Article

Unexpected Encapsulation of Selected Polycyclic Aromatic Hydrocarbons by β -Cyclodextrin Studied Using UV-Vis Spectrophotometry, Micro-Planar Chromatography and Temperature Dependent Inclusion Chromatography

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Abstract: This research communication significantly extends our previous studies focusing on the temperature effects related to the unexpected chromatographic behavior of 1-acenaphthenol in the presence of native β -cyclodextrin (β -CD) additive, working under thin-layer chromatographic (TLC) conditions. We have applied complementary and orthogonal techniques including (i) temperature-controlled ultraviolet-visible (UV-VIS) spectroscopy, (ii) thermostated microplanar high-performance chromatography (micro-HPTLC) and (iii) temperature-dependent inclusion chromatography based on high-performance liquid chromatography (HPLC) to investigate the retention behavior of related host molecules. Particularly, various symmetric and asymmetric molecules were tested, such as: naphthalene and its derivatives including acenaphthylene, acenaphthene and selected dimethynaphthalenes: 1,8-DMN, 1,5-DMN, 2,3-DMN and 2,6-DMN. Reported raw experimental data, particularly performed in liquid phase and detected by UV-Vis spectrophotometry, may suggest that solubility changes of the supramolecular complexes studied and differences in total analysis time between TLC and HPLC separation can trigger strong retention of target components in planar chromatographic systems. This was also supported by principal component analysis (PCA) of the multi-source data obtained. It is hoped that the reported analyses enable the adjustment of phenomenological models describing liquid chromatography retention and the solubility behavior of low-molecular mass guest molecules, controlled by supramolecular interactions with selected macrocycles. It should be noted that the reported phenomenon, specifically supramolecular complexes precipitation, may have a number of practical applications. This can be used to improve the efficiency and selectivity of planar and/or microfluidic systems. On the other hand, precipitation via host-guest interactions may be applied for highly selective water purification technological processes that will be designed for the removal of given organic micropollutants.

Keywords: β -cyclodextrin; polycyclic aromatic hydrocarbons; micro-planar chromatography; high-performance liquid chromatography; supramolecular interaction; temperature effects; principal component analysis; symmetric and chiral chemicals

1. Introduction

Cyclodextrins (cyclic oligosaccharides, CDs) belong to group of donut-like-shaped molecules. Inclusion properties of native cyclodextrins were recognized and extensively investigated for more than 60 years [1,2]. They have still number of practical applications, particularly in separation science, mainly to improve the efficiency and resolution of chromatographic or electrophoretic systems as well as the active parts of supramolecular sensing devices [3]. The main advantages of CDs are: (i) simple and non-expensive production involving green chemistry biosynthesis, (ii) very low or virtually no toxicity for environment, animals and humans (if delivered per os) and (iii) good solubility in polar solvents (including water or dimethyl sulfoxide (DMSO)). Unique physicochemical properties of CDs result from their three-dimensional shape. An important issue is the outside location of polar hydroxyl groups on the donut surface [4–6]. In terms of complexation ability, cyclodextrins' internal cavity is chiral and relatively non-polar. This enables strong interaction with a number of non-polar organic compounds and selectivity in their enantiomeric forms (or properly shaped parts of large guest molecules) in comparison to different non-soluble in water macrocycles like calixarenes or macrocyclic antibiotics [5]. The solubility of native cyclodextrins is strongly affected by temperature and the presence of organic co-solvents [3]. Particular molecules, e.g., methanol (if added to water at any concentration) significantly decrease CDs solubility. Several solid additives may increase CDs solubility in water, like urea or co-solvents including ethanol or acetonitrile (if mixed with water at a moderate concentration, usually less than 50%) [7].

Results of our past research have indicated that in multicomponent liquid phase environment, the host-guest supramolecular complexes formation, based on CDs guest molecules, is still not exactly recognized. For example, solid complexes can be observed for native β -cyclodextrin and *n*-alkanes (*n*-propane, *n*-butane) but not for their *n*-alcohol derivatives [3]. Under particular conditions and subambient temperatures, the retention of complexed by CDs target analytes can be shorter than the retention of uncomplexed analytes at elevated temperatures [8–10]. This is fairly uncommon, considering classical chromatographic systems composed of plain binary mobile phases and *n*-alkanes-based stationary phases, where solutes retention increases if the temperature decreases. If cyclodextrins are added to HPLC mobile phases, some interesting effects can be observed. In particular, deviation magnitude on relevant Van't Hoff plots as well as the temperature point at which the deviation begins depend on the target molecule stereochemistry and cyclodextrin cavity shape/size [11]. Most recently, we have studied unexpected differences between planar and column liquid chromatographic behavior of 1-acenaphthenol and its complexes with β -cyclodextrin at subambient temperatures [12]. Reported experimental work was focused on host-guest supramolecular complex creation between β -cyclodextrin and a racemic mixture of 1-acenaphthenol. The starting point was the observation of strong retention of 1-acenaphthenol at subambient temperatures and under planar chromatographic conditions, where β -CD was added to the mobile phase. The experiments have involved a liquid phase composed of plain 35% acetonitrile in water (v/v) or modified with β -CD. Separations were conducted at different temperatures ranging from 0 to 90 °C. The behavior of supramolecular complexes was investigated using various analytical protocols involving: (i) common non-forced flow thin-layer chromatography (RP-18 TLC and micro-TLC RP-18W HPTLC), (ii) high-performance liquid chromatography (HPLC based on C-18 and C-30 columns), (iii) UV-Vis spectrophotometry and (iv) optical microscopy. In the present work this phenomenon was studied more closely, particularly naphthalene and several additional naphthalene derivatives as the guest molecules were investigated including acenaphthylene, acenaphthene and selected dimethylnaphthalenes: 1,8-DMN, 1,5-DMN, 2,3-DMN and 2,6-DMN. It is hoped that the reported data enable the adjustment of phenomenological models describing liquid chromatography retention and the solubility behavior of low-molecular mass guest molecules, controlled by supramolecular interactions with selected macrocycles.

2. Materials and Method

Acenaphthene analytical standard was purchased from POCh (Gliwice, Poland). 1-Acenaphthenol (racemic mixture), acenaphthylene, naphthalene, 2,6-dimethylnaphthalene, 2,3-dimethylnaphthalene, 1,8-dimethylnaphthalene and 1,5-dimethylnaphthalene were product of Aldrich-Chemie (Steinheim, Germany). β -Cyclodextrin (für biochemische Zwecke) and liquids like acetonitrile and methanol (LiChrosolv; HPLC grade) were obtained from Merck (Darmstadt, Germany). Sodium nitrate (NaNO_3) which was used as a dead time marker for high-performance liquid chromatography investigations was received from POCh (Gliwice, Poland). Sulphuric acid (95% p.a.) was obtained from Chempur (Piekary Śląskie, Poland). Chromatographic experiments were conducted using double-distilled tap water that was used as the component of binary mobile phases.

2.1. Chromatography

Stock solution of acenaphthenol was prepared in methanol at a concentration of 1 mg mL^{-1} whilst NaNO_3 was dissolved in water and β -cyclodextrin in 35% (*v/v*) acetonitrile in water mixture. Stock solutions were diluted with HPLC mobile phase composed of acetonitrile:water, 35:65, *v/v* enabling the final injection of solutions at a concentration of $50 \text{ } \mu\text{g mL}^{-1}$ (except NaNO_3 for which concentration $10 \text{ } \mu\text{g mL}^{-1}$ was required). For micro-TLC/HPTLC studies, analytes mass $1 \text{ } \mu\text{g/spot}$ was investigated; in particular, $1 \text{ } \mu\text{L}$ of stock solution was manually transferred to microplates start line. Thin-layer and HPLC mobile phase were prepared as acetonitrile:water mixtures (35:65, *v/v*), which were used as plain eluents or after the addition of β -CD (10 mM).

Planar and column chromatographic separations were carried out using micro-TLC and HPLC and systems described before [12,13]. Briefly, this equipment involved:

(i) temperature controlled micro chamber unit allowing horizontal separation on $5 \times 5 \text{ cm}$ microplate. An insulated oven was connected to an external thermostat (Ultra-Low Refrigerated Circulator FP51-SL, Julabo, Seelbach, Germany). For this setup, ethanol was used as a heat transferring fluid. Chromatographic mobile phase (0.5 mL volume, approximately) was injected after 15 min of temperature equilibration. Planar separation was conducted under unsaturated chamber conditions and stopped as the eluent front reached the opposite edge of plate (real developing distance was 45 mm). For separation, glass HPTLC RP-18 WF₂₅₄S plates were applied (Merck, Darmstadt, Germany).

(ii) column separation was carried out using a HPLC pump LC-10ADvp HPLC pump, Rheodyne 7725i, injector (Rohner Park, CA, USA) equipped with analytical loop ($20 \text{ } \mu\text{L}$). This system was connected to a SPD-M20A photodiode array detector (DAD; analytical wavelength range from 190 to 700 nm) working with data acquisition software LC Solution (version 1.21 SP1; 2002–2005) installed on the external PC computer manufactured by Shimadzu (Suzhou New District, Suzhou, China). Mobile phase was pumped through the column with a 1.0 mL min^{-1} flow rate. Separation process (HPLC column temperature) was controlled by water jacket, which was connected to an external thermostat (Nestlab RTE7; Thermo Electron Corporation, Newington, NH, USA). Temperature ($30 \text{ } ^\circ\text{C}$) of detection flow-through cell of HPLC-DAD detector was independently controlled by an internal thermostat, which was part of the DAD detector. HPLC separations were performed using a Supelcosil LC-18 analytical column ($10 \text{ cm} \times 4.6\text{-mm ID}$ and $5\text{-}\mu\text{m}$ particle size; Supelco, Bellefonte, PA, USA).

2.2. TLC detection and Data Acquisition

Target components spots on micro-TLC plates were detected using a sulphuric acid:water mixture (1:3 *v/v*). After separation, eluent residue on the plate was dried at room temperature (30 min.) and the plate was immersed in detection liquid (for 1 s, approximately). The wet plate was then immediately moved to the air circulating oven and heated for 15 min ($70 \text{ } ^\circ\text{C}$).

Spots patterns on micro-plates were recorded by Canon EOS1100D digital SLR camera using Tamron 55–200 lens (equipped with HAMA filter; UV 390/52 mm). Pictures were acquired from a distance of 94.5 cm under different conditions: visible light (F16; $\frac{1}{4}$ s) and UV 254 nm (F16; 8 s) as well as UV 365 nm (F16; 30 s). The acquisition system contained a ring of 12 LED lamps (JDR, SMDHLCW-250; 3.5 W; 6400 K; 250 Lumens, Sanico Electronics, Warszawa, Poland). For UV light, two linear UV 365/254 nm light sources: VL-6.LC (Vilber Lourmat, CEDEX, France) were applied. Images of micro-TLC plates included in this paper were modified to increase the contrast for spots visual evaluation. In particular, a global manual balance filter was applied. This modification was performed using ImageJ software (ver. 1.48 Wayne Rasband, National Institutes of Health, Bethesda, MD, USA; <http://rsb.info.nih.gov/ij>).

2.3. UV-Vis Spectrophotometry and Data Computations

Hewlett Packard (one beam spectrophotometer, HP-8453, Fed. Waldbronn Rep. of Germany) was used for the recording of UV-Vis absorption spectra of the investigated liquid samples. All measurements were performed using a standard 1 cm thick quartz cell and this analysis was carried out under temperature-controlled conditions. The given temperature was maintained by a handmade anti-frosting thermostatic module that was described previously [14]. The temperature of this device was controlled by using external thermostat (Nestlab RTE7; Thermo Electron Corporation, Newington, NH, USA).

Recorded UV-Vis spectra of Polycyclic Aromatic Hydrocarbons (PAHs) and PAHs/ β -CD complexes were considered as multidimensional vectors without identification and measurement of single peaks. These data were inspected with a principal component (PCA) multivariate statistical procedure using XLSTATPro 3DPlot (version 2008.2.01; Addinsoft, Paris, France).

3. Results and Discussion

Previous research reported by our group has indicated that under various TLC conditions (including stationary phase types, chamber geometry, development or saturation modes) non-typical chromatographic behavior (extremely high retention) of 1-acenaphthenol at subambient temperatures may be observed [12]. According to present knowledge, the reported results were counter to currently existing retention models explaining column chromatographic retention of host-guest complexes. Typically, where strong interaction of analytes with macrocyclic mobile phases additives is possible, the retention of target compounds is shortened at subambient temperatures. This is valid for the systems where macrocyclic additives are not strongly retarded by stationary phase and therefore, supramolecular interactions may predominantly occur in the mobile phase [15–21]. Such conditions are fulfilled if e.g., 35% of acetonitrile/water binary mixture is applied as a mobile phase [22–24]. In the case of C18 column filled and soaked with this eluent, β -cyclodextrin is barely retarded by *n*-alkane chains attached to the stationary phase surface. Therefore, this macrocyclic additive may migrate close to the retention of the dead volume marker. The consequence of such a chromatographic phases setup is a low retention of supramolecular complexes involving β -CD.

To explain the phenomenon of a strong retention of supramolecular complexes at subambient temperatures under planar chromatographic conditions several experiments were previously proposed and tested [12]. Particularly: (i) acenaphthenol chromatography under various instrumental modes, (ii) cyclodextrin retention analyzed under different conditions: as an analyte or eluent additive, (iii) chromatographic plate development time using different eluents and temperature settings, (iv) various columns including C-18 and C-30, (v) UV-Vis spectrophotometry at different temperatures and (vi) microscopic inspection of precipitated crystals of acenaphthenol/ β -CD complex. Analysis of the collected data has revealed that most probable reasons for unexpected TLC retention of 1-acenaphthenol in the presence of β -CD in mobile phase can be associated with: (i) significant changes of solubility of the host-guest complexes formed, (ii) precipitation kinetics of the solid complex and (iii) differences in overall analysis time between TLC and HPLC chromatography [12].

In the present research, naphthalene and its several derivatives as the guest molecules were selected to study this phenomenon more closely. In particular, acenaphthylene, acenaphthene and selected dimethylnaphthalenes: 1,8-DMN, 1,5-DMN, 2,3-DMN and 2,6-DMN were studied as target compounds (Figure 1). From this set of PAHs molecules, acenaphthylene, 1,8-DMN, 2,3-DMN and 2,6-DMN were selected for the micro-TLC experiment, due to the detection ability of such compounds under fluorescence conditions. Results that are presented in Figure 2 confirm that the retention behavior of acenaphthylene and 1,8-DMN at subambient temperatures is similar to the previously investigated 1-acenaphthanol. Moreover, it can be seen that there is no difference in the retention of 2,3-DMN and 2,6-DMN for both mobile phases (with and without cyclodextrin) and the whole range of temperatures investigated. This strongly supports the concept that the high retention of 1-acenaphthanol, acenaphthylene and 1,8-DMN observed under planar chromatographic conditions may be due to a favorable fit to the macrocycle host molecule present in the mobile phase. As was documented previously, the precipitation phenomenon observed in liquid phase (acetonitrile:water 35:65, *v/v*) may cause strong retention regarding the relatively long run time of TLC separation [12]. Considering the results of the UV-Vis experiment focusing on the crystallization phenomenon observed previously for 1-acenaphthanol (in the presence of β -cyclodextrin and measured in solution at room temperature; 20.0 ± 0.1 °C), in the present study the number of temperatures were investigated. As a result, the batteries of UV-Vis spectra were recorded for all of the target components. Figure 3 contains an example of such a dataset for the selected component of interest, namely acenaphthylene. To compare target components behavior at different temperatures, principal component (PCA) computations were performed based on the initial matrix composed of 26,880 elements. Namely, 128 objects (PAHs molecules under different temperatures of 0–70 °C and solvents with or without a cyclodextrin additive) and 210 variables (absorbance values measured for individual wavelengths from 190–400 nm) were considered. This enabled multivariate computations allowing objects' grouping and their relationships comparison. Resulting PCA graphs are presented in (Figure 4). From these data it is clear to see that significant differences in UV-Vis spectra concern similarly structured PAHs: 1,8-DMN, acenaphthene and acenaphthylene that were analyzed in the presence of a cyclodextrin additive. It should be noted that under given experimental conditions a spectrophotometer detects solvent turbidity caused by supramolecular complex precipitation. Therefore, this phenomenon cannot be observed for 1-acenaphthanol because the analytical protocol was designed to eliminate precipitation for this molecule, according to the experiment conducted previously [12]. The results of this experiments may help to select potential host molecules that should be eliminated from the liquid phase using interaction with a given macrocycle. For example, based on the objects' grouping within the presented PCA graph, naphthalene can be also considered as a guest molecule, which can be eliminated from the liquid phase using a cyclodextrin additive at subambient temperature.

The observed precipitation phenomenon may be critical for the quantification of analytes by HPLC involving cyclodextrins as the mobile phase additives. Therefore, previously reported data generated by our team concerning a number of analytes (mainly steroids and non-steroidal endocrine modulators) using temperature-dependent inclusion chromatography were carefully re-examined [10,25,26]. It has been found that the precipitation problem may concern the mobile phases modified with native β -CD and selected analytes including e.g., progesterone and 20α -progesterone, which was visualized on Figure S1 in the Electronic Supplementary Material of the previous paper [12]. Due to this finding, the HPLC experiment was conducted for naphthalene and its derivatives involving mobile phases with and without β -CD additive (Figure 5). Calculated trajectories of the peak areas in the temperature domain (bottom graph on Figure 5) support the concept of supramolecular complexes precipitation at subambient temperatures for given guest molecules (1-acenaphthanol, 1,8-DMN, acenaphthylene, acenaphthene and naphthalene). As was proven previously, these compounds may strongly interact with cyclodextrin and therefore, they can precipitate at subambient temperatures in both static (solutions) and dynamic (chromatographic mobile phase) conditions.

Figure 1. Chemical structures of target compounds investigated.

Figure 2. Planar chromatographic behavior of selected polycyclic aromatic hydrocarbons (PAHs) at different temperatures on RP18WF₂₅₄S HPTLC plates using mobile phases with and without β-cyclodextrin additive. Lanelabels: 1-acenaphthenol (A); acenaphthylene (B); 1,8-DMN (C); 2,3-DMN (D); 2,6-DMN (E).

Figure 3. Changes in UV-Vis spectra of acenaphthylene at different temperatures. Spectra acquisition was performed 15 min after reagents mixing (2 µg/mL acenaphthylene, 10 µL methanol, 5 mL 35% acetonitrile).

Figure 4. Differences in grouping of investigated objects (PAHs UV-Vis spectra from 190 to 400 nm at different temperatures) using solvents without (A) and with β -CD additive (B) observed within PCA factor scores 2D space.

Figure 5. Effects of temperature and cyclodextrin additive on column chromatographic (HPLC) retention (top) and peaks areas (bottom) of PAHs investigated. Analytes labels: (1) 1-acenaphthenol, (2) naphthalene, (3) acenaphthylene, (4) acenaphthene, (5) 1,8-DMN, (6) 1,5-DMN, (7) 2,3-DMN and (8) 2,6-DMN.

4. Conclusions

In this study, significant differences between planar (micro-TLC) and column (HPLC) chromatographic behavior of naphthalene and selected PAHs at subambient and elevated temperatures using several eluents modified with β -CD have been demonstrated. Given that experimental data strongly suggest that solubility changes in the supramolecular complexes studied and differences in overall analysis time between planar and column separation, may result with strong retention of target analytes in TLC systems. We documented that under particular conditions (enabling long retention time of β -CD/host molecule complexes), the quantification of target components using a HPLC involving β -CD in mobile phase may be significantly affected by the precipitation of the host-guest complex created. This phenomenon may occur at subambient temperatures (close to 0 °C). However, such a disadvantage may be reduced if more soluble hydroxypropyl- β -CD instead of native β -CD is applied.

Solubility properties of supramolecular host-guest complexes with native β -CD seem to be important for the optimization of chromatographic retention and separation driven by host-guest interaction. PCA graphs based on spectroscopic experiments involving target molecules and macrocyclic compounds may be used to predict strong host-guest interactions. Moreover, supramolecular complex precipitation combined with plate or bar solid phase extraction can be applied as a very selective method for the fractionation or separation of low-molecular mass components involving micro-chromatography or microfluidic devices.

The observed phenomenon of the strong retention of supramolecular complexes on solid thin layers seems to be applicable for e.g., selective and high throughput separation performed by microchromatographic or microfluidic devices as well as fractionation or extraction processes for bioanalysis or environmental studies (using e.g., bar extraction systems). It is hoped that the results of our research can be useful for the design of smart purification systems for green chemistry elimination of organic micropollutants from sewage water and drinking water using cyclodextrins as active molecules.

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