Short Communication

Analysis of C_{60} and C_{70} fullerenes by high-performance liquid chromatography

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ABSTRACT

Some common solvents were roughly classified into four classes according to their decreasing solubility for fullerenes. Good solvents should be used in designing a practical liquid chromatographic separation method. Several stationary phases were tested to separate C_{60} and C_{70} fullerenes. They included ODS-, C_6H_5-, NH_2- and CN-silica, silica gel and three synthesized ones containing mono-/di- nitrobenzamidopropyl functionalities. All the stationary phases studied, with the exception of silica gel, had some retention and separation capability for C_{60} and C_{70} fullerenes. The three nitrobenzamidopropyl group-containing packings showed more pronounced retention and were capable of being used with good solvent added in the mobile phase. 3,5-Dinitrobenzamidopropyl chemically bonded phase, the most retentive packing, was used as stationary phase in HPLC for the analysis of C_{60} and C_{70} fullerenes. It was shown that with toluene–light petroleum as mobile phase this method was rapid and accurate.

INTRODUCTION

Fullerenes, which were first proposed by Kroto *et al.* [1] in 1985 and produced in bulk by Kraetschmer *et al.* [2] in 1990, have stirred a flurry of research in physics, chemistry and materials sciences. These all-carbon molecules are arranged as polyhedra with hexagonal and pentagonal faces [1,3]. They are formed in an electric arc with graphite as electrodes. C_{60} and C_{70} fullerenes, being the most abundant species isolated from a crude soot product, have been characterized in detail, *e.g.*, by NMR, Fourier transform IR, MS and UV–VIS spectra [4–6].

To isolate C_{60}, C_{70} and other higher fullerenes is a challenging task because of their poor solubility in most organic solvents [4]. Many authors have attempted to do so. Most of them used LC with silica gel [4], alumina [4,6] and chemically bonded phases, *e.g.*, ODS [7–10], 2,4-dinitro-anilinopropyl (DNAP) [5], Pirkle type [11] and multi-legged phenyl [12] and others [13], as stationary phases and *n*-hexane, toluene, *p*-xylene, dichloromethane, methanol, acetonitrile, etc., or mixture of these, as mobile phases. MS was and still is frequently used to identify and quantify the mixture of fullerenes. Field desorption (FD), laser desorption (LD) and fast atom bombardment (FAB) are the most effective ionization sources in MS, while electron impact (EI) and desorption chemical ionization (DCI)

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are not appropriate [14]. MS is very sensitive, however it has the drawback that it is not a routine method and requires an experienced operator. The low volatility of fullerenes may in some cases cause discrimination of the sample and pollution of the ionization source. HPLC is an easy, effective and accurate routine method. In this paper, an HPLC method for separating fullerenes was devised with 3,5-dinitrobenz-amidopropyl (DNBAP) chemically bonded phase as packing and toluene–light petroleum as mobile phase. Based on the mechanism of electron donor–acceptor interaction, fullerenes can undergo more pronounced interaction with this bonded phase, which permits a good solvent, *e.g.* toluene, to be added to the mobile phase to increase the sample load as well as the sensitivity of the method.

EXPERIMENTAL

Apparatus

A Vista 56 liquid chromatograph (Varian) with a UV-100 LC detector, a Valco six-port injector with 10-μl sample loop and a VISTA 401 recorder was used.

Detection was performed either at a wavelength of 254 nm using n-hexane or light petroleum as mobile phases or at 330 nm with toluene added in the mobile phase.

All separations were carried out at ambient temperature.

Columns

Packings of ODS-, NH$_2$-, C$_6$H$_5$- and CN-silica were purchased from Tianjin Second Reagent Factory, China. Silica gel was purchased from Qingdao Oceanic Chemical Factory, China.

3-Nitrobenzamidopropyl (mNBAP), 4-nitrobenzamidopropyl (pNBAP) and DNBAP silica gel chemically bonded phases were synthesized according to Felix and Bertrand [15] with some modification. The NH$_2$-silica gel was produced by reacting silica gel (10 μm) with γ-aminopropyltrimethoxysilane (Chemical Factory attached to Wuhan University, China). Then NH$_2$-silica gel was modified to mNBAP, pNBAP or DNBAP with 3-nitrobenzoyl, 4-nitrobenzoyl or

3,5-dinitrobenzoyl chloride, respectively. All the packings were packed into 15 × 0.4 cm I.D. stainless-steel columns using a conventional high-pressure slurry packing procedure.

Materials and reagents

Raw fullerene materials (consisting of about 85% C$_{60}$ and 15% C$_{70}$) were extracted from soot with toluene as previously described [1,16]. All reagents were of analytical grade and were used as obtained.

RESULTS AND DISCUSSION

The solubility of fullerenes in various solvents

One of the basic requirements of a suitable mobile phase in a liquid chromatographic system is good solubility for the solutes to be separated. Thus, a knowledge of the solubility of fullerenes in various solvents is of great importance to optimizing chromatographic systems, including both the mobile phase and the stationary phase. A few papers on this subject have been published [4]. A spot plate test was carried out. For a good solvent the colour turned to wine red or brown red, depending on the extent of solubility of fullerenes in the solvent tested; for a fair solvent the colour turned to pinkish; for a poor solvent the solution became slightly pinkish; and finally for a bad solvent the test solvent remained colourless. Thus, solvents can be divided into four categories according to their decreasing solubilities as follows:

(1) Good solvents: benzene, toluene, ethylbenzene, *o*-xylene, *m*-xylene, *p*-xylene, 1,3,5-trimethylbenzene, 1,2,4,5-tetramethylbenzene, tetralin, carbon disulphide.

(2) Fair solvents: n-hexane, light petroleum (b.p. 60–90°C), n-octane, chloroform, dichloromethene, dichloroethane, carbon tetrachloride.

(3) Poor solvents: decane, cyclohexane, diethyl ether, ethyl acetate, tetrahydrofuran.

(4) Bad solvents: n-pentane, methanol, ethanol, 2-propanol, acetone, acetonitrile, water, dimethyl sulphoxide.

Recently a study which quantitated the solubility of buckminsterfullerene in several organic solvents was reported [17].

It can be concluded form our simple experiment that:

(1) It is advisable to use a good solvent, such as benzene or toluene, as the mobile phase or as one of the components of a mixed-solvent mobile phase to increase sample load, which may in turn increase the sensitivity of the analytical method or the throughput of the preparative method.

(2) Reversed-phase liquid chromatography is not ideal for fullerene separation, though it is the most commonly used mode in HPLC. This is because of the poor solubility of fullerenes in water, methanol, acetonitrile and tetrafuran, etc., which are widely used in reversed-phase liquid chromatography.

(3) Normal-phase liquid chromatography is preferred for the analytical or preparative separation of fullerenes.

Retention behaviour of C_{60} and C_{70} fullerenes on some commercially available packings

Packings commercially available are easily accessible and of high quality, and are thus preferable when a new separation method is to be developed. ODS-, CN-, NH$_2$-, C$_6$H$_5$-silica and silica gel were tried first. n-Hexane was used as the mobile phase since it has distinct solubility for fullerenes and is one of the least polar solvents. The results (see Table I and Fig. 1) demonstrated that, of the five packings tested, both ODS- and CN-silica gave baseline separa-
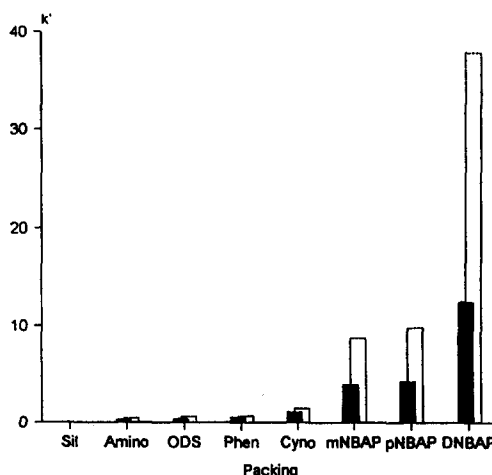

Fig. 1. Capacity factors for (■) C_{60} and (□) C_{70} on different packings. Sil = silica gel; ODS = octadecylsilyl; Phen = phenyl; Cyno = CN.

tion of C_{60} and C_{70}, NH$_2$- and C$_6$H$_5$-silica gave partial separation, while silica gel was incapable of achieving separation because of its insignificant retention of C_{60} and C_{70}. The selectivity factors (α) between C_{70} and C_{60} on these packings seemed to be sufficiently large (see Table I and Fig. 2), which is easily understandable because of the relatively large difference in the molecular masses of the fullerenes to be separated. Ajie et al. [4] claimed that silica gel did produce a definite separation of fullerenes. This difference in observation can be attributed to the probable difference in the activity of silica gel

TABLE I

CAPACITY FACTORS, k', AND SELECTIVITY FACTORS, α, FOR C_{60} AND C_{70} ON DIFFERENT PACKINGS

$k' = (t_R - t_0)/t_0$, $\alpha = k'_{C_{70}}/k'_{C_{60}}$. The retention time of toluene was used as t_0.

No.	Packing	Eluent	$k'_{C_{60}}$	$k'_{C_{70}}$	α
1	Silica gel	n-Hexane	0	0	—
2	NH$_2$-Silica	n-Hexane	0.32	0.44	1.37
3	ODS-Silica	n-Hexane	0.36	0.60	1.67
4	C$_6$H$_5$-Silica	n-Hexane	0.55	0.66	1.20
5	CN-Silica	n-Hexane	1.04	1.42	1.36
6	mNBAP	n-Hexane	3.87	8.72	2.25
7	pNBAP	n-Hexane	4.31	9.80	2.27
8	DNBAP	n-Hexane	12.5	37.8	3.02
9	DNBAP	n-Hexane–toluene (90:10, v/v)	4.78	10.8	2.26

packings used in the two laboratories. A common drawback of these commercially available packings is their low retention for C_{60} and C_{70}, compared with the three packings containing a nitrobenzamidopropyl group, shown below. Thus, these packings have low tolerance towards the addition of good solvent to *n*-hexane.

Retention behaviour of fullerenes on synthetic packings

Cox *et al.* [5] used DNAP and Jinno *et al.* [8] devised and synthesized multi-legged phenyl chemically bonded stationary phases for separating C_{60} and C_{70}. Welch and Pirkle [13] evaluated the retention behaviour of C_{60} and C_{70} using ten HPLC stationary phases, including some specifically designed for recognition of the fullerenes, and they claimed that a novel tripodal π-acidic stationary phase designed for simultaneous multipoint interaction with buckminsterfullerene provided the greatest retention and the greatest separation for the C_{60}/C_{70} mixture.

At the beginning of this study 2 years ago, it was postulated that fullerene molecules might possess certain aromaticity and they might show electron-donor properties. As nitrobenzamidopropyl functionalites can show pronounced interaction with some aromatic hydrocarbons [18,19] it is worth trying this type of packing. Only one simple step was needed for the synthesis of mNBAR, pNBAP and DNBAR packings from NH_2-silica packing, and high overall yields could be obtained even by novices. These packings gave k' values ranging from 3.87 to 12.5 for C_{60} and from 8.72 to 37.8 for C_{70} (Table I and Fig. 1). The separation factors, α, between C_{70} and C_{60} became larger than 2 on mNBAP and pNBAP, and reached 3.02 on DNBAP. Evidently, mNBAP, pNBAP and DNBAP are more suitable for the separation of C_{60} and C_{70} than the commercially available packings. In addition, DNBAP interacts with fullerenes more than mNBAP and pNBAP. In fact, the best-performing stationary phase DNBAP proposed is similar in structure to the stationary phase VI in ref. 13, although they differ by one methylene group, *i.e.* Welch *et al.* [13] introduced a dinitrobenzamidobutyl (DNBAB) group. Moreover, our synthetic route seems to be much simpler.

In this study, a fraction of good solvent was intentionally introduced into the mobile phase to increase the sample load and improve the separation. By so doing, the mobile phase became more soluble for fullerenes. The detection wavelength, however, had to be changed to 330 nm and the detection limit of fullerenes became somewhat higher than when only *n*-hexane was used as mobile phase with the detection wavelength at 254 nm. Nevertheless, the overall sensitivity of the analytical method can still increase as a result of the increasing sample load.

HPLC analysis of C_{60} and C_{70}

Selection of mobile phase. For LC separation of C_{60} and C_{70}, the solubility of fullerenes is the most important factor to be considered. With DNAP as the stationary phase, Cox *et al.* [5] used an *n*-hexane to 50% dichloromethane–*n*-hexane gradient as the mobile phase, while Nondek and Kuzilek [20] improved the solubility of fullerenes by using a more soluble mobile phase, an *n*-hexane–benzene binary mixture. With ODS-silica as the stationary phase, Diederich *et al.* [7] used toluene–acetonitrile, while Yan *et al.* [9] used methanol–benzene, methanol–toluene and methanol–*m*-xylene. Cui *et al.* [10] examined methanol–diethyl ether as the mobile phase with four different polysiloxane stationary phases, including ODS- or phenyl-substituted stationary phases.

In this paper, a good solvent, toluene, together with light petroleum was used as the mobile phase. In our experiment, the light petroleum consisted mainly of *n*-hexane with a small fraction of *n*-heptane (*ca.* 10%) and had the same elution capacity for fullerenes as pure *n*-hexane. However, light petroleum is much cheaper than *n*-hexane. This mobile phase was found to have good solubility for C_{60} and C_{70} fullerenes.

To optimize the LC system, mobile phases of different ratios of toluene–light petroleum were tested. Fig. 3 displays the relationship of the fullerenes' capacity factors (k') *vs.* ratio of toluene–light petroleum. The k' values decreased as the content of toluene in the mobile phase increased. Separation of C_{60} and C_{70} can thus be carried out under up to 30% toluene
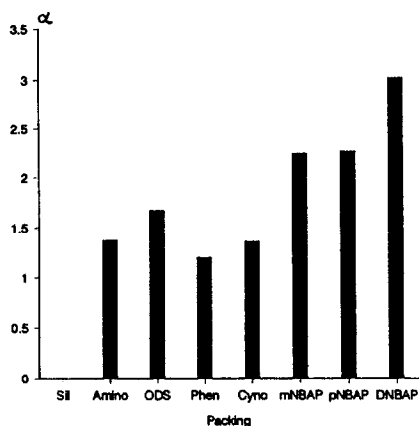
Fig. 2. Selectivity factors for C_{70} to C_{60} on different packings.

content on a newly packed column with an efficiency of 20 000 plates per metre. When the column efficiency deteriorated the column could still be used for a long time by reducing the toluene content in the mobile phase. Fig. 4 is the chromatogram of the analysis of C_{60} and C_{70} using DNBAP stationary phase.

Calibration of C_{60} and C_{70} fullerenes. Quantitative analysis of C_{60} and C_{70} fullerenes is very useful in optimizing the experimental parameters

for producing carbon arc soot, selection of proper extraction solvents and determining the purity of separated fullerenes. Using the HPLC system established [DNBAP as stationary phase and toluene–light petroleum (20:80, v/v) as mobile phase], pure C_{60}(>99.9%) and C_{70}(>99.5%) were obtained in our laboratory, and their solutions at various concentrations were prepared and injected. The regression equations of the calibration curves of C_{60} and C_{70} are, respectively:

$$C_{60}: \log A = 6.576 + 0.9521 \log C, \ r = 0.99998$$

$$C_{70}: \log A = 6.188 + 0.9621 \log C, \ r = 0.99977$$

where A presents peak area (μV s) and C is sample concentration (mg/ml) injected. It can be seen that the absorption coefficient of C_{60} is larger than that of C_{70} at a wavelength of 330 nm. Thus, the real percentage of C_{60} and C_{70} in the mixture should be calculated by multiplying their areas by correction factors. The HPLC
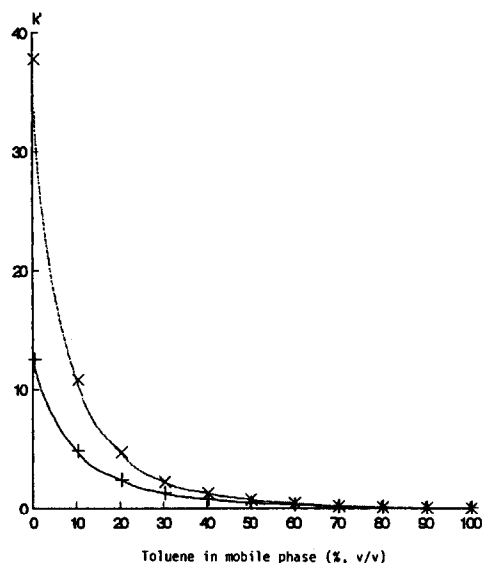

Fig. 3. Relationship between capacity factor (k') vs. toluene in mobile phase. The experimental conditions are the same as in Fig. 4, except for the mobile phase. $+ = C_{60}$; $\times = C_{70}$.
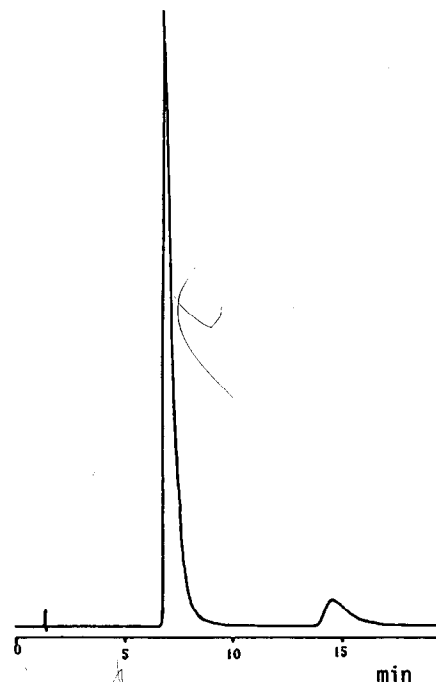

Fig. 4. Chromatogram of the separation of C_{60} ($t_R = 7.16$ min) and C_{70} ($t_R = 14.63$ min). Column: DNBAP–silica column (150 × 4 mm I.D.). Mobile phase: toluene–light petroleum (10:90, v/v), 1.0 ml/min. Detection wavelength: 330 nm.

method proposed for analysing C_{60} and C_{70} is more convenient than MS, and the column has already been used for thousands of injections for routine analysis for about 18 months.

As the production of C_{60} is becoming easier as the result of several innovative studies [21–25], the proposed chromatographic system is less useful for the preparation of C_{60} and is at present restricted to analytical use. The chromatographic system has, however, been modified with the use of o-xylene instead of toluene in the mobile phase to increase fullerene solubility and scaled up to prepare satisfactorily pure C_{70} (purity ca. 99.5%) and C_{84} (purity ca. 99.5%) fullerenes. Reports of these studies are now in preparation.

CONCLUSIONS

This paper compared eight packings and demonstrated an HPLC system for the analysis of C_{60} and C_{70} fullerenes with DNBAP chemically bonded phase as the stationary phase and toluene–light petroleum as the mobile phase.

ACKNOWLEDGMENTS

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