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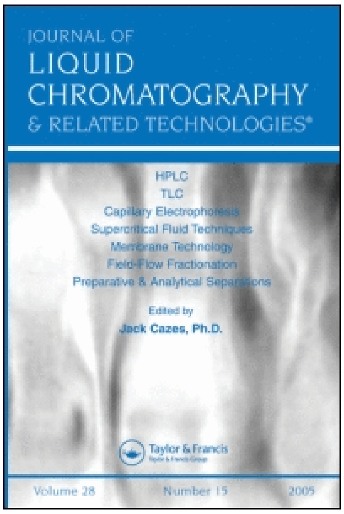

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SEPARATION BEHAVIOR OF COMMON FULLERENES IN CYCLODEXTRIN-HPLC BASED ON COMPUTATIONALLY-DERIVED INTERACTION ENERGIES

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ABSTRACT

Molecular modeling and basic thermodynamic considerations are used to explain previously reported HPLC retention behavior of C_{60} and C_{70} using a γ-cyclodextrin stationary phase. Evidence thatinclusion complex formation between the fullerene and γ-cyclodextrin is not essential to resolve C_{60} and C_{70} is presented. Computationally-derived interaction energies alone do not correlate with the observed HPLC retention behavior. However, interaction energy values combined with mobile phase solubility data, non-inclusion interactions, and a discussion of entropy changes due to phase transfer provide a more thorough explanation of retention in this separation system.

INTRODUCTION

Fullerenes have unique chemical and physical properties Recently, this has stimulated considerable research in chemistry and physics [1]. For further advancement in fullerene research, methods of creating and isolating the various fullerene compounds are essential. The generation of fullerenes is well documented but the separation of the less common fullerenes (higher than C_{60}) from C_{60} is still being developed [2, 3].

Numerous reports of the separation of C_{60} and C_{70} have appeared [4-11]. These include HPLC separations using a C_{18} bonded phase with an n-hexane mobile phase [4], gel permeation columns with a dichloromethane/cyclohexane mobile phase [5], monomeric and polymeric C_{18} bonded phases with toluene/methanol or toluene/acetonitrile mobile phases [6], and γ-cyclodextrin (γ-CD) chemically bonded to silica with an n-hexane/toluene mobile phase [7]. Electrochromatography using a 50 μm inner diameter capillary column packed with 3 μm C_{18} particles has also provided efficient separation of C_{60} and C_{70} [8].

Cyclodextrins (CDs) have proven to be a powerful separation selector when bonded to a stationary phase or as free-flowing mobile phase additives [12-14]. CDs are cylindrically-shaped macrocyclic sugar molecules that possess an axial hydrophobic cavity and an outer hydrophilic surface. The most common CDs are comprised of six (α-CD), seven (β-CD), or eight (γ-CD) glucopyranose units [15]. These molecules provide discrimination based on solute size, shape, hydrophobicity, or can even act as chiral selectors. Cabrera and coworkers [7] and Armstrong and Gasper [16] have reported the use of cyclodextrins in the separation of fullerenes. However, these authors present no or only general explanations of the interaction between the fullerene solutes and the cyclodextrin phase. A more thorough understanding of the molecular interactions responsible for the observed separation behavior should be possible using interaction energy calculations obtained by way of molecular modeling.

Recently, we have reported correlations between computationally-derived interaction energies and the CD-modified micellar electrokinetic capillary chromatography (MECC) performance of numerous benzo(a)pyrene isomers [17]. We have also successfully compared retention behavior of several derivatized amino acid enantiomers in CD-modified capillary zone electrophoresis (CZE) to interaction energies derived by molecular modeling [18]. In these reports, correct elution order for geometrical and optical isomers was predicted based on molecular modeling studies.

Correlations between molecular modeling data and separation performance in HPLC have also been reported [19, 20]. In relation to this article, Arnold et al. compared the retention times of several solutes using a β-CD bonded column to computationally-derived interaction energies of the corresponding β-CD inclusion complexes. They were able to correlate interaction energies and HPLC retention times within a given series of similarly substituted benzenes.

In this paper we present molecular modeling studies of C_{60} and C_{70} with γ-CD in order to further explain the HPLC retention behavior of these compounds reported by Cabrera et al. [7]. Our data indicates that interaction with the CD phase is not the most important factor in the resolution of C_{60} from C_{70}. Instead, it is differences in the interactions of the fullerenes with the "weakly solubilizing" mobile phases that are employed that leads to the observed separation behavior. Other evidence that supports this conclusion arises from molecular modeling studies of C_{60} and C_{70} with the smaller, β-CD, as well as HPLC separations using a β-CD column and solubility studies.

EXPERIMENTAL

Molecular Modeling Studies:

Apparatus

An Evans & Sutherland (Salt Lake City, UT, USA) workstation was used to run the SYBYL 6.0 molecular modeling software developed by Tripos Associates, Inc. (St. Louis, MO, USA). The structure of C_{60} was imported from the Computational Chemistry List Archives, while C_{70} was constructed using the BUILD routine of the software. Both were minimized using Gasteiger-Huckel charges with a convergence limit of 0.05 kcal/mol [17, 21]. These minimized structures were used in all subsequent operations. β- and γ-CD structures were generated using crystallographic coordinates imported from the Cambridge Crystallographic Database (Cambridge, England). Centroids were defined for fullerenes and CDs as the point marking the center of mass of the molecule.

Procedure

SYBYL programming language (SPL) was used to generate a routine that permitted operator control of the relative positioning of the fullerene and CD molecules [21]. Specifically, the SPL routine allowed the fullerene's position to be systematically altered relative to the CD cavity. The initial and final positions of the fullerene relative to the CD (as defined by the distance between the centroids of the molecules), translational increments, and rotational increments could all be specified within the SPL routine (see Table I notes for specific values). Energies of the complex were computed using the Tripos force field of SYBYL, which includes considerations of van der Waals, electrostatic, and hydrogen bonding forces between guest (fullerene) and host (CD). In some instances, the MINIMIZATION function of SYBYL was employed starting at selected of these positions, with the maximum number of iterations defined as 100,000 to insure complete minimization.

The energies of the minimized, free fullerene and CD were subtracted from the computed energy of the complex to give an interaction energy at each translational/rotational position. In this manner, an interaction energy matrix was generated. As stated above, some matrices were created using complexes that were minimized using the MINIMIZATION routine (although this was a very computationally intensive procedure). The matrices were then treated in different manners; e.g., a simple statistical mechanical partition function (Z) calculated by summing over all the values in the matrix using the equation:

$$Z = \sum e^{-E / kT} \qquad (1)$$

where E is the interaction energy, k is the Boltzmann constant, and T is defined as 298 K.

HPLC Separations :

Chemicals

HPLC grade solvents were used in all work and were purchased from Baxter Scientific (McGaw Park, IL, USA). Fullerene samples (pure C_{60} and the C_{60}/C_{70} mixture) were purchased from Fluka (Ronkonkoma, NY, USA).

Apparatus

Chromatographic separations were performed with an Astec Cyclobond I column (250 mm X 4.6 mm, 5μm d_p) (Advance Separations Technologies Inc., Whippany, NJ, USA). The chromatographic system consisted of a Millipore Model 510 HPLC pump (Millipore, Waters Chromatography Div., Milford, MA, USA) with a 20 μL injection loop and a Spectroflow 757 absorbance detector, operated at 334 nm, (Kratos, Ramsey, NJ, USA). Data acquisition was handled with Peak Simple software (SRI Instruments, Torrance, CA, USA).

Procedure

A mobile phase that was 70:30 (n-hexane:toluene) was flowed at a rate of 1.0 mL/min. The C_{60}/C_{70} fullerene mixture (approximately 10:1 ratio in concentration) was dissolved in toluene and injected. A separate solution of pure C_{60} was used for peak identification.

Solubility determination:

Apparatus

Absorbance measurements were made using an HP Model 8452A (Palo Alto, CA, USA) photodiode array spectrophotometer.

Procedure

Saturated solutions of C_{60} and the C_{60}/C_{70} mixture were prepared by placing the respective solids in 2 mL of hexane and sonicating for 2 hrs. After allowing the solutions to cool to room temperature, they were centrifuged and the supernate was transferred to a cuvette to perform absorbance measurements. These measurements were made in triplicate and the average of the three was used in concentration calculations.

RESULTS AND DISCUSSION

Cabrera et al. present a separation of C_{60} and C_{70} (C_{70} eluting second) using a γ-CD bonded silica column and an n-hexane mobile phase with various concentrations of toluene present [7]. They achieved the best efficiency, in the shortest amount of time, employing a 70:30 (v/v) n-hexane/toluene mobile phase. In that report, they speculate that the resolution of C_{60} and C_{70} is due to the size and shape of C_{70} being more favorable for inclusion complex formation with γ-CD. Based upon interaction energies calculated by molecular modeling (see below), the following discussion presents a more detailed, and quite different, explanation for the observed chromatographic behavior.

In our molecular modeling studies, each fullerene was systematically translated and rotated with respect to the cavity of the CD. An interaction energy was calculated at each of these positions. The lowest (most favorable) interaction energies resulted from the inclusion complex structures depicted in Figure 1. The smaller cavity of β-CD allows for minimal inclusion of C_{60} (Figure 1a), while the larger γ-CD includes the guest molecule to a greater extent (Figure 1b). γ-CD can also act as a host for the C_{70} fullerene if it is oriented in the manner shown in Figure 1c. These structures show that both C_{60} and C_{70} fit into the γ-CD cavity. The ability of a solute molecule to occupy the cavity of a CD is the primary basis of Cabrera et al., and many other researchers' explanations of CD-aided separation behavior. While this is an obvious starting point in describing CD separation systems, it is probably more accurate to consider differences in interaction energies of the individual solutes with the CD. For it is the strength

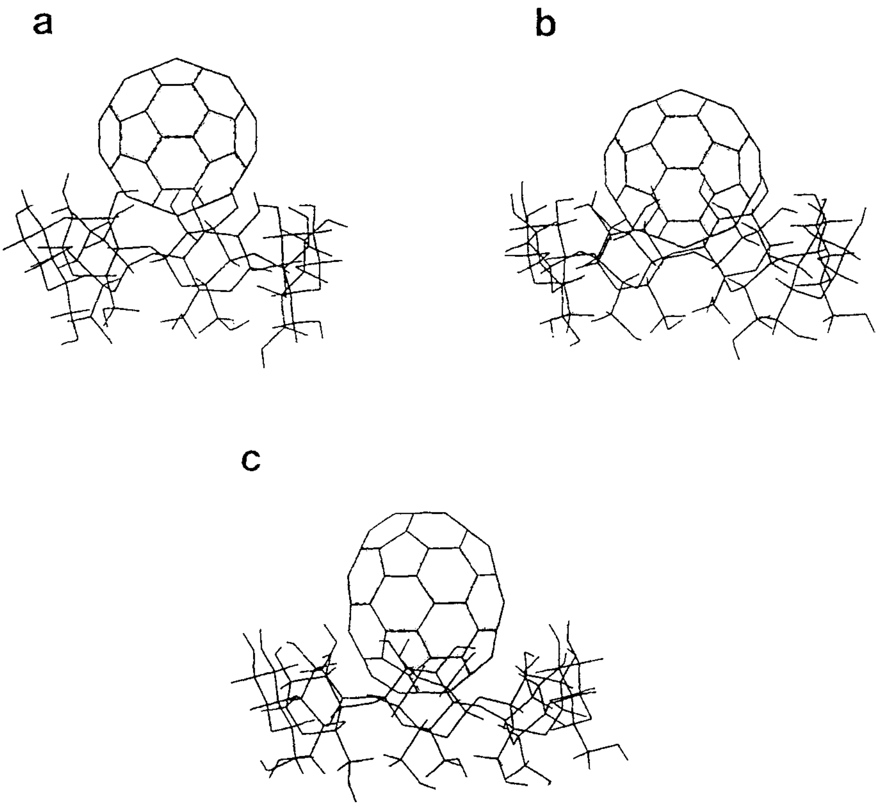

FIGURE 1. Depictions of the lowest energy (most favorable) inclusion complexes. a) C_{60} and β-CD, b) C_{60} and γ-CD, and c) C_{70} and γ-CD.

of a solutes interaction (by inclusion complex formation or non-inclusive association) with the CD that is important. However, in the case of C_{60}/C_{70} separation using γ-CD, our studies reveal that neither molecular fit nor interaction energy differences are major contributing factors (see below).

Interaction energies were calculated using several different methods which were successful in predicting elution order in our

TABLE 1
Molecular Modeling Data

	Z [a]		Z w/min		5 low[d]		5 low[e] w/min	
	γ	β	γ[b]	β[c]	γ	β	γ	β
C_{60}	2.5E20	6.0E16	1.3E22	8.0E22	-25.8	-21.7	-30.3	-30.4
C_{70}	5.1E19	5.8E16	1.5E21	2.1E19	-25.6	-21.5	-27.5	-25.1
C_{70}[f] (side)	6.6E18	5.0E18	5.2E20	1.1E22	-24.4	-24.3	-27.1	-28.8

a) Partition function, calculated using equation (1), over the following translational/rotational space:
Translation: beginning, -7.0 Å, ending, +2.0 Å, increment, 0.25 Å
Rotation: beginning, 0^0 , ending, 360^0, increment, 15^0

b) Partition function, calculated using equation (1), with minimization at each postion:
Translation: beginning, -5.5 Å, ending, -4.0 Å, increment, 0.5 Å
Rotation: beginning, 0^0, ending, 90^0, increment, 5^0

c) Same as b) but with the following matrix:
Translation: beginning, -6.5 Å, ending, -4.5 Å, increment, 0. 5 Å
Rotation: beginning, 0^0, ending, 90^0, increment, 5^0

d) Average of the 5 lowest interaction energies (kcal/mol) resulting from the following matrix:
Translation: beginning, -10.0 Å, ending, +5.0 Å, increment, 0.25 Å
Rotation: beginning, 0^0, ending, 360^0, increment, 15^0

e) Average of the 5 lowest interaction energies (kcal/mol) with minimization at each position:
Translation: beginning, -6.5 Å, ending, -4.0 Å, increment, 0.5 Å
Rotation: beginning, 0^0, ending, 90^0, increment, 5^0

f) This row of data was obtained with the C_{70} molecule oriented such that its longer dimension is parallel to the lip of the CD's cavity.

with β- and γ-CD are presented in Table 1. The partition function and the averages of the five lowest interaction energy values within the energy matrix consistently indicate a slightly more favorable interaction for C_{60} with γ-CD. From this trend, it seems that C_{60} should actually interact more strongly with the γ-CD stationary phase and thus elute slightly after C_{70}. This contradiction between calculated interaction energies and the separation behavior presented in reference 7 is evidence that the enthalpic interaction strength of these common fullerenes and γ-CD is not the sole factor that dictates their separation.

Other factors that must be considered when describing the mechanism by which this fullerene separation occurs include: 1) the entropy change associated with phase transfer (mobile phase:CD phase) of the fullerene, 2) non-inclusion interactions between the fullerene and the CD stationary phase, 3) the enthalpy (as estimated in this report by solubility) of the fullerenes in the mobile phase. These factors can be explained in terms of the fundamental thermodynamic relationship:

$$\Delta G = \Delta H - T\Delta S = - RT \ln K_p \qquad (2)$$

where ΔG, ΔH, and ΔS are the differences in Gibbs free energy, enthalpy, and entropy, respectively, which are associated with the transfer of solute from one phase to the other, R is the universal gas constant, T is the temperature, and K_p is the partition coefficient of the solute. The computations used to generate data in Table 1 consider only the enthalpy of each fullerene when associated with γ-CD. Mobile phase enthalpies are estimated by solubility studies (see below).

In our previous work, we ignored entropy considerations since the solute molecules that we were studying were all non-symmetric and structurally similar [17, 18]. However, in the case of C_{60} and C_{70}, individual entropy values in solution are expected to be different due to a greater degree of symmetry in the C_{60} molecule. Upon interaction (inclusion complex formation) with γ-CD, the entropy of each of these solutes will decrease (negative ΔS). Moreover, it is logical to expect that, due to a greater reduction of rotational freedom, the decrease for C_{60} will be larger. For this reason, consideration of entropy should better provide a correlation with the observed separation behavior since it would serve to diminish ΔG to a greater extent for C_{60}. Although Cabrera et al. carried out C_{60}/C_{70} separations at more than one temperature, insufficient retention data was provided in their report to generate a van't Hoff plot to compute ΔS values.

Another event that is important in the study of this separation system is non-inclusion interaction between the fullerene and γ-CD. It has been shown that guest molecules can interact with the lip of the CD's cavity to an appreciable extent [22]. This type of interaction could be contributing to the separation of C_{60} and C_{70} using γ-CD. Non-inclusion interactions are predicted by our molecular modeling studies. Specifically, the interaction energy values become increasingly negative (i.e. favorable) as the fullerene is moved closer to the cavity of the CD. However, just prior to inclusion of the fullerene into the CD, these values are only slightly less favorable (2-3 kcal/mol) than those at the lowest energy (inclusion complex) positions.

Other evidence of non-inclusion interactions is seen by the magnitude of the interaction energies for the sideways insertion of C_{70}. In this orientation, the C_{70} molecule cannot form an inclusion complex with γ-CD. However, the average of the 5 lowest interaction energy values is only one kcal/mol greater (less favorable) than those associated with inclusion (see Table 1). Furthermore, neither C_{60} nor C_{70} fit into the β-CD cavity but the interaction energies calculated for these systems are similar to those for γ-CD inclusion.

The discussion above indicates that inclusion complex formation is not the only possible mechanism for the separation of C_{60} and C_{70} using γ-CD. In fact, it appears that the γ-CD stationary phase functions in a manner that is similar to other organic stationary phases that have been successful in separating these fullerenes. HPLC separations employing monomeric and polymeric C_{18} stationary phases and electrochromatography using C_{18} particles provide resolution of C_{60} and C_{70} (with a k' ratio of 2.0 (C_{70}:C_{60}) in the later case) [6, 8].

Our own HPLC experimental results provide further evidence that inclusion complex formation is not essential in separating C_{60} and C_{70}. Even though significant inclusion of C_{60} or C_{70} into β-CD is not possible (see Figure 1), these compounds were resolved using a β-CD column. We obtained a k' ratio (C_{70}:C_{60}) of 1.9, using the same chromatographic conditions as Cabrera et al.; who obtained a k' ratio of 2.6 with a γ-CD column.

A solubility study was performed in order to roughly estimate the relative enthalpies of these fullerenes in the HPLC mobile phase. We measured the absorbance of saturated solutions of C_{60} and the C_{60}/C_{70} mixture using hexane as a solvent; as this is the mobile phase used by Cabrera et al. The absorbance of the C_{60} solution was subtracted from that of the mixture to obtain the absorbance of C_{70}. Previously reported extinction coefficients of these fullerenes (in toluene) and absorbance values at 406 nm and 474 nm (the wavelengths of major peaks for C_{60} and C_{70} respectively) were used to calculate an approximate concentration of these fullerenes in hexane [23]. The concentration determined for C_{60} (5.9×10^{-5} M) is nearly twice that of C_{70} (3.4×10^{-5} M). It is worth noting that the HPLC separations reported herein and by Cabrera et al. were performed near the solubility limit of these fullerenes in the hexane mobile phase employed. The greater solubility of C_{60} corresponds to the HPLC retention behavior (C_{60} eluting prior to C_{70}) observed by Cabrera et al. Although this is a very rough method of determining solubilities, the appreciable difference in the calculated values supports the idea that differences in interactions with the mobile phase, not inclusion complex formation, are primarily responsible for the C_{60}/C_{70} separation observed by Cabrera et al.

CDs have been successfully employed in the separation of higher fullerenes. However, in explaining the mechanism by which separation occurs, molecular fit is not the only factor to be considered. It has been shown that mobile phase interactions, non-inclusion interactions, and possibly entropy changes due to phase transfer are also important.

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