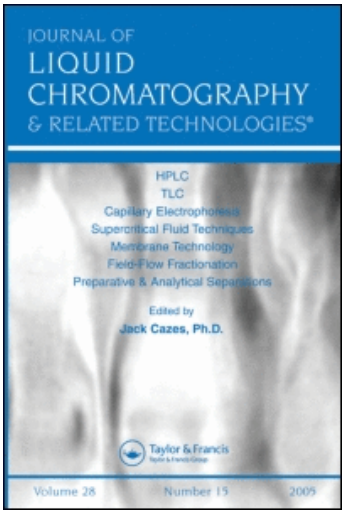

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A Comparative Study of Buckminsterfullerene and Higher Fullerene Separations by HPLC

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A COMPARATIVE STUDY OF BUCKMINSTERFULLERENE AND HIGHER FULLERENE SEPARATIONS BY HPLC

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ABSTRACT

Several HPLC columns that have been recommended for the separation of fullerenes were compared. Each column (i.e., stationary phase) had a different optimum mobile phase. In general, mobile phases consisted of binary mixtures of a "good" fullerene solvent and a "poorer" fullerene solvent. Reversed phase stationary phases with alkyl (aliphatic) substituents produced superior analytical separations for all fullerenes. However, stationary phases with aromatic substituents were better for preparative separations. Increasing the proportion of the "poor" fullerene solvent in the mobile phase generally increased resolution and retention but decreased the mass load. Injecting too high a concentration of fullerenes in any column caused peak splitting to occur. The fullerene retention order was the same on all columns and with all mobile phases. The fullerene separation ability of all columns tended to deteriorate with time. Overloading the column or doing preparative separations greatly accelerated the deterioration process. It is believed that irreversible adsorption of fullerenes or fullerene by-products is responsible for the degradation of column performance.

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INTRODUCTION

The number of high performance liquid chromatography (HPLC) stationary phases for the separation and purification of fullerenes and their derivatives has increased tremendously over the past few years. The first fullerene separations were reported on neutral alumina and silica gel.[1-5] Subsequently several commercially available alkyl and aromatic HPLC stationary phases were examined.[6-17] Also, several stationary phases (some of which are very costly) were developed solely for the purpose of separating fullerenes.[18-22] Thus far, over fifteen liquid chromatographic stationary phases have been used in the separation of fullerenes. Duplication, overlap, and discrepancies exist when comparing the claims and selectivities of these columns. Most of the available stationary phases give a more than adequate separation of C_{60} and C_{70} fullerenes provided the optimum mobile phase is used in each case. However, separation problems can arise in separating the higher fullerenes, specific isomers, fullerene derivatives, and fullerene-metal complexes. The recent demand for larger quantities of fullerenes has led to an additional challenge of separating fullerenes on a preparative basis.[23-25]

It has been suggested in the literature that some columns are better than others in regard to the aforementioned separations. However, to our knowledge, no one has compared different stationary phases regarding efficiency, loading, stability, and higher fullerene separation ability. A greater understanding of these separations will allow rational selection to be made based on specific needs.

Buckminsterfullerene and higher fullerenes (sometimes known as buckyballs) are known to be produced in appreciable quantities via graphite

vaporization with a laser, or by resistive heating of graphite, and in toluene or carbon disulfide soxlet extracts of carbon soot materials.[1,3,23-24,26-28] Approximately 85% of the fullerene material produced is C_{60} and 10% is C_{70}. Higher fullerenes are only 3-5 % by weight of the mixture. This percentage may vary significantly with different fullerene production and extraction methods.

Fullerenes are known to be most soluble in chlorinated benzenes, carbon disulfide, toluene, methylene chloride, and chloroform. Only 0.043 mg/ml of C_{60} can be dissolved in n-hexane and 0.00 mg/ml in methanol and most other polar solvents.[29] These solvents are frequently used as mobile phases in HPLC, therefore, the solubility limitation is a problem. Even toluene can solublize only 2.8 mg/ml.[29] Ruoff et. al. has determined the room temperature solubility of C_{60} as a function of solvent properties (i.e., refractive index, dielectric constant, Hildebrand solubility parameter, molecular size, and hydrogen bonding strength) in over 46 solvents using calibrated HPLC.[29] Although both polar and non-polar stationary phases have been used to separate fullerenes, the mobile phases are fairly limited to the less polar solvents because of the solubility properties of fullerenes. So, even though a reversed phase stationary phase may be used for a separation, typical reversed phase mobile phases (hydro-organic solvents) cannot be used.

Numerous chromatographic techniques for the purification of fullerenes as well as several different stationary phases have been evaluated. The first chromatographic fractionations were achieved by classic column chromatography utilizing neutral alumina and silica gel, respectively.[1-5] A simple method incorporating soxlet extraction and liquid chromatography was evaluated, however, it was found to be impractical

since the recovery yields were quite low.[30-31] Other stationary phases utilized without much success were the graphite, polystyrene gel, and other gel permeation stationary phases.[32-34]

The first aromatic stationary phase used to separate fullerenes was the 3,5-dinitrobenzoylphenylglycine (DNBPG) column. Hawkins and co-workers believed the support's π-acidic dinitrobenzamide groups would interact with the π-basic groups of the aromatic "soccerball-like" structures.[10] Up to 0.5 mg of C_{60} and C_{70} fullerenes were baseline resolved in under 30 minutes on this semi-preparative sized chiral stationary phase. This was the first report of a stationary phase that could purify milligram quantities as opposed to the lower levels reported previously. As a result, it was suggested that the aromatic phases were more efficient at separating fullerenes. This assumption remained unchallenged while other charge-transfer phases were utilized for these separations.[11] In 1991, Cox et. al. studied the retention mechanism of C_{60} and C_{70} using dinitroanilinopropyl (DNAP) silica.[12] They found that the retention of C_{60} and C_{70} resembled that of flat planar polyaromatic hydrocarbons (PAH's) such as tyriphenylene and benzo[a]pyrene, respectively. The successful use of the phenylglycine based stationary phase as well as the DNAP stationary phase led Jinno and co-workers to synthesize a "multi-legged" phenyl phase bonded to silica gel.[18] Isolation of C_{60} and C_{70} was achieved using capillary liquid chromatography, however, this method is not suitable for large scale purifications.[18]

In 1991, Diederich et. al. were the first to isolate higher fullerenes using column chromatography to fractionate and collect C_{60}, C_{70}, and a higher fullerene sample. The higher fullerene fraction was then reinjected onto a silica-gel semi-preparative support to further separate the higher

fullerene mixture by HPLC utilizing a mass spectrometer for detection.[4] Information on C_{76}, C_{84}, C_{90}, C_{94}, and a stable oxide of D_5h-C_{70} was found. Later that year, Diederich was the first to chromatographically isolate an additional isomer of C_{78} on a Vydac C_{18} phase.[6] The direct injection of fullerene soot on semi-preparative and eventually analytical C_{18} phases followed.[7-8,15] Baseline resolution of C_{60}, C_{70}, and a few higher fullerenes was observed.

A preparative recycling method was proposed by Kikuchi et. al. which isolated higher fullerenes previously reported as well as two additional members of the fullerene family.[35] Small portions of C_{82} and C_{96} were found utilizing preparative HPLC with CS_2 as a mobile phase. This repetitive method took 6 cycles to partially resolve C_{60} and C_{70} and 30 cycles for the partial separation of the higher fullerenes.[35] This technique was very tedious and time consuming and therefore not applicable to large scale purification.

Welch and Pirkle, in 1992, investigated a few commercially available π-basic stationary phases and synthesized several other π-acidic phases for fullerene as well as PAH recognition.[19] Altogether, ten phases were analyzed. The tripodal ligand phase, known as the Buckyclutcher I stationary phase, provided the highest selectivity compared to any of the other phases analyzed.

Recently, other charge-transfer phases were investigated. A semi-preparative tetrachlorophthalimidopropyl-modified silica column (TCPP) separated a 1 mg mixture of fullerenes, baseline resolved a few higher fullerenes, and provided an isomeric separation of C_{78}.[17] Diack, Compton, and Guiochon reported that the 2-(2,4,5,7-tetranitro-9-fluorenylideneamino-oxy)propionic acid (TAPA) chiral stationary phase was a stronger electron acceptor than the DNBPG phase.[14] C_{60} and C_{70} were baseline resolved and

these studies indicated the PAHs were stronger electron donors than the fullerenes. Furthermore, a dynamic temperature study involving van't Hoff plots proved that increasing the temperature of the TAPA column resulted in an increase in retention.[14] Therefore, fullerene adsorption on this stationary phase is entropy driven and endothermic. This trend is unusual in classical chromatography.[14] These results were in agreement with those of Pirkle and Welch regarding their DNBPG phase.[12] In 1993, Kibbey and co-workers, synthesized several metalated and unmetalated tetraphenyl-porphyrin- (TPP-) stationary phases for the separation of C_{60} and C_{70} fullerenes.[9] Highly selective separations were acheived using neat toluene as the mobile phase.

Another chiral stationary phase used in the separation of fullerenes is the γ-cyclodextrin (γ-CD) chemically bonded to silica. γ-cyclodextrin is made up of eight D-glucose molecules and is a cyclic oligosaccharide composed of glucopyranose units bonded through α-(1,4)-linkages. The hydrophobic interior cavity is approximately 10 angstroms in diameter and is known to form inclusion complexes with various structural compounds. C_{60} is known to be approximately 12 angstroms and cannot be totally included inside the cyclodextrin cavity. Although we were unable to separate any fullerenes on the native γ-cyclodextrin, Cabrera et. al. reported the isolation of C_{60} and C_{70} in neat hexane and in hexane/toluene.[16] It was claimed that partial inclusion and various interactions at the mouth of the cavity provided retention and isolation of C_{60} and C_{70}. We were able to obtain extremely efficient fullerene separations using chiral and non-chiral derivatized cyclodextrin bonded stationary phases. The multi-modal (R)- and (S)-naphthylethylcarbamate-β-cyclodextrin (RN-β-CD and SN-β-CD) and the 3,5-dimethylphenyl-β-cyclodextrin stationary phase baseline resolved C_{60}, C_{70}, (and higher

fullerenes) C_{76}, C_{78}, and C_{84}.[13] The RN-β-CD phase was eliminated from our study because of the high cost of chiral phases and the wide variety of other successful buckyball stationary phases available. Most of the chiral stationary phases previously mentioned (i.e., γ-CD, RN-β-CD, DNBPG, TAPA) offer a high degree of selectivity, however, none of them are considered effective preparative purification methods because of the high cost and small conversion yield.

Many of the aforementioned stationary phases were not significantly, if any, better than the separations achieved on various conventional C_{18} supports. It is well known that many variations exist among octadecylsilica (ODS) phases (i.e., monomeric, polymeric, encapped, etc.). The earlier success of these stationary phases led to a comparison study involving several C_{18} stationary phases. Two groups simultaneously studied the differences between monomeric and polymeric ODS solid supports.[7-8] Jinno's results were in agreement with those of Anacleto and Quilliam. They determined that the polymeric ODS phases were better stationary phases for distinguishing between geometric isomers while the monomeric phases were better in distinguishing differences in fullerenes based upon carbon number or molecular weight. Jinno also showed that one C_{78} isomer eluted before C_{76} and in turn described a molecular shape dependence.[8] This was the only report concerning the elution of a higher molecular weight fullerene prior to a smaller one.

Recently, we evaluated a variety of different, organic, biphasic solvent systems to be used in conjunction with centrifugal partition chromatography (CPC) for the preparative fractionation of fullerenes.[36] This method was found to purify approximately 100 times the amount of fullerenes (per batch) as compared to previously reported HPLC methods. Preparative separations of fullerenes are obviously important. Presently,

fullerenes are being used as lubricants and some are known to have superconducting properties at 18-28 ° K when complexed with certain metals.[37-40] Various research groups have demonstrated that C_{60} may also have possible biological activity.[41-42] Computer modeling has shown that the water soluble derivative of C_{60} may have use in blocking the active site of the open-ended cylinder of the HIV protease.[41-42]

In this study, we compare the selectivity, efficiency, resolution, loadability, column deterioration, and higher fullerene separation ability of various commercially available aliphatic and aromatic stationary phases. Fullerene samples were obtained from various sources and include both toluene and carbon disulfide extracts of fullerene soot materials. In addition to comparing the various fullerene separation methods, we also evaluated commercially available fullerene samples for purity.

EXPERIMENTAL

Chemicals. All solvents used were of HPLC grade and obtained from Fisher Scientific (St. Louis, MO). Pure fullerene standards of C_{60}, C_{70}, and C_{84} were purchased from either Polygon Enterprise (Waco, TX), MER Corporation (Tuscon, AZ), Fluka (Ronkonkoma, NY), or Texas Fullerene (Houston, TX). All other fullerene samples were provided by IBM at the Almaden Research Center (San Jose, CA).

Instrumentation. The chromatographic studies were performed using the following Shimadzu (Columbia, MD) equipment: a LC-6A pump, a SCL-6B system controller, a SPD-6AV UV/VIS variable wavelength spectrophotometric detector, and a CR601 chromatopac integrator. The system also included a Rheodyne (model 7125) injector.

Stationary Phases. A total of nine chromatographic stationary phases were evaluated and include the following: native γ-cyclodextrin (γ-CD), (R)-naphthylethylcarbamate-β-cyclodextrin (RN-β-CD), (S)-naphthylethylcarbamate-β-cyclodextrin (SN-β-CD), high loading (3,5-dimethylphenyl)-β-cyclodextrin (DMP-β-CD), low loading (DMP-β-CD), reverse-phase C_{18}, Buckyclutcher I, Buckysep-RP, and ChromSpher Fullerene. All stationary phases consist of 5 μm diameter particles while column dimensions were 250 x 4.6 mm. The high and low loading (DMP-β-CD), the (RN-β-CD), the (SN-β-CD), and the Astec C_{18} (monomeric-encapped ODS) were donated by Advanced Separations Technology Inc. (Whippany, NJ). Typical eluents for these columns mainly consisted of acetonitrile and added interchangeably as modifiers were methylene chloride, chloroform, and toluene. The Buckyclutcher I stationary phase was purchased from Regis Chemical Company (Morton Grove, IL) and evaluated with the recommended hexane/toluene mixture as a mobile phase. A detailed description of the structure and preparation procedures of the aromatic Buckyclutcher I stationary phase is described elsewhere.[19] Phenomenex (Torrance, CA) supplied the Buckysep-RP alkyl column and recommended an acetonitrile and toluene mixture as the mobile phase. The aromatic ChromSpher Fullerene packing was purchased from Chrompak (Raritan, NJ) and separation was achieved using the recommended combination of isooctane and toluene. Analysis on all columns occurred at 1 ml/min with the exception of the ChromSpher packing which was used at 3 ml/min as recommended by the company.

The C_{18} stationary phase as well as the Buckysep-RP column consisted of an alkyl chain bonded stationary phase while the packing of all other columns contained bonded aromatic functional groups. All fullerene

samples were dissolved in toluene. The solution concentrations of fullerenes used for analytical separations were 0.10 mg/ml, while preparative separation studies used solutions of 5 mg/ml. Between 2 and 500 µl of the analyte was directly injected to the column depending on the type of study being done. Because the mobile phase composition plays a vital role in the retention of fullerenes and different mobile phases were used with each stationary phase, all columns were compared by appropriately adjusting the eluents so that comparable k' values were obtained. All other chromatographic parameters (i.e., absorbance, attenuation, chart speed, etc.) were held constant.

RESULTS AND DISCUSSION

The solvent systems used to separate fullerenes on an analytical scale are generally very different from those that are used to separate fullerenes on a preparative basis.[9,13-14,17] Fullerenes are most soluble in carbon disulfide and in chlorinated benzenes, however, the odor, volatility, viscosity, and toxicity limits the use of these solvents in HPLC. Two component solvent systems used as mobile phases in the separation of fullerenes usually combine a "good" and/or "moderate" fullerene solvent with a "poor" fullerene solvent. C_{60}, for example, is not soluble in "poor" solvents such as acetonitrile, methanol, ethanol, isopropanol, etc.[29] Examples of "good" fullerene solvents are toluene and chloroform which dissolves 2.8 mg/ml and 0.16 mg/ml, respectively.[29] Moderate fullerene solvents such as hexane (dissolving 0.043 mg/ml C_{60}) and isooctane have intermediate solubility properties.[29] While the distinction (i.e., the dividing line) between "good" and "moderate" fullerene solvents is somewhat subjective, there is little question as to the negligible solublizing properties

of "poor" fullerene solvents. Interestingly, this approach of using "good/poor" solvent combinations is somewhat analogous to a technique developed several years ago for the LC and TLC fractionation of polymers by molecular weight.[43-47] Varying the amount of the "poor" fullerene solvent in the system enables one to control retention and resolution (see Figure 1). As the proportion of the "poor" fullerene solvent in the mobile phase increases, C_{60} and C_{70} are retained longer and the resolution increases. Our results are in good agreement with those reported by Kibbey, Guiochon, and Herren on other stationary phases.[9,13-14,17] When too much of a "poor" fullerene solvent is used or too high a concentration of fullerenes is injected, some fullerenes come out of solution or phase separate until enough solvent passes through to re-elute them. When this phenomenon occurs, double peaks are formed. This "peak doubling" effect occurred with every column tested when the fullerene solubility limit in the mobile phase was reached. The peak doubling effect is shown in Figure 2.

By in large, fullerene separations appear to be more affected by the solvent composition of the mobile phase than by stationary phase chemistry. Regardless of which stationary phase (i.e., non-polar, aliphatic reversed phase, and aromatic reversed phase) or mobile phase is used the same retention order for fullerenes is obtained. However, every stationary phase seems to have a different optimum mobile phase composition. The solvent compositions of the Astec C_{18} and the Buckysep-RP stationary phases were the most similar. Both stationary phases used similar mobile phase ratios (within 5 %) of acetonitrile mixed with toluene.

The HPLC separation of C_{60} and C_{70} has become by far the most widely studied and reported fullerene analytical methodology. Most stationary phases baseline resolve C_{60} and C_{70} provided the optimum mobile

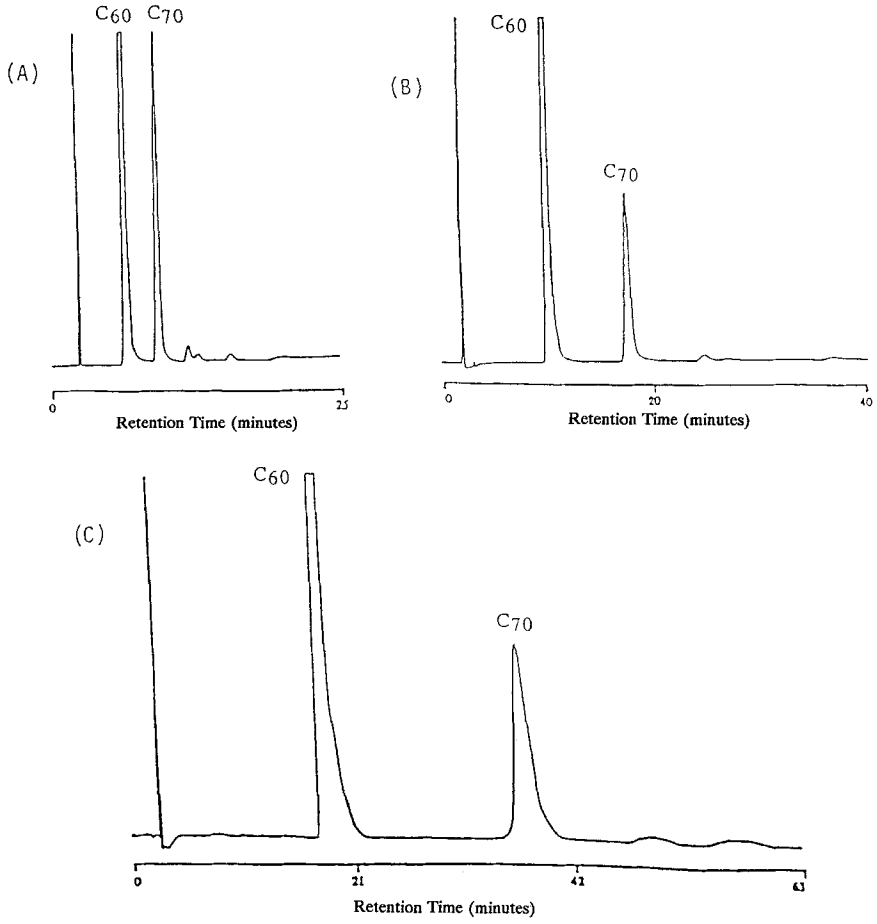


Figure 1. Chromatograms showing how the retention and selectivity can be controlled by altering the composition of the "poor" fullerene solvent. All three separations were performed on a high loading DMP-β-CD column and eluted with a acetonitrile/toluene mixture. Chromatogram (A) used a 60:40 mixture, chromatogram (B) a 70:30 mixture, and chromatogram (C) was eluted with a 80:20 ratio of acetonitrile/toluene (v/v). Separations were carried out at 1 ml/min and a 310 nm.

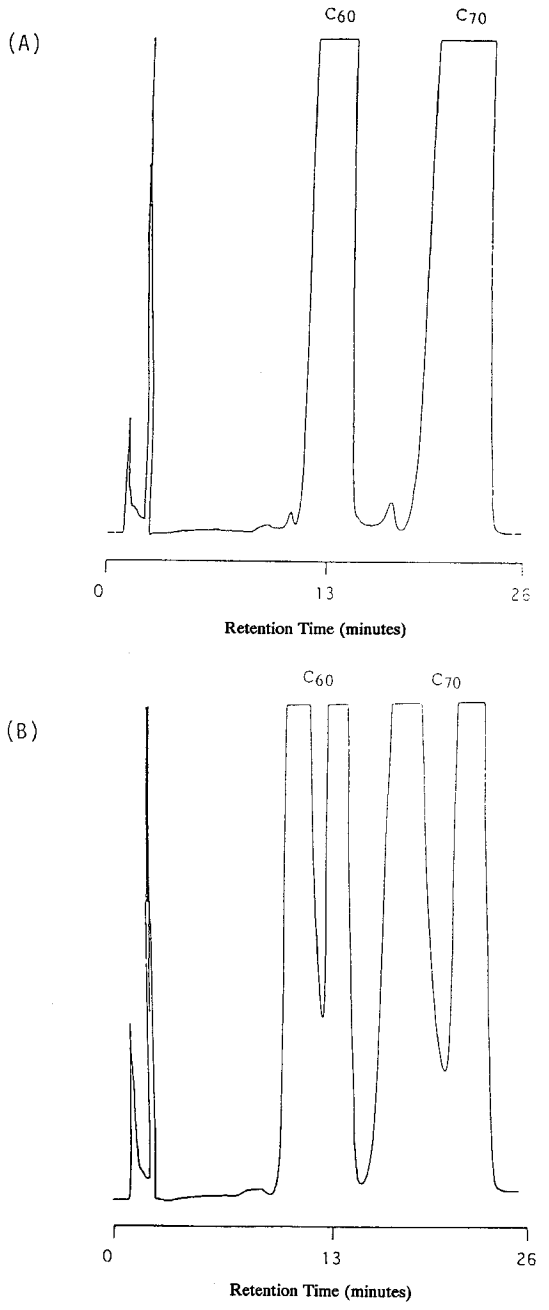


Figure 2. Representative chromatograms showing the "peak doubling" phenomena on an aliphatic C_{18} stationary phase. 0.30 mg of C_{60} and C_{70} was separated in chromatogram (A) and 0.40 mg was isolated in (B). Separations were achieved with a 45:55 ratio of acetonitrile/toluene (v/v) at 1 ml/min and at 310 nm.

phase is used. Without proper analytical methodology, the ability to ascertain the exact composition and purity of the fullerene soot starting material would be limited. The determination and quantitation of higher fullerenes (i.e. C_{76}, C_{78}, C_{84}, etc.) would be difficult and the exact purity would have to be determined by other instrumental techniques. Figure 3 shows the analytical separation of commercially available "pure" samples of C_{70} and C_{84}. In chromatogram A, small portions of C_{60} are found in the "pure" C_{70} sample. The sample in chromatogram B ("pure" C_{84}) shows significant levels of C_{60}, C_{70}, C_{76}, and C_{78}. To stress the importance of the analytical methodology, Table 1 shows the exact purity obtained in a few commercial fullerene standards. MER Corporation produced the purest C_{60} sample (99.9 %) while the Polygon Enterprise C_{70} sample was only 94.9 % pure. At the time of this study, Polygon Enterprise was the only company producing C_{84}.

Columns that are good for analytical separations are not necessarily good on a preparative basis. Although several other stationary phases were examined (See Experimental), Tables 2 and 3 show the results of loading studies for the two best analytical stationary phases and the two best preparative stationary phases, respectively. The optimum analytical stationary phases were the n-alkyl Astec C_{18} and the Buckysep-RP. Note that both of these stationary phases have surface bonded alkyl chains. It is evident from the data in Table 2 that good efficiency, selectivity, and resolution was observed with both of these phases when up to 0.30 mg mixtures of C_{60} and C_{70} fullerenes was injected. When the concentration of fullerenes injected exceeded 0.3 mg on the C_{18} column, "peak doubling" occurred (see Figure 2) as a result the solubility limitation of fullerenes in these solvent systems. The same results occurred on all of the other alkyl chain stationary phases evaluated.

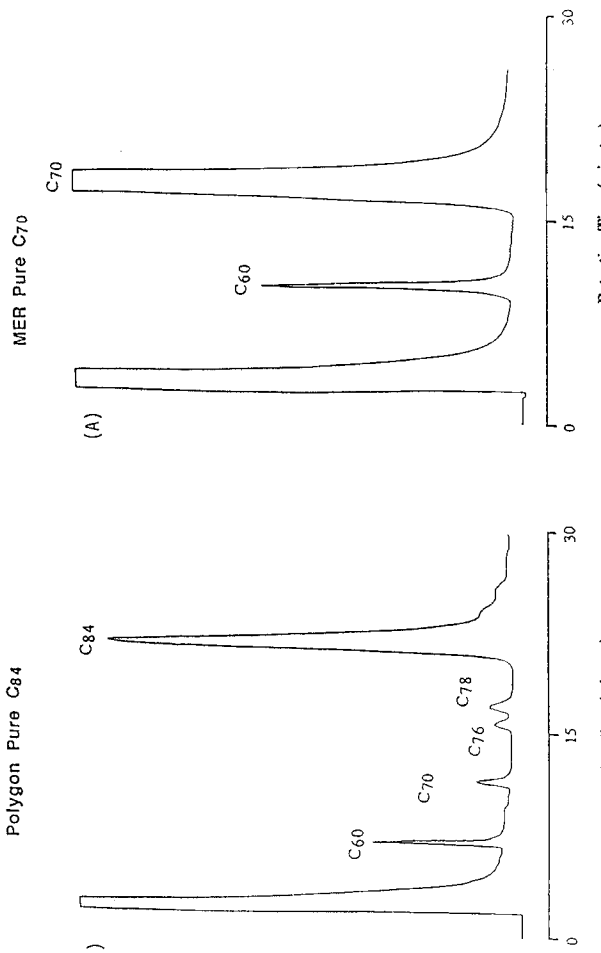


Figure 3. Liquid chromatographic separations of pure fullerene standards obtained from two commercial companies. A high loading DMP-β-CD column was used in (A) and a low loading DMP-β-CD column in (B). The mobile phase in (A) consisted of 70:30 acetonitrile/chloroform mixture (v/v). A 60:40 combination of acetonitrile/chloroform (v/v) was used as the eluent for chromatogram (B). Both separations were performed at a flowrate of 1 ml/min and at 254 nm. Chromatogram (A) (MER Corporation pure C_{70}) has a detectable level of C_{60}. The pure sample of C_{84} (Polygon Enterprise) has disclosed levels of C_{60}, C_{70}, C_{76}, and C_{78}.

Table 1. % Purity of Commercial Fullerene Standards [a,b]

Company	C_{60}	C_{70}	C_{84}
MER Corporation	99.9[c]	98.6[d]	-
Polygon Enterprise	98.9[c]	94.9[d]	97.8[e]
Texas Fullerene	99.3[c]	97.2[d]	-
Fluka	99.7[c]	98.4[d]	-

[a] The pure fullerene standards may differ slightly on a batch to batch basis
[b] The percent purity was quantitated by determining C_{60}, C_{70}, and C_{84} extinction coefficients at 254 nm and 310 nm using a UV/Vis Spectrometer and Beer's law
[c] The chief contaminating homologue is C_{70}
[d] The chief contaminating homologue is C_{60}
[e] The chief contaminating homologues are C_{60}, C_{70}, C_{76}, and C_{78}

As can be seen by the data in Table 3, the aromatic ChromSpher Fullerene and Buckyclutcher I stationary phases were the two best columns for separating fullerenes on a preparative basis. Approximately 2.5 mg of C_{60} and C_{70} was baseline resolved on both stationary phases. Unlike conventional HPLC, the efficiency of the ChromSpher column appeared to increase when greater sample quantities were injected. The chromatogram in Figure 4 shows the difference in the analytical and preparative separation of C_{60} and C_{70} on the ChromSpher column. Although the efficiency of a small 0.1 mg injection was fairly poor compared to the best analytical separations (Table 2 and Figure 1), there was little or no decrease in the efficiency and resolution when a larger

Table 2. Optimum Analytical Separation of C_{60} and C_{70}

Column: Astec C_{18}	Mobile Phase Conditions: 55% Toluene / 45% MeCN			
amount injected (mg)	k_1'	α	Rs	n
0.01	3.80	1.68	10.07	8464
0.05	3.81	1.63	7.82	4975
0.08	3.82	1.64	6.72	3857
0.10	3.83	1.64	6.00	2820
0.20	3.41	1.69	4.04	1244
0.30	3.43	1.72	3.47	872

Column: Buckysep-RP	Mobile Phase Conditions: 60% Toluene / 40% MeCN			
amount injected (mg)	k_1'	α	Rs	n
0.01	3.10	1.73	8.86	6099
0.05	3.25	1.71	7.86	3686
0.08	3.28	1.73	7.68	3257
0.10	3.44	1.72	7.54	2869
0.20	3.50	1.75	6.44	2244
0.30	3.95	1.72	2.27	644

sample (1 mg) was injected. Nevertheless, 3 mg of C_{60} and C_{70} fullerenes caused the peaks to begin to split on the Buckyclutcher I phase and 3.5 mg caused "peak doubling" on the ChromSpher packing. For all columns, it appears that the loading ability is mainly a function of how much C_{60} and C_{70} fullerenes can be dissolved in the mobile phase. The ChromSpher fullerene column was the "best" column examined for these separations.

Table 3. Preparative Separations of C_{60} and C_{70}

Column: Buckyclutcher I Mobile Phase Conditions: 65% Hexane / 35 % Toluene				
amount injected (mg)	k_1'	α	Rs	n
0.05	3.85	2.69	5.30	990
0.10	3.63	2.64	4.85	812
0.20	3.67	2.67	4.54	580
0.30	3.97	2.89	4.21	576
0.40	3.71	2.67	3.93	355
0.50	3.17	2.60	3.36	204
1.0	1.78	2.52	3.09	118
2.5	1.47	1.60	2.71	21

Column: ChromSpher Fullerene Mobile Phase Composition: 95% Isooctane / 5% IPA				
amount injected (mg)	k_1'	α	Rs	n
0.05	4.70	3.20	3.72	297
0.10	4.51	3.14	3.70	276
0.20	4.45	3.02	3.77	269
0.30	4.35	2.97	3.80	256
0.40	4.21	3.01	3.76	180
0.50	4.04	2.88	3.78	112
1.0	3.87	2.75	3.75	107
2.5	3.23	2.42	3.70	89

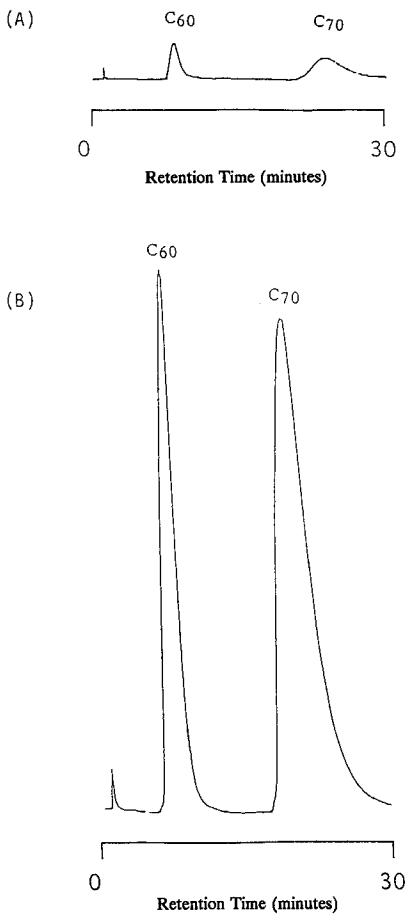
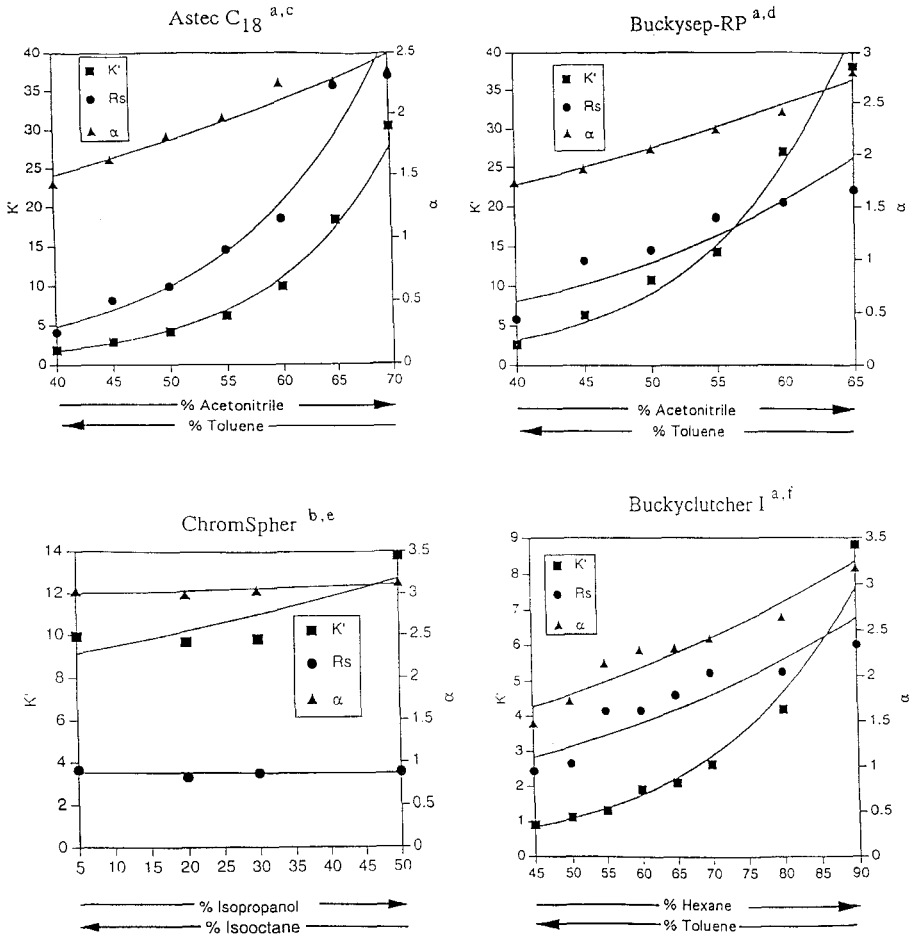


Figure 4. Chromatograms showing how the efficiency and resolution of was only slightly effected when the concentration of the fullerenes sample increased on the ChromSpher fullerene stationary phase. In (A), 0.1 mg of C_{60} and C_{70} was separated in under 30 minutes. In chromatogram (B), the concentration was increased and 1 mg of C_{60} and C_{70} was separated. Separations were carried out at 3.0 ml/min with a 95:5 isooctane/ isopropanol eluent (v/v). Detection was set at 310 nm.

The mobile phase used was a 95:5 (v/v) mixture of isooctane/isopropanol. Neither of these solvents alone would be categorized as "good" fullerene solvents. It is possible that more fullerenes can be dissolved in the two component isooctane/isopropanol solvent mixture than can be dissolved in either neat solvent.

Figure 5 shows that the retention time, selectivity, and resolution of fullerenes usually can be manipulated by controlling the mobile phase composition.[13-14] The ChromSpher column was unusual in that it produced the least variation in these parameters when the mobile phase composition was changed. Even the resolution and α value remained relatively constant at all solvent compositions (Figure 5). The retention, resolution, and selectivity increased with increasing proportions of the "poor" fullerene solvent for all other columns. Clearly the mobile phase composition affects retention, selectivity, and analyte mass load (via solubility). Unfortunately, there is usually a trade-off between selectivity and loadability in these systems. Higher proportions of the "poorer" fullerene solvent tend to increase the peak to peak distances often producing exceptional analytical separations on high efficiency columns (Figure 5). However, these mobile phase mixtures cannot solubilize enough of the fullerenes to make production scale separations practical. Columns that use better fullerenes solvents (or solvent ratios) often produce poorer analytical separation because of lower efficiency and selectivity. However, the analyte mass on these columns can be increased substantially before peak splitting occurs and with much less deterioration in the efficiency (Figure 5). Hence they tend to be superior for preparative separations. It should be noted that the optimum fullerene mobile phase for one type of column does not usually produce the best results on another column. This



[a] Separations were carried out at 1.0 ml/min flowrate.
[b] Separations were carried out at 3.0 ml/min flowrate.
[c] The recommended mobile phase compostions were between 60:40 acetonitrile/toluene (v/v) and 40:60 acetonitrile/toluene (v/v).
[d] The recommended mobile phase compostion was 50:50 acetonitrile/toluene (v/v).
[e] The recommended mobile phase composition was 95:5 isooctane/isopropanol (v/v).
[f] The recommended mobile phase composition was 50:50 hexane/toluene (v/v).

Figure 5. Multiple graphs showing how the change in mobile phase composition altered the selectivity (α), capacity factor (k'), and the resolution (Rs) on the C_{18}, Buckysep-RP, ChromSpher Fullerene, and Buckyclutcher I stationary phases. Mobile phase solvents used in this study were recommended by the respective companies. An exponential curve fitting program was used on all graphs according to the following equation: f(x) = 9.284512E-2*exp(4.897805E-2*x).

is particularly true when going from an aliphatic-type bonded stationary phase to an aromatic-type and vice versa.

Another problem in comparing fullerene separations reported in the literature is that the authors use different fullerene preparations and different detection methods. It is possible that some reports of certain columns being superior to others (because of their ability to separate one or another small fullerene component and/or isomers) may in fact be the result of the sample composition and/or detection sensitivity. The problem of sample composition and consistency is illustrated in Figure 6. The first chromatogram shows the separation of a toluene extract of fullerenes, the second is a carbon disulfide extract of the same starting material, and the third chromatogram shows the separation of a sample of scandium complexed metallofullerenes. Note that there are different minor peak components in these chromatograms. Clearly both the presence and proportion of many peaks are dependent on the manufacturing and/or extraction process (Figure 6). It is also apparent that there are many additional smaller peaks (Figure 6 C) that can be seen if sensitive detection methods are used. Hence the ability to find many of the minor components and later eluting "higher fullerenes" may be as much of a detection problem as a separation problem.

Some recent papers have focused on higher fullerenes. Peters and Jansen reported a new method of synthesis which produces greater concentrations of higher fullerenes than the contact arc method.[48] Figure 7 shows the higher fullerene separation obtained with our higher fullerene sample on two aliphatic and two aromatic stationary phases. Clearly the Buckysep-RP and the Astec C_{18} phases baseline resolved C_{76}, C_{78}, and C_{84}. The poor resolution of higher fullerenes on the aromatic phases limits the possibility of using these stationary phases to the purify large quantities of

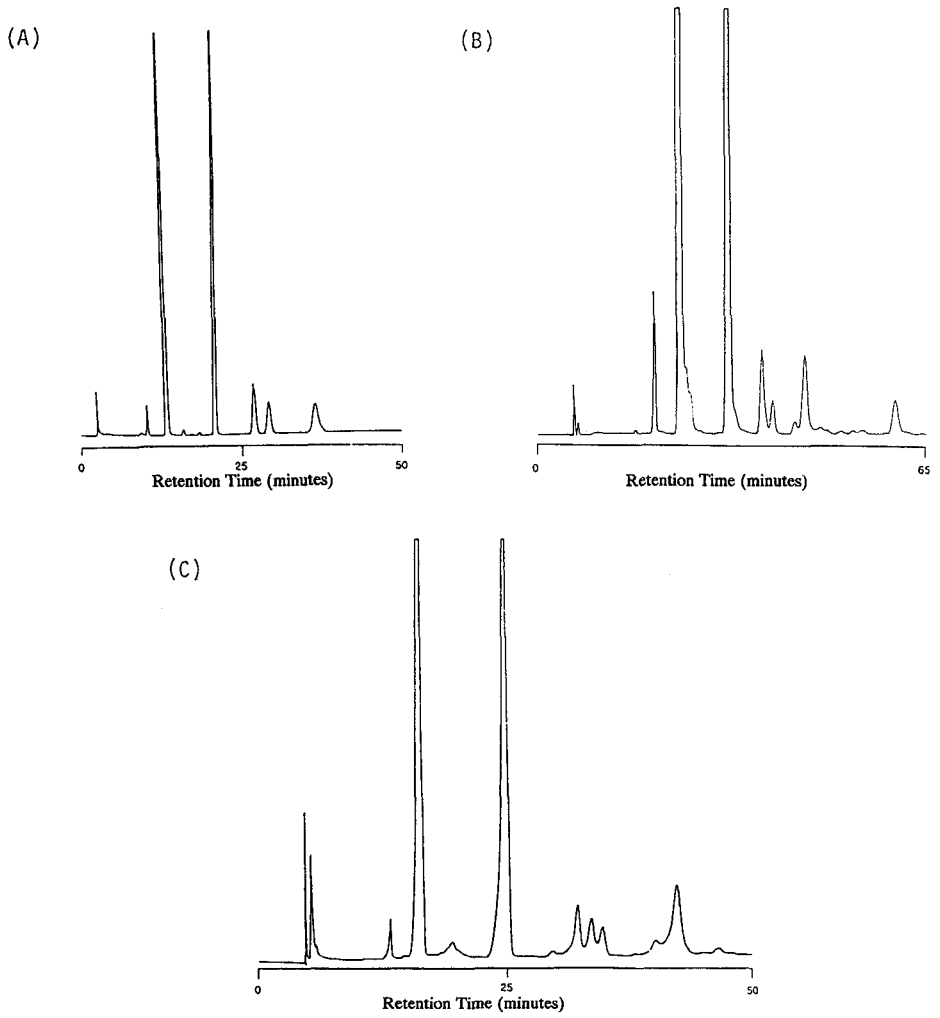


Figure 6. Multiple chromatograms showing the changes in the higher fullerene (C_{76} and greater) ratio produced when different production and extraction method are used. All fullerene samples were analyzed on the Astec C_{18} stationary phase. Chromatogram (A) is a toluene extract evaluated with a 50:50 acetonitrile/toluene mixture (v/v) while chromatogram (B) is a carbon disulfide extract of fullerenes examined with a 55:45 ratio of acetonitrile/toluene (v/v). Chromatogram (C) is a scandium complexed metallo-fullerene sample analyzed with a 50:50 acetonitrile/toluene mixture (v/v).

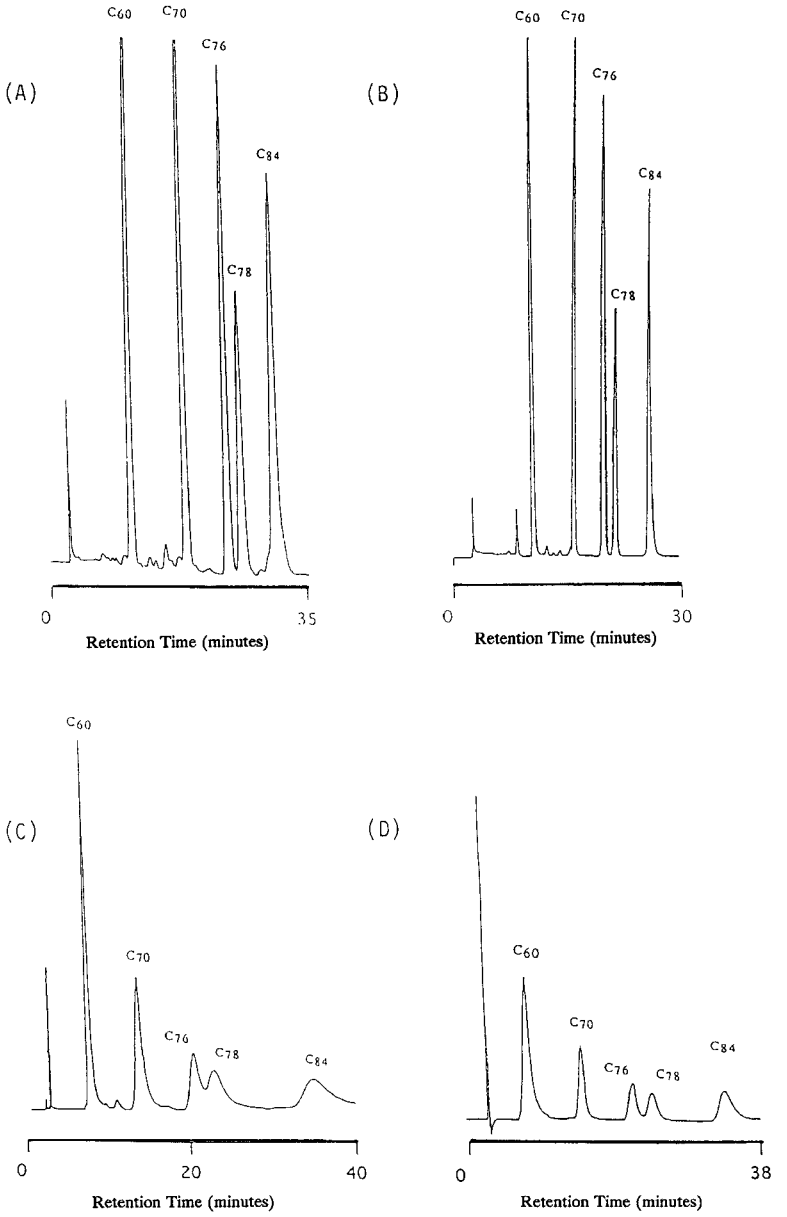


Figure 7. Chromatograms showing the higher fullerene separation on (A) Buckysep-RP, (B) Astec C_{18}, (C) Buckyclutcher I, and (D) 3,5-dimethylphenyl-β-cyclodextrin stationary phases. Clearly, the alkyl phases baseline resolved the higher fullerenes. Different solvent ratios were used to obtain similar k' values. All separations were carried out a 1 ml/min and at 310 nm.

higher fullerenes. The ChromSpher column is not featured in this study because after pumping the recommended 95:5 isooctane/isopropanol (v/v) at 3.0 ml/min, the higher fullerenes were irreversibly retained making this stationary phase a poor choice for the isolation of higher fullerenes. Possibly a solvent system other than the one recommended by Chrompak would elute fullerenes in reasonable time span. Another alternative is to increase the column temperature. Chrompak has shown the partial resolution of some higher fullerenes at 40 °C.[20] In this case, the fullerene adsorption appears to be similar to that of more conventional chromatographic separations which are enthalpy driven and exothermic.

Several isomeric forms of some of the higher fullerene allotropes have been reported.[2] In 1991, Diederich and coworkers reported the first chromatographic isomeric separation of C_{78}.[4,6] With the higher fullerene sample discussed above, no isomeric separation was obtained on any of the columns analyzed. The Buckyclutcher I phase has been said to separate specific isomers in a previous publication[22], however, in our hands, no isomeric separations were found. Of course it is not known if any isomers were present in this sample to begin with or whether they were at high enough levels for our detection method.

As yet there have been no significant published discussions on the deterioration or changes in HPLC columns used for the separation of fullerenes over a period of time. After several months of daily use, all columns investigated in this study deteriorated. It should be noted that for any fullerene separations with any stationary and mobile phase combination, there always seems to be irreversible adsorption to the column. Consequently, retention characteristics of all columns used in fullerene separation change with time. We have found that the retention, selectivity, efficiency, and loadability of every column tested in this study

became worse with time. When doing analytical separations, these parameters decrease slowly (after 100-500 injections). However, when doing loading studies, larger amounts of fullerenes are injected and the separation properties degraded after relatively few injections. It is not known whether the fullerenes themselves, their degradation products, coextracted contaminants or some combination of these three things are irreversibly binding to the columns and degrading the separation. However this does occur on all columns and with all mobile phases tested.

CONCLUSIONS

In general, aliphatic stationary phases separated C_{60} and C_{70} fullerenes better on an analytical scale while the aromatic stationary phases were more effective at purifying larger quantities of these fullerenes. The optimum solvent conditions for one column are not the same for another. The optimum mobile phases used with the aromatic stationary phases tended to solubilize greater quantities of fullerenes while the optimum mobile phases for the alkyl stationary phases did not solubilize a great quantity of fullerenes. The aliphatic-type bonded stationary phases produced analytical fullerene separations of greater efficiency, shorter retention times, better sensitivity, and greater resolution for a larger range of fullerenes. Higher molecular weight fullerenes were baseline resolved on the alkyl stationary phases but not always on the aromatic phases. Therefore, a good technique to purify large quantities of higher fullerenes is still needed. Increasing the percentage of "poor" fullerene solvent in the mobile phase tends to enhance retention and selectivity. However, it decreases the amount of fullerenes that can be dissolved in the mobile phase which detracts from preparative separations. The retention order of

the fullerenes is similar on all columns and with all mobile phases. The separation performance of all columns degrade with time due to irreversible adsorption of fullerenes and/or their associated contaminants.

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