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Evaluation of stationary phases for the separation of buckminsterfullerenes by high-performance liquid chromatography

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ABSTRACT

The high-performance liquid chromatographic separation of the buckminsterfullerenes C_{60} and C_{70} was studied using different chromatographic systems. Normal-phase (silica), non-aqueous reversed-phase (C_{18}-bonded silica and graphitized carbon black) and charge-transfer complexation with dinitrobenzoylphenylglycine (DNBPG) and 2-(2,4,5,7-tetranitro-9-fluorenylideneaminooxy)propionic acid (TAPA) were considered as retention mechanisms. Mobile phases of different compositions, but all based on hydrocarbons (toluene, hexane, heptane) as the main solvent, were used. Although it was found that the adsorption of the fullerenes on all the other phase systems is exothermic, their interactions with the charge-transfer phases exhibit an unusual endothermic behavior. The retention times of C_{60} and C_{70} on these phases increase with increasing temperature. The adsorption enthalpy and entropy derived from the Van 't Hoff plots confirm this rare temperature dependence. The adsorption enthalpy is positive whereas the adsorption entropy is strongly positive.

INTRODUCTION

The separation and purification of the buckminsterfullerenes by preparative high-performance liquid chromatography (HPLC) remain a challenging proposition, although their analytical separation is easy and straightforward. A large number of chromatographic separation methods have been described previously. These methods involve the use of gravity, HPLC or Soxhlet extraction liquid chromatographic techniques and the use as stationary phases of silica [1], alumina [2,3], reversed phases on C_{18}-bonded silica [4] or graphite [5], multi-legged phenyl-bonded phases [6] and separation on gel permeation phases [7].

The applicability of these different approaches

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for preparative purposes is limited, however, by the low throughput generally observed. An efficient separation on a conventional analytical column can be maintained only with sub-milligram amounts. The transposition of the optimized techniques to the semi-preparative (ca. 10-mg injection) or preparative level on a wide column (ca. 100–1000-mg injection on a ca. 5 cm I.D. column) is rendered difficult by some serious limitations due to the complexity of the matrix and the poor solubility of its components in the usual solvents.

The two common techniques available for the generation of fullerenes, laser vaporization and electric discharge, produce a complex soot containing C_{60} and C_{70} in a ratio of approximately 9:1, and varying but small amounts of higher mass fullerenes [4,8]. This is an unfavorable concentration ratio for purification by preparative chromatography. In overloaded elution chromatography, the two components of a binary mixture compete for access to the finite number of adsorption sites on the surface of the stationary phase during their migration along the column. The ease of the separation of the components of the mixture, their production rates and their recoveries depend strongly on their concentration ratio in the original mixture or feed.

As shown theoretically [9] and later proven experimentally [10], the purification of a 1:9 mixture is easier than that of a 9:1 mixture because the displacement effect of the lesser retained by the more retained component sharpens the boundary between the two component bands, improves their resolution and thus increases the recovery. At the same time, the presence of a high concentration of the lesser retained component decreases the adsorption of the more strongly retained one by crowding its molecules out of the surface. This is the "tag-along" effect [9]. The intensity of the displacement and tag-along effects depends on the concentration ratio of the two components and on the column efficiency [9]. The former is greater with a 1:9 mixture and the latter with a 9:1 mixture. Therefore, if one is interested in producing both components in a single step, the purification of a 9:1 mixture is much more difficult than that of a 1:9 mixture, because the displacement effect is weak, and the intense tag-along effect leads to a poor recovery [9]. With a 9:1 mixture, touching band separation is preferable to more strongly overloaded conditions, as it avoids recycling with only a small loss of production [4,10,11]. Finally, it does not seem possible to find a chromatographic system with which the heavier and more polarizable C_{70} would be eluted before C_{60}.

The different members of the fullerene series are insoluble in the most common solvents used in liquid chromatography. They are only slightly soluble in benzene (1.4 mg/ml C_{60}) and toluene (2.2 mg/ml C_{60}). For safety reasons, the latter is the most common solvent used to dissolve them. Some higher boiling solvents, such as mesitylene or pyridine, have been suggested [12] for the extraction of fullerenes from soot, but they are inconvenient for use in routine analytical or preparative chromatography because of their viscosity or odor, and because of increased difficulties in recovering the purified fractions. As already proven [4], however, the use of benzene or toluene as eluent results in very low or nearly negligible retention factors on most phases.

To overcome this limitation, a weaker solvent (linear alkanes, dichloromethane, ethyl acetate, acetonitrile or alcohols) is mixed with toluene to insure an adequate retention, while the sample solvent used is pure toluene or benzene in order to permit the injection of narrow bands of concentrated solutions, and insure an adequate throughput. However, it has been demonstrated both theoretically and practically [13,14] that chromatographic abnormalities (e.g., band deformations, band splitting) can result from the use of a sample solvent having a higher elution strength than the mobile phase. The effect is especially important under overloaded conditions and when the sample solvent is the essential component of the feed. There is often no noticeable effect under analytical conditions because the sample solvent is quickly diluted in the eluent. In contrast, in preparative applications this hinders the purification process and decreases the recovery [13].

A remedy to the first limitation, that origina-

ting from the composition of the feed, should be found in a better understanding of the parameters involved in the process of generation of the fullerenes. The solution to the second is in the development of new types of stationary phases, more selective and more strongly retentive of the fullerenes, thus allowing the use of a stronger eluent compatible with the natural sample solvent. Considerable progress has been made along this line. Charge-transfer chromatography [15] offers a logical and promising approach to the separation of fullerenes.

It has been known for more than a century that certain pairs of organic compounds can form stable molecular complexes [16] by electron transfer from a high electronic density center (*i.e.*, planar polyaromatic compounds) to an electron-deficient center. Chromatographic methods based on the formation of such complexes between an immobilized selectant acceptor and a solute donor or *vice versa* have been termed electron donor–acceptor (EDA) or charge-transfer chromatography [15]. Aromatic or heterocyclic compounds with strongly electron-attracting groups (*i.e.*, NO_2, Cl, Br) are good π-electron acceptors, whereas electron-releasing groups (*i.e.*, CH_3, CH_3O, NH_2) enhance the electron donor properties of the cycle.

For a given electron acceptor, the stability of the EDA complex, and thus the retention time in a given chromatographic system, depend on the intensity of the electron-releasing property of the donor. Consequently, the retention factors of the two components and their separation factor for a given mobile phase concentration should depend on the difference in their respective electron-donor abilities. Based on the evidence that the magnetic susceptibility of C_{70} is twice that of C_{60} [17] and that their second ionization potentials are different [18], it is to be expected that these two molecules should exhibit markedly different electron-donor abilities and can be separated on a suitable stationary phase. The only possible limitation could be that charge-transfer adsorbents are known for their high degree of planar recognition. Thus, strong retention cannot be expected.

Cox *et al.* [19] were the first to separate C_{60} and C_{70} by charge-transfer chromatography using dinitroanilinopropyl-bonded silica as selectant, and a linear gradient of hexane–dichloromethane. They showed that C_{60} (which has twenty aromatic rings, but is not planar) behaves as triphenylene, a planar five-membered aromatic compound, whereas C_{70} elutes somewhere between benzopyrene and coronene. This illustrates the effect of the non-planarity of the analyte on the retention mechanism. Other selectants have also been proposed [20]. Recently a new family of charge-transfer stationary phases, termed "Buckyclutcher", were synthesized [21]. These phases use tripodal dinitro derivatives as selectants. They are reported to exhibit good retention and a high selectivity for the C_{60} and C_{70} fullerenes, even when a 50:50 hexane–toluene solution or pure toluene is used as the mobile phase.

In this paper we describe the chromatographic behavior of the C_{60} and C_{70} fullerenes on (*R*)-(−)-2-(2,4,5,7-tetranitro-9-fluorenylideneaminooxy)propionic acid (TAPA), a chiral stationary phase used earlier for the separation of carbohelicenes [22], and synthesized in our laboratory; we discuss the retention mechanism on this selectant and the optimization of the separation of C_{60} and C_{70} in terms of mobile phase composition and temperature, and finally we compare the behavior of the same compounds on dinitrobenzoylphenylglycine (DNBPG), silica, ODS and graphitized carbon black (GCB).

THEORY

The Gibbs free energy, ΔG, for the equilibrium of a solute in a chromatographic system is related to the retention factor through the partition coefficient, K, by the following equations:

$$\Delta G = -RT \ln K \tag{1}$$

$$k' = FK \tag{2}$$

where R is the universal gas constant, T is the absolute temperature, F is the phase ratio and k' is the retention or column capacity factor.

The dependence of the retention factor on the column temperature can be derived from the following fundamental equations of thermodynamics and the previous relationships:

$$\Delta G = \Delta H - T\,\Delta S \qquad (3)$$

$$\ln k' = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} + \ln F \qquad (4)$$

where ΔH and ΔS are the molar enthalpy of transfer from the selectant to the eluent and the associated change of the molar standard entropy, respectively.

Because the phase ratio is close to 1 in these phase systems $[F = (1 - \epsilon_T)/\epsilon_T$, with the total porosity of the column, ϵ_T, being approximately 0.5), $\ln F$ can be neglected in eqn. 4, and

$$\ln k' = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \qquad (5)$$

Therefore, if the retention mechanism remains the same over the temperature range considered and the enthalpy is constant (*i.e.*, if we neglect the difference in molar heat capacity of the fullerenes at infinite dilution in the two phases), the enthalpy and entropy of transfer can be derived from the slope and the intercept of a semi-logarithmic plot of the experimental capacity factors *versus* the ratio $1/RT$, commonly referred to as the Van 't Hoff plot.

EXPERIMENTAL

Instrumentation

An HP1090 liquid chromatograph (Hewlett-Packard, Palo Alto, CA, USA) equipped with a multi-solvent-delivery system, an automatic sample-injection system, a photodiode-array detector, a temperature control system, and a data station was used.

Preparative separations were conducted on a Prochrom (Nancy, France) LD-50 axial compression column, with a *ca.* 20 cm × 5 cm I.D. column, fitted to a Biotage (Charlotteville, VA, USA) pumping system.

Columns

A 250 × 4.6 mm I.D. and a 100 × 4.6 mm I.D. column were laboratory-packed with 17.8-μm IMPAQ RG2010 C_{18} silica (PQ, Conshohoken, PA, USA) and with LiChrosorb Si60 silica (EM Separations, Gibbstown, NJ, USA), respectively. In both instances, we used the same standard slurry packing method, with methanol at 6000

p.s.i. (1 p.s.i. = 6894.76 Pa). The efficiencies of these columns were 1900 and 2200 theoretical plates, respectively.

A 250 × 4.6 mm I.D. DNBPG column and a 100 × 4.6 mm I.D. GCB column were obtained from J.T. Baker (Phillipsburg, NJ, USA) and Shandon Scientific (Runcorn, UK), respectively. The efficiency of the DNBPG column was 8000 plates and that of the GCB column 1000 plates.

The synthetic route for the preparation of the chiral TAPA stationary phase is illustrated in Fig. 1. This material was prepared in our laboratory by means of the two-step synthesis reported previously [22]. A 100 × 4.6 mm I.D. column

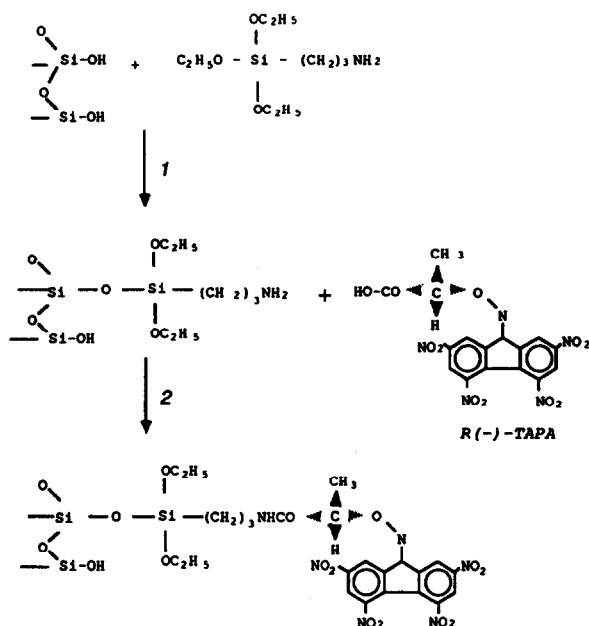
Fig. 1. Synthetic route for the preparation of the chiral (R)-(-)-2-(2,4,5,7-tetranitro-9-fluorenylideneaminooxy)-propionic acid (TAPA) adsorbent. Step 1: 1 g of activated (4 h at 120°C) LiChrosorb Si60 was first coupled to 1.3 mM of 3-aminopropyltriethoxysilane. The reaction was conducted in toluene, using a refluxing system at 120°C with stirring for 16 h. The aminated silica gel was washed with toluene, methanol and acetone and then dried under vacuum (2 h at 120°C). Step 2: the asymmetric moiety, TAPA, was linked to the aminated silica using dicyclohexylcarbodiimide (DCC) as coupling agent [23]. The reaction was conducted in dry chloroform for 8 h at room temperature. The resulting silica-bonded phase was washed with chloroform, acetone and methanol and dried (1 h at 110°C). The microanalysis of the material gave a carbon content of 12.6%. A 100 × 4.6 mm column was packed using the balanced-slurry technique.

was packed with this phase. The column efficiency was 1600 plates.

Eluent and samples

The different solvents used were all of HPLC grade and were used without further purification.

The samples of fullerenes were obtained from the extraction of a laboratory-made soot using boiling toluene [4]. The purified standard compounds were obtained by preparative chromatography, using a dual silica–alumina column system in the gravity mode, with hexane–toluene (95:5, v/v) as the mobile phase.

Chromatographic measurements

The eluent flow-rate was maintained at 1 ml/min in all experiments. The column efficiencies were measured at room temperature, using standard solutions of C_{60}. The columns void times (t_0) were determined by injecting a small plug of pure toluene and recording the perturbation signal. Under these conditions only very minor variations of t_0 were observed in the temperature range studied.

Determination of Van 't Hoff plots

The Van 't Hoff plots were determined by measuring the capacity factors of the fullerenes over the temperature range 25–95°C when toluene, heptane or their mixtures were used as the mobile phase and 25–70°C when methanol was used. Two different standard solutions (10 g/l) of C_{60} and C_{70} were injected in duplicate for each temperature point. When experiments were completed at a given temperature, the column was allowed to reach equilibrium at the next temperature by flushing it with a constant stream of mobile phase for 35 min prior to making an injection.

RESULTS AND DISCUSSION

Because the retention and separation factors and the column efficiency depend on the eluent composition and the column temperature, we need to optimize them both in order to obtain the best resolution or the maximum production rate. Accordingly, different mobile phase compositions were studied, depending on the type of interaction involved (e.g., adsorption, charge transfer). In all instances, one of our principal goals was the achievement of a high selectivity and a sufficient retention factor at the highest possible toluene concentration in the mobile phase. Heptane was preferred to hexane as the weak solvent, because of the possibility of conducting experiments in a wider temperature range.

Normal- and reversed-phase adsorption chromatography

The use of silica in either the gravity or the HPLC mode has been advocated in the literature for the purification of the fullerenes. With the various stationary phases investigated in this study, however, all the mobile phases used, whatever their composition, resulted in very low retention factors and a poor selectivity for C_{60} and C_{70}. Hence it seems that while silica can purify fullerenes from the polycyclic aromatic hydrocarbons present in the soot, it cannot separate the fullerenes.

Conversely, nearly irreversible adsorption of the fullerenes was observed on the GCB column, even with pure toluene or chloroform as the mobile phase. Heavier, more polarizable solvents such as tetralin and xylene were not considered because of the potential difficulties in eliminating them from the mobile phase after the separation has been achieved.

Fig. 2 shows a plot of the logarithm of the retention factors of C_{60} and C_{70} versus the volume fraction of toluene in methanol, on the C_{18} column. Attempts to use heptane–toluene solutions as the mobile phase resulted in a poor selectivity and a low retention. As can be seen in Fig. 2, the retention factors and the separation factor of the two fullerenes decrease rapidly with increasing toluene content of the mobile phase. While the physico-chemical phenomena responsible for retention on chemically bonded C_{18} are not fully understood, this observation can be explained by the solvophobic theory [24], or on the basis of the solubility of fullerenes in the mobile phase [25]. As far as analytical separations are concerned, the C_{60} and C_{70} fullerenes are easily resolved, with a separation factor up to 2.4, using binary mixtures of methanol and
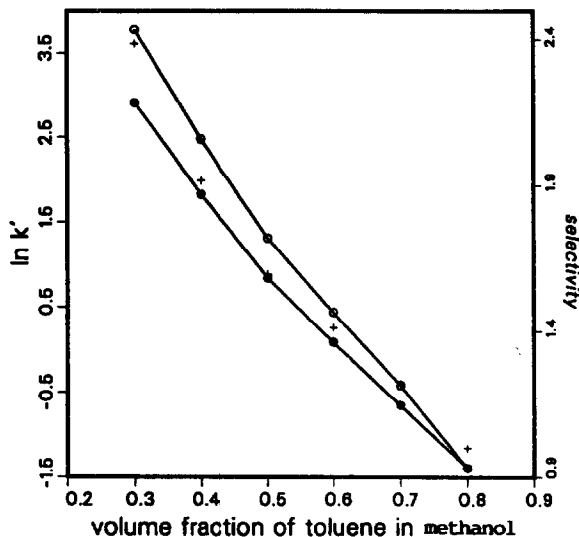
Fig. 2. Plots of the logarithmic retention factors of (●) C_{60} and (○) C_{70} fullerenes against the volume fraction of toluene in methanol. + = Corresponding selectivity. Experimental conditions: column, C_{18} (25 × 0.46 cm I.D.); temperature, 25°C; flow-rate, 1 ml/min; detection, UV at 384 nm; amount injected, 20 μl (10 mg/ml solution in toluene).

toluene as the eluent and toluene as the sample solvent. However, as will be shown later, complications quickly arise when one increases the amount injected.

Charge-transfer chromatography

In Figs. 3 and 4, we show the plots of the logarithm of the retention and separation factors of C_{60} and C_{70} on the DNPBG and the TAPA columns, *versus* the volume fraction of toluene in heptane. As often reported in the literature, quasi-linear plots were obtained in both instances. For a given eluent composition (*e.g.*, 50:50), the retention factors of C_{60} (k_1') and C_{70} (k_2'), and their selectivity, α, are larger with the TAPA column ($k_1' = 1.71$, $k_2' = 3.43$, $\alpha = 2$) than with the DNBPG column ($k_1' = 0.26$, $k_2' = 0.35$, $\alpha = 1.35$). TAPA is a stronger electron acceptor than DNBPG.

The chromatograms in Fig. 5 illustrate well the retention behavior of C_{60} and C_{70} on the TAPA column and the decrease in their resolution with increasing toluene concentration in the mobile phase.
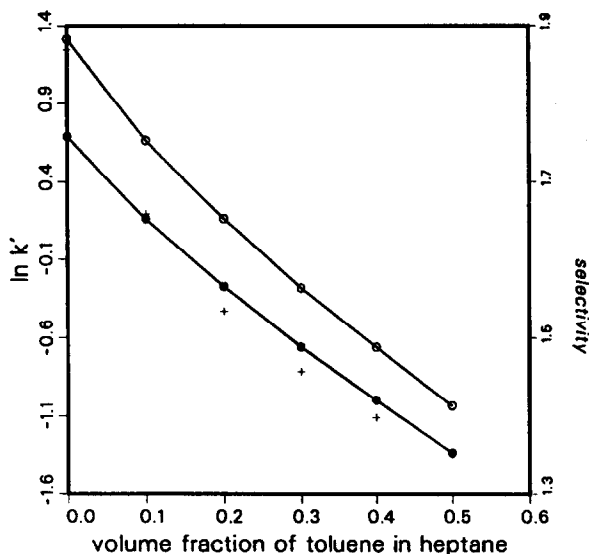

Fig. 3. Plots of the logarithmic retention factors of (●) C_{60} and (○) C_{70} fullerenes against the volume fraction of toluene in heptane. + = Corresponding selectivity. Column, DNBPG (25 × 0.46 cm I.D.); other conditions as Fig. 2.

Influence of temperature on the separation

Our main interest in studying preparative separations of the fullerenes by charge-transfer chromatography lies in the existence of a second
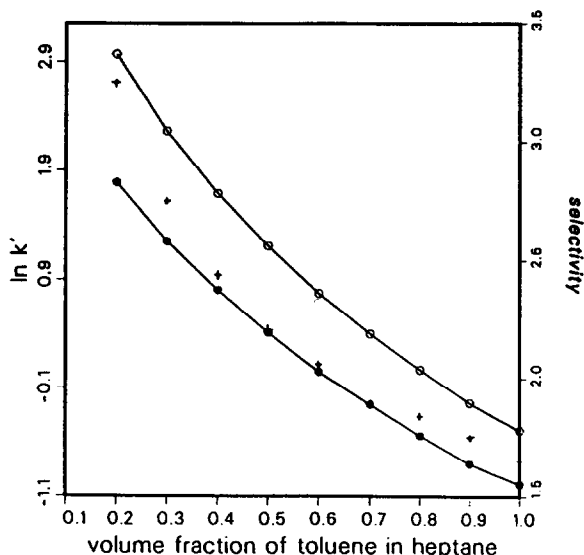

Fig. 4. Plots of the logarithmic retention factors of (●) C_{60} and (○) C_{70} fullerenes against the volume fraction of toluene in heptane. + = Corresponding selectivity. Column, TAPA (10 × 0.46 cm I.D.); other conditions as in Fig. 2.
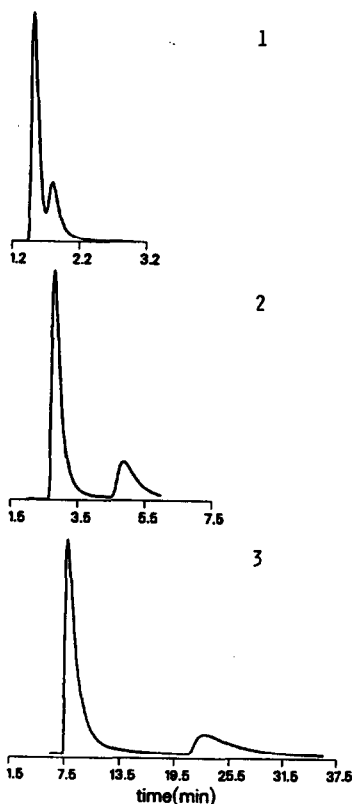
Fig. 5. Chromatogram illustrating the variation in retention time and the selectivity between C_{60} and C_{70} with the mobile phase composition. 1 = Pure toluene; 2 = toluene–heptane (50:50); 3 = toluene–heptane (20:80). Conditions as in Fig. 4.

Fig. 6. Van 't Hoff plots of the retention factors for C_{60} and C_{70} [$1000/RT$ in cal^{-1} mol (1 cal = 4.184 J)]. The retention data were measured on a 16.7-μm C_{18} column (25 × 0.46 cm I.D.) at an eluent [toluene–methanol (50:50)] flow-rate of 1 ml/min. The detector setting was 360 nm for C_{60} and 384 nm for C_{70}.

degree of freedom of the separation related to their peculiar behavior when the column temperature is changed. Figs. 6–8 show the Van 't Hoff plots obtained for C_{60} and C_{70} on the ODS column with methanol–toluene (50:50) (Fig. 6), the DNBPG column with heptane–toluene (90:10) (Fig. 7), and the TAPA column with heptane–toluene (50:50) (Fig. 8). Because of the temperature limitations encountered in the use of the different solutions, and owing to the different boiling points of the solvents used, the temperature range investigated was 25–75°C for Fig. 6 and 25–95°C for Figs. 7 and 8.

The Van 't Hoff plots are all linear in the temperature range studied. The correlation coefficients for a linear fit of the experimental data to the Van 't Hoff plots (eqn. 5) were ≥0.989. The same trends were observed for the different
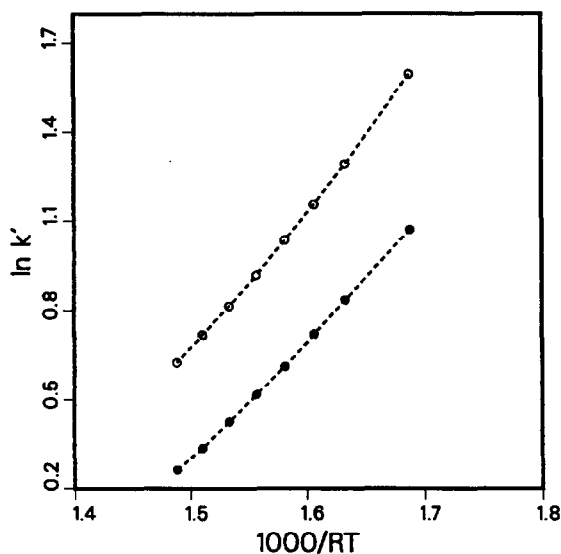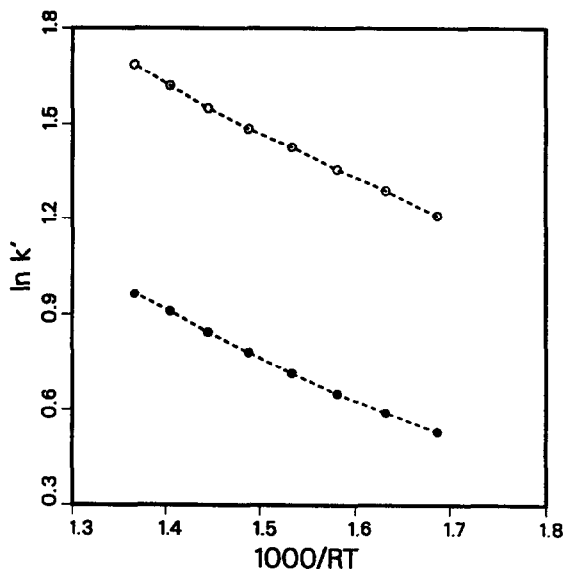

Fig. 7. Van 't Hoff plots of the retention factors for C_{60} and C_{70} ($1000/RT$ in cal^{-1} mol). The retention data were measured on a commercial analytical DNBPG column (25 × 0.46 cm I.D.) at an eluent [toluene–heptane (10:90)] flow-rate of 1 ml/min. The detector setting was 360 nm for C_{60} and 384 nm for C_{70}.
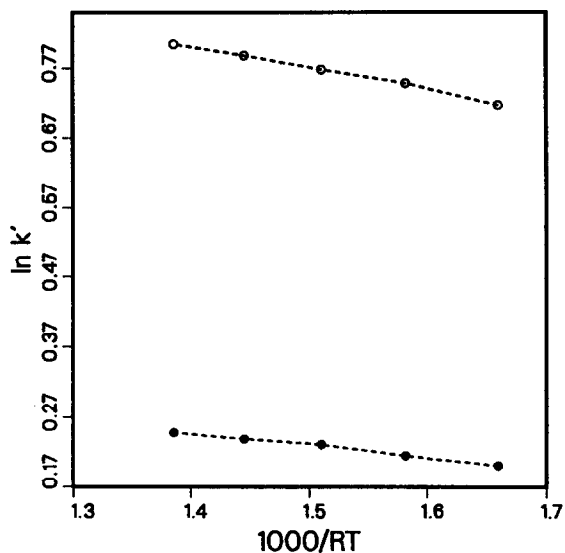
Fig. 8. Van 't Hoff plots of the retention factors for C_{60} and C_{70} ($1000/RT$ in cal^{-1} mol). The retention data were measured on the laboratory-made TAPA column (25×0.46 cm I.D.) at an eluent [toluene–heptane (50:50)] flow-rate of 1 ml/min. The detector setting was 360 nm for C_{60} and 384 nm for C_{70}.

solvents, using pure or binary mixtures of various compositions. This suggests that there is a single, or at least a strongly predominant, retention mechanism [26] that does not depend on the solvent composition for the series of solvents studied. This conclusion is consistent with the fact that C_{60} and C_{70} have a single conformation, but it has been shown that they exhibit different phase transitions at low temperatures [27] and one could have expected a non-linear Van 't Hoff plot due to solute–solvent association [28].

As usual in chromatography, the Van 't Hoff plot for the system of ODS with methanol–toluene (50:50) exhibits a positive slope (Fig. 6). Both the adsorption enthalpy and entropy are negative. The adsorption process is exothermic and, as a consequence, the retention times of both solutes decrease with increasing temperature. In contrast, a negative slope is observed for the Van 't Hoff plots obtained with the two charge-transfer systems investigated, DNBPG (Fig. 7) and TAPA (Fig. 8). Therefore, in these two instances the complexation enthalpy and the associated entropy are positive and the heat of complexation is negative. The retention times of

C_{60} and C_{70} on the two charge-transfer stationary phases increase with increasing column temperature.

These results substantiate some earlier work done on DNBPG with dichloromethane–hexane (10:90) as the eluent [29]. This unusual temperature dependence was interpreted by assuming that the analytes associate in solution. The oligomers would dissociate when the column temperature increases, allowing more interactions between the selectant and the fullerene members [30]. Indeed, C_{70} seems to undergo some reorganization in an hexagonal close-packed crystal conformation, but a similar behavior has not yet been proven in the liquid phase [27].

It is important to ascertain whether this peculiar behavior is an intrinsic property of the fullerenes, possibly related to their rigid three-dimensional structure and their unique aromatic character. In this connection, we measured the temperature dependence of the retention factors of different planar, non-planar and helicoidal polycyclic aromatic compounds, including cis- and trans-stilbene, anthracene, phenanthrene, benzo[e]- and benzo[a]pyrene and hexacarbo-helicenes, on the TAPA column. All their retention times decrease rapidly with increasing temperature, suggesting that all these electron-donor compounds undergo an exothermic complexation process on TAPA, whereas the interaction of TAPA with the fullerenes is endothermic. This result suggests that, owing to their non-planar structure and weak aromaticity, the fullerenes are much weaker electron donors than polycyclic aromatic hydrocarbons.

Another interesting feature of Figs. 7 and 8 is that the separation factor of the fullerenes remains virtually independent of the column temperature. A temperature increase does not modify the degree of non-planarity recognition of the selectants. However, noticeable improvements in the experimental band shapes were observed. This can be explained by faster mass-transfer kinetics at higher temperatures.

A negative heat of transfer or a non-linear Van 't Hoff plot is generally related to the effects of solvent or solvent additive [31], to the existence of two different conformers or to a dual reten-

tion mechanism [28,32] for a solute. The two fullerenes C_{60} and C_{70} have a unique conformation, and can behave as both electron acceptor and electron donors. The electron affinity of C_{60} is known to be 2.6 eV. It is improbable that, with the high concentration of toluene in the mobile phases used, a change in column temperature can greatly affect its equilibrium concentration in the stationary phase. Thus, the negative slope of the Van 't Hoff plot can probably be attributed to a high solvation of the solutes at low temperatures.

Practical applications

To illustrate the above discussion, Fig. 9 shows an analytical separation obtained with the injection of 20 μl of a binary mixture of C_{60} and C_{70} (20 mg/ml in toluene) (top) and the chromato-

gram obtained with an overloaded injection (200 μl of the same solution), in the same ODS column, with methanol–toluene (50:50) as the mobile phase (bottom). A temperature increase of 50°C results in a decrease of *ca.* 5 min in the retention time (dashed line). The profile obtained at 25°C (solid line) is characterized by a pronounced shoulder and a steep rear profile, probably owing to the effect of the sample solvent, as discussed previously [13].

Figs. 10 and 11 illustrate the effect of the column temperature on the separation of the two fullerenes, using DNBPG [injection volume 100 μl; solution 10 mg/ml; mobile phase heptane–toluene (90:10)], and TAPA [same conditions, except mobile phase composition heptane–toluene (50:50)]. Increasing the temperature from 25°C (solid line) to 95°C (dashed line) results in an increase in the retention time. The magnitude of the effect is more pronounced with the TAPA column because of the higher donor ability of the selectant and the higher retention factors. With the DNBPG column, splitting of
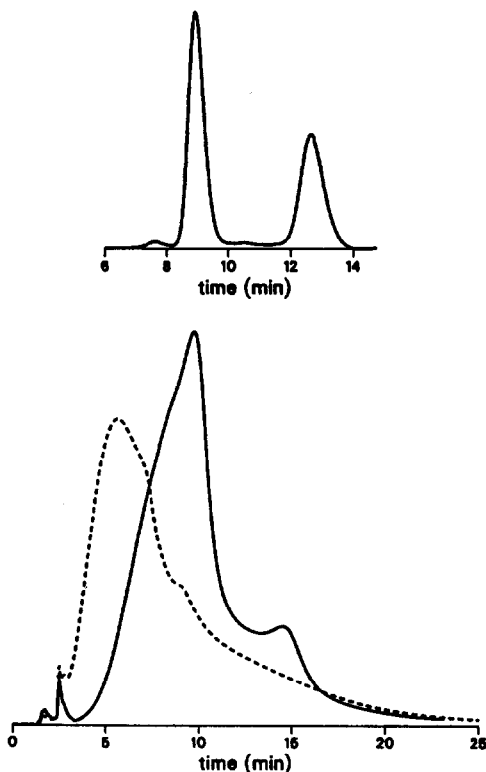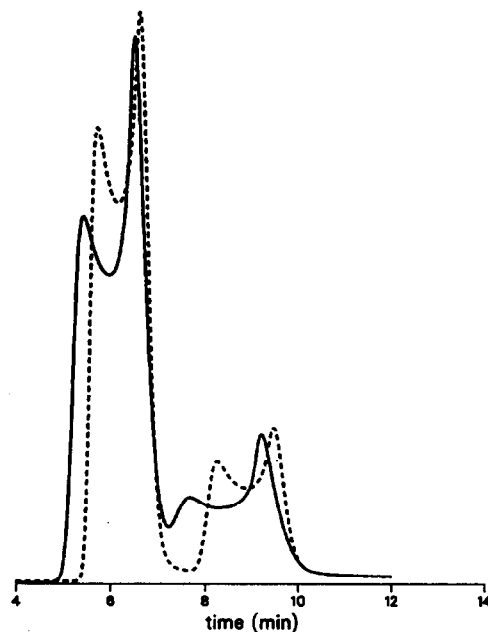

Fig. 9. Separation of C_{60} and C_{70} on a C_{18} bonded silica stationary phase. (top) Analytical injection of a mixture (*ca.* 9:1) of C_{60} and C_{70} at ambient temperature. (bottom) Effect of temperature on the overloaded chromatogram (200 μl of a 20 mg/ml solution) of the same mixture. The temperatures were 25°C (solid line) and 75°C (dashed line) and the experimental conditions as in Fig. 6.

Fig. 10. Effect of temperature on the experimental elution profiles of a mixture (*ca.* 9:1 ratio) of C_{60} and C_{70}. Volume injected, 100 μl (10 mg/ml solution). Column and conditions as in Fig. 7, except for the temperature: solid line at 25°C and dashed line at 95°C. Both C_{60} and C_{70} are eluted as bimodal peaks.

this limitation, the poor experimental approach consisting in the use of a stronger sample solvent (*e.g.*, pure toluene or benzene) to insure the injection of a narrow band of concentrated solution and an adequate throughput has been adopted by different workers (*e.g.*, [29]). As explained above (*cf.*, Fig. 10), the resulting chromatogram exhibits severe abnormalities. The complex band interaction pattern observed at high loading [13] does not permit the achievement of an adequate throughput. It is clear that, in order to improve this situation, we need to find more retentive stationary phases and better solvents than toluene.

ACKNOWLEDGEMENTS

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