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**Performance Report Covering Previous Grant Period (9-94 - 12-97)**

This report summarizes the work accomplished in our laboratories over the previous three years. This work was supported by the Department of Energy, Office of Basic Energy Science (DE FG02 88ER13819). During the funding period we have 23 monographs published or in press, 1 book chapter, 1 patent issued and have delivered 28 invited seminars or plenary lectures on DOE sponsored research. This report will cover the work that has been published (or accepted).



During this funding period five graduate students finished advanced degrees (Ph.D or M.S.). In addition seven undergraduate students (all of whom have graduated or are scheduled to graduate this year) worked on these projects at various times over the last three years.

The most notable aspect of our work involves the successful development and understanding of a new class of fused macrocyclic compounds as pseudophases and selectors in high performance separations (including high performance liquid chromatography, HPLC; capillary electrophoresis, CE; and thin layer chromatography, TLC). We have considerably extended our chiral biomarker work from amber to crude oil and coal. In the process of doing this we've developed several novel separation approaches. We have finished our work on the new GSC-PLOT column which is now being used by researchers world-wide for the

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analysis of gases, light hydrocarbons and halocarbons. Finally we completed basic studies on immobilizing a cyclodextrin/oligosiloxane hybrid on the wall of fused silica, as well as a basic study on the separation behavior of buckminsterfullerene and higher fullerenes.

The following is a list of completed scholarly works (papers, book chapter and patent) resulting from DOE sponsored research (copies have been sent with the renewal proposal):


1. D. W. Armstrong, and J. Zukowski, *J. of Chromatogr. A* **666** (1994) 445-448.

2. D. W. Armstrong, K. Le, G. L. Reid, III, S. C. Lee, K. K. Beutelmann, M. Horak, P. Tran, *J. of Chromatogr. A* **688** (1994) 201-209.

3. Y. Tang, Y. Zhou and D. W. Armstrong, *J. of Chromatogr. A* **666** (1994) 147-159.

4. D. W. Armstrong, and Y. Zhou, *J. of Liq. Chromatogr.* **17(8)** (1994) 1695-1707.

5. D. W. Armstrong, E. Y. Zhou, S. Chen, K. Le, and Y. Tang, *Anal. Chem.* **66** (1994) 4278-4282.

6. S. Chen, Y. Liu, D. W. Armstrong, J. I. Borrell, B. Martinez-Teipel, and J. L. Matallana, *J. of Liq. Chromatogr.* **18(8)** (1995) 1495-1507.

7. K. H. Ekborg-Ott, and D. W. Armstrong, *Chirality* **8** (1996) 49-57.

8. D. W. Armstrong, Y. Liu, and K. H. Ekborg-Ott, *Chirality* **7** (1995) 474-497.

9. M. P. Gasper, and D. W. Armstrong, *J. of Liq. Chromatogr.* **18(6)** (1995) 1047-1076.

10. M. P. Gasper, A. Berthod, K. Talabardon, and D. W. Armstrong, *J. of Liq. Chromatogr.* **18(5)** (1995) 1019-1034.

11. D. W. Armstrong, and K. L. Rundlett, *J. of Liq. Chromatogr.* **18(18&19)** (1995) 3659-3674.

12. K. L. Rundlett, and D. W. Armstrong, *Anal. Chem.* **34** (1995) 2088-2095.

13. K. L. Rundlett and D. W. Armstrong, *J. of Chromatogr. A* **721** (1996) 173-186.

14. A. Berthod, Y. Liu, C. Bagwill, and D. W. Armstrong, *J. of Chromatogr. A* **731** (1996) 123-137.

15. M. P. Gasper, A. Berthod, U. B. Nair and D. W. Armstrong, *Anal. Chem.* **68(15)** (1996) 2501-2514.

16. K. L. Rundlett, M. P. Gasper, E. Y. Zhou, and D. W. Armstrong, *Chirality*, **8** (1996) 88-107.

17. D. W. Armstrong, E. Y. Zhou, J. Zukowski and B. Kowmowska-Ceranowicz, *Chirality* **8** (1996) 39-48.

18. D. W. Armstrong, G. L. Reid, III and M. P. Gasper, *J. Microcolumn Separations* **8(2)** (1996) 83-87.

19. A. Berthod, U. B. Nair, C. Bagwill, D. W. Armstrong, *Talanta* **43** (1996) 1767-1782.

20. D. W. Armstrong, *L.C. GC* (1997) S20-S28.

21. D. W. Armstrong, K. H. Gahm, and L. W. Chang, *Microchemical Journal* **57** in press (1997).

22. U. B. Nair and D. W. Armstrong, *Microchemical Journal* **57** in press (1997).

23. A. Berthod, X. Wang, K. H. Gahm, and D. W. Armstrong, *Geochim. Geophys. Acta* in press (1997).

Book Chapter

1. A. Berthod, C. D. Chang and D. W. Armstrong, "Operating the Centrifugal Partition Chromatography" in Chromatographic Science Series, Ed. A. F. Foucault, Vol. 68, 1995, pp. 1-24.

Patent

1. D. W. Armstrong. United States Patent, Patent Number 5,629,424; Date of Patent May 13, 1997, Title: Stereoselective Adsorptive Bubble Process.

## Summary of Results

*I. Macrocyclic Antibiotics as Separation Agents*

In the approximately three years of this grant cycle, macrocyclic antibiotics (particularly the phenolic-glycopeptides) have gone from being an unknown factor in separations to the most rapidly growing chiral selector in HPLC and capillary electrophoresis (CE). In this time span more stereoisomeric separations were accomplished by HPLC and CE with this new class of chiral selectors than with all others combined. More importantly we are beginning to understand the mechanism of molecular recognition for these macrocycles.

The structurally related glycopeptide antibiotics, vancomycin, ristocetin A, and teicoplanin, can all be used as chiral selectors CE. Both experimental and modeling studies were done to elucidate their similarities and differences. There are identifiable morphological differences in the aglycon macrocyclic portions of these three compounds. In addition, there are other structural distinctions that can affect their CE enantioselectivity, migration times, and efficiency. Teicoplanin is the most distinct of the three and is the only one that is surface active. Its aggregational properties

appear to affect its enantioselectivity among other things. The similar but not identical structures of the three glycopeptides produce similar but not identical enantioselectivities. This leads to the empirically useful "principle of complementary separation", in which a partial resolution with one chiral selector can be brought to baseline with one of the others. Overall, ristocetin A appears to have the greatest applicability for CE enantioseparations.

When using the glycopeptides in CE it was found that adding sodium dodecyl sulfate to the run buffer increased efficiency by over one order of magnitude, decreased analysis times, and reversed the elution order of the enantiomers. This allows for control of the retention order as well as the resolution of enantiomers in complex mixtures in a single run. A mechanism was proposed which explains all of the observed effects and was verified experimentally. Since the macrocyclic antibiotic is present in both the micelle and in free solution, previously proposed micelle-selector models are, at best, limiting cases. A generally equation was derived which can be used to describe all possible interactions, including those with the capillary wall, if needed. Also, it was shown that electrophoretic mobilities and not migration times must be used to calculate binding constants of a solute to the micelle, the chiral selector, or both. Furthermore, it is shown that a neutral marker molecule cannot be used to accurately correct mobilities that have been altered due to changes in solution viscosity.

While this work utilized the antibiotic micelle system, the general conclusions and theory apply to most other analogous CE systems as well.

The glycopeptide antibiotic teicoplanin was shown to be a highly effective stationary phase chiral selector for the resolution of underivatized amino-acid and imino-acid enantiomers. Fifty four of these compounds (including all chiral protein amino acids) as well as a number of dipeptides were resolved. Hydro-organic mobile phases are used and no buffers or added salts are needed in most cases. Hence the resolved analytes are easily isolated in pure form, if needed, by evaporation of the solvent. The effect of pH, organic modifier type and amount were evaluated. The enantioselective separation mechanism was examined using both molecular modeling and retention data. The strongest stereoselective interaction appears to be for the carboxy-terminal end of D-amino-acids. In the case of peptides, it is not necessary for these to be a D-, D-, interaction with the teicoplanin chiral stationary phase. It now appears that the teicoplanin chiral stationary phase (CSP) is the method of choice for resolving native amino acids and peptides.

Over the last several decades a variety of techniques have been developed to determine apparent equilibrium constants for molecular association in solution (e.g., to micelles, proteins, cyclodextrins, antibiotics, etc.). The relationships describing binding isotherms appear in several

forms and have been given several different names. It is well known that most of these expressions are closely related and that some may be more advantageous than others for experimental or statistical reasons. In the case of electrophoresis, association constants are calculated from the relationship between ligand concentration and the measured electrophoretic mobility of the solute. This relationship has appeared in many forms that have been used numerous times at least since 1951. Recently they have reappeared in identical or slightly rearranged versions in several capillary electrophoresis (CE) studies. Some of these methods require the measurement of electrophoretic mobility of the solute-ligand complex, a value that often cannot be accurately measured. Some systems require correction or normalization procedures in order to negate any changes in solute mobility that are not due to binding. The relationship between the various expressions that can be used to calculate binding constants with CE was demonstrated. The advantages, limitations and proper use of the various approaches were decerned. Examples were given for both achiral and chiral analytes.

Adsorptive bubble separation methods are known to be useful for processing large amounts of material at a relatively low cost. These techniques have been used to enrich components from both heterogeneous and homogeneous solutions. There is a need for economical process-scale enantiomeric separations. Thus far there has been little evidence to support

the feasibility of using an adsorptive bubble process to enrich enantiomers. We demonstrate that foam-forming chiral collectors can be used in conjunction with an inexpensive glass device to enantiomerically enrich some pharmaceutically important compounds as well as derivatized and underivatized amino acids. Factors that appeared to affect this water-based separation include the following: (a) column length, (b) column geometry and packing, (c) gas flow rate, (d) concentration of the collector and the racemate, (e) nature of the collector, (f) temperature, (g) pH, (h) reflux time, (i) foam dryness and (j) the presence of other materials in the sample (e.g., miscible organic solvents, salts, etc.). The macrocyclic chiral collectors used in this study are known to be able to associate with chiral analytes via hydrophobic inclusion complexation, and hydrogen-bonding interactions and charge-charge interactions among others.

Two different amine-functionalized β-cyclodextrins were evaluated as chiral selectors in capillary electrophoresis. The first selector was a monosubstituted 6-ethylenediamine-derivatized β-cyclodextrin, and the other was quaternary ammonium hydroxypropyl β-cyclodextrin. The former compound was more widely useful as a chiral selector and had less effect on the electroosmotic flow than the latter compound. Both tended to resolve anionic compounds. The primary attractive interaction between these host chiral selectors and their enantiomeric quests were charge-charge (ionic) and hydrophobic inclusion. Additional interactions involved

hydrogen bonding and/or steric repulsions. The cationic cyclodextrins were not as widely useful in resolving anionic compounds as was vancomycin. However, they tended to be more stable and were comparatively transparent to near-UV light.

*III. Quantitation and Resolution of Chiral Biomarkers in Samples of Geochemical Importance.*

Forty chiral substituted tetralins, indans, and benzosuberans were synthesized and resolved by gas chromatography using a chiral stationary phase. The substituent groups include hydroxy, keto, methyl, and methoxy moieties. These types of compounds are known to be biological markers in fossil fuels as well as important intermediates in organic synthesis. Total ion current mass spectrometry was used to detect and identify the separated analytes. Two different derivatized cyclodextrin chiral stationary phases (having somewhat different enantioselectivities) were used. The ring size of the analyte appears to affect enantioresolution. The MS relative ion abundance and/or fragmentation patterns can be used to distinguish between enantiomeric and diastereomeric analytes.

Chiral recognition in liquid chromatography (LC) generally requires relatively strong interactions between the analyte and the chiral stationary phase, often in combination with steric interactions. Hence most LC enantiomeric separations favor compounds that have hydrogen bonding groups, aromatic rings, strong dipoles, possibilities for $\pi$-$\pi$ or charge

transfer interactions, etc. Compounds with little or no functionality are usually difficult to resolve by LC methods. Conversely gas chromatography (GC) has been very useful in resolving compounds with limited functionality, including hydrocarbons. In this sense GC has been very complementary to LC methodologies. The first enantiomeric separation of hydrocarbons (i.e., monoterpenes) by reversed-phase LC was accomplished. It appears that chiral recognition results largely from "shape-selectivity" (i.e., the tight fit of a hydrophobic moiety into a hydrophobic cavity) with few other substantial contributing interactions. Small amounts of methyl *tert*-butyl ether greatly enhanced the separation efficiency. All commercial samples of the monoterpenes, a-pinene, camphene and limonene were found to contain significant quantities of enantiomeric impurities.

Among the more prevalent chiral monoterpenoid compounds in conifers are α-pinene, β-pinene, and smaller amounts of camphene and limonene. The most prevalent chiral monoterpenoid compounds in fossilized resin (referred to as amber) appear to be borneol, isoborneol, and camphene. Most of these compounds had easily measured enantiomeric excesses. The borneol and isoborneol in some amber samples have pronounced enantiomeric excesses despite the fact that they are tens of millions of years old. The enantiomeric ratios of the monoterpenoids in different ambers vary tremendously and often are distinct. However, in

any single amber sample, the stereochemistry (absolute configuration) of the excess monoterpenoid enantiomers appears to be identical. The camphene in amber may be a secondary reaction product formed over time, possibly from the dehydration of borneol. Although a compound's original stereochemistry can be preserved, it also may diminish with the number and type of chemical transformations over geological time. The monoterpene enantiomeric ratios in modern conifer resins vary tremendously. Future stereochemical studies were outlined that could provide the data necessary for more exact geochemical interpretations and possibly for obtaining pertinent paleobiological information.

Indans and tetralins are considered biological markers (biomarkers). These C9 and C11 hydrocarbons are present in small amounts in organic geological samples. Methyl substituted indans or tetralins may possess a stereogenic center (carbon). Thus they can exist as enantiomers, and, in the case of disubstituted entities, also as diastereoisomers. The concentrations of 1-methylindan, 1,3-dimethylindan, 1-methyltetralin and 2-methyltetralin were determined in 16 crude oil sample of different sources and in 14 coal samples of different sources and ranks. Deuterated homologues were synthesized as standards to spike the samples and to assure accurate quantitative analysis. A procedure using HPLC fractionation followed by GC/MS analysis allowed the determination of μg/g (ppm) amounts of these compounds in oils. The concentration of

substituted indans and tetralins was 3-4 orders of magnitude less in coal than in crude oil. The select ion mass spectrometry (SIM) mode in GC/MS and the deuterated standards allowed detection of the much lower amounts (ng/g,ppb down to pg/g, ppt) of these compounds in coal samples. The stereochemistry of the biomarkers was determined and the relationship between their relative concentrations and the location and type of the deposits was examined. Racemic mixture of the indans and tetralins studied were found in all samples of oil and coal. It is postulated that there is an inverse relationship between the retention of stereochemical configuration and the molecular weight of hydrocarbons in crude oil. The "chiral retention of configuration cut-off" is thought to be between molecular weights of 146 and 208. An excess of *cis*-1,2-dimethylindan was found in all oil samples (average *cis/trans* ratio: 3/2). The 2-methyltetralin concentration was found to be about twice that of 1-methyltetralin in all oil and coal samples. Similar concentration correlations were found for the indan derivatives in oils and coals.

*IV. Gas-Phase Separations*

Halocarbons are usually separated using gas-liquid chromatography (GLC) using relatively long columns. Most of the more volatile chlorofluorocarbons can be better resolved by gas-solid chromatography (GSC), however, some of these compounds react with highly active

stationary phases. Particularly reactive are the replacement chlorofluorocarbons that are not fully halogenated or fluorine substituted. A new, less-active GSC stationary phase was found to be sufficiently inert to effectively separate the lower molecular weight chlorofluorocarbons in addition to the larger more polar halocarbons. These GSC columns also were used for analyses of the halocarbon content of refrigerator insulation. It was found that percent levels of specific halocarbons remained in the insulation decades after it was manufactured. Consequently, the destruction and disposal of old refrigerators could release significant quantities of halocarbons to the atmosphere. Commercial halocarbon preparations were sometimes found to contain significant quantities of other halocarbon impurities.

A single, relatively short-gas-solid porous layer open-tubular chromatographic column was used to separate aliphatic hydrocarbons, aromatic hydrocarbons and some inorganic gases ($O_2$, CO and $CO_2$) found in automobile exhaust. The column's performance and longevity did not appear to be affected by the presence of water or carbon dioxide in the samples. The concentrations of the emission gases varied considerably with changes in air/fuel ratio, coil voltage and use of catalytic converters. The results of the analyses were compared with those obtained using a commercial emission analyzer ("sniffer").

Approximately 90 chiral compounds were resolved by capillary GC on the three different cyclodextrin-based, wall-immobilized capillary columns. Despite similarities in their structure and make-up, these stationary phases often displayed different enantioselectivities. Also their selectivities were different from wall-coated varieties of neat alkyl or alkyl-acyl derivatives of cyclodextrin. It is apparent that the immobilization chemistry affects selectivity as well as stability and efficiency. While the variety of different cyclodextrin-based capillary columns may be disconcerting to many, the practical result is a net increase in the number and types of compounds that can be resolved as well as their expanded usefulness in other capillary techniques.