Final Report
DE-FG02-96ER14609
University of Tennessee

INTRODUCTION

The diagram in Figure 1 depicts the flow of research performed under our Department of Energy, Basic Energy Sciences supported program as it has evolved during the first 30 months of the current 3-year grant period. The underpinning theme of our work is the rational design and development of molecular recognition systems in chemical separations and analysis. There have been, however, some subtle and exciting shifts in our research paradigm during this period. Specifically, we have moved from mostly separations research to a good balance between separations and spectroscopic detection for separations. This shift is based on our perception that the pressing research challenges and needs in capillary electrophoresis and electrokinetic chromatography relate to the persistent detection and flow rate reproducibility limitations of these techniques (see page 1 of the accompanying Renewal Application for further discussion). In most of our work molecular recognition reagents are employed to provide selectivity and enhance performance. Also, an emerging trend is the use of these reagents with specially-prepared nano-scale materials. Although not part of our DOE BES-supported work, the modeling and synthesis of new receptors has indirectly supported the development of novel microcantilevers-based MEMS for the sensing of vapor and liquid phase analytes. This fortuitous overlap is briefly covered in this report. Several of the more significant publications (publication list – Pg.7-8) that have resulted from our work are appended. To facilitate brevity we refer to these publications liberally in this progress report. Reference is also made to very recent work in the Background and Preliminary Studies Section of the Renewal Application.

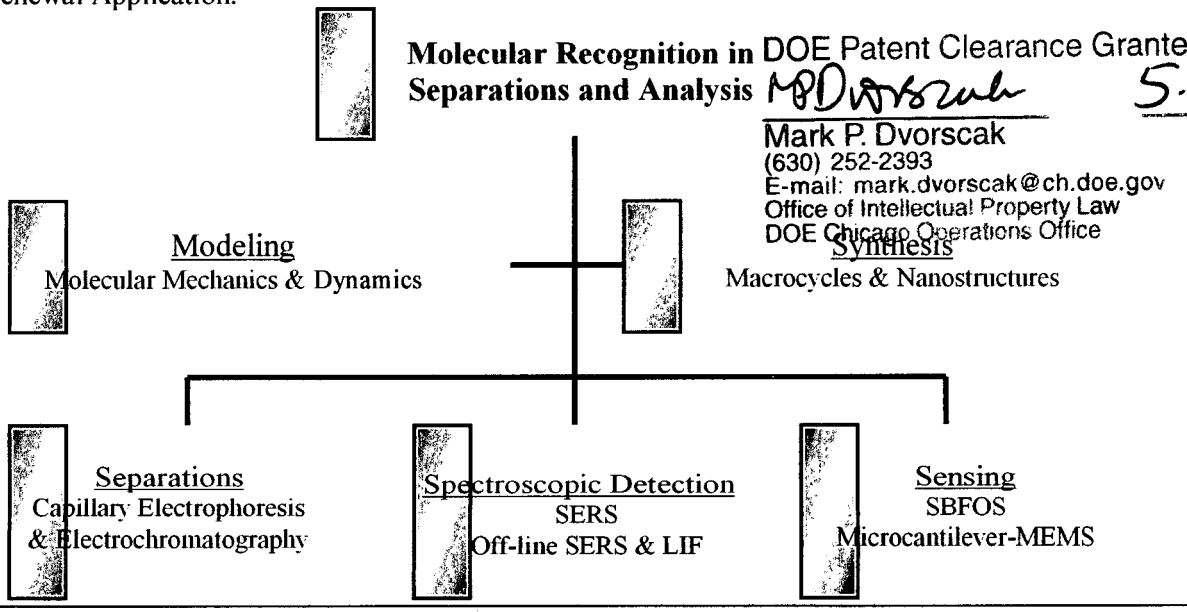

Figure 1. Flow chart showing how molecular recognition work involves the synthesis of new cyclodextrins receptors and in some instances these are used with fabricated nano-structured materials. Molecular modeling is used to guide the creation of these reagents. The utility of these reagents are explored for CE and various spectroscopic detection and sensing methods of analysis.

BRIEF SUMMARY OF RESULTS

Molecular Modeling

We have refined our grid search molecular mechnics (GSMM) approach to determining descriptive conformers for interactions of polycyclic aromatic hydrocarbons with commercially-available and newly-synthesized cyclodextrins (CDs).[4,15,22] By a process we refer to as comprehensive minimization, we

are able to reduce a large number of starting grid positions to a relatively small number of energetically favorable conformers.[Figs. 2-4, Ref. 15] The number of starting positions in the grid that "quench" to a single conformer also provides entropic information. The interaction energy for a solute–CD pair is determined using all the grid data; $IE = \Sigma\, g_i e_i \exp\{-e_i/kT\} / \Sigma\, g_i \exp\{-e_i/kT\}$. Correct elution orders have been predicted but our simulations of separations have shown only fair resemblance to actual capillary electrophoresis separations in the limited cases investigated.[Fig. 6, Ref. 15] With tweaking of parameters, such as the effective ε of the medium, the correlation between experimental and computational distribution coefficients was 0.984.[15] Unfortunately, we cannot improve on these methods of simulating separations without substantial increases in computing power. With the comprehensive minimization approach, fine grid increments, and consideration of all possible solute-CD orientations, it typically takes one to several days to determine a single solute-CD interaction energy. Moreover, we perform these calculations without consideration of the solvent because computation time increases by orders of magnitude when the solute-CD complex is solvated during minimization.

Using molecular computer assisted design (MolCAD) programs and quick (low level minimization) grid searches we have been successful in grossly interpreting the separation behavior for chiral separations of amino acids [22] and in targeting new CDs for synthesis.[23] For example, the MolCAD electrostatic surfaces for a commercially available per-sulfato-β-CD (Ren. Appl., Fig. 7) showed a highly truncated, electronegative cavity due to the sulfato groups at the C6 position. The sulfato-CD was not particularly successful in separations of nitro-PAHs. When computationally constructed and investigated using MolCAD, the cavity of a per-carboxymethyl-β-CD analog seemed more accommodating for these compounds. A quick docking experiment supported the prediction that a carboxymethyl analog would also afford better inclusion. Thus, we synthesized the carboxymethyl-CD cavitand using established, multi-step procedures and discovered that it interacted more strongly with these compounds and provided for better separations as well.[23, below]

A comparison of GSMM and simulated annealing molecular dynamics (SAMD) for the interpretation of the separation shown in Ref. 15 was conducted. The results are discussed in relation to Figure 6 in the Renewal Application. The jury is still out on whether the SAMD approach will provide more conformational freedom and yield better correlations. However, it is clear that the SAMD approach deals better with stoichiometry issues (Ren. Appl., Fig 6) and cases where clear-cut grid search protocols are not possible. Research related to the later situation includes very recent efforts to explain unusual isotherm behavior for LC separations of (+) and (-) – Trogers Base enantiomers using Chiral Pack2 as the stationary phase (Ren. Appl., Pg. 13). Under overload conditions, the effective retention of the (+) enantiomer was found to increase in the presence of the (-), contrary to common competitive isotherm expectations. The Guiochon group postulated (Ren. Appl., Ref. 147) a preferred +,+,- complex, versus the +,+,+ complex, with this stationary phase and our preliminary SAMD modeling data in Fig. 2 provides some support.

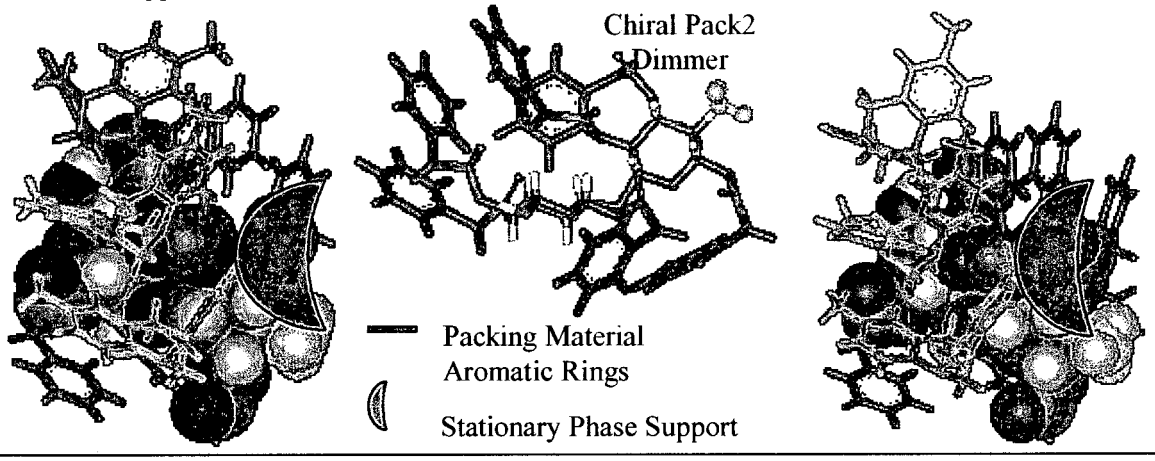

Figure 2. The +,+, - complex at far left was 1.3 Kcal/mol more stable than the +,+,+ complex at far right.

Syntheses of Cyclodextrin Derivatives

The native, commercially available derivatized, and our modified CDs remain favored molecular recognition reagents.[2,4,5,7,11,15,17,18,20,22-24] The synthetic schemes that we have used to functionalize CDs are based on multi-step procedures established in the literature (Ren. Appl., Pg.14). The reaction schemes to put carboxymethyl groups at the C6 position (see **6**) and mercapto groups at the C6 position (see **8**) are aimed at providing mobility for CE experiments and a means to attach to gold or silver surfaces in sensing or spectroscopic work, respectively. The functionalization at the C2 and C3 positions is aimed at modifying selectivity for the solutes of interest. Intermediates in the process such as the methoxy substituted compounds (see **4**) have utility as neutral running buffer additives in CE[22] and the protected one (see **3**) is sufficiently volatile to permit vapor deposition on sensor surfaces.[24] Thus far we have synthesized relatively pure forms of the following single isomer CDs: hexakis-6-mercapto-α-CD (HM-α-CD), heptakis-6-mercapto-β-CD (HM-β-CD), heptakis-(2,3-dimethyl-6-carboxymethyl)-β-CD (HDMCM-β-CD), octakis-(2,3-dimethyle-6-carboxymethyl)-γ-CD (ODMCM-γ-CD), heptakis-(2,3-diacetyl-6-carboxymethyl)-β-CD (HDACM-β-CD) and the various intermediates in route to the fully carboxylated α-, β- and γ-CDs.

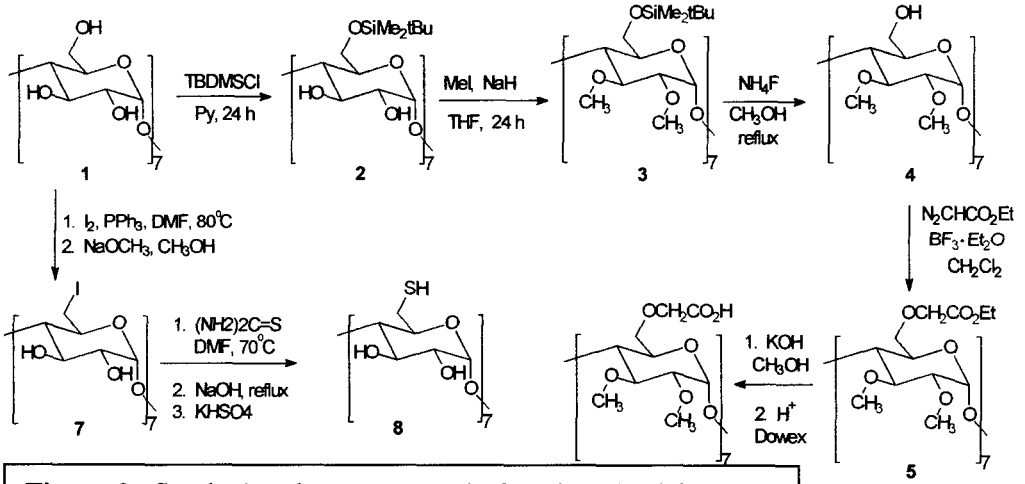

Figure 3. Synthetic schemes to certain functionalized CDs.

Separations – CD Modified CE

The new synthetic products were compared to commercially available CDs for separations of substituted napththalenes [23] and DNS amino acids.[22] Figure 4 shows the superior performance of our HDMCM-β-CD product (Fig. 4B) relative to two other single isomer CDs for separations of dihydroxy naphthalene compounds (substitution patterns are given in Fig. 4B). The single-substituted carboxymethyl product (Fig. 4A) exhibits a very narrow elution window because of its single charge and concomitant low mobility. The heptakis-sulfato commercial analog of our product (Fig. 4C) shows rather poor interaction (low capacity factors) for the solutes. The effects were even greater for dinitronaphthalene compounds.[4] We are currently "sparing" with reviewers of Ref. 23 concerning the success of our synthesis of HDMCM-β-CD. Additional spectroscopic information (MALDI-TOF-MS) has been generated that confirms that while the product is not pure (≤ 90% purity), it is indeed HDMCM-β-CD. Another advantage of our product not shown in Figure 4 is that it is conducive to reproducible flow and seems to perform predictably when used with other CDs. The importance of being able to combine CDs with predictable results was covered extensively in the appended Ref. 4.

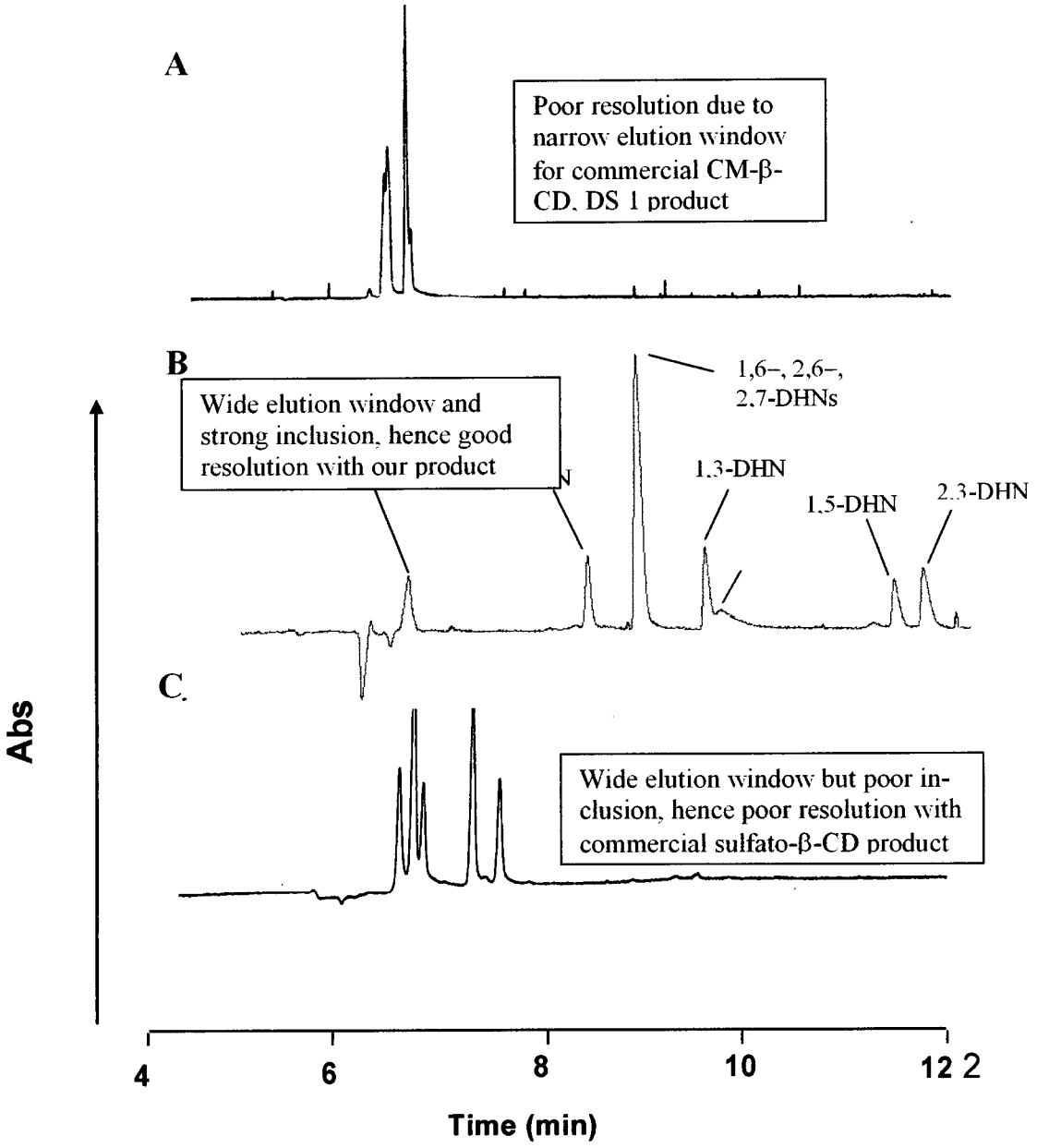

Figure 4. Capillary electrokinetic chromatographic separations of dihydroxynaphthalene compounds using three different single isomer, anionic CDs as running buffer additives.

Spectroscopy/Detection

We have pioneered the development of a modification of electrospray, referred to as electrofilament (EF), as a means to efficiently transfer the effluent of CE capillaries onto moving planar substrates.[6, 11, 21] Off-column efficiencies as high as 160,000 theoretical plates/meter have been achieved. In addition to facilitating surface enhanced Raman scattering (SERS) detection (see below), we have also employed this approach with laser induced fluorescence (LIF) detection [6] and as a means to transfer CE effluent to TLC plates for two-dimensional separations.[11] Figs. 3 - 5 in Ref. 11 provides illustrations of the performance capabilities of the off-column EF approach in terms of separation efficiency, LIF detection, and the ability to perform post-CE second dimension separations.

Recently, we demonstrated for the first time on-column CE detection with surface enhanced Raman spectrometry (CE-SERS).[8] This was accomplished by including < 1.0 w/v% silver colloidal particles in the CE running buffer. The silver nanoparticles were formed by the simple reduction of silver nitrate with sodium citrate. Although the instrumental system and silver particle size were far from optimized, a spectrum obtained on-column and on-the-fly for rhodamine 6G yielded nine distinct and characteristic bands with S/N as high as 20 for 10^{-16} moles injected.[Ref., Fig. 4] The effects of applied voltage, laser power and wavelength, column rinsing, and other parameters are presented in Ref. 8. The on-column approach is relatively straightforward but it can require careful control of running buffer conditions and the columns must be rinse between each run. Also, for some compounds, peak position and shape are altered when separations are performed with silver particles in the running buffer.

It is for this reason that we have also explored the use of EF to efficiently deposit CE bands onto SERS substrates that we have prepared by nebulizing the silver colloidal solutions onto frosted glass plates and allowing them to dry under nitrogen.[21] Ren. Appl., Fig. 5 Shows the performance of this system and provides further information on the approach. A significant advantage of the off-column approach is that the substrate can be treated post-deposition to improve spectral quality. Ref. 21, Fig. 4 shows this effect. In addition to rinsing to remove running buffer, unusual treatments such as submersion in liquid nitrogen can be performed.[19, Ren. Appl., Fig. 2] The fundamental and practical development of CE-SERS is the subject of the Renewal Application and considerable additional information on our results to date and future plans on this topic can be found therein.

Sensing

Utilizing some unique microfluidic approaches our sensing work has included coupling capillary electrophoresis with LIF remote fiberoptic detection.[1,3,5] Separations-based fiber optic sensing (SBFOS), using rationally designed and optimized CE systems, was demonstrated for both process monitoring and environmental monitoring.[1,5] Through the use of an extended pathlength capillary detection module to enhance detectability and an offset fluidic injection module to improve quantitative reproducibility (Ref. 5, Figs. 1 and 2), it was possible to monitor aflatoxins at levels < 10 pptril and accommodate real ground water matrices.[Ref. 5, Tab. 2 and Fig. 5]

We have other support and work with a group at Oak Ridge National Laboratory toward the development of microcantilever (*MC*)-based micro-electro-mechanical-systems (MEMS) for sensing of chemicals.[14,17,18,24] We recognized that certain of the molecular recognition cavitands (CDs) we employ for chemical separations and CE-SERS work should be useful reagents to impart selectivity to these MEMS devices.[17,20] In particular, the mecapto compounds (see **8** in Fig. 3) can be covalently attached to gold and silver nanoparticles for SERS applications but also to gold coated *MC*s for sensing applications.[17,18] Both NMR and IR studies confirm that Compound **3** in Fig. 3 can be vapor deposited without decomposition as thin films onto sensor surfaces.

Renewal Application (Pg. 18) provides a close up view of a *MC* with and a simplified diagram of the optical arrangement used to measure the deflection is found below in Figure 5. The response of a *MC* sensor depends on the differential stress created on the opposite sides of the cantilever due to interactions with analytes. This creates an obvious benefit to immobilizing receptor phases such as CDs on one side of the cantilever. With very thin layers of receptors there are fundamental limitations as to the levels of response and dynamic range of *MC*s.[18]. We have circumvented this limitation by nanostructuring one side of the cantilever using approaches including electrostatic attachment of 20 nm gold beads or co-evaporating gold and silver then dissolving the silver to form a colloidal-like surface. The nanostructured surface provides much greater surface area and introduces new modes of analyte induced stress.[17,18] This nanostucturing in conjunction with a SAM of HM-β-CD was shown to enhance *MC* response by two orders of magnitude and resulted in an impressive limit of detection for DMN of 140 pptrill.[17] Micrographs of these nanostructured surfaces and further discussion of this approach to sensing can be found in the appended Ref. 17. A fortuitous additional outcome of the nanostructured cantilever surface is that it does exhibit some SERS activity.

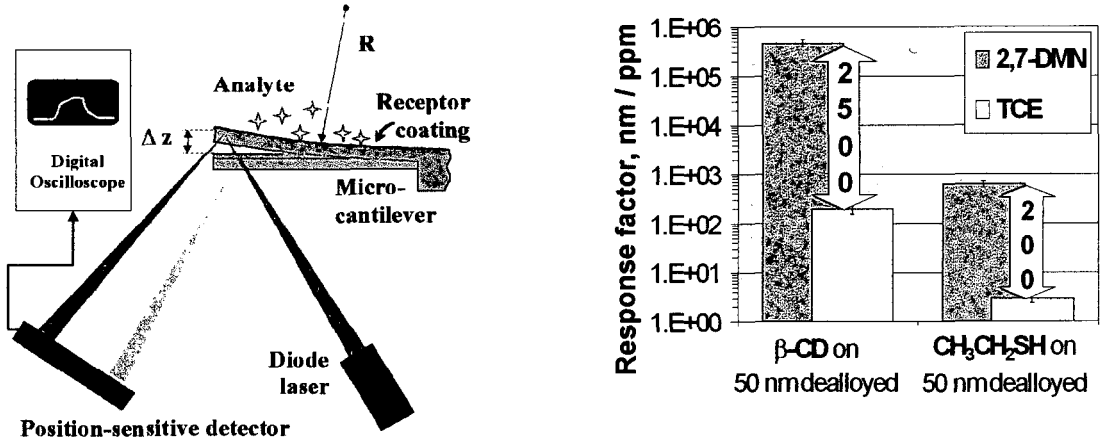

Figure 5. A depiction of the optical arrangement for MC measurements is shown at left and the responses of a nanostructured MC with SAMs of a simple alkane and HM-β-CD to 2,7-dimethyl naphthalene and tetrachloroethylene are shown.

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