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Evaluation of newly synthesized and commercially available charged cyclomaltooligosaccharides (cyclodextrins) for capillary electrokinetic chromatography

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Abstract—A highly new charged cyclodextrin (CD) derivatives, (6-*O*-carboxymethyl-2,3-di-*O*-methyl)cyclomaltoheptaoses (CDM-β-CDs), was synthesized and characterized as anionic reagents for capillary electrophoresis (CE) in an electrokinetic chromatography mode of separation. Substitution with dimethyl groups at the secondary hydroxyl sites of the CD is aimed at influencing the magnitude and selectivity of analyte–CD interactions, while substitution by carboxymethyl groups at the primary hydroxyl sites provides for high charge and electrophoretic mobility. Full regioselective methylation at the secondary hydroxyl sites was achieved in this work, while substitution at the primary hydroxyl sites generated a mixture of multiply charged products. The separation performance of CDM-β-CD was evaluated using a variety of analyte mixtures. The results obtained from commercially available negatively charged cyclodextrins, heptakis(2,3-di-*O*-methyl-6-*O*-sulfo)cyclomaltoheptaose (HDMS-β-CD) and *O*-(carboxymethyl)cyclomaltoheptaose (CM-β-CD) with an average degree of substitution one (DS 1), were compared to CDM-β-CD using a sample composed of eight positional isomers of dihydroxynaphthalene. Four hydroxylated polychlorobiphenyl derivatives, a group of chiral and isomeric catchecins, and chiral binaphthyl compounds were also separated with CDM-β-CD. The effect of adding neutral β-cyclodextrin (β-CD) into the running buffer containing charged cyclodextrins was investigated and provided evidence of significant inter-CD interactions. Under certain running buffer conditions, the charged cyclodextrins also appear to adsorb to the capillary walls to various degrees

Keywords: Heptakis(6-*O*-carboxymethyl-2,3-di-*O*-methyl)cyclomaltoheptaose; Neutral positional isomers; Capillary electrophoresis; Chiral separations; Characterization

1. Introduction

The use of macrocyclic reagents in capillary electrophoresis (CE) as running buffer additives has been proven to improve separation selectivity for a variety of structurally similar analytes. Examples of these macrocyclic molecules are cyclodextrins (CDs), crown ethers,[1] calixarenes,[2] and certain classes of antibiotics.[3] The most popular group of macrocyclic running buffer additives are the cyclodextrins due to their unique selectivity,[4] fair to good water solubility, and an ability to produce many CD derivatives via synthetic methods. They are extensively used for separations of a variety of analytes in CE.[5–11]

Injected analytes migrate in CE within a narrow-bore capillary based on their intrinsic electrophoretic mobility or an acquired mobility involving association with

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a charged reagent added to the CE running buffer. The intrinsic or acquired mobility is generally further modified by the mobility (flow) of the running buffer, referred to as an electroosmotic mobility. In most cases, the electroosmotic mobility is toward the cathode side of the CE system. When association with a running buffer additive is involved, an elution window, within which injected analytes may elute, is often observed. Such CE approaches are electrochromatographic in nature with the running buffer additive functioning as a pseudo-chromatographic phase. The separation power of CE techniques is large if high efficiency and wide elution windows are observed.

CDs are derivatized for several reasons: for example, to vary solubility, change complexation properties, and introduce certain functional groups.[12,13] Derivatization of CDs in CE is targeted to influence complexation properties, introduce a charged group, or increase solubility in the CE running buffer. Both charged and neutral CD derivatives have been synthesized and employed for electrophoretic separations of a variety of analytes.[6,14] Unfortunately, the resulting products are generally a complex mixture with varying degrees and loci of substitution. The presence of many hydroxyl groups in the CD molecule with similar reactivity complicates the synthesis of CD derivatives as single-isomer reagents. Single-isomer products, however, will perform more predictably and reproducibly when used as CE running buffer additives.

Synthetic strategies can be devised for the synthesis of single-isomer CD derivatives.[13] As an example of one approach, the primary hydroxyl groups can be initially regioselectively reacted with a protecting group. Subsequently, the secondary hydroxyl groups are reacted to produce the desired selectivity and to protect them from fuctionalization during further synthetic steps. Then, the primary hydroxyl groups are deprotected and fully functionalized with a charged group. Following this type of strategy, Vigh and co-workers reported the synthesis of a series of highly charged, single-isomer, sulfo CDs that were shown to be superior reagents for CE separations of a variety of chiral molecules.[5,15,16]

In this study, we report the synthesis of a set of (6-*O*-carboxymethyl-2,3-di-*O*-methyl)cyclomaltoheptaose (CDM-β-CDs) congeners variously substituted at O-6 and illustrate their utility for separations of a variety of analytes. The synthesis of the CDM-β-CDs was complicated due to the inefficiency of the reaction between the primary hydroxyl groups of β-CD and ethyl diazoacetate in one of the latter steps of the synthesis. Thus, the CDM-β-CD was produced as a mixture of several isomers. The separation performance of the CDM-β-CD thus produced was compared to the results obtained from commercially available negatively charged cyclodextrins, heptakis(2,3-di-*O*-methyl-6-*O*-sulfo)cyclomaltoheptaose (HDMS-β-CD) and *O*-(carboxymethyl)cyclomaltoheptaose (CM-β-CD) with an average degree of substitution of one (DS 1), by using eight positional isomers of dihydroxynaphthalene (DHNs). Further, four hydroxylated polychlorobiphenyl (PCB) congeners, a group of chiral and isomeric catechecins, and chiral binaphthyl compounds were successfully separated. The limitations and advantages of all these charged CDs are discussed in terms of separations, and capillary surface and inter-CD interactions.

2. Materials and methods

2.1. Synthesis of (6-*O*-carboxymethyl-2,3-di-*O*-methyl)-cyclomaltoheptaose

The synthetic scheme to produce CDM-β-CD is shown in Scheme 1. ^{13}C, ^{1}H, and 2D NMR experiments that is, COSY and HSQC, were performed using Varian Inova 600 MHz and Mercury 300 MHz NMR instruments for the characterization of the materials. All intermediates and final product gave ^{1}H and ^{13}C NMR spectra that matched the literature as cited.[17–19]

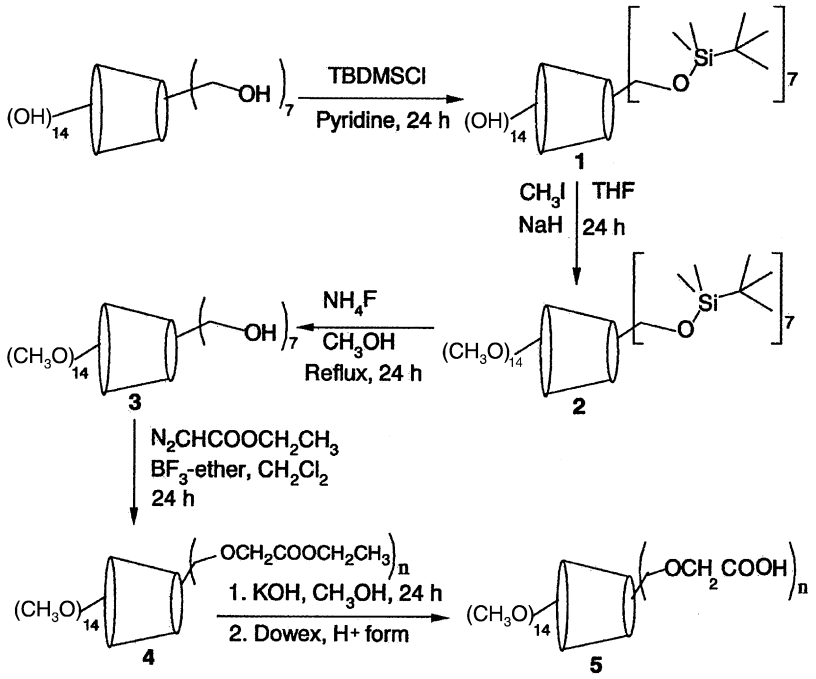
2.1.1. Heptakis(6-*O*-*tert*-butyldimethylsilyl)cyclomaltoheptaose (1). β-Cyclodextrin was treated with *tert*-butylchlorodimethylsilane according to the previously reported procedure.[17]

2.1.2. Heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)cyclomaltoheptaose (2). Intermediate **1** was methylated by first treating it with NaH and then with iodomethane as previously described.[18] This product was purified by preparative column chromatography on silica gel using an *n*-hexane–EtOAc mobile phase.

2.1.3. Heptakis(2,3-di-*O*-methyl)cyclomaltoheptaose (3). The chromatographically purified intermediate product **2** was treated with NH_4F overnight to remove the *tert*-butyldimethylsilyl group.[19] Product **3** that was obtained was purified by preparative column chromatography on silica gel using a $CHCl_3$–MeOH mobile phase.

2.1.4. (6-*O*-Ethoxycarbonylmethyl-2,3-di-*O*-methyl)cyclomaltoheptaose (4). Compound **3** was treated with ethyl diazoacetate in the presence of $BF_3{\cdot}OEt_2$.[19] The resultant material was chromatographed on silica gel using a $CHCl_3$–MeOH mobile phase.

2.1.5. (6-*O*-Carboxymethyl-2,3-di-*O*-methyl)cyclomaltoheptaose (5). The ester units in intermediate **4** were hydrolyzed by treatment with 1 M KOH solution.[19] The final product **5** was collected as a potassium salt, and passage of the product through Dowex 50W-X4 column until the eluent was neutral provided the carboxylic acid.



Scheme 1. The synthesis scheme for (6-*O*-carboxymethyl-2,3-di-*O*-methyl)cyclomaltoheptaose.

2.2. Materials

Sodium hydride, ethyl diazoacetate, boron trifluoride etherate, iodomethane, ammonium fluoride, potassium hydroxide, Dowex 50W-X4, 1,5-, 1,6-, 1,7-, 2,3-, 2,6-, and 2,7-dihydroxynaphthalene (1,5-, 1,7-, 2,3-, 2,6-, and 2,7-DHN), and (±)-1,1′-bi-2-naphthol were obtained from Aldrich Chemical Co. (Milwaukee, WI). 1,4-DHN and phthalic acid were obtained from Eastman Chemical Co. (Rochester, NY), and 1,3-DHN was obtained from Pfaltz & Bauer Co. (Stamford, CT). β-CD, (+)-catechin (C), (−)-epicatechin (EC), (−)-epigallocatechin (EGC), and tris(hydroxymethyl)aminomethane (Tris) were obtained from Sigma Chemical Co. (St. Louis, MO). 2-Chloro-4-hydroxybiphenyl (4-OH-2-PCB), 3-Chloro-4-hydroxybiphenyl (4-OH-3-PCB), 2,5-dichloro-2-hydroxy (2-OH-2,5-PCB), and 2,5-dichloro-3-hydroxy (3-OH-2,5-PCB) were obtained from AccuStandard, Inc. (New Haven, CT). *O*-(Carboxymethyl)cyclomaltoheptaose (CM-β-CD) and the sodium salts of heptakis(2,3-di-*O*-methyl-6-*O*-sulfo)cyclomaltoheptaose (HDMS-β-CD) were obtained from Regis Technologies, Inc. (Morton Grove, IL). The CE buffers were prepared with distilled, deionized water, and reagent grade NaH_2PO_4 (Sigma), and adjusted to the required pH with NaOH or H_3PO_4.

2.3. Preparation of running buffer

A common running buffer (10 mM phosphate, 8 mM CD, pH 5.0) was used as the basis for all CE separations. Although the phosphate buffer has weak buffer capacity at pH 5.0, the separation of the analytes was best at this pH. Fresh running buffer was frequently used to offset changes occurring in pH with run times. The identity of the CD, however, was varied throughout with the exception of the buffer used for indirect detection. The pH of the solutions was adjusted with dilute NaOH or H_3PO_4 and monitored with an Orion Model SA520 pH meter operating under a two-standard calibration. The running buffer was sonicated, filtered through a 0.22 μm nylon filter, and finally placed under vacuum to remove dissolved gases. To slow the growth of microorganisms, solutions were stored in the refrigerator.

2.4. Sample preparation

The samples were prepared by pipeting small amounts of the analytes in water (in methanolic stock solutions if not sufficiently soluble in water) into the running buffer, or a mixture of the running buffer with 20–40% MeOH. Figure 1 shows the analytes used in this study. Sample solutions contained solutes at concentrations ranging from 10^{-6} to 10^{-5} M.

2.5. Measurement of EOF

In order to investigate the degree of apparent CD–capillary wall interactions and its consequences on electroosmotic flow (EOF), 8 mM solutions of CDM-β-CD and HDMS-β-CD in 10 mM NaH_2PO_4 at pH 5.0 were prepared, and mesityl oxide (MO) was injected as a neutral marker three times without any flush procedures between injections. The run time for each injection was 20 min.

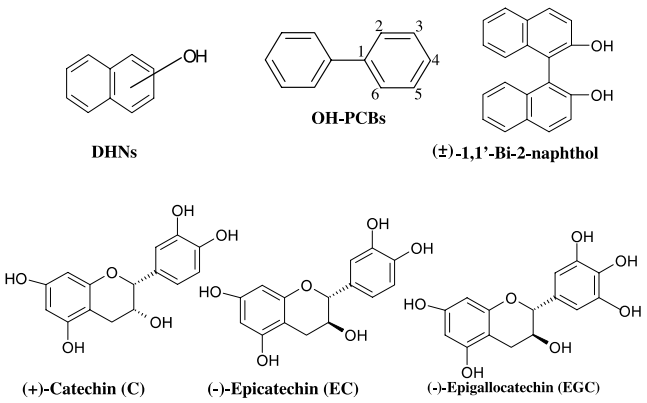


Figure 1. Structures of the analytes used in test separations.

The observed electrophoretic currents of the CDM-β-CD and HDMS-β-CD running buffer solutions were matched by adding an appropriate amount of solid NaCl.

2.6. Apparatus

All CE experiments were performed using a Hewlett-Packard HP^{3D}CE automated capillary electrophoresis instrument interfaced to an HP Pentium I personal computer. Fused silica capillaries (50 μm i.d. × 360 μm o.d.) were obtained from Polymicro Technologies, Inc. (Phoenix, AZ). The capillaries were cut to a total length of 48 cm, and approximately 1 cm of polyamide coating centered at 39.5 cm was removed to create an optical detection window.

2.7. Separation conditions

Injection was achieved by the application of 10 mbar of pressure for 6 s to the inlet buffer vial. The applied potential for all CE experiments was 15 kV. UV absorbance detection was performed at wavelengths ranging from 205 to 254 nm for all the test solutes. All experiments were conducted at room temperature (24 ± 1 °C).

2.8. CE with indirect detection for the determination of the mobility and isomeric purity of charged CDs

Because CDs do not absorb radiation appreciably in the UV region, indirect absorbance detection was used to determine both the electrophoretic mobility and isomeric purity of the synthesized CDM-β-CD. A 20 mM aqueous solution of phthalic acid (background electrolyte) was prepared, and its pH was adjusted to pH 8.5 with Tris. The buffer was sonicated, filtered, and degassed as described above. A 600 V/cm field was applied, and absorbance was monitored at 254 nm during the separation. The conditions for injecting CDM-β-CD were identical to those described above for the analyte mixtures. An electropherogram for CE with indirect detection is shown in Figure 2. The negative peaks are migrating zones wherein background electrolyte is displaced by the negatively charged CDM-β-CD isomers.

2.9. Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS)

Mass spectral data were obtained with a Voyager DE TOF (PerSeptive Biosystems, Framingham, MA). The following conditions were used for MALDI-TOF experiments: reflectron mode, 20 kV accelerating voltage, 75% grid voltage, 0.050 guide wire voltage, and 140 μs delay time. 2,4,6-Trihydroxyacetophenone (THAP) was used as matrix for the experiments. A 10 mg/mL stock solution of matrix components was prepared in $CHCl_3$. An aliquot (~10 μL) of this stock solution and the CD derivative solution were mixed and applied onto the target stage.

2.10. Safety

Because almost all solutes are severe irritants and their effects on living organisms are not known, caution should be exercised while working. Disposable latex

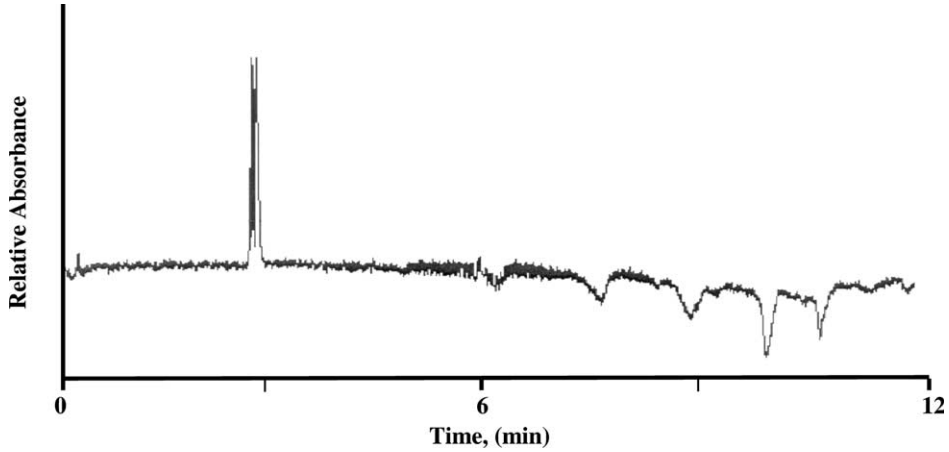


Figure 2. CE with indirect detection electropherogram of injected CDM-β-CD (see Materials and methods section for details).

gloves were worn while working with all solvents and reagents, and waste sample solutions were properly disposed.

3. Results and discussion

The presence of chemically fairly similar hydroxyl groups at the 2-, 3-, and 6-carbon positions of CDs complicates their selective modification. The primary hydroxyl groups at the 6-position are less hindered than the ones at the 2- and 3-positions. Comparing the secondary hydroxyl sites, the groups at the 3-position are least accessible. The subtle reactivity difference at 6-, and the 2- and 3-positions can be invoked in attempts to selectively modify CDs via regioselective reactions. The C-6 hydroxyl groups can selectively react with electrophilic reagents such as *tert*-butylchlorodimethylsilane (TBDMSCl) under anhydrous conditions due to their easier accessibility.[13] Even though this reagent targets the hydroxyl groups at the 6-position, its use in excessive amount results in undesired modification of the hydroxyl groups at other positions, particularly at C-2. After the hydroxyl groups at the 6-position are blocked, the hydroxyl groups at the 2- and 3-positions can be fully reacted with groups that impart the desired analyte–CD selectively. Optimization of reaction conditions such as temperature, choice of reagent, and solvent are important. Note that complications can arise if the cyclodextrins form complexes with reagents used for modification reactions.

We have previously used neutral CDs in conjunction with anionic CDs for CE separations of neutral analytes.[11,20] Since separations were governed by the distribution of the analytes between the various types of CDs in the running buffer, we refer to this technique as cyclodextrin distribution CE (CDCE). In principle, CDCE is a powerful separation technique that allows for fine-tuning of the running buffer to achieve optimal separations by adjusting the types and relative concentrations of the CDs employed. A fairly wide elution window is desired, and this can be accomplished when the charged CD has a large mobility (possesses a large negative charge) and EOF is made moderately slow.[4,20] Under the desired conditions, the negatively charged CD exhibits a mobility that is opposite in sign and nearly equal to electroosmotic mobility, and detection of all neutral analytes is at the cathode side of the system. Importantly, the method most commonly employed to reduce EOF is to reduce the running buffer pH to protonate many of the silanol groups on the surface of the capillary.

Although the sulfo-based highly charged CDs provide for elegant separations of many species,[15,17,21–23] a couple of limitations were encountered when they were used with the CDCE technique for separations of moderately hydrophobic analytes. First, at the pHs that produced desirable elution windows, EOF was erratic and generally decreased dramatically with time. We and other researchers have ascribed this to hydrogen-bonding interactions between the charged CD and silanol groups on the capillary wall,[20,24,25] thereby influencing the zeta potential that governs the magnitude of EOF. For example, it was found that using a running buffer employing 8 mM HDMS-β-CD at pH 5, a neutral EOF marker (MO) increased in migration time from 4.4 to 9.6 to 15.9 min for three injections made in succession. When single injections are performed, or higher pHs are used, the flow irregularity may not be problematic.

The wall interaction problem may be related to the very strong hydrogen-bonding capabilities of the sulfo groups, and this property may have been responsible for a second observation. When using the CDCE technique, predicable separations require that the CDs do not strongly interact with each other; that is, the CDs must act independently to influence analyte migration. However, CDs in aqueous systems are known to weakly aggregate through a network of weak hydrogen bonds.[26] For CDCE, as neutral CD concentration is increased relative to charged CD, analytes increasingly interact with the more rapidly migrating (at EOF rate) neutral CD and the observed analyte migration times will decrease.[20] However, it was observed that for analytes known to interact with β-CD, increasing β-CD concentration relative to HDMS-β-CD in CDCE had little effect on the observed migration times. It was postulated that interactions between the sulfo groups of the charged CD and the secondary hydroxyls of the β-CD could limit access to the cavity of the CD.

The CDM-β-CD was synthesized as described in the experimental section. ^{13}C and ^{1}H NMR chemical shifts of intermediates and final purified product were found to be in good agreement with previously reported chemical shifts.[17–19] The isomeric purity of the final charged CD was monitored using CE with indirect detection and 2D NMR experiments. During the synthesis procedure, the isomeric purity of each intermediate was also monitored with MALDI-TOF MS. The MALDI spectrum in Figure 3 indicates that heptakis(2,3-di-*O*-methyl)cyclomaltoheptaose (**3**) was successfully synthesized and purified as a single isomer. The calculated and measured base-isotope m/z values for Na^+ quasimolecular ion cluster are $m/z_{cal} = 1353.58$ and $m/z_{meas} = 1354.05$, and for K^+ quasimolecular ion cluster are $m/z_{cal} = 1369.69$ and $m/z_{meas} = 1370.02$, respectively.

The intermediate, single-isomer heptakis(2,3-di-*O*-methyl)cyclomaltoheptaose (**3**) was used as starting material for the synthesis of the highly charged CD derivative. Because our goal was to synthesize a highly charged single-isomer β-CD derivative, it was important

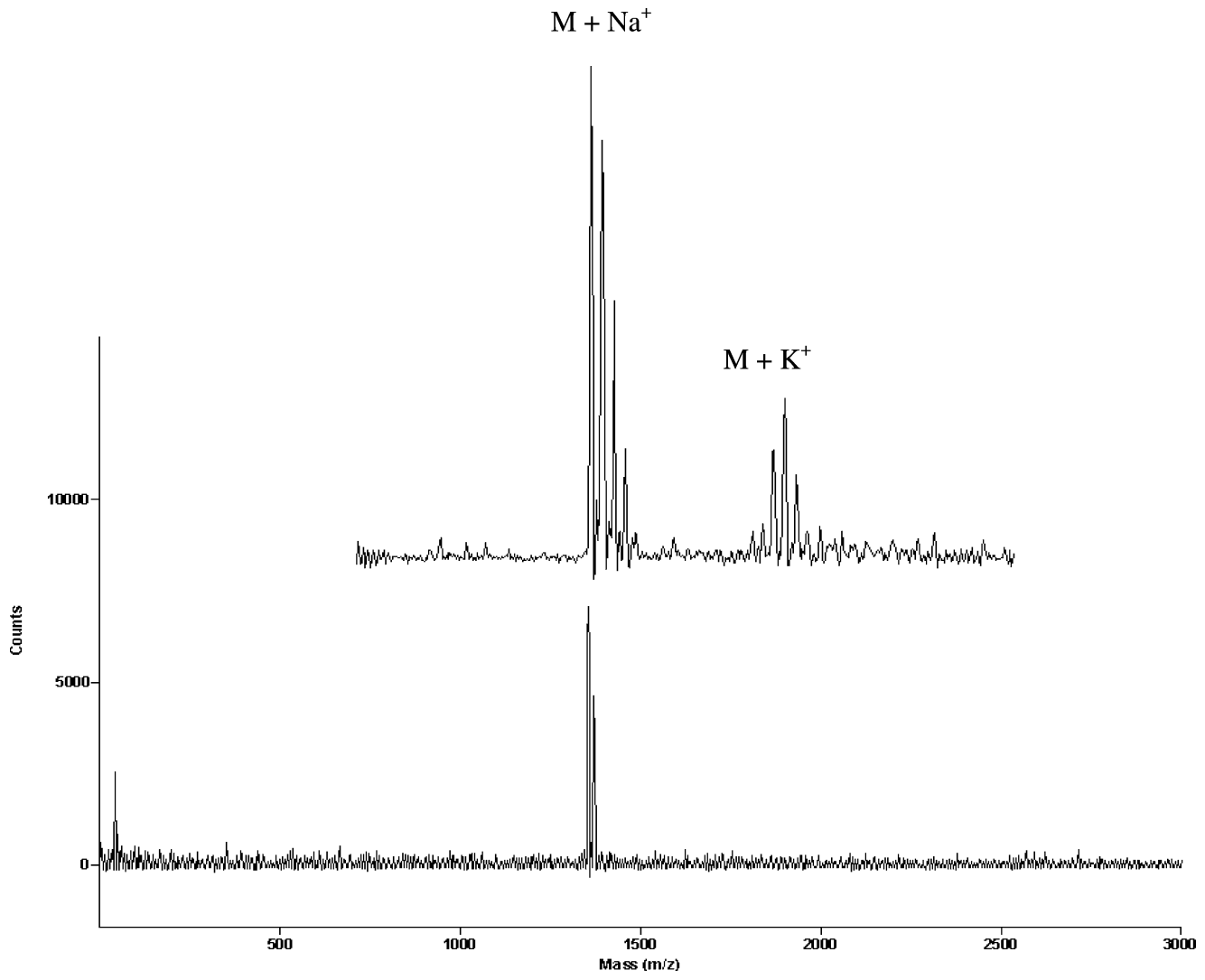


Figure 3. High resolution MALDI-TOF mass spectrum of heptakis(2,3-di-*O*-methyl)cyclomaltoheptaose (**3**) in THAP as matrix using chloroform as matrix solvent.

to start with a single isomer starting material. As seen in Scheme 1, the step after the synthesis of **3** involves the attachment of an ethoxycarbonylmethyl group to the primary hydroxyl groups of β-CD. Although reported in the literature,[19] it was found that this reaction was not efficient for attachment of an ethoxycarbonylmethyl group to the primary hydroxyl groups. Even though this reaction step was repeated several times for the same batch, it was observed to not completely functionalize the primary hydroxyl groups. This could be due to difficult accessibility of the hydroxyl groups as the reaction proceeds. The two techniques, MALDI-TOF MS and CE with indirect detection, were used to monitor the isomeric purity of the final product. Attempts to obtain an interpretable MALDI spectrum for the final product were not successful. However, the indirect detection electropherogram seen in Figure 2 indicates that the final product is composed of several isomers with differing degrees of substitution, and the degree of substitution at the primary hydroxyl sites is denoted with '*n*'.

The possible interactions of CDM-β-CD with the capillary wall or neutral native CD were also experimentally evaluated. Using the same procedure described in the EOF delay experiment, tests were performed using an 8 mM CDM-β-CD running buffer. The neutral EOF marker (MO) was found to increase in migration time from 5.87 to 6.19 to 6.37 min for three injections made in succession. Thus the MO peak was delayed only about 9% with the use of CDM-β-CD, but 350% with the use of HDMS-β-CD as the running buffer additive (see above). These results show that interaction of CDM-β-CD with the capillary wall is less severe than those interactions experienced when using HDMS-β-CD at the pHs required for CDCE.

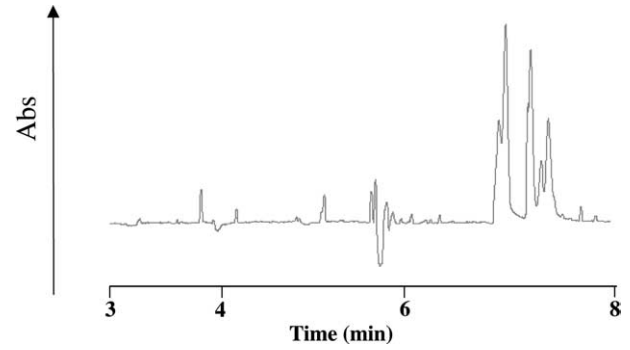


Figure 4. The effect on a separation of DHNs of adding 8 mM β-CD into 8 mM CDM-β-CD running buffer. The separation conditions are the same as described for Figure 2.

A CDCE system was applied to the separation of DHNs. Figure 4 is a separation employing 8 mM of both β-CD and CDM-β-CD. Association with the β-CD, which is migrating with EOF, should decrease the migration time of the solutes. Also, a large enough ratio of neutral to charged CD should converge the analytes at the effective migration time of EOF. By comparison of Figure 4 with Figure 5B, it is clear that such control of separation behavior is possible with a CDCE system employing β-CD and CDM-β-CD.

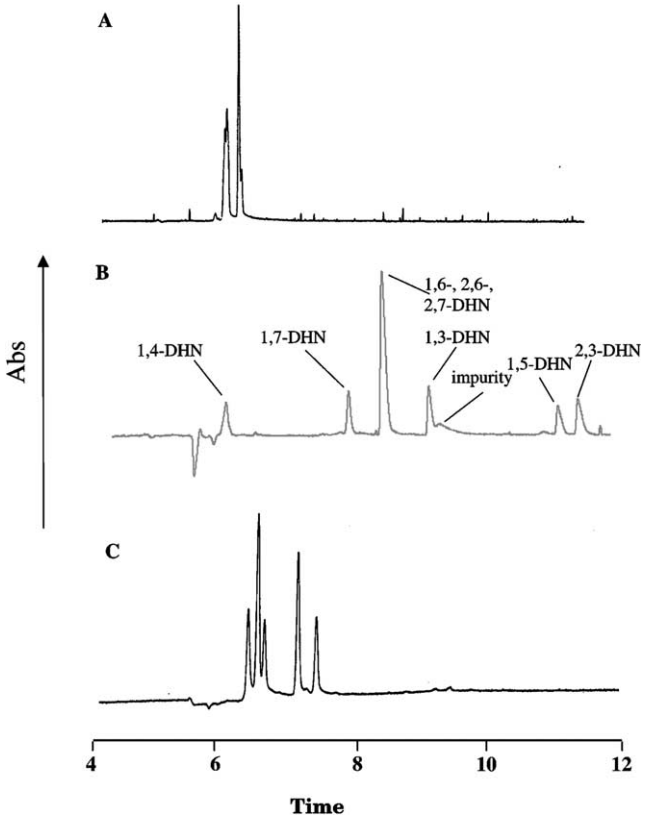


Figure 5. The separation of eight isomers of dihyroxynaphthalene (DHNs) with (A) CM-β-CD, (B) CDM-β-CD, (C) HDMS-β-CD. The running buffer: 8 mM CD in 10 mM phosphate buffer at pH 5.0. Capillary 50 μm i.d., 39/48 cm effective/total length. Detection wavelength: 225 nm. Applied voltage: 15 kV.

A variety of structural isomers and chiral analytes were chosen to evaluate the efficacy of CDM-β-CD as a resolving agent in CE. These compounds are neutral at the running buffer pHs used in these experiments. Most of the analytes have a hydrophobic moiety that should fit into the cavity, and a substitution pattern, which should allow but influence inclusion. Therefore, it is reasonable to expect some separation of these structurally similar, neutral species using charged CDs as resolving agents.

Figure 5 shows the separation of eight DHNs with CM-β-CD (A), CDM-β-CD (B), HDMS-β-CD (C). The singly charged CM-β-CD, which produces a very narrow elution window, was not very effective at separating the eight DHNs despite what are expected to be reasonably large inclusion constants for these analytes. However, six well-resolved peaks for the eight DHNs were generated with CDM-β-CD. The elution order shown in Figure 5B provides an indication of the relative affinity of the DHNs for the CDM-β-CD cavity. 2,3-DHN was the species with the greatest exposure of the naphthalene moiety and eluted last (greatest affinity for the CD cavity). Conversely, hydroxyl groups in the 1- and 4-positions reduced the apparent binding constant of 1,4-DHN in CDM-β-CD. 1,4-DHN may be sterically hindered from penetrating deep into the CDM-β-CD cavity. The co-eluting analytes 1,6-, 2,6-, and 2,7-DHN are the isomers where hydroxyl groups are separated by the greatest distance across the naphthalene moiety. Apparently the complexation constants of these solutes are too similar to provide discrimination by CDM-β-CD under these conditions. The resolving power of HDMS-β-CD is poorer than that of CDM-β-CD in this case, and the injected analytes exhibit weaker interactions (earlier migration) with the former (Fig. 5C). Due to the difficulty of reconditioning of the capillary during the multiple injections, and the fact that only five peaks appeared out of eight components in the mixture, the DHN peaks were not identified for the HDMS-β-CD case. The key points regarding CDM-β-CD seen in Figures 4 and 5 are (i) this new CD provides a wide elution window, (ii) exhibits good overall inclusion for classes of compounds such as the DHNs and effectively utilizes the window, and (iii) can be combined with other CDs with predictable effects on elution patterns.

In order to further demonstrate the separation performance of CDM-β-CD, three groups of analytes were injected. As seen in Figure 6A, four hydroxylated PCB congeners: 4-OH-2-PCB, 4-OH-3-PCB, 2-OH-2,5-PCB, and 3-OH-2,5-PCB were successfully separated with the use of 8 mM CDM-β-CD in the running buffer. The first two congeners differ in the position of the chlorine atom and last two congeners differ in the position of the

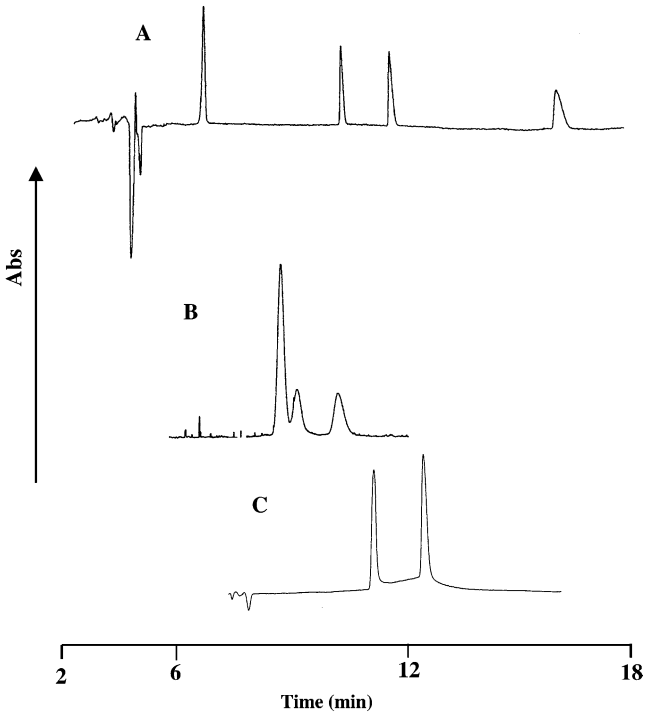


Figure 6. Separation of (A) hydroxy PCBs, (B) a group of catechins, and (C) (±)-1,1′-bi-2-naphthols. The separation conditions are the same as described for Figure 5.

hydroxyl group. Despite their close structural similarity, all of the four congeners were successfully resolved. Figure 6B shows the separation of three green tea catechins: (+)-catechin (C), (−)-epicatechin (EC), and (−)-epigallocatechin (EGC). As seen in Figure 1, C and EC are chiral and EGC has one additional hydroxyl group on the phenyl ring. Surprisingly good separation of C and EC was observed, with EGC eluting as a shoulder very close to, but later than C. The final analyte mixture studied contained chiral (±)-1,1′-bi-2-naphthols (see Fig. 6C). These analytes have two naphthalene moieties with one hydroxyl group attached to each naphthalene moiety. As seen, the separation of these chiral analytes was very successful.

4. Conclusions

A new highly charged macrocycle material, CDM-β-CD, was synthesized that utilizes carboxymethyl groups to provide mobility and methyl groups at the secondary hydroxyl face to influence selectivity during inclusion complexation. Although a single isomer with regard to substitution with the charged moiety was not obtained, full regioselective methylation with regard to substitution at the secondary sites was observed, and this should facilitate the modeling of analyte inclusion complex formation with the CDM-β-CD. Preliminary data showed that it is possible to use this CE running buffer additive for separations of a variety of analytes. From the separation results it is evident that CDM-β-CD could be a useful resolving agent for electrophoretic separations of many chiral and achiral analytes. Moreover, CDM-β-CD did not exhibit significant problems with capillary wall or inter-CD interactions. Thus reproducible migration times and an ability to utilize multi-CD running buffer systems for complex separations are possible.

Acknowledgements

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References

1. Kuhn, R.; Erni, F.; Bereuter, T.; Hausler, J. *Anal. Chem.* **1992**, *64*, 2815–2820.
2. Sun, S. X.; Sepaniak, M. J.; Wang, J. S.; Gutsche, C. D. *Anal. Chem.* **1997**, *69*, 344–348.
3. Armstrong, D. W.; Rundlett, K. L.; Chen, J. R. *Chirality* **1994**, *6*, 496–509.
4. Copper, C. L.; Sepaniak, M. J. *Anal. Chem.* **1994**, *66*, 147.
5. Cai, H.; Nguyen, T. V.; Vigh, G. *Anal. Chem.* **1998**, *70*, 580–589.
6. Chankvetadze, B.; Endresz, G.; Bergenthal, D.; Blaschke, G. *J. Chromatogr. A* **1995**, *717*, 245–253.
7. Chankvetadze, B.; Endresz, G.; Blaschke, G. *J. Chromatogr. A* **1995**, *704*, 234–237.
8. Lurie, I. S.; Chan, K. C.; Spratley, T. K.; Casale, J. F.; Issaq, H. J. *J. Chromatogr. B—Biomed. Appl.* **1995**, *669*, 3–13.
9. Ren, X. Q.; Dong, Y. Y.; Liu, J. Y.; Huang, A. J.; Liu, H. W.; Sun, Y. L.; Sun, Z. P. *Chromatographia* **1999**, *50*, 363–368.
10. Skanchy, D. J.; Wilson, R.; Poh, T.; Xie, G. H.; Demarest, C. W.; Stobaugh, J. F. *Electrophoresis* **1997**, *18*, 985–995.
11. Whitaker, K. W.; Copper, C. L.; Sepaniak, M. J. *J. Microcolumn Sep.* **1996**, *8*, 461–468.
12. Bender, M. L.; Komiyama, K. *Cyclodextrin Chemistry*; Springer: New York, 1978.
13. Khan, A. R.; Forgo, P.; Stine, K. J.; D'Souza, V. T. *Chem. Rev.* **1998**, *98*, 1977–1996.
14. Meyring, M.; Chankvetadze, B.; Blaschke, G. *Electrophoresis* **1999**, *20*, 2425–2431.
15. Vincent, J. B.; Sokolowski, A. D.; Nguyen, T. V.; Vigh, G. *Anal. Chem.* **1997**, *69*, 4226–4233.
16. Vincent, J. B.; Kirby, D. M.; Nguyen, T. V.; Vigh, G. *Anal. Chem.* **1997**, *69*, 4419–4428.

17. Fugedi, P. *Carbohydr. Res.* **1989**, *192*, 366–369.
18. Takeo, K. H. M.; Kazuhiko, U. *Carbohydr. Res.* **1989**, *187*, 203–221.
19. Kraus, T. B. M.; Zavada, J. *Carbohydr. Res.* **1997**, *304*, 81–84.
20. Culha, M.; Fox, S.; Sepaniak, M. *Anal. Chem.* **2000**, *72*, 88–95.
21. Zhu, W. H.; Vigh, G. *J. Microcolumn Sep.* **2000**, *12*, 167–171.
22. Vigh, G.; Sokolowski, A. D. *Electrophoresis* **1997**, *18*, 2305–2310.
23. Cai, H.; Vigh, G. *J. Microcolumn Sep.* **1998**, *10*, 293–299.
24. Juvancz, Z.; Jicsinszky, L.; Markides, K. E. *J. Microcolumn Sep.* **1997**, *9*, 581–589.
25. Gratz, S. R.; Stalcup, A. M. *Anal. Chem.* **1998**, *70*, 5166–5171.
26. Coleman, A. W.; Nicolis, I.; Keller, N.; Dalbiez, J. P. *J. Phenom. Mol. Recognit. Chem.* **1992**, *13*, 139–143.