Separation of monomethyl-benz[a]anthracene isomers using cyclodextrin-modified electrokinetic chromatography

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Abstract

Cyclodextrin-modified electrokinetic chromatography (CD-EKC) was investigated for the separation of 12 monomethyl-benz[a]anthracene (MBA) isomers. Combined use of a polymeric surfactant, poly(sodium 10-undecenyl sulfate) (poly-SUS), with various types of neutral cyclodextrins (CDs) [β-CD, γ-CD, dimethyl-β-CD (DM-β-CD), trimethyl-β-CD (TM-β-CD) and hydroxypropyl-β-CD (HP-β-CD)] were successful in CD-EKC separation of the MBA isomers. Baseline resolution of 10 of the 12 isomers, except for 9-MBA and 2-MBA, was achieved with γ-CD at pH 9.75. The β-CD, γ-CD, and β-CD derivatives (DM-β-CD, TM-β-CD, HP-β-CD) were found to have different resolution and selectivity. Additionally, the $t_{b}/t_{o}$ values of isomers were found to be dependent on the type and concentration of the CD additives. In general, $t_{b}/t_{o}$ values of MBA isomers decrease with an increase in the concentration of β-CD derivatives, whereas the reversed was true when the concentrations of native β-CD and γ-CD were varied. The combination of 5 mM γ-CD, 0.5% (w/v) poly-SUS, 35% (v/v) acetonitrile at a pH of 9.75 provided the best selectivity and resolution of the MBA isomers with a separation time of 110 min. However, the use of 30 mM DM-β-CD under similar EKC conditions resulted in much faster separation (ca. 16 min) of 10 MBA isomers. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Monomethylbenz[a]anthracene (MBA) isomers are of great environmental concern owing to their carcinogenicity and biologically activity [1,2]. The MBA has 12 positional isomers. The position of the methyl functional group on benz[a]anthracene molecule plays a significant role on the carcinogenicity of the compound. For instance, 7-MBA, where methyl group is located in the seventh position on the benz[a]anthracene molecule, is the most carcinogenic isomers, whereas 5-MBA has the lowest carcinogenicity [1,3–6].

Gas chromatography (GC) and high-performance liquid chromatography (HPLC) [7–10] have been used for the separation and identification of the MBA isomers. However, neither of these techniques pro-
vides separation of all MBA isomers. Due to the lack of a charge, MBAs cannot be separated in a free solution by capillary zone electrophoresis (CZE). This obstacle can be overcome by using micellar electrokinetic chromatography (MEKC), which employs a charged micelle forming surfactant (i.e., sodium dodecyl sulfate, SDS) as pseudo-stationary phase [11,12]. The technique of MEKC is more common for water-soluble analytes [13]; however, in general, polycyclic aromatic hydrocarbons (PAHs) are highly hydrophobic compounds and are difficult to separate using purely aqueous MEKC. Therefore, other separation strategies have been used to overcome this problem: the use of bile salts [14,15], addition of an organic solvent [16,17], cyclodextrins (CDs) [18–20] or urea [21] to micellar solution. Since high content of organic modifier disrupts the micelles [22,23], it is not appropriate to use very high concentrations of organic solvent (e.g., >30% acetonitrile) with normal micelles.

Native CDs function as a pseudo-stationary phase for neutral compounds when used with charged micelles in MEKC. As introduced by Terabe and co-workers [24,25], CD modified MEKC (CD-MEKC) has been demonstrated to be useful for the separation of both chiral [25–28], and achiral [18–20,24,29] compounds. However, there is a disadvantage of CD-MEKC. Normal surfactant monomers in the running buffer will likely form inclusion complexes with CD molecules [30]. Thus, complexation of free surfactant monomer with CD will possibly interfere with complexation between the analyte and the CD, which may result in a poor separation. Another disadvantage of CD-MEKC is that the concentration of surfactant used in CD-MEKC has to be above the critical micellar concentration (CMC) to achieve effective separation. Apparently, a surfactant with a high CMC requires very high concentration of a charged surfactant in the MEKC buffer. This in turn generates excess Joule heating in the capillary. The heat production will inhibit the desired optimum separation.

Polymeric surfactants have been proposed as alternatives to normal micellar systems [31–36]. The polymeric surfactants have several advantages over normal micelles. First, they do not have a CMC. Thus, the use of polymerized surfactant as pseudo-stationary phase even at low concentrations is an advantage over normal micelles. This advantage can possibly be used to minimize Joule heating. Second, covalent linkage between the individual monomers in polymeric surfactants enhances the stability of polymer in high content of organic modifiers. Furthermore, unlike a normal micellar system, the covalent linkage in polymeric surfactant diminishes the formation of normal inclusion complex between monomers of surfactant and CDs [28]. For the chiral separation of Dns-DL-amino acids by CD-MEKC, butyl acrylate–butyl methacrylate–methacrylic acid copolymer sodium salt (BBMA) was found to be superior to SDS because of the absence of monomeric surfactant in the running buffer [37].

The issue of mass transfer effects on efficiency in MEKC has been of considerable debate in the literature. Davis [38,39] and Terabe et al. [40] concluded that mass transfer kinetics could be virtually considered as an insignificant source of band broadening in MEKC. In contrast, Nielsen and Foley [41] discuss resistance to mass transfer as one of the three basic sources of band broadening in MEKC. In addition, these authors also indicate that if the adsorption–desorption process of solute molecules to and from the pseudo-stationary phase is erratic, it may result in poor efficiency in MEKC. As discussed in several of our papers [31,42,43], due to the covalent linkage of polymeric surfactants, this adsorption–desorption process of hydrophobic solutes (e.g., PAHs, MBAs) is rapid mainly because these solutes do not penetrate deep into the micellar core.

The aim of this work was to study the possibilities of using two native CDs (β-CD, and γ-CD) and three derivatives of β-CD (dimethyl-, trimethyl-, and hydroxypropyl-β-CD) in combination with a polymeric surfactant, poly(sodium 10-undecenyl sulfate) (poly-SUS), to increase the selectivity for the separation of 12 MBA isomers. At present, there are only a few reports on the separation of MBA isomers. A separation of only four MBA isomers has been reported by Ding and Fritz [44]. In our previous work [36], six out of 12 MBA isomers (1-, 4-, 7-, 10-, 11-, and 12-MBA) were baseline separated using 0.5% (w/v) poly-SUS, 35% (v/v) acetonitrile, 12.5 mM phosphate–borate buffer at a pH of 9.5. Three pairs of the MBA isomers, that is, 2-MBA/8-MBA, 5-MBA/6-MBA and 3-MBA/9-MBA, co-eluted under these conditions. In the present study,
the background electrolyte composition was the same as reported in our previous work [36]. The concentrations of native β-CD and γ-CD as well as β-CD derivatives were varied at pH of either 9.5 or 9.75 to improve the resolution and selectivity of isomers. Shorter analysis times were achieved using a combination of poly-SUS and β-CD derivatives. However, better selectivity and resolution of 12 MBA isomers were gained by a combination of 5 mM γ-CD and 0.5% (w/v) poly-SUS, 35% (v/v) acetonitrile at a pH of 9.75.

2. Experimental

2.1. Materials

The detailed syntheses and polymerization of sodium 10-undecenyl sulfate (SUS) monomer is reported elsewhere [31]. Hydroxypropyl-β-CD (HP-β-CD) with an average degree of substitution of 0.8 hydroxypropyl groups per cyclodextrin ring was purchased from Aldrich (Milwaukee, WI, USA). Heptakis(2,6-di-O-methyl)-β-CD (DM-β-CD), and heptakis(2,3,6-tri-O-methyl)-β-CD (TM-β-CD) were obtained from Sigma (St. Louis, MO, USA). The β- and γ-CDs were a gift from American Maize-Products (Hammond, IN, USA). The HPLC-grade acetonitrile was purchased from Burdick and Jackson (Muskegon, MI, USA). Disodium tetraborate and disodium hydrogenphosphate were obtained from EM Science (Gibbstown, NJ, USA) and sodium hydroxide was purchased from Curtin Matheson Science (Houston, TX, USA). MBA isomers were kindly provided by Harold Seifred (Chemical and Physical Carcinogenesis Branch, NCI, Rockville, MD).

2.2. Equipment

All of the CE experiments were performed on a Beckman P/ACE model 5510 CE instrument (Fullerton, CA, USA). A fused-silica separation capillary [57 cm (50 cm to detector)×51 μm I.D.×361 μm O.D.], obtained from Polymicro Technologies (Phoenix, AZ, USA), was installed in a capillary cartridge and thermostated at 23°C by use of a fluoroorganic fluid. The detection of MBA isomers was carried out at 254 nm.

2.3. Procedures

2.3.1. Buffer and standard preparation

The pH of the stock solution of disodium tetraborate and disodium hydrogenphosphate (12.5 mM each) was adjusted to either 9.5 or 9.75 using 1 M NaOH and used as a background electrolyte (BGE). The running EKC solutions were prepared by dissolving 0.5% (w/v) poly-SUS and an appropriate amount of CD in a 12.5 mM borate-phosphate buffer. The pH values of all BGE samples were adjusted before addition of acetonitrile, poly-SUS and CD. Appropriate amounts of acetonitrile and triply deionized water were added to make final running buffer solutions. The running EKC solutions were sonicated and filtered through a 0.45-μm Nalgene Syringe filter (Rochester, NY, USA) before use. The stock standard MBA solutions were prepared in acetonitrile at a concentration of ca. 4 mM. The final concentration of each MBA isomer in a test mixture was ca. 0.3 mM. Due to the carcinogenicity of the MBA isomers, precautions were taken while handling stock solutions. The solutions of MBA isomers were prepared and diluted in a ventilated hood and stored in a closed container in a refrigerator. Disposable latex gloves were worn while working with MBA standards and care was taken to dispose of the waste solutions.

2.3.2. Electrokinetic chromatography

A new capillary was conditioned with 1 M NaOH for about 3 h at 40°C and then washed with water for about 30 min. Between injections, the capillary was conditioned for 3 min with the running buffer. The mixture of the MBA isomers was loaded on the capillary by applying 0.5 p.s.i. pressure (1 p.s.i. = 6894.76 Pa) on the injection vial for 1 s.

3. Results and discussion

When a hydrophobic solute is introduced into the CD-EKC system, it will partition between CD and the micelle. Due to the high hydrophobic character, the MBA isomers are retained in either the CD or the
micellar phase more than in the aqueous phase. The ratio of an MBA isomer incorporated into the micellar phase depends on its hydrophobicity; however, the inclusion complex formation between an MBA isomer and CD depends on the cavity size and hydrophobicity of CD. In addition, MBA isomers may possibly exchange directly between electroosmotically migrating CD and electrophoretically migrating micelle. We believe that this latter mechanism is dominant in this study and should be governing the tration of the HP-micelle. We believe that this latter mechanism is relative retention time was noticed as the concen-
tration of all

3.1.1. Effect of concentration of β-CD derivatives

Larger retention time (t_R/t_0) values were obtained at low concentration of all β-CD derivatives (data not shown). This indicates that at low concentrations of the β-CD derivative, the interaction between MBA isomers and the poly-SUS micelle is stronger than that of the β-CD derivatives. As the con-
centration of β-CD derivatives increased from 0 to 10 mM DM-β-CD and 0 to 20 mM TM-β-CD, t_R/t_0 values decreased sharply. Further increases in concentration (>20 mM) of these two β-CD derivatives showed only a slight decrease in t_R/t_0 values. The use of DM-β-CD, as an additive to poly-SUS, reduces the t_R/t_0 values of MBA isomers to a greater extent than for TM-β-CD. A gradual decrease in relative retention time was noticed as the concen-
tration of the HP-β-CD increased from 0 to 50 mM (data not shown). The reduction in t_R/t_0 values of MBA isomers is not surprising, because these β-CD derivatives are neutral and move with the electro-
smotic flow (EOF). As the concentration of β-CD derivative increases, stronger interactions between MBA isomers and CD will occur. Thus, a faster separation (i.e., reduction in t_R/t_0 values) is expected. Furthermore, changes in selectivity of MBA isomers were observed when different concentrations of β-CD derivatives were added to the running buffer solution (Fig. 1A–C). Fig. 1A–C shows not only the change in selectivity, but also the maximum number of peaks resolved at each concentration of β-CD derivative. Overall, the maximum resolved MBA isomers at 0, 5, 10, 20, 30 and 50 mM β-CD derivative are 9, 7, 7, 8, 10, and 10 with DM-β-CD; 9, 6, 5, 7, 8, and 8 with TM-β-CD; and 9, 5, 10, 7, 8, and 8 with HP-β-CD.

3.1.2. Effect of type of β-CD derivatives

Fig. 2A–C shows the separation profiles of the MBA isomers under the optimum conditions of each β-CD derivative. The optimum concentrations were found to be 30 mM each of DM-β-CD and TM-β-CD, and 15 mM HP-β-CD. Better resolution with shorter analysis time was observed with DM-β-CD compared to TM-β-CD at the same concentration (Fig. 2A,B). The total number of peaks resolved was 10 and 8 using 30 mM each of DM-β-CD and TM-β-CD, respectively. TM-β-CD is relatively more methylated than DM-β-CD. It is more likely that MBA isomers have relatively stronger interaction with the hydrophobic cavity of DM-β-CD than with TM-β-CD, probably due to the steric effect of the methyl group on the CD molecule. It is assumed that the presence of more methyl groups may have given TM-β-CD unfavorable structure than DM-β-CD for best complexation with the MBA isomers. Hydroxy-
Fig. 1. Migration order of 12 MBA isomers as a function of β-CD derivative concentrations. (A) DM-β-CD, (B) TM-β-CD, and (C) HP-β-CD. EKC conditions: 0.5% (w/v) poly-SUS and 35% (v/v) acetonitrile in 12.5 mM each of Na₂HPO₄ and Na₂B₄O₇ buffered at pH 9.5; pressure injection for 1 s, +25 kV applied voltage; UV detection at 254 nm. Curve identification: the numbers shown on the right of each plot denote the MBA isomers.

Fig. 2. Electropherograms showing the separation of 12 MBA isomers using optimum concentration of β-CD derivatives. (A) 30 mM DM-β-CD, (B) 30 mM TM-β-CD, and (C) 15 mM HP-β-CD. Peak identifications: (1) 1-MBA, (2) 2-MBA, (3) 3-MBA, (4) 4-MBA, (5) 5-MBA, (6) 6-MBA, (7) 7-MBA, (8) 8-MBA, (9) 9-MBA, (10) 10-MBA, (11) 11-MBA, and (12) 12-MBA. EKC conditions are the same as Fig. 1 except fixed concentration of each β-CD derivatives.

Propyl groups of HP-β-CD might have prevented the MBA isomers from penetrating into the CD cavity. Thus, the MBA isomers interact more strongly with poly-SUS than with HP-β-CD. As a result, longer migration times are observed even with 15 mM HP-β-CD (Fig. 2C) as compared to 30 mM DM-β-CD or TM-β-CD (Fig. 2A,B). It should be noted that, under optimum conditions, the separation window (i.e., retention time from the first MBA peak to the last MBA peak) was wider (~15 min) with HP-β-CD than that with DM-β-CD (~2.7 min) and TM-β-CD (~2.0 min). Moreover, some selectivity differences were observed using DM-, TM- or HP-β-CD. For example, the resolution and selectivity between 4-MBA and 2-MBA and that between 1- or
7-MBA versus 11-MBA isomers is significantly higher with DM-β-CD than with TM-β-CD. In addition, 12-MBA isomer comigrated with 1- and 7-MBA as the second to last peak with DM-β-CD. On the other hand, the same isomer eluted ahead of 1- and 7-MBA but comigrated with 6- and 8-MBA isomers using TM-β-CD. Moreover, reversal in migration order of 4- and 5-MBA was observed using HP-β-CD instead of DM-β-CD or TM-β-CD. Note that, 2-MBA eluted much later (as second to last peak) with HP-β-CD than with DM-β-CD. As discussed in a number of recent review papers, the chemical modification of native CDs has significant effects on the hydrogen bond ability and physical properties as well as on the shape and size of their cavities [45–47]. Thus, it is more likely that different substituted groups on native β-CD would give different selectivity and retention behavior due mostly to steric effects of substituted groups. Nuclear magnetic resonance (NMR) spectroscopy may be helpful for a better understanding of the interaction mechanisms using β-CD derivatives. As shown in Fig. 2, a complete separation of all 12 isomers was not successful with any of the β-CD derivatives.

3.2. Separation selectivity of MBA isomers using native β-CD and γ-CD

Since MBA isomers are large hydrophobic compounds, β-CD and γ-CD were chosen for their larger cavity diameters (6.6 and 8.4 Å, respectively). As reported earlier without β-CD or γ-CD, nine of 12 MBA isomers were resolved using poly-SUS [36]. By addition of 2 mM of β-CD to the mixture of 0.5% (w/v) poly-SUS, 35% (v/v) acetonitrile and 12.5 mM each of borate and phosphate buffer containing running solution, $t_R/t_0$ value reduced slightly and a shift in migration order of some MBA isomers was observed (Fig. 3A). However, no improvement in overall resolution was observed. Although 2-MBA/8-MBA and 5-MBA/6-MBA isomer pairs were resolved at 2 mM β-CD, some other isomers co-migrated. For instance, 8-MBA co-eluted with 4-MBA and 6-MBA (fourth peak); 10-MBA co-eluted with 11-MBA (sixth peak); and 9-MBA and 3-MBA remained unresolved (last peak) (Fig. 3A,B). Addition of 5 mM β-CD increased $t_R/t_0$ values resulting in the separation of 10 MBA isomers and resolution of 9-MBA/3-MBA isomers. Note that at this concentration of β-CD, 8-MBA was also separated from 4-MBA and 6-MBA. However, still the peak pairs of 4-MBA/6-MBA and 2-MBA/11-MBA could not be resolved. Although $t_R/t_0$ values were decreased with further increases in β-CD concentration to 7 mM and leveled off at 10 mM, the number of resolved peaks was decreased to 9 and 7, respectively (Fig. 3B). The unexpected behavior of $t_0$
and \( t_r/t_0 \) using \( \geq 2 \) mM \( \beta \)-CD is not clear. One possible source can simply be the uncertainties in measuring \( t_r \) that lead to significant error in determining \( t_r/t_0 \) values (Fig. 3A and inset). It is worth mentioning that the relative standard deviation (RSD) of migration time reproducibilities in presence of \( \beta \)-CD was much higher (~10%) compared to other CDs. The RSD values for DM-\( \beta \)-CD, TM-\( \beta \)-CD, HP-\( \beta \)-CD were <2% and that for \( \gamma \)-CD was 4.6%. The optimum \( \beta \)-CD concentration was found to be 5 mM, which resulted in a separation of 10 MBA isomers (Fig. 4).

Addition of 2 mM \( \gamma \)-CD to BGE showed a decrease in \( t_r/t_0 \) values, but did not improve the resolution of the MBAs (Fig. 5A). The isomers of 12-MBA, 1-MBA, and 7-MBA eluted as the first, second, and third peak, respectively, and remained in the same order at all \( \gamma \)-CD concentrations. At 0 mM \( \gamma \)-CD, 2-MBA co-migrated with 8-MBA (fifth peak) and 5-MBA co-migrated with 6-MBA (seventh peak). The migration order changed at 2 mM \( \gamma \)-CD. For example, 8-MBA co-migrated with 11-MBA (fourth peak) and 5-MBA co-migrated with 10-MBA (seventh peak). On the other hand, 6-MBA and 2-MBA eluted separately as sixth and eighth peak, respectively (Fig. 5A,B). Upon addition of 3 mM \( \gamma \)-CD, migration order remained the same except 5-MBA, 10-MBA, and 3-MBA were resolved and eluted as the seventh, eighth, and ninth peaks, respectively, whereas 9-MBA and 2-MBA co-migrated as the tenth peak. However, at 5 mM \( \gamma \)-CD all but two of the MBA isomers were baseline resolved. The 9-MBA and 2-MBA were partially resolved (Fig. 6B). A further increase in \( \gamma \)-CD concentration to 7 mM deteriorated the resolution of some MBA isomers. As seen in Fig. 5A, an increase in \( \gamma \)-CD concentration from 2 to 7 mM caused an increase in both \( t_r/t_0 \) and \( t_0 \). The increase in \( t_0 \) can be attributed simply to an increase in viscosity as the concentration of \( \gamma \)-CD increased (see Fig. 5A, inset). In general, an increase in \( \gamma \)-CD concentration decreases the retention time [18]; however, for some hydroxy-
This behavior was explained in our previous work [36]. That is, at higher pH values, poly-SUS has a relatively more open and hydrophobic structure, which causes a stronger interaction between poly-SUS and MBA isomers, resulting in an increase in retention time. Furthermore, an increase in pH increases the ionic strength of the buffer solution, which reduces the zeta potential on the capillary surface. Similar behavior was also observed when \(\beta\)-CD was added to poly-SUS. However, under such conditions an increase in pH did increase the retention time, but did not increase the number of resolved MBA isomers (data not shown). An attempt to increase the resolution of the last two peaks (i.e., 2-MBA and 9-MBA) by addition of 0.5–2\% (v/v) 2-propanol or \(n\)-butanol to running EKC buffer (5 mM \(\gamma\)-CD, 0.5\% poly-SUS) was not successful. For example, addition of 1\% 2-propanol to the running EKC buffer (containing 34\% (v/v) acetonitrile) increased the total analysis time to ~200 min. However, a slightly better resolution of 9-MBA and 2-MBA was observed (data not shown). Further investigation is underway using different buffer composition and organic modifier type. Our ultimate goal is to further improve the resolution and to obtain shorter analysis time of the MBA isomers.

4. Conclusions

A combination of poly-SUS and three \(\beta\)-CD derivatives (i.e., DM-\(\beta\)-CD, TM-\(\beta\)-CD, and HP-\(\beta\)-CD) as well as native \(\beta\)-CD and \(\gamma\)-CD was investigated to separate 12 MBA isomers. The \(\beta\)-CD, \(\gamma\)-CD and three \(\beta\)-CD derivatives were found to have different resolution and selectivity. Additionally, the analysis time of isomers was found to be dependent on the type and concentration of the CD additives. Relatively shorter analysis times were achieved using \(\beta\)-CD derivatives comparing to native \(\beta\)-CD and \(\gamma\)-CD. This is an indication of a stronger complexation between MBA isomers and \(\beta\)-CD derivatives, which can be attributed to the fact that \(\beta\)-CD derivatives have deeper cavities compared to native \(\beta\)-CD [48]. The \(t_{Rm}/t_{Ro}\) values were decreased as the concentration of \(\beta\)-CD derivatives increased, whereas the opposite effect was observed with native \(\beta\)-CD and \(\gamma\)-CD. A combination of 5 mM \(\gamma\)-CD,
0.5% (w/v) poly-SUS, 35% (v/v) acetonitrile at a pH of 9.75 provided the best selectivity and resolution of the 12 MBA isomers. However, a total separation time was about 110 min. Alternatively, combined use of poly-SUS and DM-β-CD resulted in a relatively faster separation (ca. 16 min) of MBA isomers. This occurred only at the expense of co-migration of some MBA isomers.

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