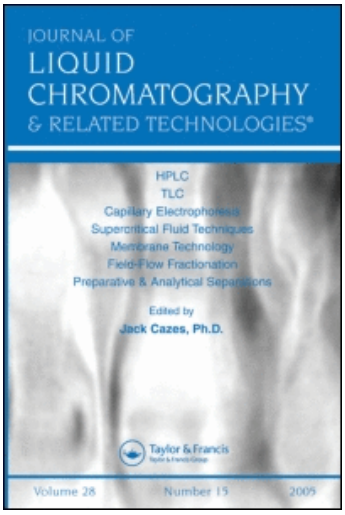

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AN EVALUATION OF THE DIFFERENTIAL PARTITIONING AND SEPARATION OF C_{60} AND C_{70} FULLERENES IN A BIPHASIC SYSTEM USING CENTRIFUGAL PARTITION CHROMATOGRAPHY (CPC)

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ABSTRACT

The partition coefficients of C_{60} and C_{70} fullerenes were measured in several different organic, 2-phase (liquid-liquid) systems using centrifugal partition chromatography (CPC). The partition coefficients of C_{60} and C_{70} were sufficiently different in some biphasic solvent systems to provide a CPC separation of these fullerenes. A phase diagram was made of the best 2-phase system for fractionating C_{60} and C_{70} fullerenes. This system contained the solvents 1,2-dichlorobenzene, isooctane, and dimethylformamide (DMF). The separation times, selectivity, and efficiency are affected by the ratios of the major solvent components, the addition of small amounts of a quaternary solvent, and the temperature (in addition to the usual instrumental parameters). Preparative separations of fullerenes were done and a maximum batch production was calculated for

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one solvent system. It appears that a significantly greater amount of fullerenes can be purified per run in this system than in corresponding HPLC systems. The rather unusual organic biphasic solvent systems developed for the fractionation of fullerenes may be useful for future separation and purification of more conventional organic compounds.

INTRODUCTION

Fullerene separations have been achieved in high performance liquid chromatography (HPLC) using several types of stationary phases. Over fifteen liquid chromatographic stationary phases have been evaluated including silica gel[1-3], alumina[4], graphite[5], monomeric and polymeric C_{18}[6-10], native and derivatized cyclodextrins[10-11], gel permeation[12-13], and aromatic charge transfer[14-21] stationary phases. To date C_{60}, C_{70}, higher fullerenes (up to C_{96}), fullerene isomers, fullerene derivatives, and most recently, metal complexed fullerenes have been successfully isolated chromatographically.[1-24] Most of the aforementioned stationary phases can baseline resolve C_{60} and C_{70} without much difficulty provided the optimum mobile phase composition for each stationary phase is used. The recent demand for larger quantities of fullerenes has led to the challenge of separating fullerenes on a preparative basis. One of the problems in the area of preparative purification of fullerenes is that they have limited solubilities in most HPLC eluents. Ruoff et. al. determined that only 0.043 mg/ml of C_{60} can be dissolved in n-hexane, 0.001 mg/ml in ethanol, and 0.00 mg/ml in acetonitrile.[25] Even toluene dissolves only 2.8 mg/ml of C_{60}.[25] Fullerenes are known to be most soluble in chlorinated benzenes, carbon disulfide, toluene, methylene chloride, and chloroform (in order of decreasing solubility).[25] The better fullerene solvents (CS_2, chlorinated benzenes, etc.) are not often used for HPLC because of their volatility, odor, viscosity, and toxicity. Although both polar and nonpolar stationary phases have been used for fullerene separations, the mobile phases are fairly limited to the less polar solvents. Consequently, even though a reversed phase stationary phase may be used, typical reversed phase mobile phases (hydro-organic solvents) cannot be used.

Many early attempts at chromatographically separating fullerenes on preparative basis involved overloading analytical (25 cm x 0.46 cm) and

semi-preparative (25 cm x 1.0 cm) length columns. In 1990, Hawkins and coworkers reported that their semi-preparative 3,5-dinitrobenzoylphenylglycine (DNBPG) column could only resolve 0.5 mg of fullerene material per unit injection.[14] Welch and Pirkle later reported that a 1.5 m x 5 cm preparative column containing the same DNBPG stationary phase would separate 100 mg of C_{60} and C_{70}.[19] In contrast, they indicated that this stationary phase is capable of normally separating 20 g of a soluble mixture of more "typical" organic compounds of the same selectivity. Herren et. al., in 1993, claimed to improve preparative separations for C_{60} and C_{70} fullerenes using a chemically bonded tetrachlorophthalimidopropyl-modified silica (TAPA).[17] Approximately 1 mg could be separated per injection. Most of the HPLC stationary phases evaluated for large scale fullerene purifications were found to be impractical because recovery yields were quite low. Other stationary phases were found to be very costly because of the large diameter and length columns needed in addition to the high cost of column hardware. Another problem with preparative chromatography is the irreversible adsorption and degradation of some fullerenes when they are associated with the stationary phase.

Recently, we did a comparison study of the selectivity, resolution, column deterioration, higher fullerene isolation, and preparative purification (loading ability) of C_{60} and C_{70} fullerenes using several commercial aliphatic and aromatic stationary phases.[10] The general trend found was that alkyl-chain bonded silica stationary phases seemed to be best for the analytical separation of fullerenes. Conversely, aromatic stationary phases were found to be better for purifying fullerenes on a preparative basis. Between 2.5 and 3 mg of C_{60} and C_{70} were resolved on the aromatic analytical columns (25 cm x 0.46 cm) before the solubility of the fullerenes in the sample solvent exceeded that of the mobile phase. Also irreversible adsorption of fullerenes or associated degradation products occurred on all of the columns tested. This results in significant decrease in column performance for both analytical and preparative applications.

Ideally, the goal of preparative chromatography is to produce the largest quantity product of the highest purity per unit time while keeping the production cost low. Column loadability (amount that can be injected per unit run), to a large extent, is dictated by the peak to peak separation or

α value.[26-27] The amount that can be injected affects the cost of purification and, therefore, must be maximized. Numerous factors affect band broadening in preparative HPLC from the physicochemical chromatographic parameters (i.e., the surface area of the stationary phase, the temperature, the number of theoretical plates, and the chemical nature of the mobile phase) to the type of distribution isotherm.[27] In general, if a compound cannot be separated in a single run under optimum conditions, the simplest and most routine method for improving the separation involves increasing the bed length of the column. Guiochon and co-workers have published a series of related studies on the theory and general optimization parameters for preparative liquid chromatography.[28-30]

Because of the solubility limitation of fullerenes in common HPLC eluents, it is possible that an optimum separation method for the purification of fullerenes would involve the principles of liquid-liquid extraction and incorporate the efficiency, resolving power, speed, and convenience of chromatography. There is a chromatographic technique that utilizes these principles. Centrifugal partition chromatography allows one to do a series of liquid-liquid extractions in the chromatographic mode. CPC is a variation of countercurrent chromatography. CPC has a liquid mobile phase and a liquid stationary phase. Consequently, problems with irreversible adsorption or degradation by a solid stationary phase are avoided. The liquid stationary phase is held in place by a centrifugal field while the liquid mobile phase is pumped through it. Detailed descriptions of the CPC apparatus and theory have been published.[31-34] Some of the more common two phase liquid systems used to separate organic compounds with the CPC apparatus are hydro-organic and water rich systems such as octanol/water and methanol/hexane/water.[35-37]

There are several advantages to using CPC over preparative liquid chromatography such as the increased sample capacity obtained due to the large stationary phase to mobile phase volume ratio and the elimination of irreversible retention by use of the dual-mode of elution.[31-38] Also, unlike other chromatographic methods, the efficiency of CPC increases at very high flow rates.[33] These characteristics makes CPC an ideal large-scale separation or purification method. The effect of analyte concentration and injection volume (as a means to increase mass load) in preparative CPC has been evaluated.[35] Unlike preparative or analytical HPLC, increasing the concentration of analyte injected (at constant volume) does not cause a

dramatic decrease in separation efficiency. Hence, in most cases, band broadening that occurs from column overloading in HPLC is not as significant in CPC. In order to purify large quantities of compounds by HPLC, immense preparative columns are required. The only limitation affecting mass load in CPC is the solubility of fullerenes in the mobile phase.

In this work, we examine several organic liquid-liquid, two phase systems and evaluate the partitioning behavior of fullerenes in them. Devising such two phase systems are not always straight forward since fullerenes are insoluble or very poorly soluble in many of the more popular chromatographic solvents (e.g., water, methanol, other alcohols, acetonitrile, etc.) that are used to form liquid biphasic systems. The fullerenes must be at least somewhat soluble in both liquid phases if the system is to be used for separations. In addition the partition coefficient (K) of various fullerenes between the liquid phases must be different. Also, for large scale separations, the fullerenes must be appreciably soluble in at least one of the phases. We demonstrate: (1) that fullerenes are amenable to such systems (2) that the selectivity of these systems can be altered or optimized, and (3) that both separations and partition data can be obtained in a liquid-liquid countercurrent chromatographic experiment.

EXPERIMENTAL

Materials. Isooctane, hexane, heptane, toluene, acetonitrile, and methyl-*tert*-butyl ether were of HPLC grade and obtained from Fisher (Pittsburgh, PA). Aldrich Chemical Company (Milwaukee, WI) supplied the dimethylformamide (DMF), dimethylsulfoxide (DMSO), N-methyl-2-pyrrolidinone (NMP), γ-butyrolactone, decalin, and 1,2-dichlorobenzene. Pure fullerene standards of C_{60} and C_{70} were purchased from either Polygon Enterprise (Waco, TX), Fluka (Ronkonkoma, NY), or provided by IBM at the Almaden Research Center (San Jose, CA).

Apparatus. All chromatographic experiments were performed on a Sanki Laboratories Inc. (Mount Laurel, NJ) Model CPC-NMF centrifugal partition chromatograph equipped with an adjustable temperature thermostat (from 15 to 35 °C). The following Shimadzu (Columbia, MD) equipment was also used: two LC-6A pumps, a SPD-6A UV/Vis variable wavelength spectrophotometric detector with preparative flow cell, and a

SCL-6B system controller. The system also included a Rheodyne injector and switching valve (models 7125 and 7010, respectively) equipped with a 1 ml injection loop. A Recorder Company 4500 series strip chart recorder (San Marcos, TX) was used to record the data. After elution, the C_{60} and C_{70} peaks were collected with an Isco Cygnet fraction collector (Lincoln, NE).

The rotor of the CPC apparatus holds up to 12 cartridges although either 3 or 6 cartridges were used in this study. A complete description of the cartridges and the CPC system was given previously.[32,35-36] Generally the cartridges are filled with the liquid stationary phase at high flow rates. The centrifugal spin rate is chosen which generates the centrifugal field and the mobile phase is slowly pumped through. When equilibrium is established, the ducts are filled with the mobile phase, the channels are filled with the stationary phase, and only the mobile phase exits the CPC apparatus.

The CPC apparatus can be used in the ascending or the descending elution mode. In the descending mode, the stationary phase is the upper or less dense liquid and the mobile phase is the more dense liquid phase. In this case, the more dense liquid (i.e., the mobile phase) flows through the stationary phase from the top of the apparatus to the bottom. When using the ascending mode the opposite occurs. The most dense liquid becomes the stationary phase and the less dense liquid is mobile phase which is pumped up through the instrument from the bottom to the top of the centrifuge. Both modes were used during the course of this study and are labeled accordingly in the appropriate tables and figures.

Procedure. Numerous organic liquid systems were evaluated with small 5 ml batch scale experiments to determine whether biphasic systems were formed and if they could be used as stationary and mobile phases in CPC. Approximately, 1 mg of fullerenes were dissolved in each liquid system and sonicated for 10 minutes. 10 µl of each layer was injected onto a liquid chromatograph equipped with a Astec C_{18} (25 cm x 0.46 cm) (Whippany, NJ) stationary phase. The peak areas of C_{60} and C_{70} in one phase were compared to the peak areas of C_{60} and C_{70} in the other phase. The biphasic system giving the largest fullerene concentration difference between the top and the bottom layers were further evaluated as stationary and mobile phases for CPC.

All biphasic ternary liquid systems used in CPC were mixed on a mechanical stirplate for 1 hour and then placed an additional 15 minutes in a ultrasound bath to ensure complete mutual saturation. The appropriate number of cartridges (i.e., the column in CPC) were filled at a high flowrate with the liquid stationary phase in the proper elution mode. With the centrifuge field spin rate between 700 and 1700 rpm in the opposite elution mode, the mobile phase was pumped in at a flowrate between 0.2-1.0 ml/min. The system was equilibrated for 1-2 hours or until no more stationary phase was displaced. The effluent is collected via a graduated cylinder so that the volume of stationary phase displaced can be measured. Each analysis required only 400 ml of solvent because the mobile phase was continuously recycled until the beginning of the first peak eluted. The most frequently used 2-phase organic system was the isooctane/dimethylformamide/1,2-dichlorobenzene. The more dense dimethylformamide/1,2-dichlorobenzene layer was used as the stationary phase while the less dense isooctane/1,2-dichlorobenzene mixture served as the mobile phase. While this unusual biphasic solvent system was determined to be an ideal system to differentially solubilize fullerenes, it is not the best system to use with this model CPC apparatus since it also tends to accentuate the erosion of the graphite disc located within the rotary seals. Optimum flowrates for this system were between 0.2 and 0.5 ml/min. Between 50 - 500 µl of a 5 or 15 mg/ml concentration of the C_{60} and C_{70} dissolved in 1,2-dichlorobenzene was injected. Detection was set at 384 nm because Guiochon and co-workers reported that C_{60} and C_{70} have the same extinction coefficients at that wavelength. [9]

The ternary phase diagram was determined by weighing specific amounts of each of the three liquids, vigorously shaking, and then allowing them to thermally equilibrate for 15 minutes. The solvent system was found to be biphasic by visual examination of the meniscus. The precise composition of each phase was ascertained by gas chromatography utilizing thermal conductivity detection.

RESULTS AND DISCUSSION

Table 1 lists several of the organic, biphasic solvent systems and experimental parameters that were varied to find an optimum liquid-liquid system to fractionate C_{60} and C_{70}. Many more solvent systems were prepared and evaluated than are shown in Table 1. However, little or no

Table 1. Partition Coefficient Determination of C_{60} and C_{70} in Various Solvent Systems

System number	Cartridge number	Elution mode	Fullerene Solvent Systems: good % v-v	moderate % v-v	poor % v-v	poor % v-v	Temp (°C)	K1 C60	K2 C70	Vr1 C60 (ml)	Vr2 C70 (ml)	Vo (ml)	Vt (ml)	α
1	2	A	1 toluene	3 hept	2 DMF		22	0.96	0.96	46	46	24	47	1
2	3	D	2 toluene	1 hept	1 DMSO		20	3.13	3.13	178	178	15	67	1
3	3	D	1 deca		1 DMF		16	0.84	0.84	60	60	24	67	1
4	3	D	3 deca	2 toluene	2 DMF	2 DMSO	17	7.62	8.38	312	340	30	67	1.1
5	3	A	3 deca	3 toluene	2 DMF	2 DMSO	17	7.33	8.22	124	132	58	67	1.12
6	6	D	4 deca	1 toluene	4 DMF		18	7.53	7.93	695	730	40	127	1.05
7	3	D	11 deca	3 toluene	8 NMP	0.2%water	16	6	6	240	240	30	67	1
8	3	D	3 DCB	10 deca	8 DMF		17	0.41	0.46	120	110	30	67	1.12
9	3	D	2 DCB	8 deca	5 DMF		17	0.45	0.52	110	100	32	67	1.15
10	3	D	1 DCB	4 deca	4 DMF		18	0.16	0.18	255	230	29	67	1.15
11	6	D	1 DCB	6 isoo	4 DMF		19	2.08	2.27	77	73	30	127	1.09
12	3	D	1 DCB	4 isoo	2 DMF		15	2.33	2.7	43.5	41	26	67	1.16
13	3	A	1 DCB	4 isoo	2 DMF	0.1%water	17	2	2.43	116	137	18	67	1.22
14	3	A	1 DCB	4 isoo	2 DMF	0.5%water	17	2.17	2.67	123	147	17	67	1.23
15	3	D	1 DCB	4 isoo	2 DMF	0.8%water	17	2.13	2.33	76	72	30	127	1.09
16	3	D	1 DCB	4 isoo	2 DMF	1 MeCN	15	1.15	1.21	61	59	22	67	1.06
17	3	A	1 DCB	4 isoo	2 DMF	0.5%MTBE	15	2.23	2.75	114	132	22	67	1.2
18	3	A	1 DCB	4 isoo	2 DMF	1% MTBE	15	2.02	2.96	118	165	17	67	1.47
19	3	A	1 DCB	4 isoo	2 DMF	5% MTBE	15	2.29	2.82	103	118	39	67	1.23
20	3	A	1 DCB	4 isoo	2 DMF	1% MTBE	20	2.34	2.61	133	146	18	67	1.15

[a]A and D correspond to the ascending and descending mode of elution, respectively. In the ascending mode the stationary phase is the more dense liquid phase and the mobile phase is the less dense liquid layer. The opposite is true for the descending elution mode (See Experimental).

[b]The solvent that dissolves the greatest quantity of C_{60} and C_{70} in each system is refered to as a "good" fullerene solvent. "Poor" fullerene solvents do not solubilize C_{60} and C_{70}, whereas "moderate" solvents dissolve small amounts of C_{60} and C_{70}. The abbreviations are as follows: hept = heptane, DMF = dimethylformamide, DMSO = dimethylsulfoxide, deca = decalin, isoo = isooctane, MeCN = acetonitrile, MTBE = methyl-*tert*-butyl-ether, and DCB = 1,2-dichlorobenzene.

[c]The partition coefficients (K = [fullerenes]sp/[fullerenes]mp) were calculated with the liquid layer solubilizing the greatest quantity of fullerenes as the stationary phase.

[d]Vr_1 and Vr_2 are the retention volume in ml of C_{60} and C_{70}, respectively. V_o is the volume (in ml) of the mobile phase and Vt is the total volume in ml of the CPC apparatus (which is dependent upon the number of cartridges used).

[e]The selectivity factors were calculated by the following equation: $\alpha = K_2/K_1$.

fractionation of C_{60} and C_{70} was observed in these systems. A few examples of the "unsuccessful" systems are included in Table 1 for illustrative purposes (i.e., system numbers 1-3 and 7 in Table 1). In each biphasic, ternary liquid system, a well known "good" fullerene solvent is combined with a "moderate" and/or "poor" fullerene solvent. The liquid in each ternary solvent system that dissolves the greatest quantity of fullerenes is denoted as the "good" fullerene solvent. Examples of "good" fullerene solvents are 1,2-dichlorobenzene, decalin, and toluene. The "poor" fullerene solvents are polar aprotic solvents such as dimethylformamide and dimethylsulfoxide. "Poor" fullerene solvents are necessary in order to obtain a two phase system and to impart selectivity to the systems. It should be noted that other polar protic and aprotic solvents such as methanol and acetonitrile also can be used to form biphasic organic systems with "good" fullerene solvents. However, these systems are not included since there was little encouraging evidence of fullerene fractionation in the early studies mentioned previously. The liquid phase in which the fullerenes were most soluble (Table 1) was always used as the stationary phase.

We previously reported on the use of CPC to determine the partition coefficients of a variety organic compounds in many liquid-liquid two phase systems.[31] The basic CPC retention equation can be rearranged so that the partition coefficient is easily determined:

$$K = [(Vr - Vt)/ Vs] + 1 \qquad [1]$$

where Vr is the retention volume in ml of the compound of interest, Vt is the total internal volume of the CPC instrument in ml, Vs is the stationary phase volume in ml, and K is the partition coefficient of the compound between the stationary phase and the mobile phase. This equation applies to the ascending elution mode. Therefore, the reciprocal (1/K) is used to determine the partition coefficient when using the descending mode of elution.

The partition coefficients of C_{60} and C_{70} fullerenes in a large number of biphasic systems also are shown in Table 1. As can be seen from this data, the various solvent systems generated a wide variety of partition coefficients. Hence the partition coefficients of C_{60} and C_{70} can be varied over an order of magnitude if desired. The highest partition coefficient measured was 8.38 and the lowest was 0.16. There does not appear to be any

correlation between the size of the fullerene's partition coefficient (K) and their selectivity (i.e., the α-values in Table 1). For example, system 4 which yields larger partition coefficients does not necessarily provide any greater selectivity than system 12 which gives small partition coefficient values. Systems 1 through 12 are representative of the initial experiments in which we hoped to find a "highly" selective system for the fullerenes. The 1,2-dichlorobenzene/isooctane/DMF solvent combination in trial 10 afforded the largest selectivity value for any three component system tested. However, as can be seen from the data in Table 1, adding small amounts of additional components or modifiers can cause the α values to change. Hence attempts were made to improve the selectivity and/or efficiency by adding different minor components. For example, the 1,2-dichlorobenzene/isooctane/DMF biphasic system is sensitive to small amounts of water. In trials 14-16, the ratio of water was varied from 0.1 percent to just under 1 percent. No basic trends were observed when attempting to improve selectivity by adding either small or large increments of water. The optimum water amount was found to be 0.5 % and provided an α-value of 1.23. Small quantities of methyl-*tert*-butyl-ether was added to the 1,2-dichlorobenzene/isooctane/dimethylformamide system as well. A trend similar to that for water was observed. The optimum methyl-tert-butyl-ether content was found to be between 0.5 % and 5 %. 1 % of methyl-*tert*-butyl-ether added to the previously determined optimum biphasic system and gave the largest selectivity value in this study (system 18, Table 1). Methyl-*tert*-butyl ether was chosen because it greatly enhanced the separation efficiency of monoterpenes hydrocarbons in reverse phase liquid chromatography.[39] It was one of the few additives studied that also seemed to enhance the efficiency of the fullerene separation.

In some cases, changing the temperature appeared to alter the retention and selectivity of the fullerenes (Table 1). Unlike HPLC, the mobile and stationary phase in CPC can alter their composition with a change in temperature (see the ternary phase diagram in Figure 1). As discussed in the preceding paragraph, even a small change in solvent composition can significantly affect the elution of fullerenes. Consequently the influence of temperature on retention and selectivity (α) is not as straight-forward in CPC as it is in HPLC (where the stationary and mobile phase composition do not change with temperature). Since the solvent composition and temperature effects are coupled in CPC, there is no way to

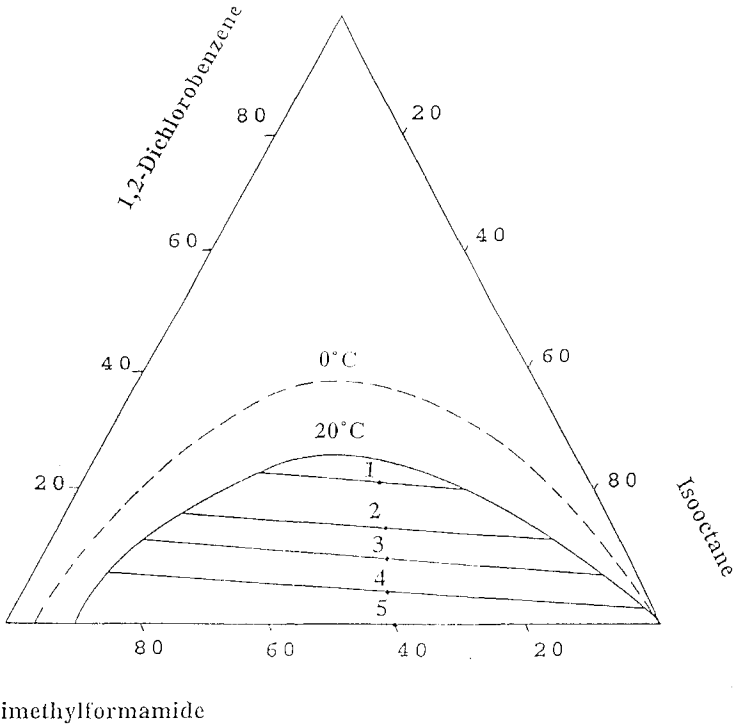

Figure 1. Ternary mass phase diagram for the isooctane/dimethylformamide/1,2-dichlorobenzene system at two temperatures, 0 °C the dotted curve and 20 °C the darkened curve. The region above the temperature curves indicates the miscible monophasic area and the region below designates the region where two layers are formed or the biphasic solution region. The tie lines were established for the 5 different chemical compositions shown in Table 2 at 20 °C.

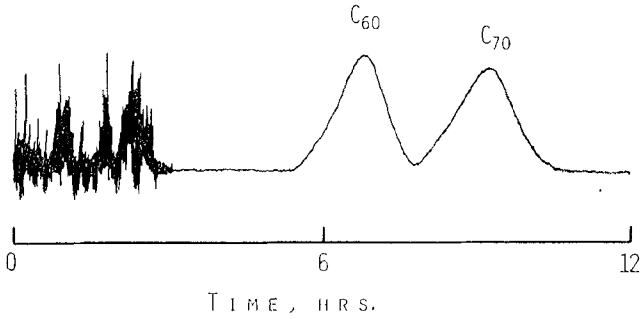

Figure 2. CPC ascending mode chromatogram showing the separation between C_{60} and C_{70}. The conditions were as follows: solvent system = 4:2:1 isooctane/DMF/DCB (v/v/v) + 1% MTBE; the isooctane-rich mobile phase ascended; flowrate 0.3 ml/min; rotation rate 1000 rpm; cartidge number 3; wavelength 384 nm; temperature 15 °C, injected volume 150 µl of a 15 mg/ml solution; and chartspeed 2 cm/hr.

predict *a priori* whether lowering the temperature will increase selectivity (α) and retention. However, the best separation in this work was obtained at 15 °C as opposed to higher temperatures (Experiment 18, Table 1, and Figure 2).

The ternary mass diagram for the isooctane/DMF/1,2-dichlorobenzene solvent system (the "optimum" system as determined from the data in Table 1) is found in Figure 1. The regions above the dashed and solid curved lines designates the monophasic area or homogeneous region (for the respective temperatures). Obviously these solvent compositions would be useless for any separation by countercurrent chromatography or liquid-liquid extraction. The region below the solid and dashed curved lines corresponds to the biphasic region which is suitable for the CPC apparatus. The dashed line designates the biphasic boundary at 0 °C and the solid line indicates the biphasic boundary at 20 °C. The tie lines are calculated according to the lever rule which is described in detail elsewhere.[37] These tie lines allow one to precisely quantitate the composition of the two phases obtained when ternary liquids become saturated with one another. As can be seen, the 1,2-dichlorobenzene partitions almost equally between the upper and lower phases. The exact compositions of the tie lines (in mass percentages or g/100g) of the two phase liquid-liquid mixtures are found in Table 2. The optimum solvent composition that was the focus of most of this study is indicated by point one. The composition is 46.4% isooctane, 31.7% DMF, and 21.9 % 1,2-dichlorobenzene (w/w).

The ternary phase diagram also shows that the waterless isooctane/DMF/1,2-dichlorobenzene system used is critically temperature dependent. It was observed that the two phases in this system become homogeneous at temperatures ~ 30 °C. As noted in the experimental section, the lower (more dense) layer of this biphasic system consists mainly of DMF and 1,2-dichlorobenzene. The denser lower layer dissolves fullerenes better than the isooctane-rich upper layer. The fullerene partition coefficients (Ks) are higher than 2.0 (See Table 1). Therefore, when the two phases are equilibrated, the fullerene concentration is more than 2 times greater in the lower DMF-rich stationary phase than in the upper isooctane-1,2-dichlorobenzene mobile phase. When this system is warmed, the bottom layer increases in volume at the expense of the top layer. This decreases the difference in the C_{60} and C_{70} partition coefficients

Table 2. Chemical Composition of Ternary Phase Diagram Tie Lines

Point[a]	DCB % w/w	Isoctane % w/w	DMF % w/w	Solubilization Temperature °C[b]
1	21.9	46.4	31.7	31
2	15	51	34	56
3	10	53	37	60
4	5	56	39	67
5	0	60	40	75

a Numbers correspond to the those on Figure 1 (ternary phase diagram). Point 1 refers to the volume ratio 1:4:2 DCB/isooctane/DMF (v/v/v).

b The biphasic system becomes monophasic at the solublization temperature.

although both fullerenes are still more soluble in the lower DMF-1,2-dichlorobenzene phase. Temperature changes also are known to alter the physicochemical properties of liquids including their density, viscosity, vapor pressure, and the partition coefficients of dissolved solutes.[31,33,37-38] All of these factors previously have been shown to affect CPC separations.

A model has been developed by Cretier and Rocca which enables one to predict the maximum sample capacity on a given preparative silica based packed stationary phase.[40-41] We have used an analogous model for CPC.[35] It was extended so that one can estimate the maximum injection volume, V_{max}, in CPC, taking into account peak symmetry and the absence of mass overload by:

$$V_{max} = Vr_2 - Vr_1 - [(W_2 + W_1) / 2] \qquad [2]$$

where Vr is the retention volume, W is the width of the peak at the base, and the subscripts 1 and 2 corresponds to C_{60} and C_{70}, respectively.[35] Using the retention volume data from Figure 2 and solving for V_{max}, one obtains:

$$V_{max} = 164.6 \text{ ml} - 117.6 \text{ ml} - [(37.8 \text{ ml} + 36.9 \text{ ml}) / 2] = 9.65 \text{ ml} \qquad [3]$$

Therefore, the maximum amount that can be separated as indicated by equation 3 is 144.8 mg (9.65 ml x 15 mg/ml) of a fullerene solution with 100 % recovery and purity per run. The above calculation was done using data generated with 3 cartridges, however, up to 12 cartridges can be loaded

into the CPC apparatus. Therefore, theoretically the quantity of fullerenes can be further increased provided the pressure limits of the system are not exceeded and the allowed degree of peak overlap does not change.

CONCLUSIONS

It is possible to formulate a variety of different liquid, organic, biphasic solvent systems that can be used to fractionate fullerenes. When used in conjunction with centrifugal partition chromatography (a type of countercurrent chromatography) one can measure partition coefficients and carry out preparative-scale separations. The current CPC separation can purify approximately 50 times the amount of fullerenes (per batch) as compared to previously reported HPLC methods. The relative mildness of this technique could make it useful in isolating greater percentages of the more labile fullerenes such as those containing metals. These novel liquid-liquid systems may be useful for separating more conventional organic compounds as well.

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