Optimization of the high-performance liquid chromatographic separation of fullerenes using 1-methylnaphthalene as the mobile phase on a tetraphenylporphyrin-silica stationary phase

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Abstract

The separation of C_{60} and C_{70} was studied on four different stationary phases. The performance of three commercial products, Buckyclutchers, PYE and PBB and of a new phase, immobilized tetraphenylporphyrin on silica, were investigated. The influence of the surface chemistry on this separation is discussed. On all four phases, good resolution is obtained with pure toluene as the mobile phase. With some of them, this result is obtained with stronger eluents, mixtures of toluene and 1-methylnaphthalene. This allows the injection of much more concentrated and larger samples than on conventional systems and gives considerably improved production rates of purified compounds. The optimization of the temperature, the flow velocity, and the mobile phase composition for the separation of C_{60} and C_{70} on the tetraphenylporphyrin bonded phase is discussed. The overloading of this stationary phase was studied empirically in mixtures of toluene and 1-methylnaphthalene. The advantage and properties of this latter solvent are illustrated. Fractions of C_{60} with 98.5% purity and C_{70} with a purity better than 99% were prepared with near total recovery yield from 5 mg samples. © 1997 Elsevier Science B.V.

Keywords: Stationary phases, LC; Mobile phase composition; Preparative chromatography; Fullerenes; Methylnaphthalene

1. Introduction

The physical and physico-chemical properties of fullerenes are still largely unknown and can only be determined accurately if pure substances are used in their determination. Thus, the development of improved methods for the separation and purification of fullerenes and their derivatives is of great importance. Although some nonchromatographic methods have been published, high-performance liquid chro-

matography (HPLC) is by far the most widely used method in this area, because of the extreme flexibility and separation power of this method. A large number of HPLC procedures have been reported for fullerene separations. They were reviewed recently by Gasper and Armstrong [1] and by Theobald et al. [2].

The analytical scale separation of C_{60} and C_{70} has been solved using a variety of chromatographic systems. This allows the solution of all current practical analytical problems. Most stationary phases studied are chemically bonded silica using simple hydrocarbon ligands, in a majority of cases octadecyl-silica. These systems give baseline resolution

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of these two compounds provided that the optimum mobile phase composition is used. The best mobile phases are either poor solvents (e.g., n-heptane) or, even better, mixtures of a good (e.g., toluene) and a poor (e.g., n-heptane, methanol, or dichloromethane) solvent of these fullerenes. It is known that the retention of any eluite on reversed-phase materials, such as C_{18} silica, decreases with increasing solubility of this compound in the mobile phase. This is in agreement with reports that common stationary phases used for analytical scale separations of fullerenes are unable to separate these compounds when a strong solvent like toluene, let alone carbon disulfide or 1-methylnaphthalene, is used as the mobile phase. This lack of resolution arises from the mere absence of retention of the light fullerenes which are eluted at the hold-up time, with negligibly small retention factors, by these strong solvents.

Accordingly, preparative scale separations of C_{60} and C_{70} are difficult. Low production rates are achieved with conventional systems because of the small capacity of the mobile phase which is limited by the low solubility of these compounds in mobile phases which have an elution strength poor enough to give a retention sufficiently important to ensure resolution (i.e., k' larger than 1 to 2). This does not impair analytical separations because the molar absorptivities of fullerenes are extremely high, allowing the detection of low concentration signals. On the other hand, the maximum concentration of the feed components in the eluent is the saturation concentration of the mobile phase. The injection of more concentrated samples dissolved in stronger solvents results in poor separations [3]. Increasing the sample volume (volume overload) is limited by the distance between the bands of the components to be resolved. The only real possibility to increase the column loadability is to use strong solvents combined with stationary phases on which the retention of fullerenes would be very high with a weak mobile phase, because of a high adsorption energy, but will be moderate in a strong solvent. The selectivity for molecular size should be conserved on these phases. Under these conditions it could be possible to inject more concentrated samples. The limit to the amount of feed injected would then be set by concentration overloading of the stationary phase, which should not be excessive [4].

Several groups have realized the strong need for such stationary phases and have actively investigated the synthesis of packing materials for preparative chromatography of fullerenes. Four different packing materials meet inequally well the requirements listed above for a preparative scale separation of C_{60} and C_{70}, the Buckyclutcher, PYE, PBB and tetraphenyl-porphyrin materials, all chemically bonded silicas. The Buckyclutcher (3,3,3-tri-dinitrobenzoyl-propyl silica) was developed by Welch and Pirkle [5]. This electron-acceptor phase retains the fullerenes through a charge transfer mechanism. Although it can separate C_{60} and C_{70} in pure toluene as the mobile phase, the retention factors and the selectivity factor are low. Stronger solvents cannot be used. Thus, this stationary phase, like the conventional C_{18} bonded silica materials, is not useful for preparative separations of fullerenes. The PYE [immobilized 2-(1-pyrenyl)ethyl-silyl silica] [6] and the PBB {immobilized 3-[(pentabromobenzyl)oxy]- propylsilyl silica} materials were developed by Kimata et al. [7]. They retain fullerenes more strongly and solutions of stronger solvents in toluene can be used as the mobile phase. Several tetraphenylporphyrin immobilized silicas were developed by Kibbey and Meyerhoff [8] and used for the separations of various polyaromatic hydrocarbons, fullerenes and endohedral metallofullerenes [9–12]. They exhibit the largest retention of fullerenes observed so far.

The goal of this paper is to study and compare the performance in preparative chromatography of the four stationary phases listed above, to discuss the optimization of the experimental parameters for the separation of C_{60} and C_{70}, to assess the production rates and purity which can be achieved, and to illustrate the considerable advantages brought by the use as a mobile phase component of 1-methylnaphthalene. The solubility of C_{60} in 1-methylnaphthalene is 33 mg/ml [13], and exhibits all the desirable properties of a mobile phase for HPLC. The overloading of this stationary phase for high production rate of pure fractions is also illustrated and discussed.

2. Experimental

2.1. Apparatus

The chromatographic separations were carried out

using a Hewlett-Packard (Palo Alto, CA, USA) HP 1090 liquid chromatograph equipped with a ternary solvent delivery system, an automatic sample injector, a UV photodiode array detector, and a data station for computerized data acquisition. When collection of selected fractions was carried out, a Gilson FC 203 (Middleton, WI, USA) fraction collector was used.

2.2. Chemicals

HPLC grade toluene was purchased from Aldrich (Milwaukee, WI, USA) and was used without further purification. 98% pure 1-methylnaphthalene (m.p.: − 22°C [15]) was purchased from Aldrich. Although this purity is low for an HPLC solvent, it is acceptable in the present case because the main impurity reported is 2-methylnaphthalene, an isomer which could be used as the mobile phase with practically the same properties, except that its melting point is slightly above ambient temperature (m.p. 36–38°C). The boiling point of 1-methylnaphthalene is 240–243°C under atmospheric pressure [15] which allows its easy removal from collected fractions by evaporation under vacuum, a conventional stage of down-processing. The mobile phase was filtered prior to use through a 5 μm pore size glass filter which seemed suitable as the columns never exhibited an increase of their head pressure at constant flow-rate. Carbon disulfide and decacyclene were obtained from Aldrich. The fullerene standard mixture of C_{60} and C_{70} used in all analytical scale experiments was received from MER (Tucson, AZ, USA). The C_{60} and C_{70} standard mixture used for loadability measurements and a 99.9% pure C_{60} standard were purchased from SES Research (Houston, TX, USA).

2.3. Columns and chromatographic conditions

A 250×4.6 mm column packed with tetraphenylporphyrin bonded silica was given by Selective Technologies (Ann Arbor, MI, USA). The preparation of the packing material was carried out based on the method developed by Kibbey and Meyerhoff [8], except that an in situ method was used to react the surface of 10 μm irregularly shaped particles of an aminopropyl bonded silica (Alltech Associates, Deerfield, IL, USA) with the tetraphenylporphyrin rings.

The nonmetallated form of this stationary phase was used. The column void volume was measured using carbon disulfide as unretained compound, dissolved in the mobile phase.

The Buckyclutcher I stationary phase was purchased from Regis Chemical Company (Morton Grove, IL, USA), the pyrenyl–ethyl column from Phenomenex (Torrance, CA, USA), and a column was laboratory-packed with a monomeric C_{18} stationary phase given by YMC (Wilmington, NC, USA). These three stationary phases were based on 5 μm silica particles. The dimensions of all three columns were 250×4.6 mm. Because of the difference in the sizes of the packing particles, the efficiency of these three columns is expected to be twice that of the former one.

The values of the capacity factors, selectivity factors and column efficiencies of each stationary phase were determined by injecting 20 μl samples of the C_{60}/C_{70} standard solution mixture, at a concentration of 0.13 mg/ml. All samples were filtered before injection through a PTFE syringe filter (0.2 μm pore size). The column temperature was controlled by the chromatograph oven.

3. Results and discussions

3.1. Stationary phase comparison

In order to compare the properties of the new preparative-scale stationary-phase candidates, we measured first the capacity factors of C_{60} and C_{70} and their separation factors using toluene as a mobile phase. For the sake of comparison with more conventional stationary phases, we selected an alkyl-bonded (YMC C_{18} monomeric) and a charge transfer (Buckyclutcher) packing material. The results of the measurements are reported in Table 1.

To interpret the results obtained, we calculated the average molecular polarizabilities of the stationary phases by the empirical method of Miller and Savchik [14] using the formula:

$$\alpha = \frac{4}{N} \left[\sum_A \tau_A \right]^2 (\text{Å}^3) \qquad (1)$$

where N is the number of electrons in the molecule, and τ_A is the atomic hybrid component for each atom in a particular hybrid configuration. The average

Table 1
Chromatographic properties of five stationary phases (mobile phase: pure toluene)

	k' C_{60}	k' C_{70}	$\alpha(C_{70}/C_{60})$
YMC C_{18} (monomeric)[a]	0	0	
Buckyclutcher[a]	0.3	0.45	1.5
PYE[a]	0.85	1.7	2
PBB[b] [7]	1.97	4.83	2.45
Tetraphenylporphyrin[b]	0.56	2.4	4.3
prepared by Meyerhoff et al. [8,11]	1.7	8.1	4.8
Tetraphenylporphyrin[a] prepared by Selective Tech.	2.5	14.3	5.7

[a] This work.
[b] Published data.

molecular polarizability obtained for C_{60} is 81.6 $\mathring{A}^3$ and that for C_{70} is 95.2 $\mathring{A}^3$, a relative difference of 14%. The polarizability of the bonded groups of the five stationary phases discussed here are reported in Table 2. Even though the direct comparison of the results is difficult owing to the differences in the degree of surface coverage by the bonded groups and in the specific surface area of the stationary phases, some conclusions are obvious. It is a well-known fact that none of the C_{18} bonded silica stationary phases (and more generally, none of the alkyl-bonded stationary phases) are capable of separating C_{60} and C_{70} with toluene as the mobile phase because neither compound is retained. This fact means that the separation ability is affected but cannot be explained entirely by the polarizability of the stationary phase. Furthermore, the ethyl–pyrene and octadecyl groups have nearly the same polarizability (Table 2); the PYE stationary phase has a 3 μmol/m^2 surface coverage and a 330 m^2/g specific surface area, values close to those of the YMC C_{18} stationary phase (17% carbon, 300±40 m^2/g). However, the former phase shows a much higher selectivity for these compounds than the latter (Table 1). This probably arises because of π-electron interactions between the polynuclear aromatic ring of pyrene and the fullerene molecules.

Data published elsewhere regarding the PBB stationary phase are also included in Table 1. This phase was prepared using the same silica as for the PYE stationary phase; the degree of surface coverage was reported to be 2.4 μmol/m^2 [7]. Although the polarizability of the bonded groups of the stationary phase is larger for the PYE than for the PBB column
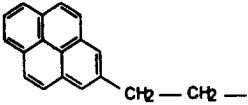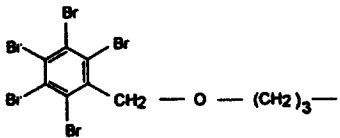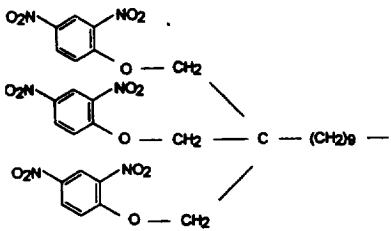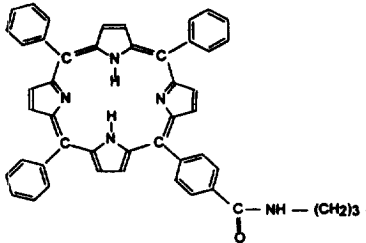
(Table 2), the retention of the fullerene compounds is stronger on the latter column (Table 1). The tetraphenylporphyrin stationary phase prepared by Meyerhoff et al. [8–12] was based on a silica having a 200 m^2/g surface area with a typical degree of surface coverage between 0.2 and 0.4 μmol/m^2. The overall polarizability of this stationary phase is much lower than that of the other stationary phases because of a much lower density, but the interaction energy is much higher and the selectivity and retentivity are larger. This is easily explained by the simple proportionality of the retention factor to the specific surface area, while it increases exponentially with the interaction energy.

3.2. 1-Methylnaphthalene as a mobile phase

In previous studies, carbon disulfide and 1,2,4-trichlorobenzene were used as the strongest fullerene solvents during the chromatographic separations of these compounds [7,11]. One author noted: "These are not the best solvents to use by any means in the separation of fullerenes in terms of solubility, safety and handling" [7]. We have found a solvent which is much better on all three accounts than those previously used, 1-methylnaphthalene.

First, there are no safety problems with this solvent. It has a low vapor pressure at room temperature, it is highly stable, and it is not toxic. Naphthalene itself is a household product (insecticide). Secondly, the solubility of C_{60} in 1-methylnaphthalene is 33 mg/ml, which makes it the best practical solvent of fullerenes. For the sake of comparison, Ruoff et al. [13] have reported that the solubility of

Table 2
Polarizability of bonded groups in stationary phases

Stationary phase type	Average molecular polarizabilities [Å³]	The functional group attached to the silica surface
C$_{18}$	33.6	CH_3—$(CH_2)_{17}$—
PYE	28.8	
PBB	32.9	
Buckyclutcher	64.98	
Tetraphenyl-porphyrin	82.98	

C_{60} is 7.9 mg/ml in carbon disulfide and 8.5 mg/ml in 1,2,4-trichlorobenzene, i.e., approximately four-fold less. Naphthalene could certainly be used in solutions in toluene, up to a relatively high concentrations, especially at temperatures above ambient. However, such solutions are prone to leave solid naphthalene deposits upon vaporization of toluene and this could be harmful to the pumps. As there does not seem to be any particular advantage to using naphthalene rather than 1-methylnaphthalene, liquid below room temperature (m.p. $-22°C$), we have used only the latter compound. The main disadvantage of this solvent is its relatively high viscosity of 3.2 cP at 25°C. This is 10% lower than the viscosity of a 50:50 isopropanol/water mixture, not uncommonly used in RP HPLC.

The viscosity of 1-methylnaphthalene at different temperatures is reported in Table 3. It was calculated from the data published by Reid et al. [15]. The viscosity of the (1:1) binary mixture with toluene was calculated based on the Arrhenius theory of the viscosity of liquid mixtures [15]. The density of 1-methylnaphthalene was calculated from the data published by Daubert and Danner [16]. Its molecular mass is 142.2, its melting point $-22°C$, its boiling point 240–243°C, its refractive index at 20°C 1.615, its density at 293 K 1.02 g/cm^3, and its UV absorbance (1 cm optical pathlength cell, with water as the reference) are 0.038 AU at 400 nm, 0.841 AU at 350 nm, 1.0 AU at 346 nm, and 2.99 AU at 330 nm. Detection was carried out at 380 nm at which wavelength the background signal was low and exhibited little noise.

The high boiling point of the solvent has both advantages and disadvantages to the chromatog-raphy. It means that the vapor pressure is low (the vapor pressure of 1-methylnaphthalene at 70°C is 20 times less than the vapor pressure of toluene at room temperature, Table 3), that there are small losses to the atmosphere from even an open container or from a fraction collector, and that there is a low degree of pollution of the atmosphere of the laboratory. It also permits column operation at a temperature higher than ambient, using more concentrated solutions since the solubility of higher fullerenes increases with increasing temperature. Finally, this solvent allows extraction of fullerenes from fullerene soot with a high recovery yield [2,17,18]. With the phase system described here, intermediate recovery of the extract would not be necessary and the solution could be injected directly into the chromatograph, after filtration. On the other hand, a high boiling point makes it more difficult to remove the solvent and recover the purified products after the separation.

3.3. Optimization of C_{60} and C_{70} separation on tetraphenylporphyrin stationary phase

The temperature dependence of the capacity factors of C_{60} and C_{70}, and of their separation factor on the chemically bonded tetraphenylporphyrin silica, with pure toluene as the mobile phase, is illustrated in Fig. 1. The Van't Hoff plots obtained are linear. The calculated enthalpies of transfer are negative and the adsorption of C_{60} and C_{70} on this stationary phase is exothermic. The selectivity factor at room temperature was found to be 5.7 and to decrease with increasing temperature, down to 4.0 at the highest temperature (70°C) used. The retention factors of both compounds decrease with increasing column

Table 3
Physical properties of 1-methylnaphthalene and toluene

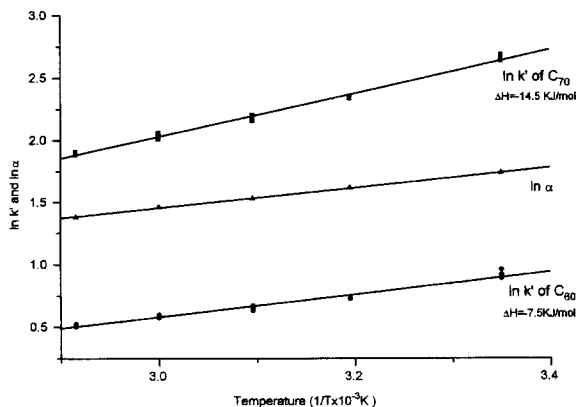| T (K)	Viscosity of 1-methyl-naphthalene (cP)	Vapor pressure of 1-methyl-naphthalene (mm Hg)	Viscosity of toluene (cP)	Vapor pressure of toluene (mm Hg)	Viscosity of toluene–1-methylnaphthalene (1:1) mixture (cP)
298	3.24	0.05	0.55	28.21	1.18
313	2.35	0.18	0.46	58.75	0.93
323	1.93	0.38	0.41	91.52	0.81
333	1.61	0.74	0.37	138.13	0.71
343	1.35	1.37	0.34	202.62	0.62

1 mm Hg = 133.322 Pa.

Fig. 1. Temperature dependence of the capacity and selectivity factors with toluene as the mobile phase. Conditions: packing material: tetraphenylporphyrin–silica; particle size: 10 μm; pore size: 60 Å; column size: 250×4.6 mm; mobile phase flow-rate: 1.0 ml/min; detection: UV, 380 nm.
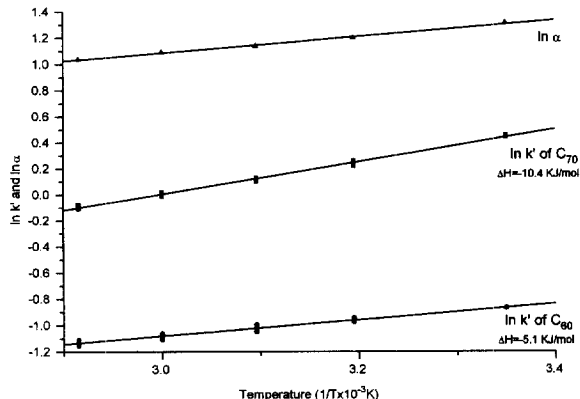

Fig. 2. Temperature dependence of capacity and selectivity factors with a mixture of toluene–1-methylnaphthalene (1:1, v/v) as the mobile phase. Same column as in Fig. 1. Flow-rate: 0.3 ml/min; detection: UV, 380 nm.

temperature. ΔH of C_{60} was found to be -7.5 kJ/mol, of C_{70} -14.5 kJ/mol. The same phenomenon was observed previously on the tetraphenylporphyrin stationary phases prepared by Meyerhoff et al. [11]. This is a conventional result, so there is no 'unusual temperature' effect by contrast with what was observed on the Pirkle-type stationary phases [19,20] or on chemically bonded TAPA columns [20]. This result is interesting, however, because it seems to be in contradiction with the apparent anomalous solubility behavior of C_{60} in toluene. It has been reported [21] that the solubility of C_{60} in toluene decreases with increasing temperature in the temperature range of 20–70°C, which would lead one to predict the opposite behavior for the retention, given the well-known relationship between retention and solubility. Further investigations are required to explain the origin of this apparent contradiction.

The Van't Hoff plots obtained for the same column with a 1:1 mixture of toluene and 1-methylnaphthalene are shown in Fig. 2. As in the previous case, these plots are straight lines. As expected, the retention factors decrease with increasing mobile phase strength as illustrated by comparing Figs. 1 and 2. When the toluene/1-methylnaphthalene mixture is used as the mobile phase, the retention factors of C_{60} were found to be 0.42 at 25°C and 0.32 at 70°C. For C_{70}, k' was 1.57 at 25°C and 0.91 at 70°C.

However, the separation factor remains high, 3.72 at 25°C and 2.82 at 70°C, allowing easy analytical separations. As demonstrated by the overlaid chromatograms shown in Fig. 3, the separation of C_{60} and C_{70} carried out with the toluene/1-methylnaphthalene mixture is complete in the entire temperature range investigated (25–70°C). The values of the enthalpy derived from the Van't Hoff plots are smaller than those obtained with pure toluene, as expected when the solvent strength of the eluent is increased. The same trend continues when the eluent is neat 1-methylnaphthalene (Fig. 4). The retention
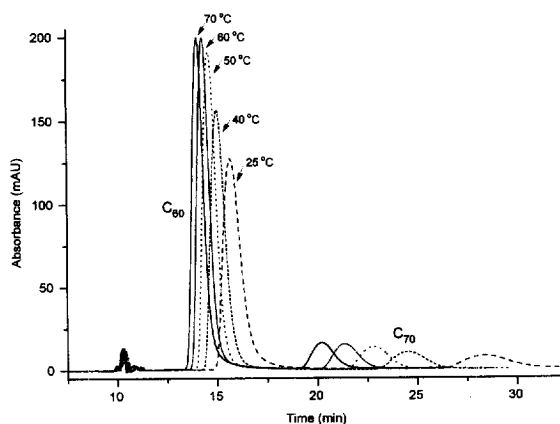

Fig. 3. Overlaid chromatograms obtained with a mixture of toluene–1-methylnaphthalene (1:1) as the mobile phase. Same conditions as for Fig. 2.
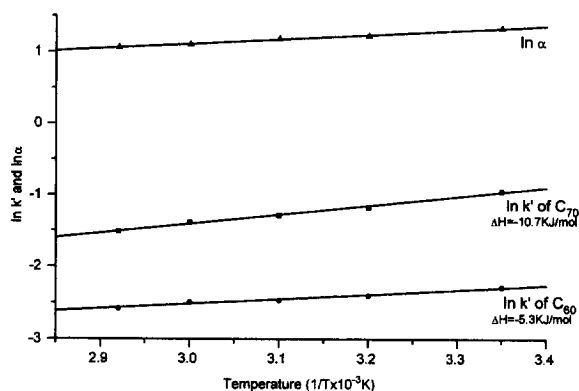
Fig. 4. Temperature dependence of the capacity and selectivity factors with neat 1-methylnaphthalene as the mobile phase. Same column as in Fig. 1. Mobile phase flow-rate: 0.3 ml/min.

Fig. 6. Overlaid chromatograms obtained with neat 1-methyl-naphthalene as the mobile phase. Same conditions as for Fig. 4.

factors of C_{60} and C_{70} become equal to 0.1 and 0.39, respectively, at 25°C (with $\alpha = 3.8$). At 70°C, these factors become respectively, 0.076 and 0.22, with $\alpha = 2.9$. In spite of the low values of the retention factors, an excellent separation of C_{60} and C_{70} is achieved at 25°C (Fig. 5). The overlaid chromato-grams recorded at different temperatures illustrate the achievement of a resolution always exceeding unity (Fig. 6). In this case also, the Van't Hoff plots are linear (Fig. 4), the adsorption enthalpies are negative and smaller than with the toluene–1-methylnaphthalene (1:1) mixture.

Because of the large viscosity of 1-methylnaph-thalene, the column efficiency is expected to be rather low which may require column operation at a low mobile phase flow-rate to bring the reduced
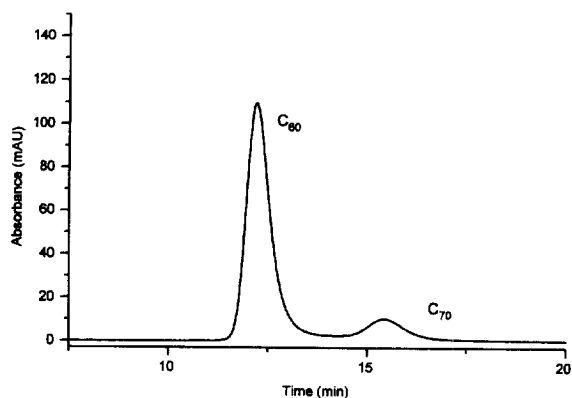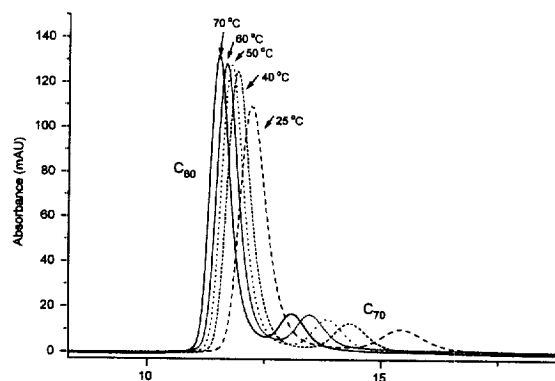
velocity closer to the conventional range giving best column performance (i.e., between 3 and 8). As a first step in a study of the optimization of the column efficiency, we measured the temperature dependence of the number of theoretical plates using toluene as the mobile phase. For the sake of comparison, efficiencies were also measured for two other com-pounds, the nonretained carbon disulfide and a high molecular mass (450.5) polynuclear aromatic hydro-carbon, decacyclene (structure in Fig. 7). Ho and Pascal [22] have characterized the nonplanar mole-cule of this compound as propeller shaped, although its degree of twist is modest. One naphthalene group is in the plane of the central ring. The angle between the mean plane of the central benzene ring and the mean planes of the two crystallographically indepen-dent naphthalene moieties are 9.3° and 7.7°, respec-tively [22].

When toluene is used as the mobile phase at 70°C, the retention factors of decacyclene, C_{60}, and C_{70} are 0.3, 1.7, and 6.6, respectively. The column shows a

Fig. 5. Separation of C_{60} and C_{70} with 1-methylnaphthalene as the mobile phase. Same conditions as for Fig. 4.
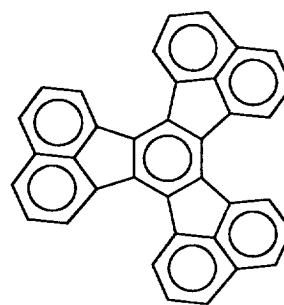

Fig. 7. Structure of decacyclene.
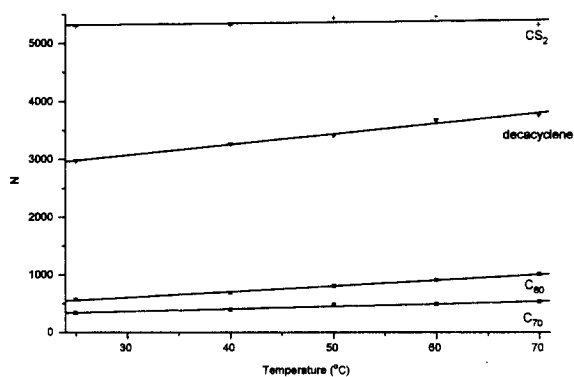
Fig. 8. Plot of the column efficiency versus the temperatures, with toluene as the mobile phase. Same conditions as for Fig. 1.
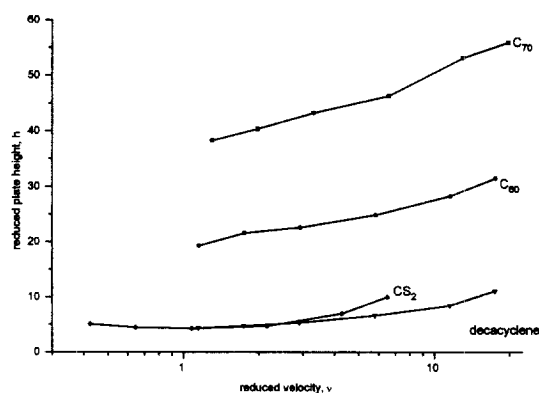

Fig. 9. Plot of the reduced plate height versus the reduced linear velocity, with toluene as the mobile phase. Same column and conditions as for Fig. 1, except temperature: 70°C.

much higher efficiency for carbon disulfide and decacyclene than for the fullerenes at a flow-rate of 1.0 ml/min, at all temperatures (Fig. 8). The column efficiency increases slowly with increasing temperature for the two fullerenes and for decacyclene and remains constant for carbon disulfide. It decreases rapidly with increasing retention factor. The number of theoretical plates of the column at 70°C was to be 5340 for carbon disulfide (suggesting a well-packed column), 3780 for decacyclene, 1010 for C_{60} and 530 for C_{70}. To reach higher efficiency for the fullerenes we determined the velocity dependence of the column efficiencies for the two fullerenes with toluene as the mobile phase.

The calculation of the reduced mobile phase velocity is necessary for a proper assessment of the dependence of the column efficiency on the mobile phase velocity. This calculation, in turn, requires an estimate of the molecular diffusivity of the compounds studied. These coefficients were derived from the conventional equation of Wilke and Chang [23]:

$$D_{A,B} = 7.4 \times 10^{-8} \frac{\sqrt{\psi_B M_B}\, T}{\eta_B V_A^{0.6}} \qquad (2)$$

where V_A is the molar volume of the liquid solute at

its normal boiling point, M_B is the molecular mass of the solvent, η_B is its viscosity, and ψ_B is a constant which accounts for solute–solvent interactions. In nonpolar solvents, this coefficient is unity. We used the Van der Waals volumes of C_{60} and C_{70} calculated from the Van der Waals radii by Affholter et al. [24]. These data are in good agreement with the Van der Waals volumes published by Adams et al. [25] who generated these data using 1.76 Å as the Van der Waals radius of a carbon atom.

Table 4 shows the physical properties of fullerenes. The molar volume of decacyclene was calculated based on the density published by Ho and Pascal [22] and was found to be 314 cm³ mol⁻¹. The molecular diffusivities of C_{60} and C_{70} in toluene at 70°C were found to be 2.26×10^{-5} and 2.00×10^{-5} cm²/s, respectively. Those of decacyclene and carbon disulfide were found to be 2.28×10^{-5} and 6.13×10^{-5} cm²/s, respectively.

A plot of the reduced column plate height (h) versus the logarithm of the reduced velocity (ν) is illustrated in Fig. 9. With a minimum value of $h = 4$ corresponding to $\nu = 1$, the plot suggests that the

Table 4
Physical properties of fullerenes

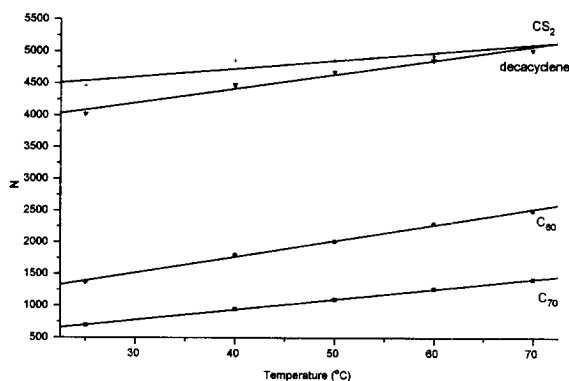|	Van der Waals radii; (Å)	Van der Waals volumes (Å³) [24]	Van der Waals volumes (Å³) [25]	Van der Waals volumes (cm³ mol⁻¹) [24]
C_{60}	5.02	530	549	318
C_{70}	6.94; 7.80	650	646.3	390

Fig. 10. Plot of the column efficiency versus the temperature, with the mixture of toluene–1-methylnaphthalene (1:1) as the mobile phase. Same conditions as for Fig. 2.

column is reasonably well packed but that mass transfer are surprisingly sluggish in this case. The curves corresponding to the two fullerenes are well above those for carbon disulfide and decacyclene. These curves have not yet reached their minimum at a reduced velocity as low as 1. This suggests values of the c coefficient of the Knox equation above unity, orders of magnitude larger than usually found. Using a flow-rate near the optimum value (0.3 ml/min), we measured the temperature dependence of the number of theoretical plates in toluene–1-methylnaphthalene (1:1) (Fig. 10) and in 1-methylnaphthalene (Fig. 11). The results show a much stronger temperature dependence for the fullerenes than when pure toluene is the mobile phase. The efficiency for
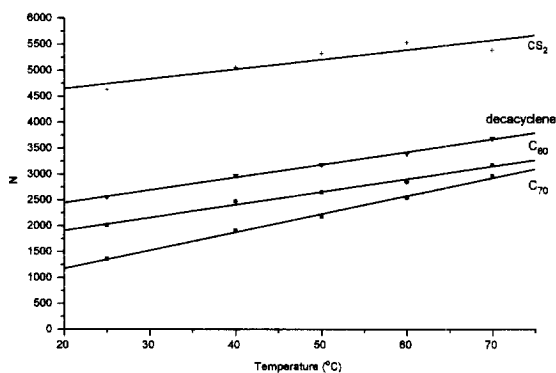

Fig. 11. Plot of the column efficiency versus the temperature, with 1-methylnaphthalene as the mobile phase. Same conditions as for Fig. 4.

C_{70} in pure 1-methylnaphthalene at 70°C exceeds 2500 theoretical plates, which becomes acceptable, although still low.

3.4. Column overloading

A 20.4 mg/ml solution of C_{60} and C_{70} in a toluene–1-methylnaphthalene (1:1) mixture was prepared. This solution contains also some fullerene epoxide. The initial content of $C_{60}O$ relative to C_{60} was 2.4% measured at 330 nm and 3.3% measured at 380 nm, assuming that the absorptivities of the two compounds are equal. The published spectrum of $C_{60}O$ [26] cannot be used for calibration. This result shows that the two compounds have different spectra. However, the lack of a source of pure $C_{60}O$ prevents proper calibration of the detector response. 250 µl of samples of this solution were injected onto the 250×4.6 mm tetraphenylporphyrin column, with the same toluene–1-methylnaphthalene (1:1) mixture as the mobile phase, at room temperature, with a flow-rate of 0.3 ml/min. The column was loaded with a 5.1 mg sample of the C_{60}–C_{70} mixture. To check the separation ability of the column under such conditions, nine fractions were collected between the peaks of C_{60} and C_{70}. The peaks of the purified C_{60} and C_{70} fractions were also collected. The positions of these fractions are shown in Fig. 12. These collected fractions were stored under nitrogen and an aliquot was reinjected into the 250×4.6 mm PYE column and analyzed using toluene as the mobile phase at room temperature and with a flow-rate of 1.0 ml/min. The analytical chromatograms are shown in Fig. 13.

All the collected fractions coming from the tail of the C_{60} peak (fractions 2–7) contain two compounds other than C_{60}. The bigger peak can be assigned to the epoxide, $C_{60}O$, based on the UV spectrum recorded during its elution [26]. None of these fractions is contaminated with C_{70}. However, the main fraction containing the purified C_{60} is also contaminated with the epoxide but no effort had been made to eliminate this component, ubiquitous in fullerene analysis. The concentration of this compound in the purified C_{60} can be estimated only on the basis of its relative peak area, which is ~1.5% of the C_{60} peak area. The fractions collected from the C_{70} peak are contaminated with some amount of C_{60}
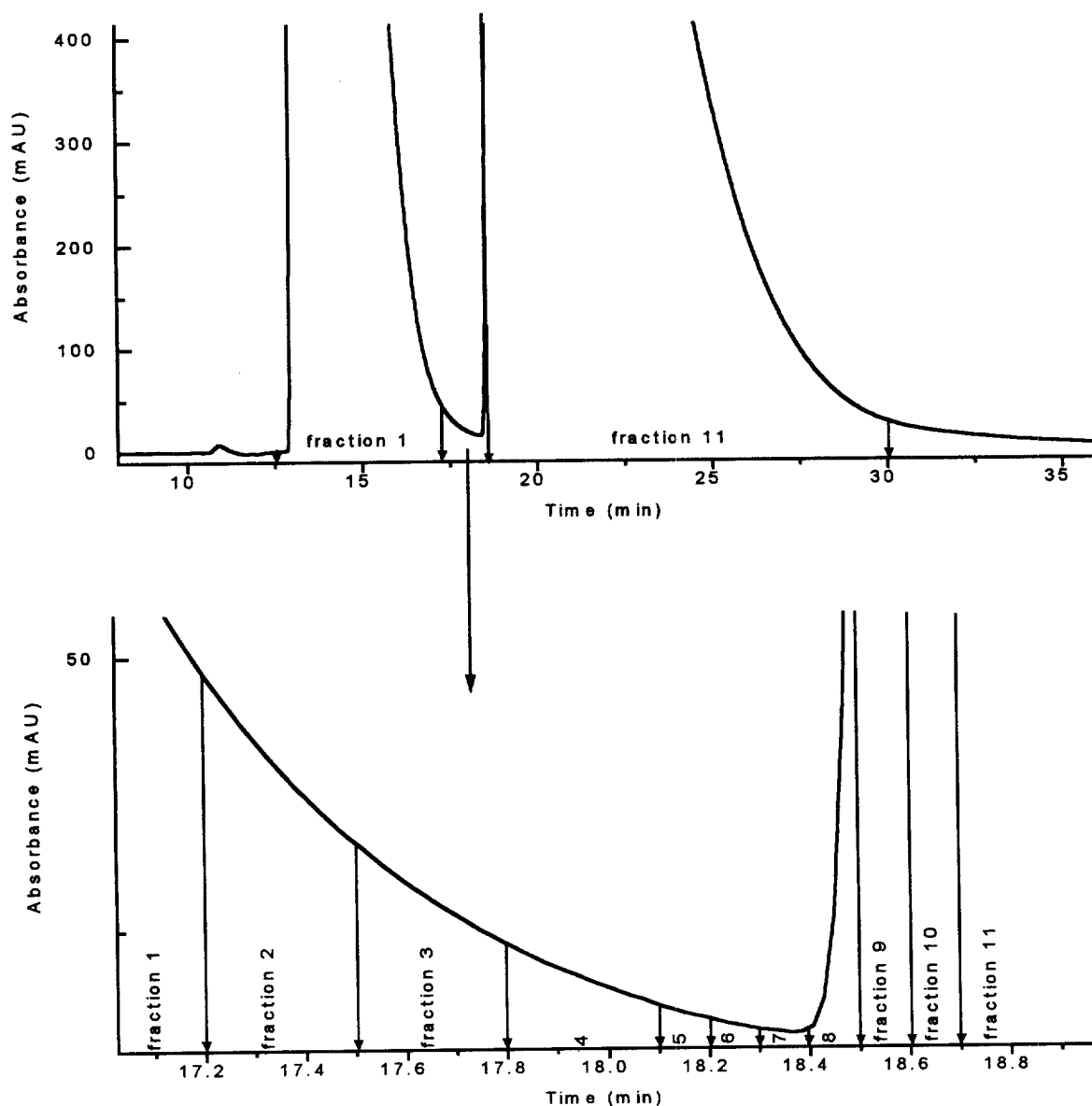
Fig. 12. Position of the collected fractions. Same column and conditions as for Fig. 2, except temperature: 26°C; detection: UV, 590 nm.

and also with a small amount of $C_{60}O^{1}$. The main C_{70} peak contains the same impurities. Based on the calibration of the detector for both fullerenes, the

concentration of C_{60} in the C_{70} fraction was found to be 0.7%. An excellent purification of fullerenes can be done under the experimental conditions described, with a 100% recovery yield, and the separation of more than 5 mg in a single run.

These results were compared to those obtained with the PYE stationary phase. Because of the low separation factor (1.3) obtained under analytical

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[1] Note that $C_{60}O$ can be eliminated from a C_{60} solution by percolating it on an alumina column, as suggested by Creegan [26]. Using a hexane–toluene (8:2) solution, we have verified that this procedure allows a five-fold reduction of the initial $C_{60}O$ concentration.
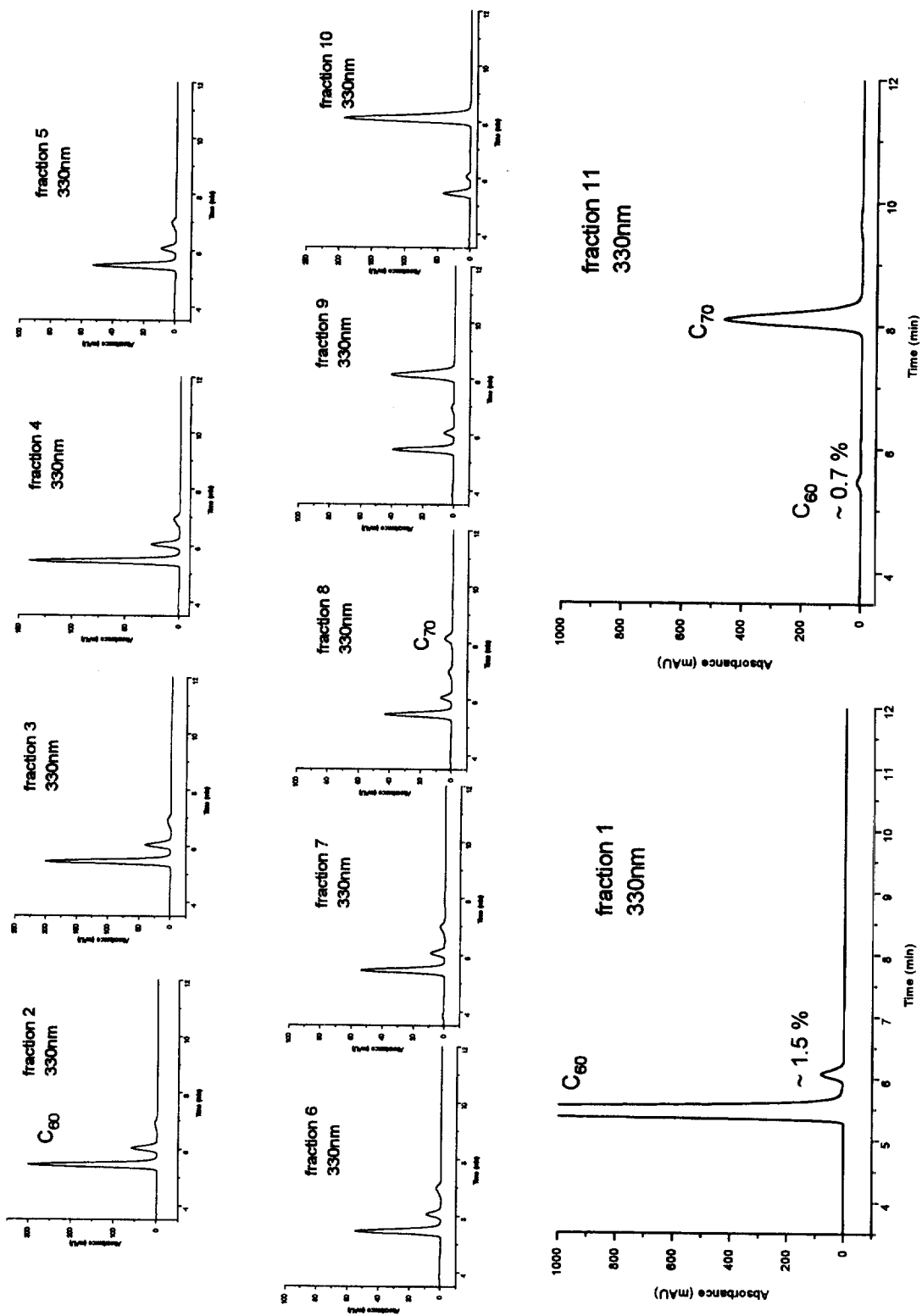
Fig. 13. Chromatograms of the collected fractions. Conditions: packing material: PYE; particle size: 5 μm; pore size: 120 Å; column size: 250×4.6 mm; mobile phase: toluene; flow-rate: 1.0 ml/min; detection: UV, 330 nm; temperature: 26°C.

conditions with the toluene–1-methylnaphthalene (1:1) mixture as the mobile phase, loadability measurements were carried out with toluene. The sample being poorly soluble in this mobile phase, there are two ways to overload the column, (1) by loading the sample dissolved in a solvent of higher elution strength than the mobile phase; or (2) by injecting large volumes of the solution of the sample in the actual mobile phase. Experiments were arranged to inject the same amount of feed sample with both methods. Fig. 14 demonstrates the concentration overloading achieved with the tetraphenylporphyrin column (for which the sample solvent was the mobile phase) and the overloading of the PYE column when using a sample solvent (toluene–1-methylnaphthalene, 1:1) with an elution strength higher than that of the mobile phase (toluene). There is a considerable amount of peak broadening on the PYE column, much more than on the tetraphenylporphyrin column.

To find out why, the experiment on the PYE

Fig. 15. Chromatograms obtained under conditions of column overloading of the PYE stationary phase using a sample solvent with a higher elution strength than the mobile phase. Variable volumes of a constant concentration solution. Experimental conditions: column size: 250×4.6 mm; mobile phase: toluene; flow-rate 0.3 ml/min; detection: UV, 590 nm; injection: C_{60}/C_{70} sample prepared in toluene–1-methylnaphthalene (1:1); sample concentration: 21 mg/ml.
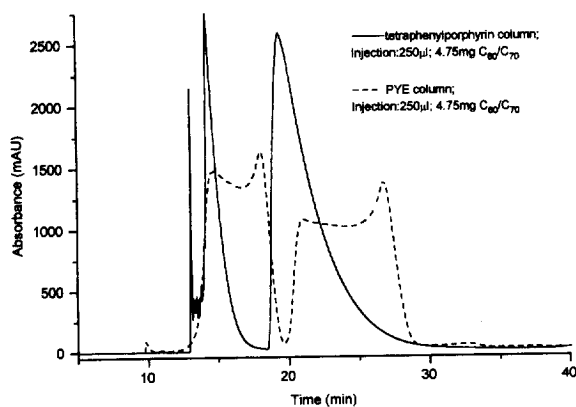

Fig. 14. Chromatograms obtained under (1) conditions of concentration overloading of the tetraphenylporphyrin column (solid line), and (2) of column overloading of the PYE stationary phase using a sample solvent with a higher elution strength than the mobile phase (dashed line). Experimental conditions on tetraphenylporphyrin stationary phase: column size: 250×4.6 mm; mobile phase: toluene–1-methylnaphthalene (1:1); flow-rate 0.3 ml/min; detection: UV, 590 nm; injection: 250 μl of C_{60}/C_{70} sample prepared in toluene–1-methylnaphthalene (1:1); sample concentration: 19 mg/ml. Experimental conditions on PYE stationary phase: column size: 250×4.6 mm; mobile phase: toluene; flow-rate 0.3 ml/min; detection: UV, 590 nm; injection: 250 μl of C_{60}/C_{70} sample prepared in toluene–1-methylnaphthalene (1:1); sample concentration: 19 mg/ml.

column was repeated with different volumes (10, 20, 50, 100 and 200 μl) of the same feed (21 mg/ml of crude fullerenes in a toluene–1-methylnaphthalene (1:1) mixture). These loading experiments were also
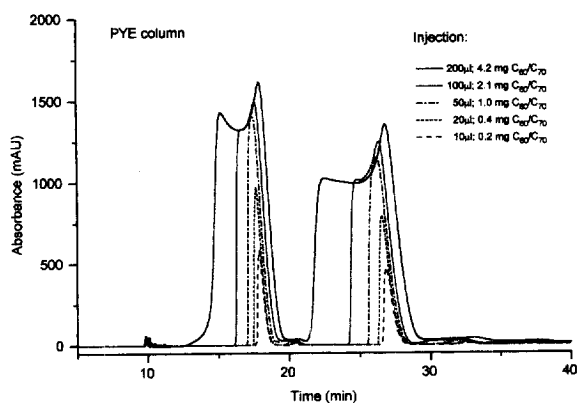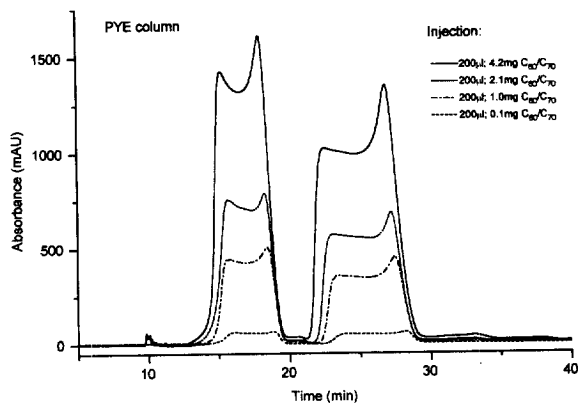

Fig. 16. Chromatograms obtained under conditions of column overloading of the PYE stationary phase using a sample solvent with a higher elution strength than the mobile phase. Constant volume of a variable concentration solution. Experimental conditions: column size: 250×4.6 mm; mobile phase: toluene; flow-rate 0.3 ml/min; detection: UV, 590 nm; injection: 200 μl C_{60}/C_{70} sample prepared in toluene–1-methylnaphthalene 1:1; sample concentrations: 21 mg/ml, 10.5 mg/ml, 5.25 mg/ml and 0.5 mg/ml.

carried out using the same sample volume (200 µl) but different amounts (0.1, 1.0, 2.1 and 4.2 mg) of the fullerene mixture, obtained by changing the feed concentration. Based on the results (Fig. 15 Fig. 16) of these experiments, we conclude that the peak deformation and broadening observed in Figs. 14–16 originates from both an overloading of the stationary phase (i.e., a nonlinear isotherm) and the effect of a high concentration of the strong solvent in the mobile phase, which results in a local, temporary reduction of the retention factor of the sample component [3]. This latter effect is the most important factor under the conditions used for Fig. 14.

In a second series of experiments, the column was loaded with 2 ml of a toluene solution of the fullerene feed, at a concentration of 2.4 mg/ml (Fig. 17). The flow-rate was raised to 1 ml/min. The band broadening originates from the large injection time (2 min) which is significant compared to the retention time of the two fullerenes and large compared to the width of the analytical peaks obtained under the same conditions. The important observation is that, no matter which loading method was used, baseline separation of the bands of C_{60} and C_{70} cannot be achieved while it is when the same amount of the mixture is injected on the tetraphenylporphyrin column.
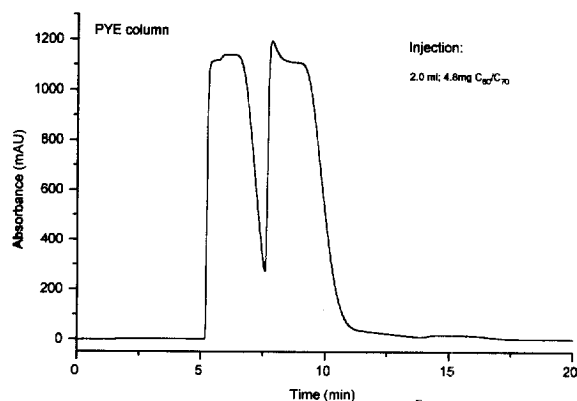

Fig. 17. Chromatogram obtained under conditions of volume overloading of the PYE stationary phase. Experimental conditions: column size: 250×4.6 mm; mobile phase: toluene; flow-rate 1.0 ml/min; detection: UV, 590 nm; injection: C_{60}/C_{70} sample prepared in toluene; injected volume: 2.0 ml; sample concentration: 2.4 mg/ml; loaded amount: 4.8 mg.

4. Conclusion

The results reported here show that preparative separations of fullerenes are advantageously carried out on stationary phases which retain strongly these compounds. This allows the use of good solvents which are also strong eluents. The separation factor is higher than with conventional systems and the column loadability is considerably increased. More specifically, using large size ligands which can interact with a large fraction of the surface of the fullerene molecule is a key to solving several important problems: increasing selectivity, improving column loadability for preparative separation or trace analysis, and improving shape recognition to better separate geometrical isomers, and, eventually achieve enantiomeric separation.

There are better solvents of fullerenes than 1-methylnaphthalene [13]. The concentration of saturated solutions of C_{60} in chloro- and phenylnaphthalene are of the order of 50 g/l, i.e., nearly 50% larger than in 1-methylnaphthalene. However, the viscosity of these solvents is also higher, causing increasingly serious problems unless the columns are operated at temperatures above 100°C. Furthermore, chloro-naphthalene is an organochlorinated solvent. As such, it is subject to more severe and costly regulations. Finally, the data in Figs. 12 and 13 show that the column capacity is controlled as much by the capacity of the stationary phase than by that of the mobile phase. The production rate with these other solvents would be increased in proportion to the decrease in elution time but the increased solubility would probably not allow the use of larger samples. At the present time, improvements in production rate would come essentially from an increase in the monolayer capacity of the adsorbent, i.e., from the density of the bonded ligands (at least as long as these ligands would not sterically hinder each other).

Finally, our results show that, of all stationary phases currently available for fullerene separations, immobilized tetraphenylporphyrin on silica exhibits by far the best performance for preparative applications. The other phases exhibit sufficiently large separation factors only with relatively weak solvents in which the fullerene solubility is poor. This drastically limits the production rate which can be

achieved. Immobilized C_{18} does not separate C_{60} and C_{70} with pure toluene as the eluent. Immobilized 3,3,3-tri-dinitrobenzoyl-propyl on silica and the PYE column do not separate C_{60} and $C_{60}O$ with any eluent stronger than toluene. With all mobile phases used, the relative retention of the two pairs studied (C_{60} and C_{70}, C_{60} and $C_{60}O$) and the production rate of purified C_{60} are markedly lower on these two phases than on the immobilized tetraphenylporphyrin column. Although the separation of the second pair of components (C_{60} and $C_{60}O$) remains uneasy, it is possible on this last phase with 1:1 solutions of toluene and 1-methylnaphthalene. Without special attention being paid to the achievement of this separation, the $C_{60}O$ content was reduced by half. An order of magnitude increase in the production rate of 99% pure C_{60} and C_{70} can be achieved.

Acknowledgments

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