elution chromatography was first discovered as a preparative process by Tswett [21] for the fractionation of chlorophylls, it is not suprising that preparative SFC was proposed at the

very beginning of the development of SFC by Klesper *et al.* [23], 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 tool in comparison with preparative gas chromatography, which is unsuitable for heavy and thermolabile compounds, and preparative liquid chromatography, in which fraction–eluent separation is difficult and costly.

As shown in Fig. 2, 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.*, in large-scale production).

The main other mode is frontal chromatography, which is substantially similar in many instances to processes called adsorption–desorption by chemical engineers. In fact, supercritical fluids have appeared as promising desorption agents as their adaptable solvent power permits

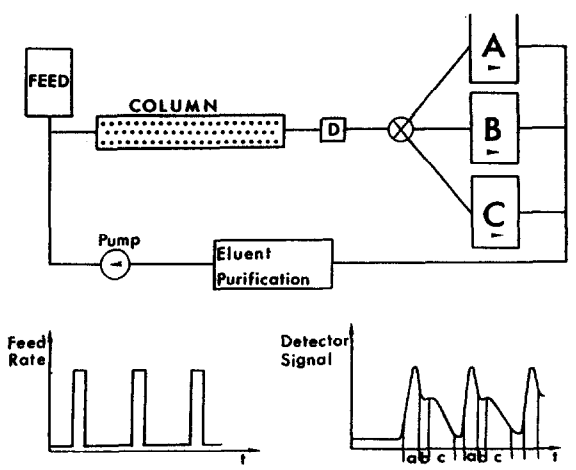

Fig. 2. Preparative elution chromatography: general flowsheet and concentrations of feed and products *versus* time.

successive desorption of different components from the adsorbent and final solvent–product separation prior to solvent recycling is easy.

Prior to detailing the technology and applications of these processes, we shall discuss the methods available for the evaluation of the basic parameters encountered in the description of flow through porous media and chromatographic interaction modelling.

4. HYDRODYNAMICS OF SUPERCRITICAL FLUIDS THROUGH POROUS MEDIA

4.1. Pressure drop [23,24]

Since the pioneering work of Darcy, the description of fluid flow through porous media was essentially based on empirical correlations obtained from numerous experimental results and empirical models, as the resolution of rigorous fluid mechanics with such a complicated geometry is impossible except for very simple cases.

For laminar flow, a very accurate pressure drop correlation was derived through the Kozeny–Carman equation, based on the representation of the porous bed as unconnected cylindrical pores of unique diameter and length equal to $l = L\theta$, where L is the bed length and θ the tortuosity factor, in which the fluid motion is described by Poiseuille's law:

laminar flow condition:

$$Re_g = \frac{\rho u}{(1-\varepsilon_e)a_p\mu} \ll 1 \tag{2}$$

pressure drop estimation:

$$\frac{\Delta P}{L} = h_k \mu u a_p^2 \cdot \frac{(1-\varepsilon_e)^2}{\varepsilon^3{}_e} \tag{3}$$

For most granular beds, it has been proved that $h_k = 4.5 \pm 1$.

For turbulent flow ($Re > 1$), good accuracy is obtained with the Burke–Plummer equation, based on the representation of the porous bed by unconnected cylindrical pores of unique diameter the axis of which is a broken line; supplementary energy losses with each direction change are expressed by:

$$\frac{\Delta P}{L} = K\rho u^2$$

leading to

$$\frac{\Delta P}{L} = h_B \rho u^2 a_p \cdot \frac{1-\varepsilon_e}{\varepsilon^3_e} \quad (4)$$

where the Burke–Plummer constant h_B was determined to be *ca.* 0.3 from numerous experimental results.

The addition of eqns. 3 and 4 leads to the well known Ergun equation, which has long been used with success for convex particles (no hole like in a Raschig ring), which is always the case for chromatography:

$$\frac{\Delta P}{L} = A\mu u + B\rho u^2 \quad (5)$$

In fact, it cannot be used in this "integrated" form if the fluid is compressible (gas, supercritical fluid) as the specific gravity ρ and viscosity μ vary along the column. Hence, in order to estimate the pressure drop in the general case, the following system has to be solved:

system I:

$$\frac{dP}{dz} = -A\mu u - B\rho u^2 \quad (6)$$

$$u = Q/(\rho\Omega) \quad \text{mass conservation} \quad (7)$$

$$\rho = f(P, T) \quad \text{equation of state} \quad (8)$$

$$\mu = g(P, T) \quad \text{viscosity dependence} \quad (9)$$

Even if some thermal effects exist in chromatographic processes, it is generally possible to assume that the operation is isothermal.

Eqn. (8) is often expressed by

$$\rho = \frac{MP}{ZRT} \quad (8a)$$

where the compressibility factor $Z = Z(P, T)$ is given by the equation of state. Moreover, Reid *et al.* [25] recommend evaluating the viscosity by

$$\mu(P, T) - \mu_0(T) = \frac{1}{\xi} \cdot h(\rho_r) \quad (9a)$$

where $\mu_0(T)$ is the viscosity at low pressure and temperature T and ξ is defined from the molar mass (g mol^{-1}) and critical parameters (K and atm):

$$\xi = T_c^{1/6} M^{-1/2} P_c^{-2/3}$$

The function h of the reduced specific gravity ($\rho_r = \rho/\rho_c$) depends on the fluid nature (non-polar or polar) [26,27].

In a previous paper [23] related to SFC using small-diameter stationary phases (<50 μm), we showed that it is possible to predict the experimental results with acceptable accuracy by choosing the Peng–Robinson equation of state and viscosity correlations recommended by Reid *et al.* [25] (for details, see refs. 23 and 24). This modelling is general as these correlations for eqns. 8 and 9 are not restricted to one fluid, even if they are not very precise as shown in Fig. 3 (from ref. 23).

In further and unpublished work, extended experimental results were compared with predictions based on other pairs of correlations 8 and 9, especially with specific and very precise experimentally based correlations for carbon dioxide; as the accuracy of pressure drop predictions from experimental data was not significantly better whilst no extrapolation to other fluid could be made, we strongly recommend the first choice. However, such modelling should not be used when the eluent is a mixture of a non-polar "classical" supercritical fluid (*e.g.*, CO_2, halocarbons) and a polar co-solvent (*e.g.*, alcohol, amine), except if only a very rough estimation is required.

In contrast of some publications considering that a high pressure drop is a considerable drawback of SFC, our experience has shown that acceptable pressure drops (<20 bar) are obtained on efficient preparative columns, even packed with small-diameter particles (10–30 μm); this pressure drop is the more acceptable as the classical "hardware" used in supercritical technology is commonly designed for service pressures up to 250–300 bar. It should be emphasized that the low viscosity of supercritical fluids, in comparison with classical liquid solvents, leads to very low pressure drops (<1 bar) through a fixed bed (catalyst, adsorbent) of "large" particles ($d_p > 200$ μm) in most in-

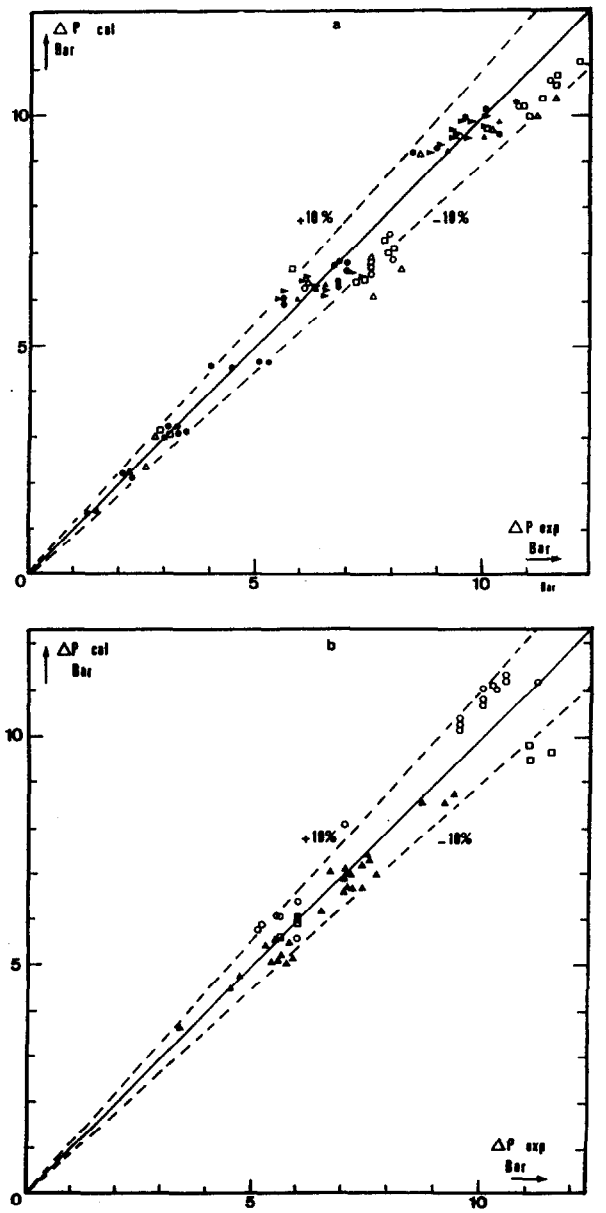

Fig. 3. Experimental *vs.* calculated pressure drop. (a) Eluent, carbon dioxide. Two columns, RP-18 silica 300 × 4 mm I.D., Temperature: □ = 20; ○ = 25; △ = 30; * = 35; ▶ = 40; ▲ = 45°C. (b) Column, C_3-NH_2 silica, 300 × 4 mm I.D., 20°C < T < 45°C. Eluents: ○ = CO_2; ▲ = CHF_3; □ = N_2O.

stances. Moreover, we stress the importance of "extra-packing" effects on the pressure drop in process chromatography, especially the contribution of the inlet and outlet frits. In our experience, inlet frit plugging must be prevented especially to avoid maldistribution (see below) by both the eluent and feed filtering and also by eluent choice in order to avoid possible precipitation. Outlet frit plugging is often much more difficult to avoid, especially when the stationary phase particle diameter distribution is wide: the smaller particles tend to migrate within the bed and to accumulate near the frit and, in certain cases, inside the frit itself; this is the reason why much attention must be paid to the "tail" of the diameter distribution and its evolution when several packings are successively performed with the same phase.

4.2. *Eluent residence time distribution [24]*

The eluent residence time distribution (RTD) plays a key role in chromatographic performance; it can be obtained through the classical Dirac injection method and described with several types of models that will be investigated below. The first parameter of RTD is the classical t_0, first-order momentum of the distribution. As eluents are always "small" molecules, they have access to both external (ε_e) and internal (ε_i) porosity of the stationary phase, so t_0 is given by integration:

$$dt = \frac{\varepsilon_T \Omega}{Q/\rho} \cdot dz \tag{10}$$

where

$$\varepsilon_T = \varepsilon_e + (1 - \varepsilon_e)\varepsilon_i$$

In SFC, the experimental evaluation of t_0 is not easy as it is difficult to find a solute that is completely unretained on the stationary phase; the use of bromotrifluoromethane (CF_3Br) [28] permits UV detection and leads to lower retention times than other classically used solvents (*e.g.*, hexane, benzene, chlorinated solvents [23]). The CF_3Br retention times appeared to be correctly correlated with the values obtained by modelling with eqn. 10 and system I with the same equation of state and viscosity correlations as used for pressure drop predictions (see Fig. 4). Moreover, a few experimental results obtained with a non-porous glass bead packing and benzene as solute are also well correlated with the values obtained by such modelling, confirm-

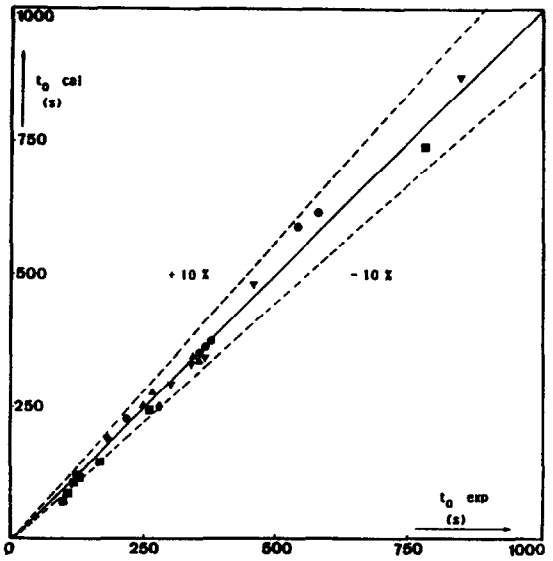

Fig. 4. Experimental *vs.* calculated eluent residence time. Eluent, carbon dioxide. Column, Hypersil, 235 × 4.6 mm I.D., Tracer, F13 B1 (CF_3Br). Temperature: ● = 40; ▲ = 45; ▼ = 50; ◆ = 55; ■ = 60°C.

ing its validity in the case of strictly zero retention. Whatever the care taken to design and pack a granular bed, the residence time distribution is never a perfect Dirac distribution and the flow must never be considered as "piston": the deviation from this ideal situation leads to a key contribution to peak broadening; even if this contribution is not as prominent in preparative as in analytical columns, it is of major interest to describe this RTD in order to analyse the different contributions to peak broadening in order to try to improve it when possible. In fact, very promising results were obtained recently regarding preparative HPLC columns [24] and were extended to preparative SFC [29].

The general technique of linear chromatographic modelling (derived from chemical engineering methods) was extensively described by Villermaux [30]. This powerful method, based on the theory of transfer functions (in the Laplace domain), allows the derivation of many more or less refined models exactly adapted to the problem to be solved. This method was used to describe a PSFC column through a complex model taking into account packing dispersion, radial velocity profile, mass transfer and extra-packing effects. This model is detailed in the Appendix and will be discussed below in relation to packing techniques [29].

5. RETENTION PHENOMENA

Retention phenomena have been described by various workers [31–39] who tried to correlate capacity factors with thermodynamic parameters. The first detailed analysis was proposed by Van Wasen and Schneider [31], based on two assumptions: thermodynamic equilibrium of a solute i between the mobile and stationary phases, and high dilution of solute i in both phases, permitting the use of Henry's law. This leads to

$$\left(\frac{\partial \ln \rho k_i'}{\partial P}\right)_T = \left[\frac{\partial}{\partial P}\left(\ln \frac{x_i^{stat}}{x^{mob}}\right)_T\right] = \frac{V_{i\infty}^{mob} - V_{i\infty}^{stat}}{RT} \quad (11)$$

where ρ is the eluent specific gravity, k_i' the capacity factor of solute i $[k_i' = (t_{Ri} - t_0)/t_0]$, x_i^{stat} and x_i^{mob} the molar fractions of solute i in the stationary and mobile phase, respectively, and $V_{i\infty}^{stat}$ and $V_{i\infty}^{mob}$ the molar volumes of solute i in each phase at infinite dilution.

In fact, eqn. 11 was used in order to evaluate the molar volumes from retention times; it was confirmed [31] that according to the theoretical predictions of Wheeler [32], $V_{i\infty}^{mob}$ is proportional to the isothermal compression coefficient at the vicinity of the critical point:

$$V_{i\infty}^{mob}/RT \approx -\lambda_i \left(\frac{\partial \ln \rho}{\partial P}\right)_T \quad (12)$$

where λ_i is a positive constant.

If $V_{i\infty}^{stat}$ is assumed to be much smaller than $V_{i\infty}^{mob}$, which has been indirectly proved [36], eqn. 11 reduces to

$$\left(\frac{\partial \ln \rho k_i'}{\partial P}\right)_T = -\lambda_i \left(\frac{\partial \ln \rho}{\partial P}\right)_T \quad (13)$$

which can be integrated as

$$k_i' = \rho^{-n_i} f_i(T) \quad (14)$$

where $n_i = \lambda_i + 1$.

Eqn. 14 appears to be accurate to explain the observations of many workers [15,18,19] accord-

ing to whom the capacity factors are strongly dependent on the eluent specific gravity, especially when the temperature range is narrow. This relationship was tested in hundreds of experimental runs [38,39] with naphthalene and derivatives, naphthoquinone and bromothiophene isomers (mono-, di-, tri-, tetra-) using either carbon dioxide or fluoroform as eluent on an RP-18 silica gel stationary phase; for each component, we obtained the parameters n_i, a_i and b_i when writing $f_i(T) = \exp(a_i - b_i T)$, ρ being estimated by the Peng–Robinson equation of state as the average specific gravity along the column. Moreover, the unexpected discrepancies observed in retention times when using helium head-pressure carbon dioxide as eluent on both normal- and reversed-phase silica gel columns were quantitatively explained by this pressure dependance of capacity factors [40].

It is also possible to connect these parameters to solubility data; in fact, the equilibrium of a solute i between the mobile and stationary phases can be expressed by

$$\left(\frac{\partial \ln a_{i\text{sat}}^{\text{mob}}}{\partial P}\right)_T = \frac{V_{i\text{pure}}^{\text{solid}} - V_{i\text{sat}}^{\text{mob}}}{RT} \tag{15}$$

where $a_{i\text{sat}}^{\text{mob}}$ is the solute activity in the mobile phase (at saturation) and $V_{i\text{pure}}^{\text{solid}}$ and $V_{i\text{sat}}^{\text{mob}}$ are the solute molar volumes in the solid considered as pure and in the mobile phase, respectively; eqn. 15 is surprisingly isomorphic with eqn. 11. If it is assumed that Henry's law as used by Van Wasen and Schneider [31] can be used until saturation (which, in most cases, corresponds to low molar fractions), it is possible to write

$$\left(\frac{\partial \ln x_{i\text{sat}}}{\partial P}\right)_T = \frac{V_{i\text{pure}}^{\text{solid}} - V_{i\infty}^{\text{mob}}}{RT} \tag{16}$$

Assuming the partial molar volumes of the solute in pure solid form and adsorbed on the stationary phase are equal under the same conditions, or are small in comparison with the partial molar volume of the solute in the mobile phase, it is possible to combine eqns. 11 and 16 as

$$\left(\frac{\partial \ln \rho k_i'}{\partial P}\right)_T = -\left(\frac{\partial \ln x_{i\text{sat}}}{\partial P}\right)_T \tag{17}$$

showing the relationship between capacity factor and solute concentration at equilibrium in the mobile phase.

Moreover, if $x_{i\text{sat}}$ is evaluated from Chrastil's correlation (eqn. 1) [17], it appears that

$$\left(\frac{\partial \ln k_i'}{\partial P}\right)_T = -\alpha_i\left(\frac{\partial \ln \rho}{\partial P}\right)_T \tag{18}$$

which can be integrated as

$$k_i' = \rho^{-\alpha i} g(T) \tag{19}$$

which is of the same form as eqn. 14, as has been extensively demonstrated by numerous experimental results; in addition, for the system CO_2–naphthalene, the α_i obtained by Chrastil [17] from equilibrium measurements was surprisingly very close to the n_i obtained from chromatographic results [36,38]: $\alpha_i = 3.403$ and $n_i = 3.42$.

Hence this development shows that equilibrium data and retention times can be easily correlated; on the other hand, eqns. 14 and 19 permits the extrapolation of capacity factors and retention times from a few experimental results to a wide range of operating parameters, which is extremely important when optimizing production cycles and process design. However, although no assumption has been made regarding the type of stationary phase (normal or bonded), experimental confirmation was obtained only on bonded silica, so extension to normal-phase materials should not be made until further experiments have been performed.

On the other hand, few workers have tried to establish the total adsorption isotherms:

$$C_{i\text{A}} = f(C_{i\text{F}}) \tag{20}$$

where $C_{i\text{A}}$ and $C_{i\text{F}}$ are concentrations of solute i in the adsorbent and in the fluid, respectively. In fact, if the capacity factors k_i' are sufficient for the determination of the retentions of highly diluted solutes, the total isotherm is required for peak simulation on a preparative scale where a linear behaviour is seldom found. Isotherm determination is already difficult with liquid eluents and is much more complex with supercritical fluids if a high precision is required. Some valuable work was published by Yonker and Smith [34] by a method based on mass isotope

tracer pulse chromatography, which is known to be not very precise; other isotherm curves were established for phenol and pesticides on activated carbon in the presence of supercritical CO_2 [41,42] and expressed through Toth or Freundlich equations (see below).

As we consider that further progress with SFC processes requires multi-component isotherm modelling, we are now developing new equipment for such determinations, in parallel with a non-linear simulation model; the method is based on desorption front analysis after equilibrium of fluid, solutes and adsorbent at various solute concentrations.

6. ADSORPTION–DESORPTION PROCESSES

The specific physico-chemical properties of supercritical fluids and especially of carbon dioxide seem perfectly adapted to frontal chromatography and particularly for stepwise desorption, during which fractionation of adsorbed species can be effected by elution power modulation of the desorbent agent. In fact, supercritical fluids were first primarily used as extraction solvents from solids and, subsequently, it was proposed to achieve extraction with several steps at increasing pressures leading to extract fractionation; this has been widely described and used on a large scale in essential oil and aroma extraction and fractionation. In contrast to extraction processes, adsorption–desorption processes are based on adsorbents that must be reused for many cycles of adsorption–desorption.

Most work reported on this subject is in fact restricted to supercritical fluid desorption: a fluid mixture (mostly aqueous) is first percolated through an adsorbent bed on which some components are fixed, leading to either removal of contaminants or high-value production recoveries from the main liquid flux that is subsequently rejected (or recycled to first use); when the adsorbent is almost saturated and the component to be fixed begins to merge at the bed end, the flux is stopped and the bed is subjected to desorption by a supercritical fluid either at constant pressure and temperature if no fractionation of adsorbed components is expected or with stepwise elution (by increasing the specific gravity or pressure at constant temperature); the main interest in using a supercritical fluid instead of a liquid solvent as desorbent consists in both elution power modulation, leading to adsorbate fractionation, and easy separation of the adsorbate from the solvent prior to solvent recycling. Another definitive advantage is related to the acceptability of carbon dioxide permitting regenerated adsorbents to be used with food products (*e.g.*, drinking water). However, the major drawback of such processes is linked to the high pressure requirement for the whole plant, including the adsorbent bed vessels that often have very large volumes.

Regarding process design, several points must be considered. As several adsorbent beds are to be used alternatively for adsorption on the process fluid and then subjected to supercritical fluid desorption, the bed number, volume and dimensions and cycle duration must be optimized, taking in account the high pressure constraint. Moreover, special attention must be paid to bed packing and fluid distribution in order to limit the desorbent volume and to optimize the adsorbent use, which needs steep fronts and therefore requires a quasi-piston flow through the whole installation.

Most reported applications are related to desorption. The original patents of Modell [11–13] and further studies by several workers [41–44] are related to organic pollutant desorption from activated carbon [41–44] and polymeric adsorbents [41] used for either drinking water or residual water treatment; originally, investigators tried to find a new regeneration process for activated carbon that was intended to be used on a very large scale for the treatment of drinking water throughout the USA, which would have led to a very high consumption of adsorbent if regeneration was operated by the classical activation procedure at high temperature. This is the reason why investigators chose various pollutants (such as phenol and pesticides, *e.g.*, alachlor, diazinon) as model molecules adsorbed from very dilute aqueous solutions and desorbed by supercritical CO_2. From this important experimental work, several conclusions were drawn, as follows.

Regeneration of the adsorption capacity of the

activated carbon appears to be limited; in fact, the adsorption breakthrough curves exhibit a capacity decrease of about 50% after the first cycle but the capacity seems to be stable during further cycles as if the sites on which irreversible adsorption occurs were saturated during the first cycle, the other sites remaining perfectly reversible, as shown by alachlor adsorption on activated carbon; however, other pesticides are not as easily desorbed as alachlor and supercritical fluid regeneration appears impossible with components such as diazinon, pentachlorophenol or carbaryl [42].

Desorption curves were predicted from the classical mass balance equation:

$$\varepsilon \partial C_F/\partial t + \rho_A \partial C_A/\partial t + u_F \partial C_F/\partial z = 0 \qquad (21)$$

and the isotherm $C_A = f(C_F)$, where C_A and C_F are the concentrations of the solute on the adsorbent and in the fluid, respectively, ρ_A is the bulk adsorbent specific gravity, u_F the fluid velocity and ε the bed porosity (note that eqn. 21 neglects any mass transfer limitation and axial dispersion along the bed). Toth or Freundlich isotherms were selected to fit the experimental data with good accuracy [41,42].

For components exhibiting a high affinity with adsorbents, such as phenol, supercritical CO_2 does not offer a high potential regeneration alternative; however, supercritical CO_2 might be considered as a potential eluent for the regeneration of activated carbon loaded with organics that are not so strongly adsorbed, even if direct extraction from the aqueous solution might also need to be evaluated [41].

Finally, we consider that the most important drawback of supercritical fluid regeneration of activated carbon is due to the non-desorption of strongly adsorbed pollutants that are frequently present, leading to a continuous decrease in adsorption capacity along the successive cycles. This is probably why no large-scale plants have been developed according to this concept.

More recently, several studies were reported regarding two other types of applications: fat or oil treatment [45–47] and deterpenation of citrus essential oils [48,49]. King *et al.* [45] described the on-line deodorization of vegetable oils extracted from seeds by supercritical CO_2, by percolating the CO_2 extract mixture on an adsorbent bed; several adsorbent materials including activated carbon and polymeric material (Tenax and XAD resins) were used and they characterized the adsorption capacity as a function of the operating conditions (pressure, temperature). Their results showed that for porous polymeric solvents, morphological changes in the polymer matrix may occur with a positive effect on adsorption capacity, *e.g.*, for Tenax and XAD-2 at intermediate pressures (*ca.* 20 MPa); however, they concluded that, owing to these morphological modifications, such polymeric material seems unsuitable for long-term processing under supercritical conditions; although activated carbon exhibits a higher adsorption capacity, irreversible adsorption and slow desorption kinetics also limit its long-term application. Another similar application is related to butterfat fractionation and cholesterol removal; in fact, many investigators have recently tried to fractionate butterfat with supercritical CO_2 on packed counter-current columns; like many others, we obtained very disappointing results as the preparation of cholesterol-free butter is not possible by such a process even through a good fractionation of triglycerides according to their molecular mass is obtained: only a hard and flavourless residue containing long-chain triglycerides (over 46 carbons) is almost freed from cholesterol. Several types of adsorbents have been used for the selective removal of cholesterol from the extract–CO_2 effluent from the fractionation column. Acceptable selectivities [46] were reported on silica gel (75% or 94% cholesterol removal with CO_2 at 300 bar and 40°C with silica-to-butterfat ratios of 1:1 and 3:1 respectively); however, as described in a recent patent [47], basic adsorbents are to be preferred as flavour components, pigments and triglycerides are not adsorbed. Selective and efficient adsorption of cholesterol on calcium hydroxide or magnesium oxide was obtained on treating butterfat or lard in the presence of CO_2 at 22 MPa and 35–45°C. However, these processes will probably not be widely used for economic reasons.

Another application of supercritical fluid adsorption–desorption is in citrus oil deterpena-

tion; essences from fruit peel are highly concentrated in low-value terpenes (mainly limonene), whereas the highly aromatic oxygenated compounds are present at very low levels (a few per cent); such fractionation is extremely difficult as these products are thermolabile and leads to off-flavour compounds when subjected to heat or oxygen. Supercritical CO_2 fractionation on counter-current columns has been investigated for long but it has been shown that the selectivity is very low owing to the effect of "entrainment" due to terpenes dissolved in CO_2, which leads to very high costs (large number of theoretical plates, high reflux rate and very low productivity). Similarly to the previous case of cholesterol removal from fat, selective adsorption of oxygenated compounds appears promising; such a concept is now used on a large scale as described in a recent patent [48], the adsorbent being silica gel or activated alumina with which the crude oil is mixed, the adsorbate being recovered by CO_2 extraction, which leads to an extract with a much lower limonene concentration: according to the extraction procedures, low selectivity (limonene concentration variation <10%, high yield) for one step (high solvent power, 28 MPa and 35°C) or high selectivity but low yield for two steps (low solvent power step, 7–9 MPa and 50–70°C, followed by high solvent power step, 28 MPa and 50–70°C). Other investigators [49] reported briefly a similar process using a non-disclosed adsorbent from which lemon oil is extracted in two steps at low pressure (terpenes) and high pressure (oxygenated compounds). In our experience, such fractionation is very attractive although the problem of irreversible wax product adsorption must bé solved prior to long-term exploitation under economical conditions.

7. PREPARATIVE ELUTION CHROMATOGRAPHY

Two different types of processes and equipments have been used, according to the fractionation purpose.

When small amounts of pure products (10^{-3}–1 g) are required, bench-scale equipment directly derived from analytical apparatus has been used with the adoption of non-destructive detection, fraction collection and eluent–product separation; as detailed in a previous review [10], fraction collection has been performed in different ways: decompression at atmospheric pressure, collection at high pressure or adsorption on a solid followed by elution or dissolution in a liquid solvent. Many applications have been reported in the last 30 years, first in order to demonstrate the feasibility and selectivity of SFC [50–62] and later for chemical structure identification (MS, NMR, etc.) in most instances. Among the original studies, a two-dimensional method consisting of coupling small-scale preparative SFC with thin-layer chromatography (TLC) led to exciting results for the identification of new molecules in aroma products [53]; sample preparation for trace analysis is also often cited [51,53,54], similarly to micro-supercritical extraction [52,63]. Recent work [64] on loading effects on resolution for polyunsaturated fatty acid tryglyceride fractionation is also of interest.

Large-scale preparative SFC requires eluent recycling after fraction collection and eluent–product separation; after the first process description in the original patent [14] (1982), we undertook feasibility studies [38,39,65–68] followed by development work and applications [68–71] to valuable fractionation problems, which led to commercial development in 1990 [72].

Promising results were obtained in my laboratory in 1985, but the development work was much more complex than expected and this attractive process, permitting the production of solvent-free fractions at much lower cost than preparative HPLC, requires the resolution of many problems: eluent–fraction separation with high yields and eluent purification prior to recycling, periodic feed injection as a perfect "square" signal, column packing (efficiency, reproducibility, lifetime) and also modifier (co-solvent) injection at a perfectly constant concentration in the eluent. In fact, the easiest problems to solve were the technological ones (valves, circuit and pumps, etc.) with special attention to the computer system permitting safety and operating parameter control and logging (including an automatic cut-point control algorithm) [38,67]. Several years were spent

before reaching a commercial stage, which possibly explains why so few investigators have worked on the subject. Kosah [73] reported the elution of compounds with a supercritical eluent on several types of stationary phases (natural or not, mineral or polymeric) and discussed the technical and economic feasibility of the process on a pilot scale; Alkio *et al.* [74] modified an extraction unit to convert it into a preparative chromatograph with columns 0.3–2 l in volume eluted with supercritical CO_2 (flow-rate up to 8 kg h^{-1}). Even though few papers have been published, we know that several companies are now developing specific applications, especially of pharmaceutical interest.

Fig. 5 presents the general flow-sheet of a large-scale preparative SFC unit, and the following comments can be made. If the eluent is a pure fluid (*i.e.*, CO_2 without modifier), it is easy to design the circuit and control the process: a metering membrane pump delivers high-pressure liquid eluent at a desired flow-rate, the pressure at the column inlet being controlled through a by-pass; a loop of desired volume permits feed injection in a similar way to a classical six-port injection valve, although a proprietary system is necessary to guarantee both reproducibility and a perfect square shape of the injection signal, as any tailing may ruin the fractionation efficiency; fraction collection must be extremely efficient and could be done in high-performance

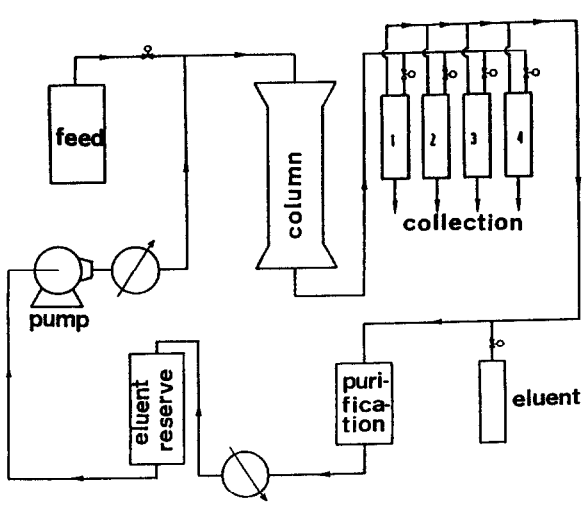

Fig. 5. General flowsheet of preparative-scale SFC.

separators [75] designed as cyclonic chambers in order to break the two-phase "mist", resulting from mixture depressurization, with heated jackets in order to supply the enthalpy for eluent vaporization; whatever this eluent–fraction separation efficiency, it is necessary to purify the eluent prior to recycling, which can easily be done by percolation through an activated carbon bed; the eluent is then liquefied through a condenser, stored in a reservoir and recycled.

Unfortunately, as discussed previously, modifiers must be used in most instances in order to improve the elution strength and drastically increase the fractionation performance even if it makes the process complex. In fact, as retention times are very sensitive to modifier concentration, this parameter must be carefully controlled, which is not easy when the eluent is recycled as in large-scale units; two complementary systems permit very reliable results to be obtained, as follows. For "low" co-solvent concentrations [76], it is recommended to "saturate" the eluent at the separator outlet through a counter-current column working at constant pressure and temperature, which leads to a constant composition according to the Gibbs law; this system also has the advantage of scrubbing the eluent to remove traces of compounds that might be entrained from the separators. It was shown to be very satisfactory in a difficult purification of a vitamin intermediate [67]. For "high" co-solvent concentrations, saturation is often difficult to use as too high temperatures might be necessary to reach the required composition; in this case, we recommend the use of the saturation system followed by direct addition of a controlled flow-rate of modifier within the liquefied eluent.

For example, with CO_2 as fluid and ethanol or methanol as modifier, the first system alone can be used to control concentrations up to 3–4% (mass) and the second for higher concentrations. Moreover, it should be noted that a patented device [75] permits the quasi-continuous removal of fractions collected in the separators and decompression to the atmosphere without product losses; this system seems essential when a modifier is used.

Chromatographic columns must be very carefully designed. In fact, most of laboratory-scale

preparative SFC has been operated on prepacked columns of diameter less than 20 mm. However, we have found that, for larger diameter columns, unacceptable dead volumes ruin the column efficiency after a few hours of elution and compression techniques must be used. Similarly to preparative HPLC, four techniques can be considered: radial compression [77], static axial compression, dynamic axial compression [78,79] and annular expansion [80]. Because of technical problems, radial compression seems not applicable to SFC. We have tried both static and dynamic compression [81]. The piston is moved with a manual screw in static compression (Fig. 6a) whereas it is moved by a head pressure of the eluent in dynamic compression (Fig. 6b). The results obtained by static compression were extremly poor, even on a 24 mm diameter column, with a reduced HETP between 10 and 20 and non-symmetrical peaks, whereas columns designed according to the dynamic axial compression concept are extremely efficient, as shown in Fig. 7; moreover, we reproduced many packing with various phases with similar performances, the best of which were obtained with an irregular-shaped 7-μm chiral phase [82] leading to an optimum efficiency of 5800 theoretical plates on a 10 cm long bed [71] (reduced HETP

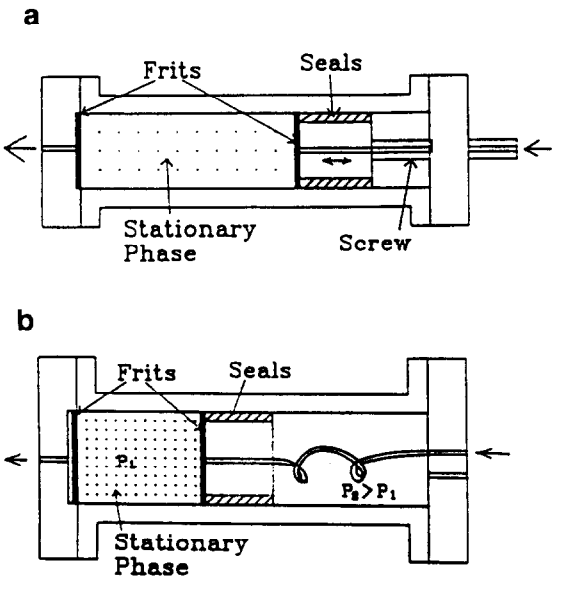

Fig. 6. Axial compression columns. (a) Static axial compression. (b) Dynamic axial compression with "floating" piston.

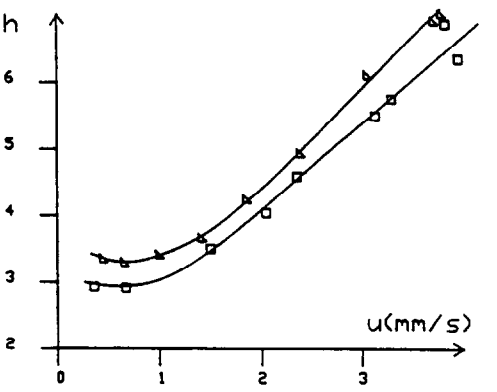

Fig. 7. Reduced HETP *versus* eluent velocity of a dynamic axial compression column. Eluent, carbon dioxide; diameter 60 mm at 20 MPa and 308 K; stationary phase, C_{18} silica, 12–45 μm (average diameter 23 μm), 300 g. Time: $\Delta = 1$; $\square = 90$ h.

ca. 2); also, the performance of such packing remained very stable during several weeks of elution.

The extreme quality of packings obtained with dynamic axial compression is confirmed by the results [29] reported in Fig. 8. As detailed in the Appendix, peak broadening for any hydrodynamic reason can be expressed by an equation leading to an increase in HETP with increased

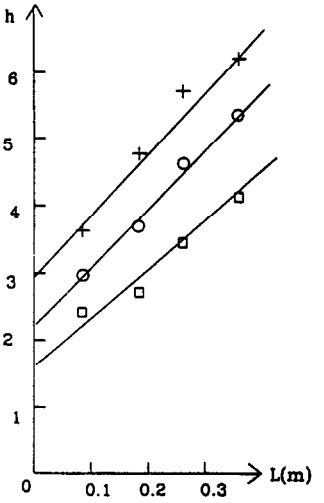

Fig. 8. Dynamic axial compression column performance. Same conditions as in Fig. 7. Reduced HETP (after elimination of extra-column effects) *versus* packed length for several eluent velocities: $\square = 1$; $\bigcirc = 2$; $+ = 3$ mm s^{-1}.

packing length, especially if the velocity distribution on a bed section is not perfectly flat. The interest in this equation is to permit the evaluation of each contribution to peak broadening and to reduce each one by a proper design (*i.e.*, elimination of extra-packing effects, frit replacement in the case of maldistribution, etc.). In fact, in Fig. 8, a linear dependence of packing efficiency (expressed as reduced HETP calculated from peak variance) [70] on bed length is clearly shown; moreover, the eluent velocity profile inside the column (obtained by model fitting from these results) is almost perfect except some wall effects that lead to slight peak tailing [29].

Obviously, transposition of the technology of preparative HPLC columns to preparative SFC columns requires many adaptations, either when the piston is moved by the pressurized eluent ("floating" piston) or by an external hydraulic jack; in fact, great care must be taken to maintain the correct strength on the piston in all situations, preventing both backward movement of the piston and crushing of the stationary phase. Finally, annular expansion might also be an efficient technique, but no experience has been reported in preparative SFC so far.

As efficient packing procedures were obtained only recently, few applications of preparative SFC have been published, apart from the numerous laboratory-scale results already cited [10]. Hence it is difficult to evaluate large-scale preparative SFC and to compare it with competing techniques such as preparative HPLC. However, the fractionation of polyunsaturated fatty acid esters [83] can be taken as a first example. After preliminary results obtained on low-efficiency prepacked columns [38,39,67], fractionation of polyunsaturated fatty acids (EPA/DHA) ester-rich mixtures was performed [69] on a dynamic axial compression column (60 mm diameter, 230 mm bed length) and the results were compared with preparative HPLC results obtained on similar feeds, as shown in Table 2. It appears that higher purities are obtained by preparative SFC and much higher feed fluxes can be injected,

TABLE 2

FRACTIONATION OF POLYUNSATURATED FATTY ACID ESTERS

Parameter	Ref. 84	Ref. 85	Ref. 69
Freed composition:			
EPA (%)	36.8	46.7	56
DHA (%)	40.9	30.5	31
Equipment	Preparative HPLC: radial compression	Preparative HPLC: dynamic axial compression	Preparative SFC: dynamic axial compression
Bed dimensions:			
Diameter (mm)	200	300	60
Length (mm)	600	300	230
Stationary phase	C_{18} silica, 55–105 μm	C_{18} silica, 12–45 μm	Silica, 10 μm
Eluent	MeOH–water (90:10)	MeOH–water (90:10)	CO_2 (14.3 MPa, 50°C)
Flow-rate (l h^{-1})	72	200	53
Injected amount (g)	90	136	7.8
Cycle duration (min)	50	19	10
Feed flux (kg h^{-1} m^{-2})	3.4	6.1	16.5
Purities (mass-%)			
EPA	86+	91–96	95–96
DHA	83+	75–85	85–97

corresponding to feed-to-stationary phase ratios 3.5 times higher. Moreover, purified products obtained by preparative HPLC must be subjected to complex treatments for elimination of residual solvent down to a very low level, whereas those contacted with CO_2 are available for clinical use.

However, any global economic comparison is not easy as no long-term experience of such fractionation is available on large-scale preparative SFC equipment. From our experience with both techniques and this fractionation problem, we conclude that the operating costs of preparative SFC are far lower (*ca.* 50%) than those of preparative HPLC (including solvent recycling and final product purification). Regarding investment costs, the comparison is more difficult because preparative SFC equipment is only now being commercialized and by only one supplier [72], whereas preparative HPLC equipment is available from several manufacturers. However, the costs of both equipment (including solvent recycling and final product treatment, which are extremely costly in preparative HPLC) designed for the same fractionation capacity will probably tend to be similar. Hence for this typical fractionation it is certain that preparative SFC will lead to overall costs that are significantly lower (20–40%) than those of preparative HPLC.

From analytical results [86,87], chiral fractionation by SFC appear to be extremely promising as the resolutions are similar to those obtained by HPLC, but the retention times are 3–5 times lower. Recent work [71,88] with a 60 mm diameter column packed by dynamic axial compression with Pirkle-type chiral phases [82] (Fig.

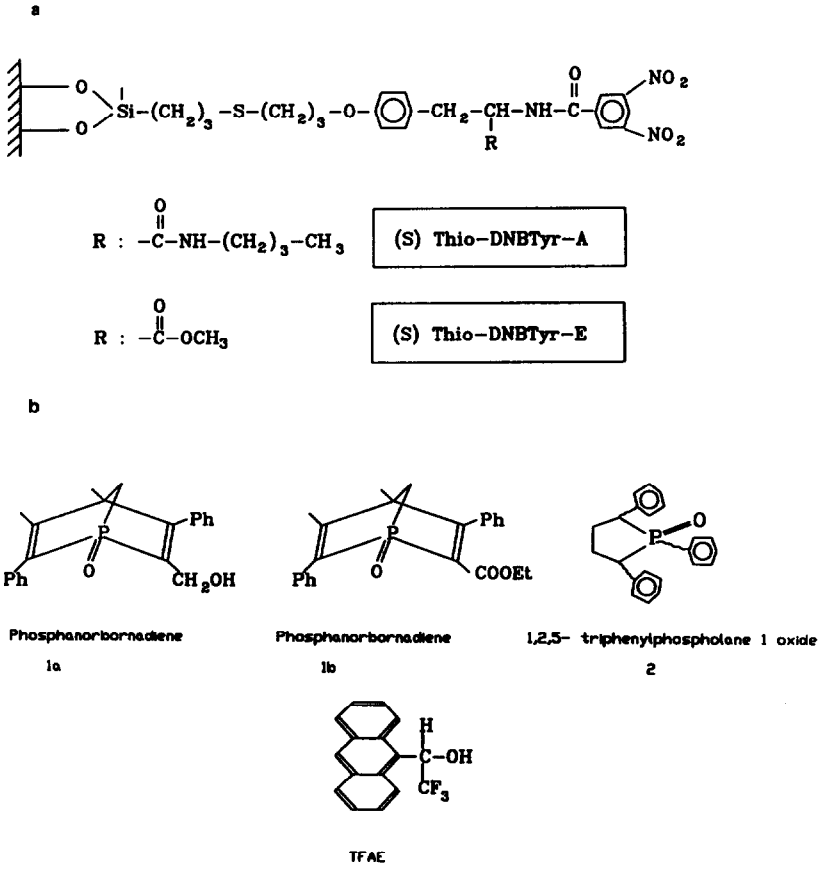

Fig. 9. Chiral fractionations. (a) Stationary phase structures. (b) Racemate structures.

9a) led to valuable results for several racemates (Fig. 9b). It should be noted that, in these cases, a modifier (ethanol) must be added to CO_2 and, as the selectivity tends to decrease when temperature is increased, the process was operated at 293 K, corresponding to a subcritical fluid phase. Very pure fractions were obtained with enantiomeric excesses between 94 and 100% for both isomers for the example shown in Fig. 10. However, as Pirkle-type phases exhibit a low capacity in relation to the small concentration of chiral sites, productivities remain low and the selectivity drastically decreases when non-chiral interactions occur after chiral site "saturation" (Fig. 11). From results obtained on TFAE fractionation, the overall cost was estimated to be *ca.* US$ 50/g of purified enantiomer for a production of 15 g per day on the equipment used for this work [88].

Before concluding this section on elution chromatography, it should be noted that most variations classically developed in preparative HPLC [20] may be applied in preparative SFC. As an illustration, recycle chromatography was used by Saito *et al.* [89] to improve the resolution of phthalate isomers; obviously, the well known recycle chromatography with "peak shaving" is an interesting variant that should always be considered.

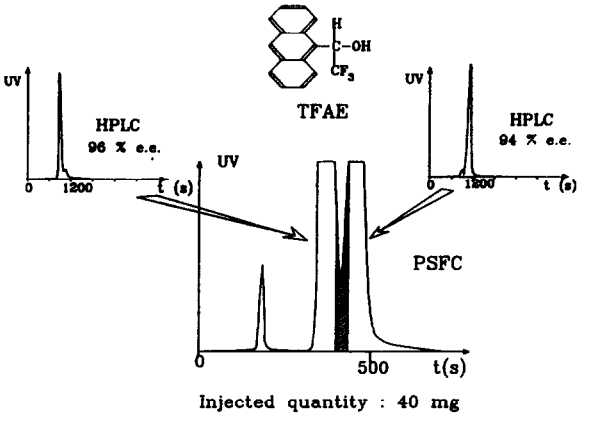

Fig. 10. Fractionation of TFAE enantiomers. SFC eluent, carbon dioxide–ethanol (96:4, v/v), 20 MPa, 293 K, flow-rate 43 l h^{-1}. Column, 76 mm × 60 mm I.D. Si-60, 10 μm, (S)-thio DNB Tyr A. HPLC analysis: column, 250 mm × 4.6 mm I.D., same stationary phase. Eluent, hexane–ethanol (96:2, v/v), flow-rate 2.5 ml min^{-1}.

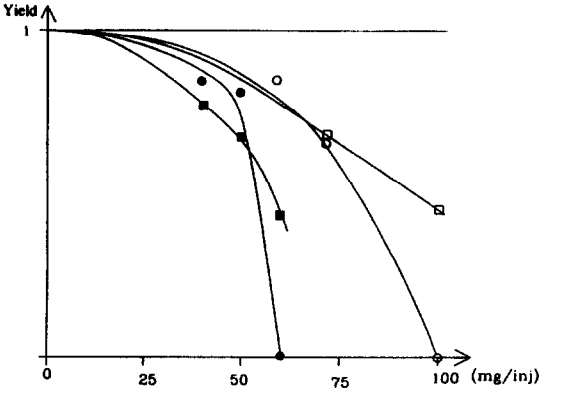

Fig. 11. Yield of pure TFAE enantiomers *versus* injection mass. Conditions as in Fig. 10 except for eluent flow-rates: ○ = (+ isomer) 19.5; □ = (− isomer) 19.5; ● = (+ isomer) 43; ■ = (− isomer) 43 l h^{-1}.

8. CONCLUSIONS

Along with simultaneous progress in liquid-phase chromatographic processes, now subject to rapid industrial development, analytical SFC —now widely accepted— and extraction–fractionation by supercritical fluids, with the related technological developments, supercritical fluid chromatography processes are attracting increasing interest after a long period of "suspicion" during which the technological drawbacks related to both high pressures and non-continuous operation and possible patent limitations restrained most investigators.

In fact, the combination of both the high selectivity of chromatographic interactions and the unique properties of supercritical fluids (*e.g.*, CO_2), will lead to promising applications in the pharmaceutical industry and possibly in pollution abatement processes during the next 10 years.

Moreover, apart from the necessary basic experimental data related to each specific application, efficient theoretical tools will be available within a few years and permit process simulation with sufficient accuracy to obtain reliable technical and economic evaluations, necessary for process optimization and further industrial development.

9. SYMBOLS

a, b Coefficients in Chrastil equation

a, a_p	Specific surface area of stationary phase/or of a particle ($m^2\ m^{-3}$)
A, B	Ergun coefficients
C, C_A	Concentration in mobile phase (mol m^{-3}, mol kg^{-1} or kg kg^{-1})
C, C_F	Concentration in the stationary phase (mol kg^{-1} or kg kg^{-1})
D, D_m	Diffusion coefficient ($m^2\ s^{-1}$)
D_{AX}	Axial dispersion coefficient ($m^2\ s^{-1}$)
d	Particle diameter, mean diameter (m)
G	Laplace transfer function
H	Height equivalent to a theoretical plate (HETP) (m)
h	Reduced HETP ($=H/d$)
k	Mass transfer coefficient ($m\ s^{-1}$)
k_B	Burke–Plummer coefficient
k_K	Kozeny–Carman coefficient
K	Partition coefficient between two phases
k'_i	Retention factor of solute i
l	Pore length (m)
L	Column length (m)
M	Molar mass (kg mol^{-1})
N	Total number of plates of the column
P	Pressure (Pa)
P_c	Critical pressure (Pa)
Pe	Peclet number
Q	Mass flow-rate ($kg\ s^{-1}$)
R	Perfect gas constant (J $mol^{-1}\ K^{-1}$)
Re_g	Particle Reynolds number
t	Time variable (s)
T	Temperature (K)
T_c	Critical temperature (K)
t_0	Eluent residence time (s)
t_{Ri}	Retention time of solute i (s)
u	Fluid velocity ($m\ s^{-1}$)
u_i	Normalized superficial velocity
V	Volume (m^3)
V_i	Molar partial volume of solute i (m^3 mol^{-1})
x_i	Molar fraction of solute i (mol mol^{-1})
z	Length variable (m)
Z	Compressibility factor

Greek symbols

α	Exponent in Chrastil's equation, variable definition in Appendix 1.
ε_e	External porosity
ε_i	Internal porosity
ε_T	Total porosity
ρ	Specific gravity ($kg\ m^{-3}$)
ρ_c	Critical specific gravity ($kg\ m^{-3}$)
ρ_r	Reduced specific gravity ($=\rho/\rho_c$)
λ_i	Coefficient in eqn. 12
μ	Viscosity (Pa s)
μ_i	Distribution time momentum of order i
θ	Bed tortuosity
ω	Surface fraction
Ω	Column section (m^2)
ν	Kinematic viscosity ($m^2\ s^{-1}$)
ξ	Parameter in viscosity estimation correlation
ΔP	Pressure drop (Pa)
τ_m, τ_p	Residence time in column heads and tubings (s)

10. ACKNOWLEDGEMENTS

The author is grateful to L. Doguet, G. Fuchs, P. Jusforgues and R.M. Nicoud for reviewing this work and for fruitful discussions.

11. APPENDIX: RESIDENCE TIME DISTRIBUTION MODELLING IN LARGE SFC COLUMNS

The mass-balance equations in a section of column for the stationary phase and the mobile phase are given by [30]

$$u \cdot \frac{\partial C_F}{\partial z} + \varepsilon \cdot \frac{\partial C_F}{\partial t} + ka(C_F - C_A/K) = D_{AX} \cdot \frac{\partial^2 C_F}{\partial z^2}$$

$$(1-\varepsilon)\frac{\partial C_A}{\partial t} - ka(C_F - C_A/K) = 0$$

where C_F and C_A are the concentration of the solute in the mobile phase and the stationary phase, respectively, D_{AX} is the axial dispersion coefficient, k is the mass transfer coefficient between the stationary phase and the mobile phase, a is the specific surface area of the stationary phase, ε is the porosity of the stationary phase, K is the partition coefficient between the two phases and u is the fluid velocity.

The solution of these equations in the Fourier domain gives the following transfer function [30]:

$$G_A(\omega) = \exp\left[\frac{Pe}{2} - \frac{Pe}{2} \cdot \sqrt{1 + \frac{4j\omega t_0(1+\alpha)}{Pe}}\right]$$

where

$Pe = \frac{uL}{D_{AX}}$ is the Peclet number and $j^2 = -1$

L is the length of the column

$t_0 = L/u$ is the zero retention time

$$\alpha = \frac{K(1-\varepsilon)}{\varepsilon(1 + t_m j\omega)}$$

$$t_m = \frac{K(1-\varepsilon)}{ka}$$

The extra-column effects can be considered as a series of two mixed cells (flow distributors) and a piston flow through the connection tubes. The transfer function of these effects in the Fourier domain is

$$G_B(\omega) = \frac{\exp(-j\omega\tau\rho)}{(1 + j\tau m/2)^2}$$

where

$$\tau_p = \frac{V_p}{Q} \text{ and } \tau_m = \frac{V_m}{Q}$$

Q is the flow-rate
V_m is the volume of the two mixed cells
V_p is the tubing volume

In order to take into account the velocity radial profile inside the column, the fluid flow is considered as the sum of n trickles in parallel (Fig. 12) where

$$\sum_{i=1}^{n} q_i = 1 \text{ (fractions of flow-rate)}$$

$$\sum_{i=1}^{n} \alpha_i = 1 \text{ (fractions of column section)}$$

The transfer function in the Fourier domain in a trickle is

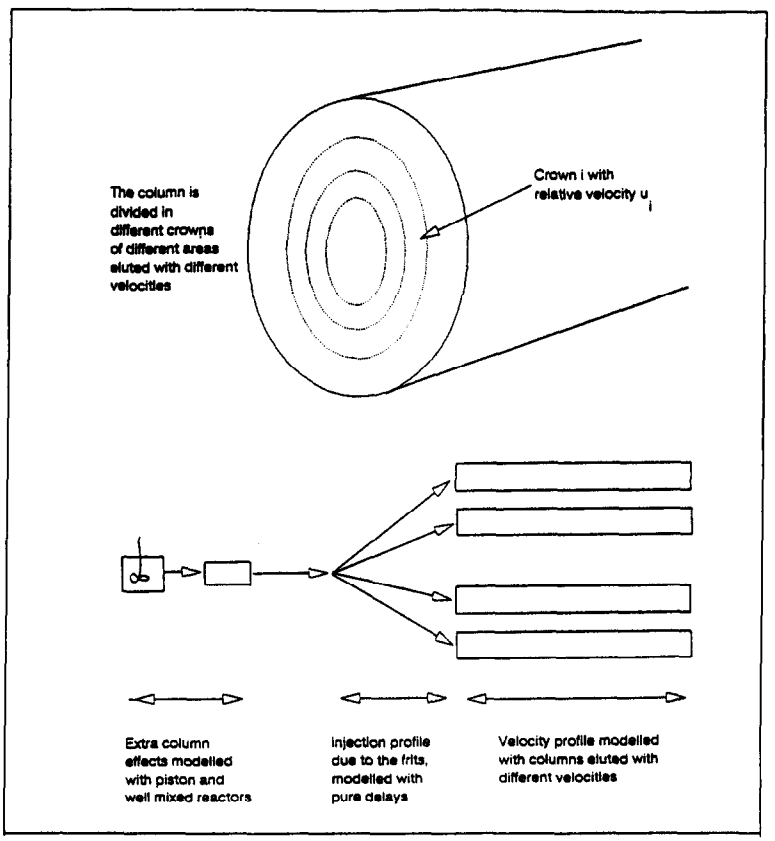

Fig. 12. Eluent flow modelling.

$$G_i(\omega) = \exp\left[\frac{Pe}{2} - \frac{Pe}{2} \cdot \sqrt{1 + \frac{4j\omega t_i(1+\alpha)}{Pe}}\right]$$

where

$$t_i = \frac{t_0 \alpha_i}{q_i}$$

The whole transfer function is then

$$G(\omega) = G_B(\omega) \sum_{i=1}^{n} q_i G_i(\omega)$$

In preparative HPLC it is possible to measure the velocity radial profile after an injection of a coloured trace. For technical reasons, this cannot be performed in preparative SFC; then the velocity profile parameters are obtained by fitting the whole model to the experimental peaks.

The Van der Laan relationship allows the estimation of HETP to be simplified:

$$H = \frac{\mu_2 - \mu_1^2}{\mu_1^2} \cdot L$$

where μ is the distribution momentum [first (μ_1) and second (μ_2) order].

Considering that t_m and t_p are negligible compared with t_0 $(1 + K')$, we obtain

$$H = \frac{2}{P_M} + \frac{2K_r}{P_M} + \frac{2K' t_m \mu_0}{(1+K')^2} + K_r L$$

where

$$K_r = \sum_{i=1}^{n} \alpha_i^2 / q_i - 1$$

and

$$P_M = Pe/L$$

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