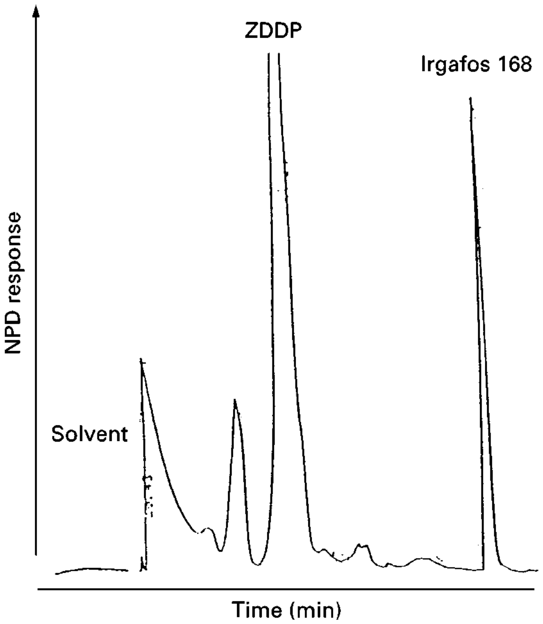

Figure 8 Chromatogram of used lubricating oil. Conditions: Diol packed column, temperature 50°C; ramp rate of 3 bar min^{-1}, NPD detection. (Courtesy of the University of Leeds.)

advantages of SFC are its speed of analysis and improved column efficiency when compared to liquid chromatography. The lower column temperature than needed for GC allows the analysis of higher-molecular-weight mixtures with carbon numbers up to C_{120} and beyond.

Further Reading

ASTM Method D5186 (1992) In: *Annual Book of ASTM Standards*, vol. 05.03, p. 855. Philadelphia, PA: American Society for Testing and Materials.

Kithinji JP, Raynor MW, Egia B *et al.* (1990) Analysis of coal-tar polycyclic aromatic hydrocarbon LC-fractions by capillary SFC on a liquid-crystalline stationary phase. *Journal of High Resolution Chromatography* 13: 27–33.

Lee ML and Markides KE (eds) (1990) *Analytical Supercritical Fluid Chromatography and Extraction*. Provo, UT: Chromatography Conferences Inc.

Medvedovici A, David F, Desmet G and Sandra P (1998) Fractionation of nitro and hydroxy polynuclear aromatic hydrocarbons from extracts of air particulates by supercritical fluid chromatography. *Journal of Microcolumn Separations* 10: 89–97.

Raynor MW, Moulder R, Davies IL *et al.* (1990) Dual capillary column supercritical fluid chromatography. *Journal of High Resolution Chromatography* 13: 22–26.

Roberts I (1995) Supercritical fluid chromatography. In: Adlard ER (ed.) *Chromatography in the Petroleum Industry*. Journal of Chromatography Series, vol. 56, pp. 305–346. Oxford: Elsevier Science.

Shariff SM, Robson MM, Myers P *et al.* (1998) Hydrocarbon group-type separations for high aromatic fuels by supercritical fluid chromatography on packed capillary columns. *Fuel* 77: 927–931.

Westwood SA (ed.) (1993) *Supercritical Fluid Extraction and its Use in Chromatographic Sample Preparation*. London: Blackie Academic & Professional.

FULLERENES: LIQUID CHROMATOGRAPHY

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Introduction

Since the first separation in 1990 of C_{60} and C_{70} using column liquid chromatography (LC), this technique has played a very important role in fullerene chemistry. LC has allowed macroscopic quantities of fullerenes (particularly C_{60}) to be isolated and purified from the processing products. Obtaining sufficient amounts of pure fullerenes has been crucial both for determining physical and chemical properties in order to investigate practical applications of this new variety of carbon, and for developing a chemistry of these spherical and polyfunctional carbon molecules. A very rich chemistry has been developed in less than a decade based mainly on C_{60}, and to a lesser extent on higher fullerenes. The starting reactives for this chemistry have been the compounds previously isolated by LC. It is now possible to bind covalently many types of compounds to the fullerene molecule.

LC is currently the method of choice for the separation, isolation and purification of fullerenes. Progress in fullerene chemistry therefore depends on the development of improved chromatographic methods, i.e. those with the highest efficacy and best resolution between the different components of fullerene mixtures, at both analytical and preparative scales, and at the lowest cost.

Contribution of LC in the Field of Fullerene Production

In early 1990, the design of LC methods was mostly geared towards isolating the most abundant fullerenes obtained from the different production methods (e.g. laser vaporization of graphite, electric arc discharge, hydrocarbon flame combustion process, pyrolysis of carbon material). Each of these methods has its own specificity, with its advantages and drawbacks. In general, the objective of these methods was to obtain C_{60} and, to a lesser extent, C_{70}. On occasions, endohedral metallofullerenes (i.e. $M@C_{60}$) were also isolated. Therefore, chromatographic methods developed for this purpose should be (semi-) preparative. Likewise, the presence 'as impurities' of higher fullerenes and metallofullerenes (in low or very low concentration), polyaromatic compounds (PACs, created during production), residual solvents, solvates (due to strong interactions between fullerenes and some solvents), fullerene adducts (mostly with oxygen), and other artefacts produced during the production must be taken into account when designing an analytical procedure.

In this case, an extraction step for pre-purification is required. The origin of the soots, which is determined by the starting carbon material and the production method, influences the extraction yields and the nature of the fullerenes extracted.

LC in the Field of Organic and Organometallic Chemistry of Fullerenes

At the same time that C_{60} and C_{70} became available in significant amounts, the organic chemistry of fullerenes started to be developed. New reactions were performed and plentiful data about C_{60} reactivity were obtained. C_{60} was initially considered to be a polycyclic aromatic hydrocarbon (PAH), and the chromatographic methods that were developed were based on previous knowledge accumulated on PAHs. Further research, to which LC contributed significantly, demonstrated that the reactivity of C_{60} is more like that of a localized polyolefin. The LC contribution to this chemistry includes separation, identification and isolation of products. LC is also used to monitor the reactions typical of fullerenes: nucleophilic additions, cycloadditions, hydrogenation reactions, and oxidation and reactions with electrophiles. In addition, different isomers, diastereomers and enantiomers have been separated (**Figure 1**). Different examples of these separations are shown in **Table 1**.

The analytical designs required to separate fullerene derivatives from organic reactions differ considerably from those used to isolate fullerenes from their production methods. Several points must be considered. First, the structural similarities of these fullerene derivatives are often an obstacle in fullerene separations. Furthermore, the matrices and relative percentages of compounds to be separated are different. Finally, some addents on the fullerene core have a dramatic influence on the solubility properties and the retention behaviour.

Separations Using Normal and Reversed Stationary Phases

In general, the poor solubility of fullerenes in most organic solvents limits the choice of mobile and stationary phases that can be used. Conventional stationary phases used for normal- and reversed-phase elution may exhibit reasonable fullerene selectivity, but only when using mobile phases in which the fullerenes are poorly soluble (e.g. *n*-hexane, dichloromethane, acetonitrile). These phases usually provide weak interactions with fullerenes when using eluents in which fullerenes are most soluble (e.g. *o*-dichlorobenzene, CS_2), which produce elution without adequate separation. Detection of fullerenes is not a problem: conventional or diode-array UV are used in the range 230 to 600 nm, depending on the cutoff of the solvent used.

Normal-Phase Elution

Open-column and low-pressure liquid chromatographic techniques, rather than HPLC, have been used for separations based on adsorption chromatography and performed with conventional normal-phase stationary phases (e.g. silica gel or alumina). In this subsection, we summarize the application of these methods, including the use of charcoal columns, to fullerene separations.

C_{60} was first separated from C_{70} using open-column LC on alumina as stationary phase, and *n*-hexane or *n*-hexane/toluene (95 : 5) as mobile phase. This method presents several disadvantages: as *n*-hexane is not a good solvent for fullerenes, a high consumption of both mobile and stationary phases is required; it is time-consuming, because several sequential separations are necessary in order to obtain sufficient amounts of C_{70} and other higher fullerenes, owing to peak tailing; and on-column degradation of C_{70} and higher fullerenes occurs (this phenomenon had been already described for PAHs). The preadsorption of fullerenes on alumina did not solve these problems.

A modification was introduced to overcome these deficiencies: the combination of Soxhlet extraction and LC on alumina, in which hot solvent was continuously recycled, allowed solvent consumption and labour to be reduced. However, the most inexpensive and efficient method for rapid separation of C_{60} and C_{70} involves an activated charcoal (Norit A) and silica gel mixture (1 : 2) as the stationary phase. As carbon

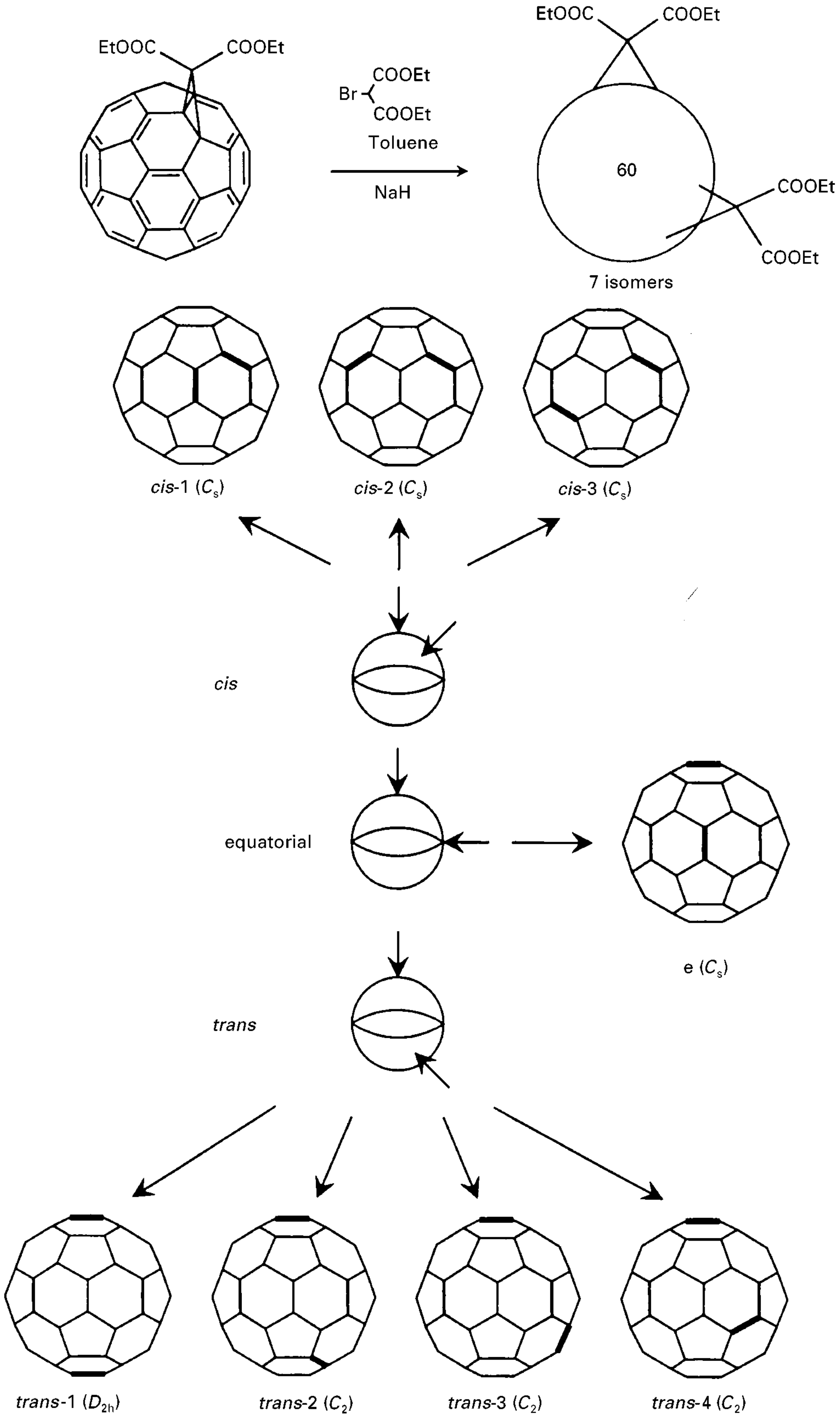

Figure 1 Positions of the ligand-carrying bonds in the eight possible regioisomers of C_{60} with symmetrical additions to 6-6 bonds and symmetry of the corresponding isomers. (Reprinted from Hirsch A (1994) *The Chemistry of the Fullerenes*, pp. 68 and 69, with permission from Thieme.)

Table 1 Some examples of LC contribution to organic and organometallic chemistry of fullerenes

Reaction mechanism category	*Reaction*	*LC contribution*
Nucleophilic additions	Hydroalkylation and hydroarylation of C_{60} and C_{70}	Monitoring of products formation; consumption of C_{60} followed from HPLC peak areas
	$C_{60} + RLi \rightarrow C_{60}R^{-} \rightarrow C_{60}HR$ (protonation)	Purification of 1,2-organodihydrofullerenes (preparative C_{18}, chloroform/acetonitrile, 60/40)
	Synthesis of [6,6]-methanofullerenes from stabilized sulfonium ylides with C_{60} or C_{70}	Monitoring of the reactions by HPLC Pure monoadducts obtained after column chromatography on silica gel Resolution of a racemic derived-amide on a chiral (S,S)-Whelk-O HPLC column
	Cyclopropanation of C_{60} and C_{70} and higher adducts (stabilization by intramolecular S_{Ni})	Isolation of seven stable regioisomeric bisadducts of C_{62} $(COOEt)_4$ of the eight possible ones (see Figure 1). Identified by NMR and HPLC elution order Isolation of chiral trisadducts of C60 and di-(ethoxycarbonyl) methylene
[4 + 2] Cycloadditions	Cycloadducts from C_{60} with: –cyclopentadiene –anthracene	Purification using GPC columns and toluene
	Cycloadducts from C_{60}-4 (4-fluoro-3-nitro benzoyl) benzo-cyclobutene with: –4,13-diaza[18]-crown-6 –1,6-bis (aminomethyl) hexane –4-amino-azobenzene	Separation of products by GPC columns and chloroform, or toluene
	Cycloadducts from C_{60} with: –2-trimethyl-silyloxy-1,3-butadiene	Synthesis through flash chromatography
[3 + 2] Cycloadditions	Synthesis of fullerene-bound dendrimers	Isolation by GPC
	Methano-bridged cycloadducts from C_{60} with diazoamides (biological importance)	Isolation on silicagel using toluene
[2 + 2] Cycloadditions	Photochemical cycloaddition of enones to C_{60}	Analysis of products using HPLC-Buckyclutcher® The corresponding enantiomers of each diastereomer for a methyl substituent of the enone have been resolved and isolated by HPLC using chiral stationary phases
Hydrogenation	Synthesis of $C_{60}H_2$ via hydroboration and hydrozirconation, and also by Zn/acid reduction	The only regioisomer formed can be isolated from the reaction mixture by HPLC
	Polyhydrofullerenes from Birch–Hückel reduction	$C_{60}H_{36}$, $C_{60}H_{18}$ obtained from the mixture using LC on silica gel in CH_2Cl_2/hexane
Oxidation and reactions with electrophiles	Oxygenation of C_{60} and C_{70}	$C_{60}O$, $C_{70}O$ and others isolated from fullerene extracts by preparative HPLC (neutral alumina)
	Thermal treatment of fullerene systems	Isolation of $C_{120}O$, $C_{120}O_2$, $C_{180}O_2$ using Cosmosil Buckyprep® and PBB® in several steps, with *o*-dichlorobenzene, and toluene
	Bisosmylation of C_{60}	Isolation of five regioisomers of C_{60} $[OsO_4(t\text{-}bupy)_2]_2$
	Asymmetrical osmylation of chiral C_{76} using chiral ligands (L*)	HPLC analysis of $C_{76}[OsO_4L^*]$ shows that two regioisomers are predominantly formed upon osmylation

compounds can adsorb fullerenes in a similar manner to alumina, silica gel is added to mitigate this effect. This system readily elutes C_{60} (using toluene, at low pressure or even slight vacuum). By adding *o*-dichlorobenzene to the solvent system, gram quantities of both C_{60} and C_{70} can be purified and a fraction enriched in higher fullerenes collected, all in a single column pass.

Silica gel, in contrast, is not able to separate parent fullerenes on its own. However, column chromatography on silica gel has occasionally been able to isolate pure C_{60} and C_{70} derived adducts from organic reactions (see Table 1). Likewise, silica gel has been used to separate PAHs cosynthesized during fullerene production.

Normal Elution Using Nonconventional Phases

γ-Cyclodextrin bound to silica gel, in HPLC mode, is mostly used for enantiomer separations. However, in the field of fullerenes, it has been used for separating

C_{60} from C_{70}. γ-Cyclodextrin is a cyclic oligosaccharide that forms complexes with fullerenes. The interaction of C_{70} with cyclodextrin is stronger than that with C_{60}, and both molecules can be separated, at analytical scale, even using *n*-hexane or *n*-hexane/toluene (70 : 30). However, the problem of fullerene solubility in these solvents has hampered further research on this stationary phase.

Bonded-Phase Sorbents

Octadecylsiloxane-bonded silica phases (C_{18}) have been used in HPLC mode for analytical-scale separations of fullerenes. Although *n*-hexane or *n*-heptane have been used as mobile phases, toluene has been mostly used, together with a counter solvent (e.g. acetonitrile, methanol) in variable proportions. Although the solubility of parent fullerenes is low in these solvent compositions in general, the use of toluene/acetonitrile provides adequate separation between C_{60} and C_{70} with selectivities (expressed as $\alpha_{C70/C60}$) near 2 for a 50 : 50 mixture. The use of toluene/methanol, in turn, shortens the analysis time in comparison with toluene/acetonitrile mixtures. Other profile phases, such as $CHCl_3$/acetonitrile on CH_2Cl_2/acetonitrile, have also proven suitable for separation of C_{60} and C_{70}.

Monomeric and polymeric C_{18} phases have also been used. They provide differences in retention for fullerenes. A ‘topological’ recognition ability of the polymeric phase has been invoked to explain these differences. Therefore, fullerenes would be separated as a function of their spherical diameters or their geometries in general.

Separations Using Charge-Transfer Stationary Phases

Considerable research has been carried out in developing supports that can provide strong interactions with fullerenes when using eluents that solubilize fullerenes. Charge-transfer chromatography is based on the donation or acceptance of electrons between the stationary phase and, in this case, the fullerene derivatives. The migration of fullerenes through the column is selectively retarded to a degree depending on the strength of the charge-transfer complex formed. Based on research on separation of PAHs, phases such as dinitroanilinopropyl silica (DNAP) or tetrachlorophthalimidopropyl silica (TCPP) were studied in the early 1990s. The latter presented adequate selectivity for C_{60} and C_{70} ($\alpha = 2.75$ in the best case).

Many of these phases, called Pirkle-type, have been synthesized and tested. In general, they are based on electron-deficient aromatic rings capable of simultaneous interaction with the spherical, electron-rich, fullerene molecules. These phases afford greater interaction with fullerenes, thus allowing the use of toluene-based mobile phases.

In the second-half of the 1990s, several of these stationary phases became commercially available and are used now in many different types of fullerenes separations, at both analytical, semipreparative or preparative scale. **Figure 2** shows, among other phases described in this work, the chemical structures of Buckyclutcher® (tri-(dinitrophenil)-silica), Cosmosil PBB® (pentabromobenzyl-silica) and Cosmosil Buckyprep® (2-[(1-pyrenyl)ethyl]-silica). The Buckyclutcher column, developed by Pirkle, can be used in both normal and reversed phase mode. The capacity of this phase to separate fullerenes seems to be due, in addition to electron donor–acceptor interaction, to a steric effect created by a cone-shaped arrangement of dinitrophenyl groups.

High-capacity stationary phases containing condensated aromatic systems (e.g. Buckyprep®, 5-coronenylpentyl-silica, COP) or heavy heteroatoms such as sulfur, chlorine or bromine (e.g. Cosmosil PBB®) showed high retentivity with excellent efficiency for separation of fullerenes, particularly Cosmosil PBB®. This phase allows the use of solvents providing high fullerene solubilities, such as CS_2 or 1,2,4-trichlorobenzene, for gram-scale separations with ordinary HPLC equipment. These solvents exceed toluene in solubility of fullerenes.

Although large aromatic systems were expected to show high fullerene retentivity according to electron donating–accepting the positive effect of heavy atoms is not correlated with the electron-withdrawing or donating properties and more research is needed on this point.

The use of the above-mentioned commercially available columns usually involves two or three steps in the process of separation, isolation and purification of fullerene derivatives. As an example, a typical scheme of proceeding may be a first step using an analytical Cosmosil PBB column® (with CS_2, *o*-dichlorobenzene or 1,2,4-trichlorobenzene as eluents) and a second step using a preparative Buckyprep® or Buckyclutcher® column (e.g. with toluene). HPLC with these columns has allowed the identification, separation and/or isolation of endohedral metallofullerenes (e.g. $M@C_{74}$, $M@C_{60}$, $M@C_{70}$), fullerenes higher than C_{60} (e.g. C_{86}, C_{92}, C_{94}, C_{120}), odd-numbered clusters (e.g. C_{119}), products derived from fullerene oxidation (e.g. $C_{120}O$, $C_{120}O_2$, $C_{180}O_2$), and the separation of isomers (e.g. $C_{60}H_4$).

Tetraphenylporphyrin (TPP)-silica based stationary phase is another promising family for fullerene separation at both analytical and preparative scale. **Figure 2** shows chemical structures of

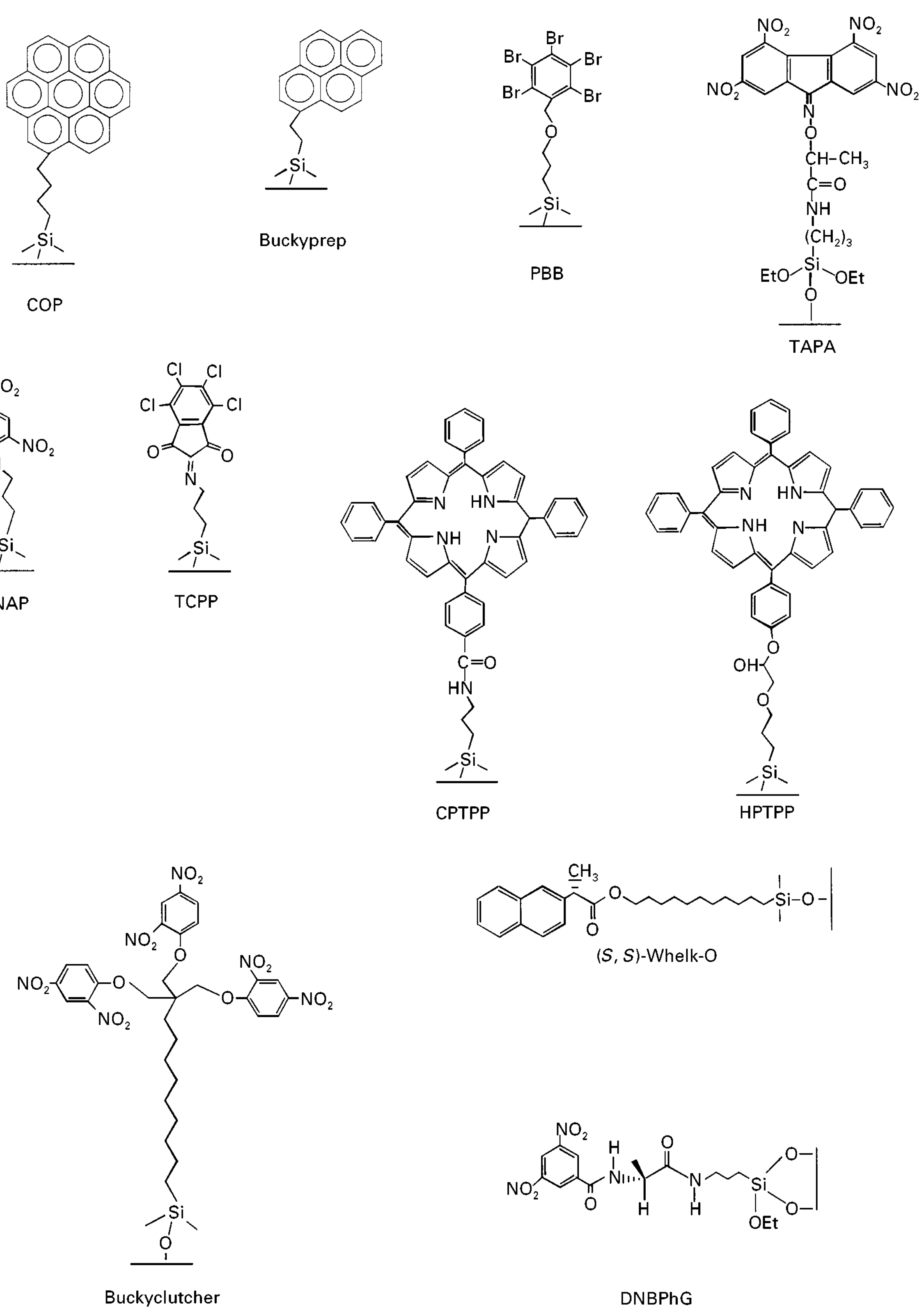

Figure 2 Some charge-transfer stationary phase used for fullerene separation (for abbreviations, see text).

[5-(*p*-hydroxyphenyl)-10,15,20-triphenyl]porphyrin-silica (HPTPP) and [5-(*p*-carboxyphenyl)-10,15,20-triphenyl]porphyrin-silica (CPTPP).

These phases have demonstrated strong retention and unmatched selectivity in the separation of C_{60} and C_{70} using strong mobile phases such as

Table 2 Fullerene selectivity, expressed as $\alpha_{C70/C60}$, of different stationary phases, using toluene as the mobile phase, under similar conditions

Stationary phase	$\alpha_{C70/C60}$
Buckyclutcher	1.5
PBB	2.5
Buckyprep	1.8
CPTPP	5.7
HPTPP	7.0

1,2-dichlorobenzene or CS_2. **Table 2** gives a rough picture of the influence under similar conditions of different stationary phases on fullerene selectivity, expressed as $\alpha_{C70/C60}$, using toluene as the mobile phase. Selectivity using toluene is the highest for all phases with regard to stronger eluents: $\alpha_{tol} > \alpha_{CS2} > \alpha_{dichlorobenzene}$. Another important point for TPP-silica stationary phases is their potential for preparative-scale separations. This includes higher fullerenes. Owing to the usually low percentage of these kinds of fullerenes in the parent mixtures, their efficient separation using conventional or commercial phases becomes difficult and usually involves several reinjections, which are both solvent- and time-consuming. Currently, TPP phases present a good resolution in the separation of higher fullerene isomers in a single column pass, using strong eluents such as 1,2-dichlorobenzene, as shown in **Figure 3**.

Enantiomer Separation

Some charge-transfer stationary phases, such as (*R*)-*N*-(3,5-dinitrobenzoyl)phenylglicine or *R*-(−)[2-(2,4,5,7-tetranitro-9-fluorenylideneaminooxy)propionic acid] (TAPA) bound to silica gel (see **Figure 2**), have been used for the separation of enantiomeric fullerene derivatives.

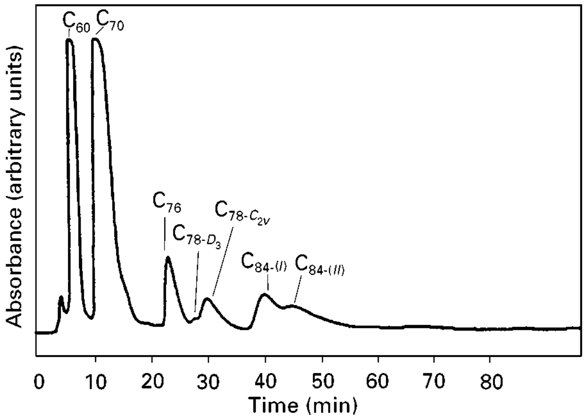

Figure 3 Separation of higher molecular mass fullerenes on a 250 mm × 4.6 mm column packed with HPTPP(4)-silica. Injection of 12 mg of fullerene soot; mobile phase = 100% 1,2-dichlorobenzene; flow rate = 0.85 mL min^{-1}; detection wavelength = 410 nm; ambient column temperature. (Reprinted from Coutant DE, Clarke SA, Francis AH and Meyerhoff ME (1998) Selective separation of fullerenes on hydroxyphenyl-triphenylporphyrin-silica stationary phases. *Journal of Chromatography A* 824: 147–157, with permission from Elsevier Science.)

Taking advantage of the fact that (*R*)-*N*-(3,5-dinitrobenzoyl)phenylglicine (DNBPhG) was able to separate fullerenes, other chiral stationary phases were designed. A stationary phase derived from S-naproxen (Whelk-O columns; see **Figure 2**) has provided successful separation of chiral fullerene derivatives (**Table 1**).

A strong charge-transfer interaction is necessary but on its own it is not a sufficient requirement for chiral discrimination. Both π-donor–acceptor interactions and one stereochemically significant interaction via hydrogen bonds are required.

TAPA-bounded silica gel has also been used for nonchiral separations (e.g. separation between C_{60} and C_{70}).

Size Exclusion Chromatography (SEC)

SEC is a molecular sieving technique that separates molecules according to their selective permeation into the gel pores on the basis of differences in their size in solution. Solute permeation into the gel increases with decreasing molecular size, resulting in later elution. By virtue of these particular characteristics of SEC, the total time of the chromatographic runs is previously known. This technique is also named GPC (gel permeation chromatography) when carried our using organic solvents.

SEC stationary phases consist of a gel, typically a three-dimensional network of cross-linked polymeric chains of controlled porosity. Compatibility between solvent and stationary phase is of prime importance. If the mobile phase is not suitable, gels can swell or shrink, often damaging the packing bed; this changes the pore distribution and hence the exclusion volume. SEC is therefore confined to isocratic elution using a limited range of pure solvents.

The differences in size between the fullerenes are sufficient to allow SEC separation. Because pure toluene can be used as the mobile phase (allowing fewer constraints for solvent recycling) and these columns have a greater lifetime than other LC columns (owing to the theoretical absence of interaction between the fullerenes and SEC stationary phase) it is possible to use SEC at preparative scale.

Polystyrene-divinylbenzene-based columns, from 10 to 100 nm pore size, have been used for fullerene separations. In spite of their low selectivity ($\alpha_{C70/C60} = 1.1$), the possibility of using automated systems with reinjection/sample collection and solvent recycling has allowed the separation of 10 g of an extract (from production) a day, yielding C_{60} with high purity. The relatively short time of SEC runs allows the frequency of injections to be increased with respect to other LC techniques.

This kind of automated system has also been used to separate and isolate metallofullerenes from empty cage fullerenes in sufficient amounts, despite their low concentration in the production mixtures (e.g. 200 mg of metallofullerenes isolated in 16 h).

However, the fact the C_{60} is eluted before C_{70} and other higher fullerenes, regardless of the mobile phase used (e.g. toluene, $CHCl_3$), means that solutes are not separated according to their size. Non-size effects (due to adsorptions and other types of interactions) have been described in the case of other relatively small molecules (e.g. PACs) with relative molecular masses lower than 1000.

Further Trends

Research is now focused on finding more selective stationary phases (mainly based on charge-transfer chromatography) to improve fullerene separation. An efficient method for separating individual fullerenes on a large (preparative) scale is still required. Most of the separation methods reported here are limited to gram scale. This has hampered the study of higher molecular mass fullerenes.

Further Reading

Ajie H, Alvarez MM, Anz SJ *et al.* (1990) Characterization of the soluble all-carbon molecules C_{60} and C_{70}. *Journal of Physical Chemistry* 94: 8630–8633.

Coutant DE, Clarke SA, Francis AH and Meyerhoff ME (1998) Selective separation of fullerenes on hydroxyphenyl-triphenylporphyrin-silica stationary phases. *Journal of Chromatography A* 824: 147–157.

Gross B, Schurig V, Lamparth I and Hirsch A (1997) Enantiomer separation of [60]fullerene derivatives by micro-column high-performance liquid chromatography using (R)-(−)-2-(2,4,5,7-tetranitro-9-fluorenylideneaminooxy) propionic acid as chiral stationary phase. *Journal of Chromatography A* 791: 65–69.

Hirsch A (1994) *The Chemistry of the Fullerenes*. Stuttgart: Georg Thieme-Verlag.

Kimata K, Hirose T, Moriuchi K *et al.* (1995) High-capacity stationary phases containing heavy atoms for HPLC separation of fullerenes. *Analytical Chemistry* 67: 2556–2561.

Pirkle WH and Welch CJ (1991) An unusual effect of temperature on the chromatographic behavior of buckminsterfullerene. *Journal of Organic Chemistry* 56: 6973–6974.

Scrivens WA, Cassell AM, North BL and Tour JM (1994) Single column purification of gram quantities of C_{70}. *Journal of the American Chemical Society* 116: 6939–6940.

Taylor R, Hare JP, Abdul-Sada AK and Kroto HW (1990) Isolation, separation and characterisation of the fullerenes C_{60} and C_{70}: the third form of carbon. *Journal of Chemical Society*, *Chemical Communications* 20: 1423–1425.

Theobald J, Perrut M, Weber JV, Millon E and Muller JF (1995) Extraction and purification of fullerenes: a comprehensive review. *Separation Science and Technology* 30: 2783–2819.

Welch CJ and Pirkle WH (1992) Progress in the design of selector for buckminsterfullerene. *Journal of Chromatography* 609: 89–101.

FUNGICIDES

Gas Chromatography

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Fungicides, a class of pesticides, are toxic substances that are used to prevent or kill the growth of fungi which are hazardous for plants, animals and human beings. Most fungicides for agricultural use are fumigated or sprayed over seeds, leaves or fruits to control and avoid a variety of economically important fungal diseases. The first fungicide of proven efficiency was the Bordeaux mixture, developed in 1882. This is a mixture of lime and copper(II) sulfate that has been used for a long time; nowadays a wide variety of compounds are used in a more selective way to fight specific fungi in specific plants. It is necessary to pay attention to their environmental impact (on water, the atmosphere, soil and food) and also to their presence in vegetables which are intended for direct human consumption. Among the important fungicides are those which are applied in greenhouses and in wine production.

There are two options for fungicide analysis: the determination of the composition of formulations, where the concentrations are relatively high, and the