MOLECULAR RECOGNITION IN PLASTICIZED POLY(VINYL CHLORIDE)

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Mixtures of poly(vinyl chloride) (PVC) with plasticizers have been used in ion-selective electrodes for many years. The same material has proven useful in solid-phase microextraction (SPME), both with and without artificial receptors. In the first study, we hypothesized that by increasing the polymer concentration in plasticized PVC membranes containing artificial receptor, the extraction selectivity of target barbiturates over similar molecules could be improved. This is verified by SPME-CE experiments. At 30%, 40%, and 50% (w/w) PVC, as polymer concentration increases, selectivity for barbiturate extraction over other cyclic imides becomes better in the presence of barbiturate receptor and worse without it. In the second study, SPME has been applied to the analysis of eight barbiturate drugs and drug analogs in serum samples. Finally, a screening method for fast evaluation of chiral selectors has been proposed based on molecular recognition in plasticized PVC membrane. The advantage of this method is that it does not require the covalent immobilization of either the analyte or the selector, and the potential selector usage is at the microgram level. The method needs to be verified prior to application to libraries of peptide mimics. *R*/*S*-N-(3,5-dinitrobenzoyl)-phenylglycine bonded to silica gel is a commercial available brush-type CSP which can resolve various racemic mixtures, such as Troger's base, 2,2,2-trifluoro-1-(9-anthryl)-1-ethanol, 1-phenyl-butanol, etc. We have used freely diffusing selectors (*R* or *S*-N-(3,5-dinitrobenzoyl)-phenylglycine and its methyl ester). The selectors are doped into plasticized PVC membranes. The test analytes are the three mentioned above. Distribution difference of the two enantiomers of the Troger's base and 1-phenyl-butanol could be detected by the extraction of plasticized PVC membrane containing the test chiral selector. The difference in the selectivities is significant ($p<0.01$) by a t-test. Thus, we have demonstrated that a partitioning experiment with a selector-doped membrane can be used to determine the efficacy of a potential chiral selector.

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1.0 INTRODUCTION

1.1 MOLECULAR RECOGNITION

Molecular recognition became a popular phrase in the early 1980s, implying a complementary "lock-and-key" type fit between or within molecules. "It covers a set of phenomena that may be more precisely but less economically described as being controlled by specific noncovalent interactions."[1] Such phenomena are crucial in biochemical systems, such as enzyme action, molecular transport, genetic information and processing, protein assembly, etc. In most cases, molecular recognition is defined as "host-guest chemistry", implying a specific intermolecular interaction of a receptor molecule with a substrate one. To get effective intermolecular interaction, receptors are generally required to be compatible with the substrate in size, shape, and charge density. An additional requirement for receptors is precise alignment of multiple binding groups on the receptor with complementary regions on the substrate to provide both orientation and selective complexation of the substrate.[2] Molecular recognition can also apply to intramolecular processes, for instance, protein folding.[3]

Modern chemical research is motivated by the prospect that molecular recognition by design could lead to new technologies.[1] Most studies are toward one of the following themes: (1) elucidation of the role of noncovalent interactions;[3-8] (2) application of molecular recognition principles to practical goals;[9-12] (3) extrapolation from biological examples.[13-18]

The noncovalent interactions of molecular recognition include hydrogen bonding, electrostatic attraction, π-π stacking, van der Waals forces, etc. Among them, hydrogen bonding gathers the most interests in the efforts toward the understanding and controlling of molecular recognition.[19-21] It is worth noting that in biological systems, hydrogen bonding usually proceeds at microscopic interfaces such as cell and protein surfaces in aqueous media,[22] while most artificial molecular recognition mediated by hydrogen bonding take place effectively only in

nonaqueous media. To compete with the solvent, molecular recognition in water requires enhancement of the driven force.[23]

In our group, molecular recognition was applied in liquid-liquid extraction[24], solid phase microextraction[25,26], and membrane transport[26] in achieving high selectivity. Selectivity has two meanings depending on the ability of a system to discriminate.[27] When the ability to discriminate is high, as in a high-resolution separation or other high-resolution technique such as atomic spectroscopy, the selectivity describes the probability that a particular signal represents the desired analyte and not an interfering species. In techniques or operations in which the resolution is poorer, the expectation is that the separation of the analyte and the interfering species is never complete. In extraction, which falls into the latter category, selectivity can be described as the ratio of the relative concentration of the analyte and the interfering species in the two phases.[28] It is a relative enrichment factor. One objective of the design of new extraction methods is to increase the selectivity of the extraction for the group of analytes desired. Other objectives are to improve speed, to manage smaller amount of samples, to improve the mechanics to allow automation, and to decrease the volume of waste solvents. [27]

To achieve the maximum selectivity, a suitable solvent is expected to bring the largest solubility and partition coefficients to the desired substrate, the receptor and their complex, while inhibiting the solubility and partitioning of interfering species. However, all of these requirements are difficult to satisfy at the same time. The extraction selectivity can be increased to some degree by carefully choosing the organic solvent according to the properties of the target analyte. In the example of solid phase microextraction of barbitals and their analogues in Chapter 3, different plasticizers are investigated and Santicizer 141 gives the best result. Generally, solvents do not have much selectivity by themselves, so molecular recognition plays an important role. As partitioning is a less-selective process and molecular recognition is a highly selective one, our hypothesis is that high extraction selectivity can be achieved by making the extraction more dependent on the molecular recognition process by application of good receptors and poor solvents. Good receptors can provide high association constants with the desired substrates and specifically enhance extraction of these substrates, while poor solvents will suppress the partition process, thus decrease extraction of the interfering species from the sample matrix.

Another concern in extraction is the associated environmental hazards of solvents due to their pyrophoric nature, volatility, and poor recovery. Attempts have been made toward solvent-free chemistry.[29-33] However, solvent plays a crucial role in the majority of extraction processes. In seeking the transformation of solvents, reusability becomes the key part. Ionic liquids have been at the cutting edge of this research.[34-37] Liquid polymer and plasticized polymer are also gathering increased interests.[38] Plasticizers are high-boiling organic solvents used chiefly to impart flexibility to a rigid plastic or polymer such as poly(vinylchloride) (PVC).[39] The plasticization of PVC accounts for the single largest usage of plasticizers.[40] In analytical chemistry, plasticized PVC has found its way into the area of sensors. This material is used in the fabrication of ion-selective electrodes (ISEs).[41] For use in ISEs, a plasticizer is normally chosen on the basis of its plasticizing ability, water immiscibility, viscosity, and receptor solubility.[41] Consequently, these properties of the plasticizer influence the overall performance of the membrane.

1.2 CHIRAL RECOGNITION

Chiral recognition is actually a branch of molecular recognition. The recognition and separation of chiral compounds are getting increased attention due to their pharmaceutical importance. To resolve racemic mixtures into two enantiomers, it necessarily requires involvement of a chiral resolving agent – a chiral selector, to form a diastereomeric complex with one of the enantiomers, or both, but with different formation constant. Enantioselectivity, α, the ratio of the binding constants in the interaction of the chiral selector with the two enantiomers, is the key leading to the resolving of racemates. Depending on different values of α, either "single step" or "multi-step" procedure can be chosen to fulfill the goal of chiral separation. In the case of "single step" procedure, in order to obtain a product with an optical purity of 98-99%, the discrimination of the enantiomers by a factor of $\alpha = 100$ is desired.[42] While in most applications, it is almost impossible to find such a chiral selector with that extremely high enantioselectivity. As a result, chromatography, standing as a "multi-step" separation method, becomes the most used tool to effectively employ a large number of chiral selectors with

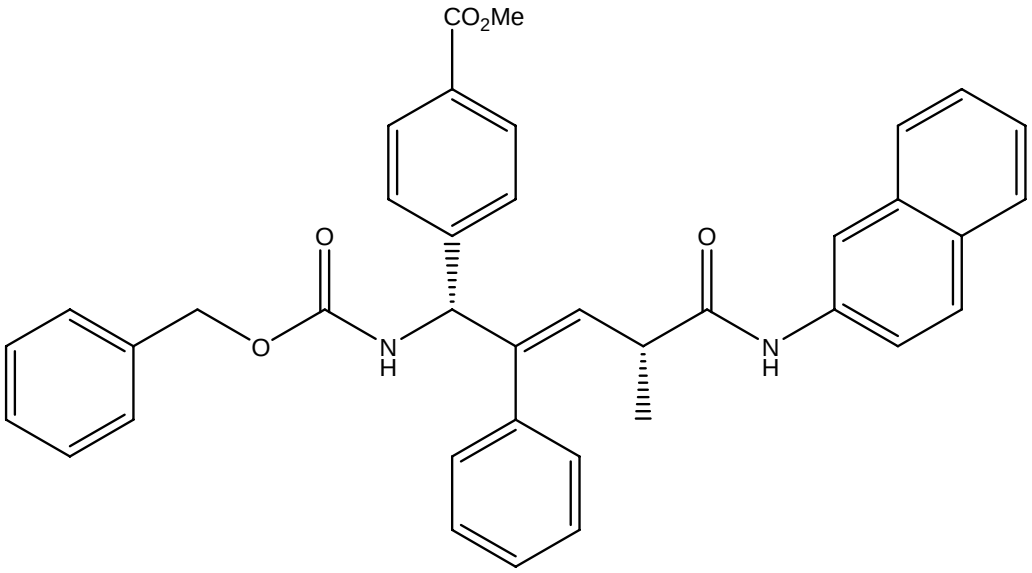
the value of α as low as 1.01. Today, the most applicable technique for chiral separation remains either liquid or gas chromatography on chiral stationary phases.

Chiral stationary phases are the most common first approach for chiral separation. The chiral selector is covalently immobilized to the solid phase and forms transient diastereomeric complexes with the enantiomers of the solute, leading to the stereochemical resolution of racemic solutes. There are basically five types of CSPs in liquid chromatography (LC), which are: Pirkle-type CSPs, cellulose-based CSPs, cyclodextrin-based CSPs, protein-based CSPs and ligand exchange CSPs.

The first commercially available LC chiral stationary phase was introduced by Pirkle in 1981.[43] It is also called as brush-type CSP. These sorts of CSPs are based on synthetic small molecules. The interactions between the solute and the CSPs involve hydrogen bonding, π-π interaction, dipole stacking, and charge transfer, etc. In order to accomplish chiral recognition, a minimum of three simultaneous interactions between CSP and solute is required. At least one of these interactions must be stereochemically controlled.[44] Comparing with cellulose, CD, and protein based CSPs, Pirkle-type have less binding sites with solutes thus chiral recognitions are more specific to give higher separation efficiencies.

"Three-point" interaction model was initiated by Easson and Stedman[45] in 1933 and resurrected by Ogston[46] in 1948. It was quickly spreading from biological area to pharmaceutical chemistry, chromatographic science and other disciplines in the 1950s. Though the accuracy and applicability of this model is still on debate, it has been the basis of the rational design of brush-type CSPs and explanation of the chiral recognition mechanism.[47] A number of other model systems were built for elucidation of the chiral recognition mechanism.[48-51]

A methodology is developing in Dr. Wipf's group for the preparation of a large (>10,000 components) library of peptide mimics. It is believed that di- and tri-peptide mimics contain the best of the properties of above mentioned "three-point-interaction" chemistry. As shown in Figure 1.1, there are more than three functional groups can be viewed as possible points for interaction with a solute and the peptide mimics are easy to immobilized to solid phase such as silica gel. In combination with the great choices from the large library, these peptide mimics have the best potential to be useful as brush-type CSPs.



$C_{38}H_{34}N_2O_5$

Mol. Wt.: 598.69

Figure 1.1 *An example of peptide mimics.*

1.3 SELECTIVITY IN SOLID PHASE EXTRACTION

Solid phase extraction (SPE) is more time efficient and less labor intensive than conventional liquid-liquid extraction methods. Another advantage of solid phase extraction is the minimal solvent consumption and sample volume required for an effective separation, particularly in the case of solid phase microextraction (SPME) in which the extraction phase is just a small coated fiber or tip. Solid phase extraction is governed by principles similar to liquid chromatography. The solid phase is a small cartridge filled with bonded silica material or other packing. Solutes in liquid or gas sample are absorbed or adsorbed onto the solid phase during extraction and desorbed later for detection. The packing materials are similar sorbents used in chromatographic columns: ion exchangers with acidic or basic ion-exchange functional groups, reversed phase packing such as resins containing C_{18}, C_8 or benzyl groups, and normal phase packing (SiO_2) [52, 53]. Polymers are also applied in SPE. For instance, polydimethylsiloxane (PDMS) is widely used in extraction of semivolatile analytes in direct and headspace modes.[54] Polyacrylate (PA),[55] polyhydroxylated polyparaphenylene (PH-PPP)[56] are also examples of SPME polymer coating.

The selectivity in SPE depends on not only the packing materials but also the choice of the sample solvents during the extraction process and the elution solvents or desorption temperature during the desorption process. When non-specific sorbents (poly(styrenedivinylbenzene), SiO_2, C_{18}) are used, the selectivity is more dependent on the elution or thermal desorption conditions as in chromatography (LC or GC). The solvents with optimized elution strength will help elute the desired analytes with small volume of solvents. The elution volume is important because it affects the SPE preconcentration efficiency and the compatibility with detection methods. In SPE-HPLC and SPE-CE, very small amount of the elution solvent is the minimum requirement for detection. In the case of polymer-sorbent based SPE, selectivity can be achieved through carefully chosen polymers. Since PDMS is less polar than PA, it is widely used for the extraction of non-polar compounds, such as benzene,[57] while the PA coating works better for polar compounds like alcohols or some pesticides.[55, 58] Various organic solvents have different affinity to molecules and could be employed to improve the selectivity of SPE. Similar to liquid-liquid extraction, introduction of receptors to SPE could greatly enhance the extraction selectivity of target analyte.[25, 26]

1.4 OUTLINE

Chapter 2 focuses on the effect of polymer concentration in plasticized Poly(vinylchloride) on molecular recognition. Chapter 3 introduces an application of solid phase microextraction followed by sample stacking-micellar electrokinetic chromatography on the medical sample analysis. The work in these two chapters was done by cooperation with senior graduate students and has been published or in revision. A screening method is proposed in Chapter 4 for fast evaluation of the enantioselectivity of potential chiral selectors. This is the main work I am focusing on and the primary topic of my later Ph.D. study. In chapter 5, a future plan is given for further research of the screening method.

1.5 REFERENCE

(1) Gellman, S. H. *Chemical Reviews (Washington, D. C.)* **1997**, *97*, 1231-1232.

(2) Chang, S. K.; Van Engen, D.; Fan, E.; Hamilton, A. D. *Journal of the American Chemical Society* **1991**, *113*, 7640-7645.

(3) Robertson, A. D.; Murphy, K. P. *Chemical Reviews (Washington, D. C.)* **1997**, *97*, 1251-1267.

(4) Stites, W. E. *Chemical Reviews (Washington, D. C.)* **1997**, *97*, 1233-1250.

(5) Davidson, S. M. K.; Regen, S. L. *Chemical Reviews (Washington, D. C.)* **1997**, *97*, 1269-1279.

(6) Mader, M. M.; Bartlett, P. A. *Chemical Reviews (Washington, D. C.)* **1997**, *97*, 1281-1301.

(7) Ma, J. C.; Dougherty, D. A. *Chemical Reviews (Washington, D. C.)* **1997**, *97*, 1303-1324.

(8) Connors, K. A. *Chemical Reviews (Washington, D. C.)* **1997**, *97*, 1325-1357.

(9) Babine, R. E.; Bender, S. L. *Chemical Reviews (Washington, D. C.)* **1997**, *97*, 1359-1472.

(10) Kool, E. T. *Chemical Reviews (Washington, D. C.)* **1997**, *97*, 1473-1487.

(11) Chow, C. S.; Bogdan, F. M. *Chemical Reviews (Washington, D. C.)* **1997**, *97*, 1489-1513.

(12) de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chemical Reviews (Washington, D. C.)* **1997**, *97*, 1515-1566.

(13) Ikeda, A.; Shinkai, S. *Chemical Reviews (Washington, D. C.)* **1997**, *97*, 1713-1734.

(14) Linton, B.; Hamilton, A. D. *Chemical Reviews (Washington, D. C.)* **1997**, *97*, 1669-1680.

(15) Zeng, F.; Zimmerman, S. C. *Chemical Reviews (Washington, D. C.)* **1997**, *97*, 1681-1712.

(16) Wallimann, P.; Marti, T.; Fuerer, A.; Diederich, F. *Chemical Reviews (Washington, D. C.)* **1997**, *97*, 1567-1608.

(17) Schmidtchen, F. P.; Berger, M. *Chemical Reviews (Washington, D. C.)* **1997**, *97*, 1609-1646.

(18) Conn, M. M.; Rebek, J., Jr. *Chemical Reviews (Washington, D. C.)* **1997**, *97*, 1647-1668.

(19) Ghaddar, T. H.; Castner, E. W.; Isied, S. S. *Journal of the American Chemical Society* **2000**, *122*, 1233-1234.

(20) Chou, H.-C.; Hsu, C.-H.; Cheng, Y.-M.; Cheng, C.-C.; Liu, H.-W.; Pu, S.-C.; Chou, P.-T. *Journal of the American Chemical Society* **2004**, *126*, 1650-1651.

(21) Ishizaka, S.; Kinoshita, S.; Nishijima, Y.; Kitamura, N. *Analytical Chemistry* **2003**, *75*, 6035-6042.

(22) Gohlke, H.; Klebe, G. *Angewandte Chemie, International Edition* **2002**, *41*, 2644-2676.

(23) Niikura, K.; Anslyn, E. V. *Journal of Organic Chemistry* **2003**, *68*, 10156-10157.

(24) Valenta, J. N.; Dixon, R. P.; Hamilton, A. D.; Weber, S. G. *Analytical Chemistry* **1994**, *66*, 2397-2403.

(25) Li, S.; Sun, L.; Chung, Y.; Weber, S. G. *Analytical Chemistry* **1999**, *71*, 2146-2151.

(26) Zhang, X.; Zhao, H.; Chen, Z.; Nims, R.; Weber, S. G. *Analytical Chemistry* **2003**, *75*, 4257-4264.

(27) Li, S.; Weber, S. G. *Applications of Solid Phase Microextraction* **1999**, 49-56.

(28) Giddings, J. C. *Unified Separation Science*, 1991.

(29) Cave, G. W.; Raston, C. L.; Scott, J. L. *Chemical communications (Cambridge, England) FIELD Publication Date:2001 Nov 7*, 2159-2169. FIELD Reference Number: FIELD Journal Code:9610838 FIELD Call Number:.

(30) Tanaka, K.; Toda, F. *Chemical Reviews (Washington, D. C.)* **2000**, *100*, 1025-1074.

(31) Cave, G. W. V.; Raston, C. L. *Chemical Communications (Cambridge)* **2000**, 2199-2200.

(32) Toda, F. *Accounts of Chemical Research* **1995**, *28*, 480-486.

(33) Toda, F. *Synlett* **1993**, 303-312.

(34) Welton, T. *Chemical Reviews (Washington, D. C.)* **1999**, *99*, 2071-2083.

(35) Wasserscheid, P.; Keim, W. *Angewandte Chemie, International Edition* **2000**, *39*, 3772-3789.

(36) Gordon, C. M. *Applied Catalysis, A: General* **2001**, *222*, 101-117.

(37) Sheldon, R. *Chemical Communications (Cambridge, United Kingdom)* **2001**, 2399-2407.

(38) Chandrasekhar, S.; Narsihmulu, C.; Sultana, S. S.; Reddy, N. R. *Organic Letters* **2002**, *4*, 4399-4401.

(39) Sears, J. K.; Darby, J. R. *The Technology of Plasticizers*, 1982.

(40) Kroschwitz, J.; Editor *Kirk-Othmer Concise Encyclopedia of Chemical Technology, 4th Edition*, 1999.

(41) Covington, A. K.; Editor *Ion-Selective Electrode Methodology, Vol. 1*, 1979.

(42) Davankov, V. A. In *Advances in Chromatography (New York, NY, United States)*, 1980; Vol. 18, pp 139-195.

(43) Pirkle, W. H.; Finn, J. M.; Schreiner, J. L.; Hamper, B. C. *Journal of the American Chemical Society* **1981**, *103*, 3964-3966.

(44) Dalgliesh, C. E. *Journal of the Chemical Society, Abstracts* **1952**, 3940-3942.

(45) Easson, L. H.; Stedman, E. *Biochemical Journal* **1933**, *27*, 1257-1266.

(46) Ogston, A. G. *Nature (London, United Kingdom)* **1948**, *162*, 963.

(47) Pirkle, W. H.; Pochapsky, T. C.; Burke, A. J.; Deming, K. C. In *Chiral Separations.*; Wilson, I. D., Ed.; Plenum: New York, 1988.

(48) Lipkowitz, K. B.; Coner, B.; Peterson, M. A.; Morreale, A. *Journal of Physical Organic Chemistry* **1997**, *10*, 311-322.

(49) Schefzick, S.; Lindner, W.; Lipkowitz, K. B.; Jalaie, M. *Chirality* **2000**, *12*, 7-15.

(50) Maier, N. M.; Schefzick, S.; Lombardo, G. M.; Feliz, M.; Rissanen, K.; Lindner, W.; Lipkowitz, K. B. *Journal of the American Chemical Society* **2002**, *124*, 8611-8629.

(51) Suzuki, T.; Timofei, S.; Iuoras, B. E.; Uray, G.; Verdino, P.; Fabian, W. M. F. *Journal of Chromatography, A* **2001**, *922*, 13-23.

(52) Nilsson, U. J. *Journal of Chromatography, A* **2000**, *885*, 305-319.

(53) Ferrer, I.; Barcelo, D.; Thurman, E. M. *Analytical Chemistry* **1999**, *71*, 1009-1015.

(54) Bruheim, I.; Liu, X.; Pawliszyn, J. *Analytical Chemistry* **2003**, *75*, 1002-1010.

(55) Lambropoulou, D. A.; Sakkas, V. A.; Albanis, T. A. *Analytical and Bioanalytical Chemistry* **2002**, *374*, 932-941.

(56) Basheer, C.; Suresh, V.; Renu, R.; Lee, H. K. *Journal of Chromatography, A* **2004**, *1033*, 213-220.

(57) MacGillivray, B.; Pawliszyn, J.; Fowlie, P.; Sagara, C. *Journal of Chromatographic Science* **1994**, *32*, 317-322.

(58) Eisert, R.; Levsen, K. *Journal of the American Society for Mass Spectrometry* **1995**, *6*, 1119-1130.

2.0 EFFECT OF POLYMER CONCENTRATION ON MOLECULAR RECOGNITION IN PLASTICIZED POLY(VINYL CHLORIDE)

2.1 ABSTRACT

Mixtures of poly(vinyl chloride) (PVC) with plasticizers have been used in ion-selective electrodes for many years. The same material has proven useful in solid-phase microextraction (SPME), both with and without artificial receptors. We hypothesized that by increasing the polymer concentration in plasticized PVC membranes containing artificial receptor, the extraction selectivity of target barbiturates over similar molecules could be improved. This is verified by SPME-CE experiments. At 30%, 40%, and 50% (w/w) PVC, as polymer concentration increases, selectivity for barbiturate extraction over other cyclic imides becomes better in the presence of barbiturate receptor and worse without it.

2.2 INTRODUCTION

Selectivity in chemical reactions and catalysis is a central focus of the field of chemistry. Only recently, however, has molecular selectivity in partitioning of solutes gained attention. There are two routes to molecular selectivity in extractions, using biomolecular recognition (e.g., antibodies and their fragments [1-4]), and using synthetic receptors [5-15] or templated materials [16-19]. We have pursued the use of hydrogen bond-based molecular recognition in nonaqueous solvents for the extraction of barbiturates from aqueous solutions. This system is of course useful in a practical sense, but it also acts as a model system for the investigation of approaches to improving the selectivity of the extraction process. The free energy for receptor (**1**)-phenobarbital (**2**) complex (Fig. 2.1) formation is strongly dependent upon the solvent in which

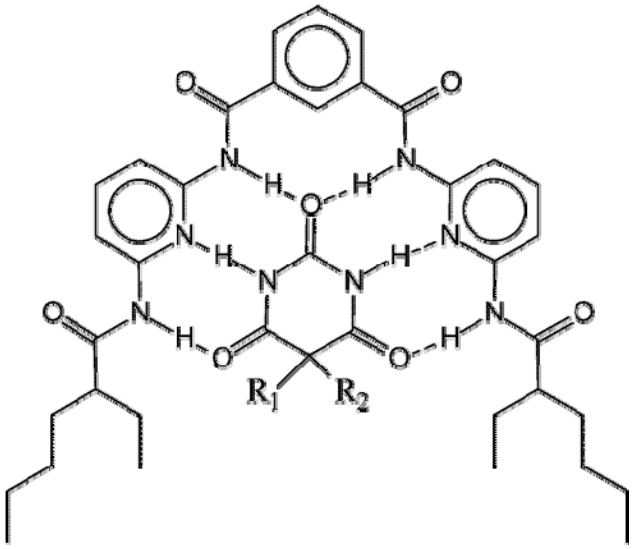


Figure 2.1 *Structures of **1**, **2**, and Their Complex.*

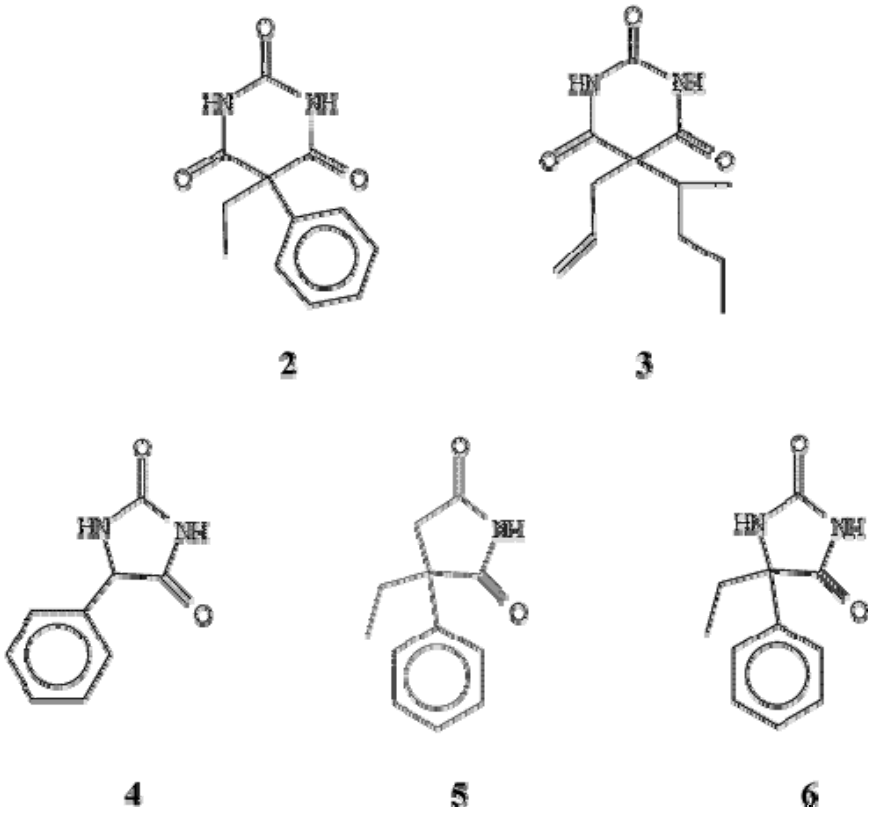


Figure 2.2 *Barbiturates and Cyclic Imides.*

the complexation occurs [13, 14]. Plasticized poly(vinyl chloride) (PVC), already successful in potentiometric sensors [20-24], has been the basis of our efforts [10, 25] in solid-phase microextraction (SPME) [26, 27]. Surprisingly, useful plasticizers come in a wide range of polarities, so the type of plasticizer controls the free energy of **1**-**2** complex formation and partitioning of **2** from aqueous solutions. For example, our earlier work showed that the aqueous/plasticizer partition coefficient for **2** changes nearly 3 orders of magnitude on changing the plasticizer from a low to a high polarity [12], yet these same solvents function as plasticizers for PVC.

In this chapter, we report on the role of the concentration of PVC in the extraction process. SPME-CE experiments led to the conclusion that high [PVC] improves selectivity.

2.3 EXPERIMENTAL SECTION

Reagents and Solutions PVC (high molecular weight, Selectophore) and dioctyl sebacate (DOS, Selectophore) were purchased from Fluka Chemical Co. (Ronkonkoma, NY). Phenobarbital (**2**) and secobarbital (**3**) (Figure 2.2) were purchased from Sigma (St. Louis, MO). 5-Phenylhydantoin (**4**) was purchased from TCI (Tokyo, Japan). DL-2-Ethyl-2-phenylsuccinimide (**5**) and DL-5-ethyl-5-phenylhydantoin (**6**) were synthesized as described elsewhere [28, 29]. HPLC grade tetrahydrofuran (THF) was purchased from Aldrich. Milli-Q (Millipore) water was used. All other compounds were AR grade or better and purchased from commercial sources. The details of the barbiturate receptor synthesis have been described elsewhere [10].

Buffer Preparation The acetic acid/sodium acetate buffer solutions (25 mM, pH 4.96-5.02) were made by mixing equimolar solutions of acetic acid and sodium acetate and adjusting the pH with concentrated acetic acid or sodium hydroxide. The phosphate buffer (25 mM, pH 11.46) was made by dissolving sodium phosphate in water and adjusting the pH with concentrated sodium hydroxide solution.

Equipment An Isco 3850 (Lincoln, NE) capillary electropherograph was used for MEKC separation as well as quantitative analysis of barbiturates and cyclic imides. A detection window was opened 40 cm from the injection end on a 65-cm fused-silica capillary (50-μm i.d., Polymicro Technologies, Inc., Phoenix, AZ). The separation buffer was 25 mM sodium dodecyl

sulfate (SDS) in 100 mM Tris solution adjusted to pH 8.09 with TAPSO and was filtered prior to usage. Standards and samples were injected by applying 0.5 psi at the end of capillary for 10 s. During separation, a potential of 23 kV was applied. The current was ~24 μA. The detection wavelength was set to 210 nm for maximum detection sensitivity for all five compounds. During these experiments, there was blockage of the capillary, which was corrected by removing a short length of it. This resulted in altered retention times. Data acquisition and integration were operated through Peak Simple (SRI Instruments, Torrance CA).

SPME probe preparation The procedure to prepare SPME probes was adapted from Li and Weber [25] and modified for better reproductivity. Stainless steel probes (o.d. 1.1 mm, Small Parts, Miami Lakes, FL) were cut into 7 cm pieces, polished with emery cloth, cleaned with a Kimwipe and acetone, and ultrasonicated in ethanol and then tetrahydrofuran (THF) for 5 min. Teflon tubes (inside diameter 1.2 mm, Small Parts, Miami Lakes, FL) were cut into 5 cm pieces and 24 of them were bound together to held eight of the above mentioned probes. About 4 cm of the probes were left out of the tubes with the ends at the same level. Poly(vinyl chloride-*co*-vinyl acetate-*co*-maleic acid) (PVAM, vinyl chloride, 86%; vinyl acetate, 13%; maleic acid, 1%) was dissolved in THF (3% w/v). The eight stainless steel probes were put into the THF solution of PVAM, removed from the solution immediately, held still and vertically for 1 min, and air-dried in the hood for at least 5 h. A PVAM primer was obtained on the probes. PVC ("very high molecular weight") was added slowly to THF to 3.6% (w/v) with stirring. Dioctyl sebacate (DOS, Selectophore) was added to the PVC solution to 7.2% (v/v). The PVAM-primed probes were put into the solution, taken out immediately, held vertically for 1 min, and air-dried for at least 5 h. The length of the coating is 3 cm from one end.

SPME operation The extraction was conducted by immersing the SPME probes into a solution (pH 5.02, 25 mM acetate buffer) containing **2-6** for 30 min. To speed up the kinetics, the probes were secured on a rotating ring disk electrode that spins at 300 rpm (Figure 2.3). At the end of the extraction, the rods were twice rinsed in DI water for 5 s while the rotating speed was maintained. The probe was then transferred into a Teflon tube (inside diameter 1.2 mm, Small Parts, Miami Lakes, FL) containing 10 μL of phosphate buffer (pH 11.46, 25 mM) and allowed to sit for 2 h. Long extraction and back extraction times were used so that the system approached equilibrium, allowing for interpretation of results in an equilibrium framework. Certainly such

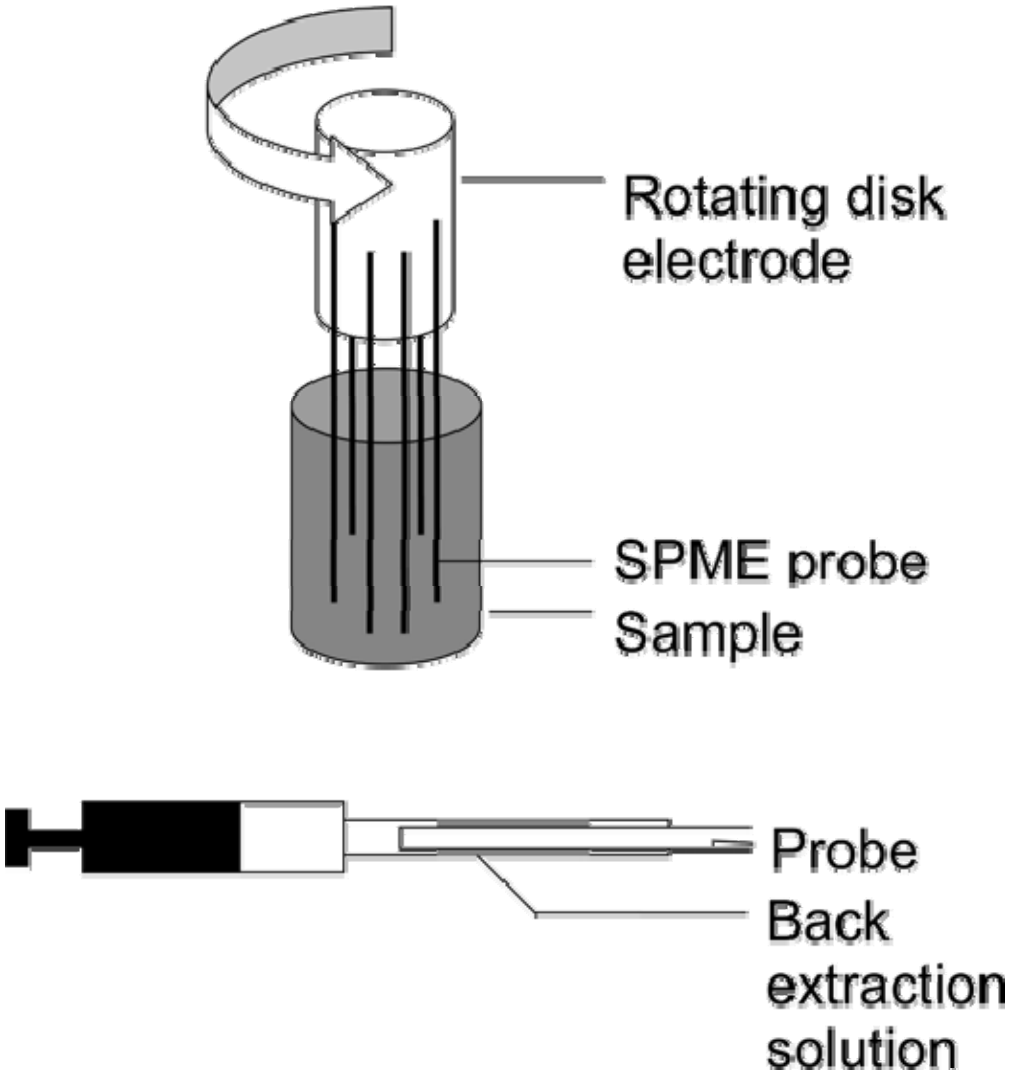


Figure 2.3 *Extraction and back extraction in SPME.*

The mass transport in the extraction is controlled by using a rotating disk electrode rotator at 300 rpm. Back extraction is into a static drop of basic solution in a Teflon tubes.

long times are not required for applications. After removing the rod from the tube at the end of the back extraction, a 1-mL syringe was used to push out the solution into a 200 μL injection vial. The MEKC separation was performed as described above.

2.4 RESULTS AND DISCUSSION

Among the compounds **2**-**6**, only **2** and **3** are complementary to receptor **1** by forming six hydrogen bonds along the latter's inner cavity. Compounds **4**-**6** were chosen because they contain a cyclic imide and the phenyl substituent as in **2**. They all possess a five-membered ring on which hydrogen bond donors and acceptors exist. However, since the hydrogen bond donors and acceptors have a different spatial arrangement compared to barbiturates, they cannot form stable complexes with receptor **1**. Clearly, the similarity of compounds **2**-**6** makes the selective extraction of **2** and **3** from a mixture a significant challenge.

We have prepared SPME probes with 30%, 40% 50%, and 60% PVC, with and without 1% **1** (receptor). Extracted solutes were quantitated by peak area in the MEKC [30] (standard chromatogram is shown in Figure 2.4). Log *P* (octanol/water partition coefficients) of the five compounds are as follows: **4** (0.32) < **2** (1.34) < **6** (1.44) < **5** (1.48) < **3** (2.17) [31, 32]. The order of elution is approximately in that same order: **4**, **6**, **2**, **5**, **3** (last), consistent with separation based on partitioning.

Figure 2.5 shows the results of extractions carried out with and without receptor **1**-doped membranes with 30%-50% PVC. In both sets of chromatograms, only signals from **2**, **3**, and **5** appear; thus, the matrix rejects solutes **4** and **6** (at the concentrations of solutes that were used). Solute **4** is expected to be a stronger weak acid than the other compounds and may, therefore, be significantly ionized at the pH of the extraction medium (pH 5). This, together with the poor lipophilicity of solute **4** in its non-ionized form, would disfavor its partitioning through the matrix. Though we have no enough data for a complete understanding, it is at least clear that the plasticized PVC matrix (with or without receptor) is not a good solvent for these hydantoins (**4** and **6**). The barbiturate signals in the bottom chromatogram (**2** and **3**) result from specific (**1**-**2** complex) and nonspecific (partitioning) extraction. The top chromatogram shows only

nonspecific extraction (note the scale difference in the two chromatograms). It is clear that the amount of *specific* extraction increases as polymer concentration goes from 30% to 50%.

We define a selectivity term *S* as

$$S = \frac{A_2 + A_3}{A_4 + A_5 + A_6} \qquad \text{(Equation 2.1)}$$

where A_2-A_6 are peak areas of the five compounds **2**-**6**, respectively. Figure 2.6 shows the selectivity of the membranes as a function of their composition. Membranes with a higher PVC concentration indeed provide a solvent system that has a better selectivity toward barbiturates. Control experiments conducted with membranes that have the same PVC concentration but without receptor actually show a slight decrease in selectivity as PVC concentration increases. The optimum composition appears to be 50% PVC. The slight decrease in selectivity in the 60% PVC membrane is not anticipated in theoretical models of selectivity [14] that assume that the value of K_p for the desired and undesired solutes are identical at all compositions. Overall, however, such a simple model does predict the general behavior observed.

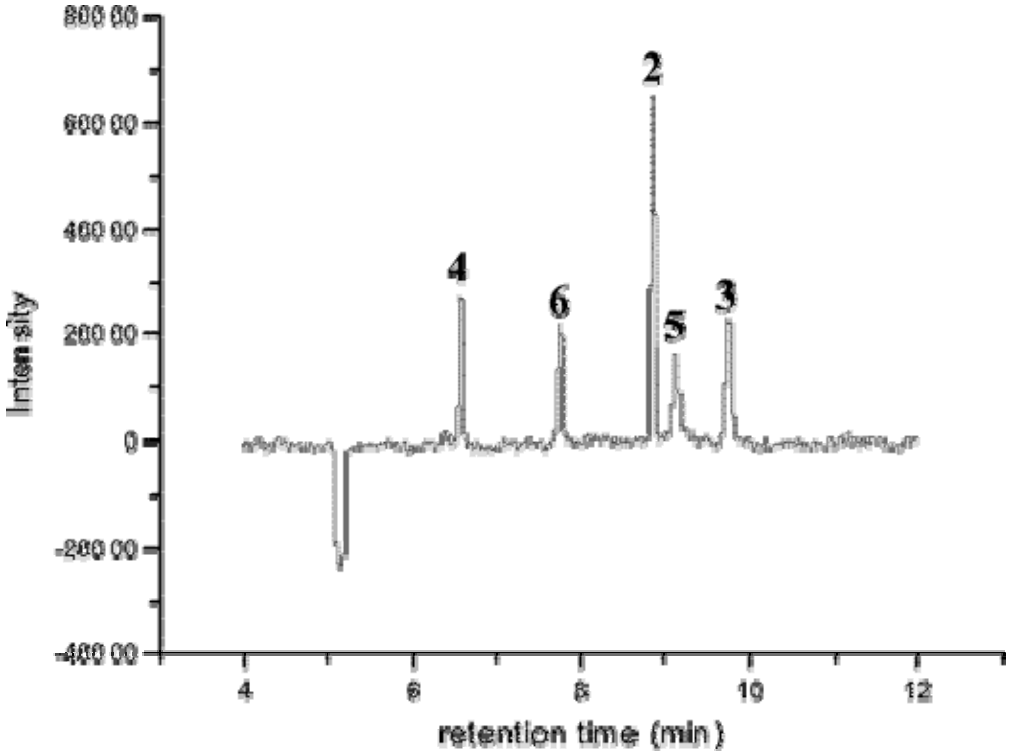


Figure 2.4 *Separation of **2**-**6** with MEKC.*

Running buffer: 25 mM SDS in 100 mM Tris solution adjusted to pH 8.09 with TAPSO. Standards and samples were injected by applying 0.5 psi at the end of capillary for 10 s. During separation, a potential of 23 kV was applied. Standard concentrations: 100 µM.

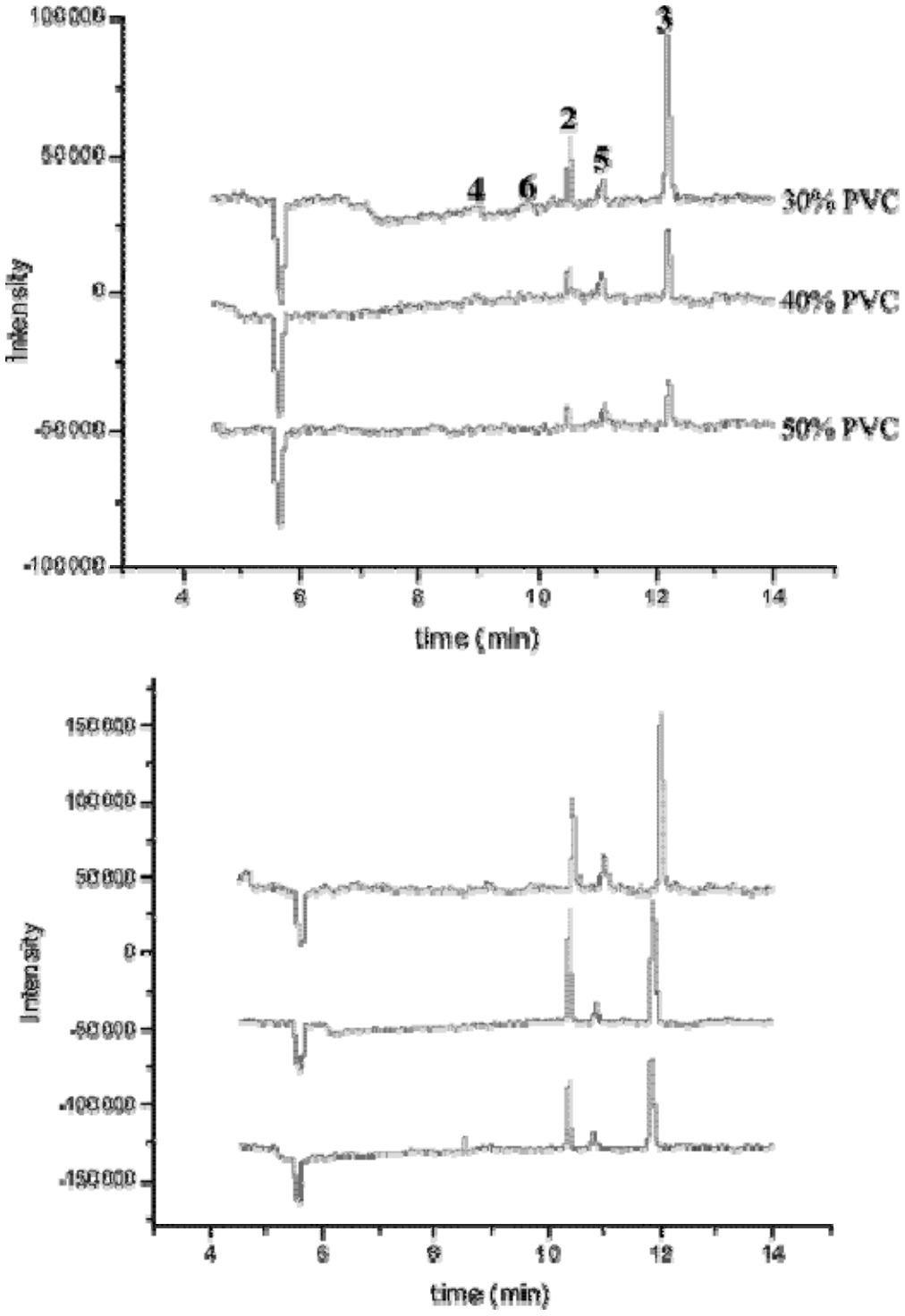


Figure 2.5 *Chromatogram of **2**-**6** extracted by SPME with plasticized PVC membranes. Top, without **1**; bottom, with **1**.*

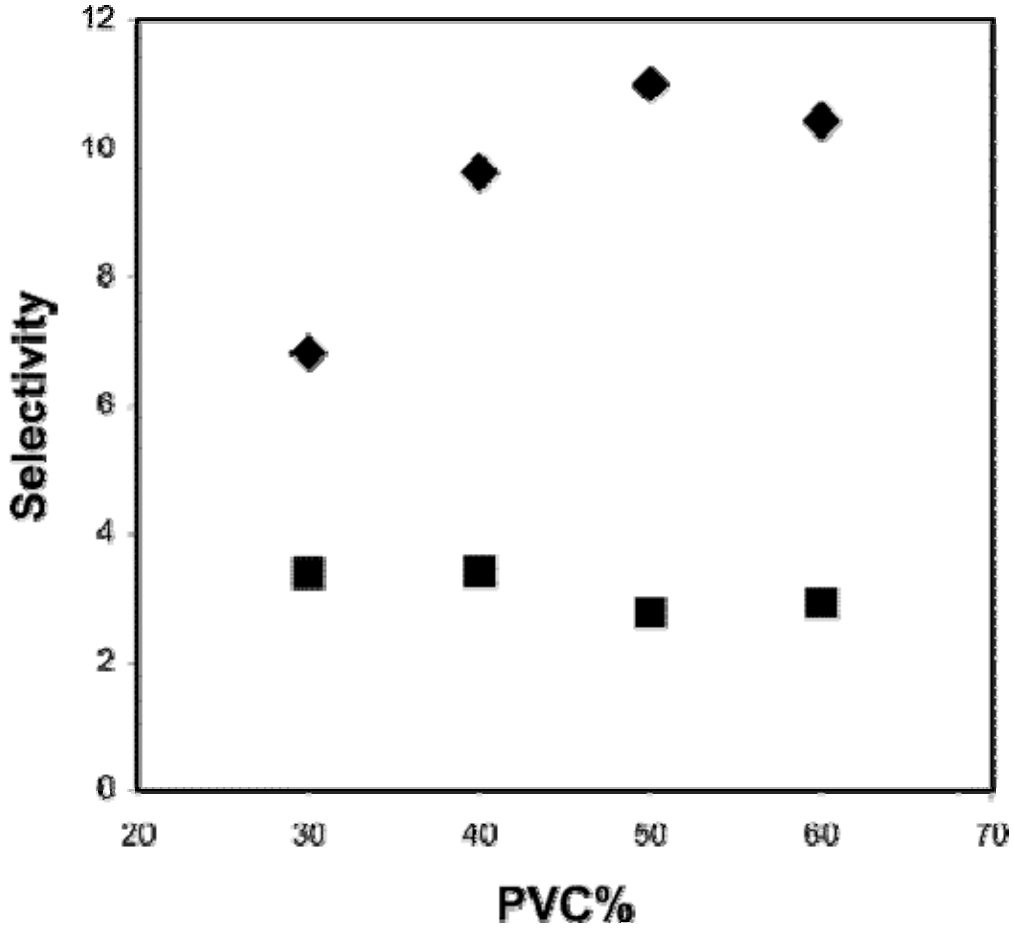


Figure 2.6 *Selectivity of membranes with different PVC percentages toward barbiturates.*
(Based on data of which Figure 2.5 is an example)
*Gray symbols: membrane with receptor **1**; black symbols, membrane without receptor **1**.*

2.5 REFERENCE

(1) Ferrer, I.; Hennion, M.-C.; Barcelo, D. *Anal. Chem.* **1997**, *69*, 4508-4514.

(2) Ferrer, I.; Pichon, V.; Hennion, M.-C.; Barcelo, D. *J. Chromatogr., A* **1997**, *777*, 91-98.

(3) Pichon, V.; Bouzige, M.; Miege, C.; Hennion, M.-C. *TrAC, Trends Anal. Chem.* **1999**, *18*, 219-235.

(4) Pichon, V.; Delaunay-Bertoncini, N.; Hennion, M.-C. *Compr. Anal. Chem.* **2002**, *37*, 1081-1100.

(5) Smith, I. D.; Simpson, C. F. *HRC-J. High-Resolut. Chromatogr.* **1993**, *16*, 530-535.

(6) Rebek, J. J. *J. Mol. Recognit.* **1992**, *5*, 83-88.

(7) Chang, S. K.; Engen, D. V.; Hamilton, A. D. *J. Am. Chem. Soc* **1991**, *113*, 7640-7645.

(8) Visser, H. C.; Reinhoudt, D. N.; Jong, F. D. *Chem. Soc. Rev.* **1994**, *23*, 75-81.

(9) Paugam, M.-F.; Bien, J. T.; Smith, B. D.; Chrisstoffels, L. A. J.; Jong, F. d.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1996**, *118*.

(10) Li, S.; Sun, L.; Chung, Y.; Weber, S. G. *Anal. Chem.* **1999**, *71*, 2146-2151.

(11) Valenta, J. N.; Dixon, R. P.; Hamilton, A. D.; Weber, S. G. *Anal. Chem.* **1994**, *66*, 2397-2403.

(12) Valenta, J. N.; Weber, S. G. *J. Chromatogr., A* **1996**, *722*, 47-57.

(13) Valenta, J. N.; Sun, L.; Ren, Y.; Weber, S. G. *Anal. Chem.* **1997**, *69*, 3490-3495.

(14) Sun, L.; Weber, S. G. *J. Mol. Recognit.* **1998**, *11*, 28-31.

(15) Zhang, X.; Zhao, H.; Weber, S. G. *Anal. Chem.* **2002**, *74*, 2184-2189.

(16) Wulff, G. *Mol. Interact. Biosep.* **1993**.

(17) O'Shannessy, D. J.; Ekberg, B.; Andersson, L. I.; Mosbach, K. *J. Chromatogr.* **1989**, *470*, 391-399.

(18) Matsui, J.; Kato, T.; Takeuchi, T.; Suzuki, M.; Yokoyama, K.; Tamiya, E.; Karube, I. *Anal. Chem.* **1993**, *65*, 2223-2224.

(19) Kempe, M.; Mosbach, K. *Tetrahedron Lett.* **1995**, *36*, 3563-3566.

(20) Rosatzin, T.; Holy, P.; Seiler, K.; Rusterholz, B.; Simon, W. *Anal. Chem.* **1992**, *64*, 2029-2035.

(21) Bakker, E.; Lerchi, M.; Rosatzin, T.; Rusterholz, B.; Simon, W. *Anal. Chim. Acta* **1993**, *278*, 211-225.

(22) Nahir, T. M.; Buck, R. P. *Helv. Chim. Acta* **1993**, *76*, 407-415.

(23) Bakker, E.; Willer, M.; Lerchi, M.; Seiler, K.; Pretsch, E. *Anal. Chem.* **1994**, *66*, 516-521.

(24) Bakker, E.; Buhlmann, P.; Pretsch, E. *Chem. Rev.* **1997**, *97*, 3083-3132.

(25) Li, S.; Weber, S. G. *Anal. Chem.* **1997**, *69*, 1217-1222.

(26) Pawliszyn, J. *Solid-Phase Microextraction Theory and Practice*; Wiley-VCH: Waterloo, 1997.

(27) Kataoka, H.; Narimatsu, S.; Lord, H. P., J. *Anal. Chem.* **1999**, *71*, 4237-4244.

(28) Diwan, B.; Rice, J. N., R.; Lubet, R.; Hu, H.; Ward, J. *Cancer Res.* **1988**, *48*, 2492-2497.

(29) Nims, R. W.; Syi, J. L.; Wink, D. A.; Nelson, V. C.; Thomas, P. E.; Jones, C. R.; Diwan, B. A.; Keefer, L. K.; Rice, J. M.; Lubet, R. A. *Chem. Res. Toxicol.* **1993**, *6*, 180-187.

(30) Terabe, S.; Otsuka, K.; Ichikawa, K.; Tsuchiya, A.; Ando, T. *Anal. Chem.* **1984**, *56*, 111-113.

(31) Lien, E. J. *J. Med. Chem.* **1970**, *13*, 1189-1191.

(32) Nims, R. W. Ph. D, The American University, Washington, DC, 1993.

3.0 AN APPLICATION OF SOLID PHASE MICROEXTRACTION FOLLOWED BY SAMPLE STACKING-MICELLAR ELECTROKINETIC CHROMATOGRAPHY ON SEDATIVE AND ANTICONVULSANT SPIKED SERUM SAMPLE ANALYSIS

3.1 ABSTRACT

Micellar electrokinetic chromatography (MEKC) and on-line concentration using normal sample stacking achieved the separation of eight barbiturate drugs and drug analogs. Separation was carried out using 90 mM Tris-TAPSO buffer containing 24 mM SDS under normal polarity. Sample stacking was best when dissolving the drug mixture in a high pH solution (20 mM NaOH solution, pH 12.10), in which the drugs have higher ionization efficiency than in the run buffer. This method has been applied to the analysis of spiked serum samples following solid phase microextraction (SPME).

3.2 INTRODUCTION

Capillary Electrophoresis (CE) is a powerful separation and analysis technique. However, the low concentration sensitivity with on-line photometric detection has been a disadvantage. The minimum detectable concentration without preconcentration lies in the micromolar range for good absorbers.[1] Some detection techniques suffer less from this problem because of better detection sensitivity, such as laser induced fluorescence,[2, 3] electrochemical detection,[4, 5] and mass spectrometric detection.[6, 7]

Higher detection sensitivity can be achieved with sample stacking. In sample stacking, a large volume of sample is injected without sacrificing separation efficiency. In the normal stacking mode in capillary zone electrophoresis (CZE),[8] the sample is prepared in a solvent that

has a lower conductivity than the run buffer. When a voltage is applied across the capillary, a greater field is developed across the sample plug, which causes the sample ions to move faster within the sample plug than in the CE run buffer. Analytes become stacked as narrow zones at the interfaces of the sample plug and the run buffer. The degree of stacking and enhancement of concentration is proportional to the ratio of the conductivity of the sample solution and the run buffer. However, laminar flow induced by the differing rates of electroosmotic flow (EOF) in the two areas results in band broadening. Calculations indicate that the best stacking result is obtained when the concentration of the sample buffer is about one tenth of the run buffer.[9]

Micellar electrokinetic chromatography (MEKC) has the ability to separate electrically neutral species.[10-12] The separation is based on the different affinities of the neutral compounds for the charged micelles in the run buffer that work as a pseudostationary phase. Stacking in MEKC is different from that in CZE, because neutral analytes do not respond to the electric field. However, stacking can be obtained through the association of neutral solutes with the charged micelles that respond to varied electric fields. Nice reviews have been published about stacking/sweeping in MEKC.[13-17] Among all the on-capillary concentration techniques, the normal stacking mode,[18-20] field amplified sample stacking,[18, 21] high salt stacking,[22-24] and sweeping[25-27] are widely used.

Barbitals and hydantoins are sedatives and anticonvulsants (structures of these compounds are shown in Figure 3.1). Phenobarbital and 5,5-diphenylhydantoin are among the prescriptions for treatment of head trauma and epilepsy.[28] A therapeutic anticonvulsant level of phenobarbital in serum is 10-25 μg/mL (43-108 μM) [http://www.rxlist.com]. With most medications, a certain level of drug is required in the blood stream to obtain the desired effect. Monitoring serum drug levels can help to ensure an effective range. HPLC,[29] CZE,[30] and MEKC[31, 32] can separate barbiturates and give quantitative analysis at the concentration of about 50 μM. Barbiturates are also frequent drugs of abuse, alone and in combination with alcohol. An overdose may result in coma and death. The implication of any concentration is more serious for short-acting than for long-acting barbiturates (e.g. phenobarbital). The toxic or lethal blood level varies with many factors and can be as low as 60 μg/mL for long-acting and 10 μg/mL for intermediate- and short-acting barbiturates (amobarbital, butabarbital, butalbital, pentobarbital, secobarbital). In presence of alcohol or other depressant drugs the lethal concentrations may be lower [http://www.labcorp.com]. It is quite useful to have a separation method for these drugs

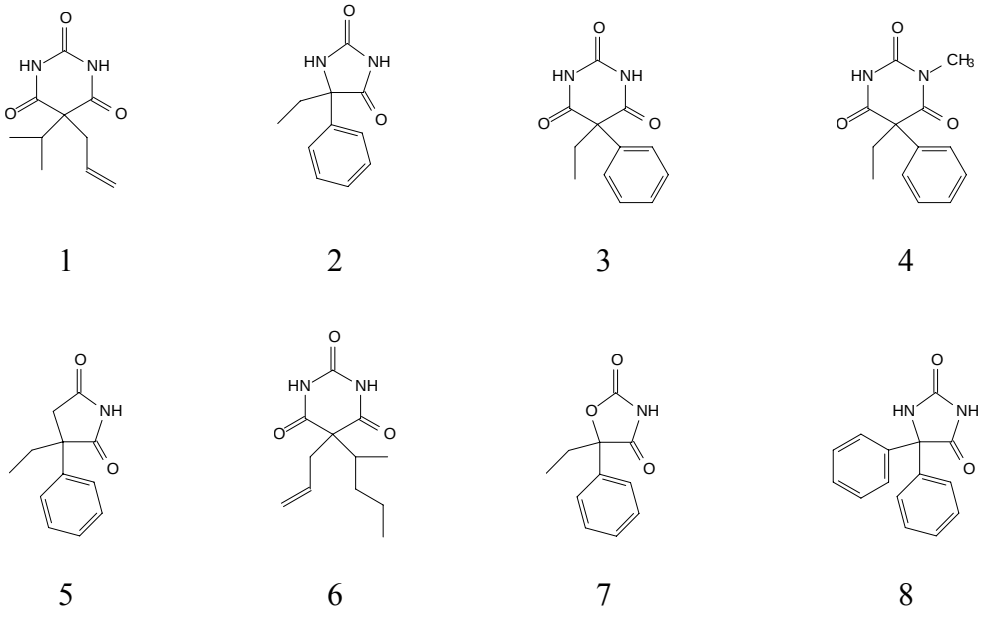


Figure 3.1 *Chemical structures of barbiturates and their analogs.*

1. aprobarbital (AB), 2. 5-ethyl-5-phenyl-hydantoin (EPH), 3. phenobarbital (PB), 4. mephobarbital (MB), 5. 2-ethyl-2-phenyl-succinimide (EPS), 6. secobarbital (SB), 7. 5-ethyl-5-phenyl-2,4-oxazolidinedione (EPO), and 8. 5,5-diphenyl-hydantoin (PH).

with a detection limit at the micromolar level. Moreover, in our studies of molecular recognition, barbiturates are among the target analytes of the artificial receptors. In order to evaluate the selectivity of the receptors, interfering drug analogues are introduced. Separation of the drugs along with their analogues is also required. The wide distribution of pK_a values of these compounds (from 5.55 to 9.17) brings difficulty for achieving a good separation and stacking at the same time by CZE. The best run buffer for separation of barbitals is around pH 8.0; however, some of the compounds are not ionized at this pH. In analysis of complicated samples, MEKC has been widely used because of its ability to separate both polar and non-polar compounds. MEKC with cyclodextrin has been applied to separate mixtures of isomers of these therapeutic drugs.[33, 34] Therefore, we investigated separation of these mixtures with MEKC and approaches to obtain sample stacking in MEKC.

3.3 EXPERIMENTAL SECTION

Reagents and solutions Tris(hydroxymethyl)aminomethane (Tris), 3-[N-tris(hydroxymethyl)-methylamino]-2-hydroxy-propanesulfonic acid (TAPSO), sodium dodecyl sulphate (SDS), aprobarbital (AB), secobarbital (sodium salt, SB), mephobarbital (MB), phenobarbital (PB), 5,5-diphenylhydantoin (PH), lyophilized bovine serum were purchased from Sigma (St. Louis, MO). PVC (high molecular weight, Selectophore) and dioctyl sebacate (DOS, Selectophore) were purchased from Fluka Chemical Co. (Ronkonkoma, NY). Santicizer 141 (90% octyl diphenyl phosphate) was a gift from Monsanto (St. Louis, MO). DL-2-Ethyl-2-phenylsuccinimide (EPS), DL-5-ethyl-5-phenylhydantoin (EPH), and 5-ethyl-5-phenyl-oxazolidinedione (EPO) were gifts from Dr. Nims (Laboratory of Comparative Carcinogenesis, Chemistry Section, National Cancer Institute at Frederick, Frederick, Maryland). Sudan III (1-(4-(phenylazo)phenylazo)-2-naphthol)) and other chemicals that are not specified were purchased from Aldrich (Milwaukee, WI). Water used in all the experiments was deionized water purified with Milli-Q A10 System (Millipore, Bedford, MA).

Buffer and sample preparation The CE run buffer was prepared by adjusting 90 mM Tris solution to pH 8.00 with TAPSO, and various amount of SDS was then added according to the requirements of the experiments. An Accumet pH meter equipped with an Orion Ross reference

electrode (Fisher Scientific) was used to measure the pH of solutions. The phosphate sample buffers were prepared by mixing 40 mM Na_2HPO_4 solution with 40 mM NaOH solution until the right pH was obtained. A series of phosphate buffers with pH values of 10.54, 11.00, 11.50, 12.00, and 12.24 were made in this way. The stock solution of the drug mixture was 100 μM for each analyte dissolved in D.I. water. In CE analysis, the stock solution was diluted by the investigated sample buffers to the desired concentrations.

The CE system and separation conditions All CE separations were per-formed on an ISCO 3850 Capillary Electropherograph (ISCO Inc., Lincoln, NE), with PeakSimple Chromatography Data System (SRI Instruments Inc., Las Vegas, NV) for data collection, and EZdata System (Q. Liang, http://www.chemilab.net) for data analysis. An uncoated fused silica capillary (Polymicro Technologies, Inc., Phoenix, AZ) with 50 μm I.D. and a total length of 70 cm (45 cm to the detection window) was employed. A detection window 0.5 cm wide was created by stripping the polyimide coating of the capillary. UV absorbance was monitored at the wavelength of 210 nm for maximum detection sensitivity for all compounds. A new CE capillary was conditioned with 1.0 M sodium hydroxide for 1 hour followed by 0.1 M sodium hydroxide for 2 hours. Before running samples, the capillary was flushed with water and then the run buffer. The capillary was conditioned with the same procedure and stored in 0.1 M sodium hydroxide everyday after experiments. Non-stacking experiments used hydrodynamic injection of 5 s at 0.5 psi vacuum; stacking experiments used hydrodynamic injections of 5 to 100 s at the same vacuum. All separations were conducted under normal polarity at 27 kV, giving an average current of 19 μA in CZE and 28 μA in MEKC. Methanol was used to determine the velocity of EOF, and Sudan III was used to determine the velocity of SDS micelles in MEKC.

SPME pretreatment and analysis of spiked serum samples The procedures for SPME device preparation, extraction, and back-extraction have been established in our lab.[30] Briefly, a stainless steel rod (1.1 mm O.D.) was dip-coated to form membranes of santicizer- plasticized PVC (Please refer to Chapter 2 for detailed procedure). For spiked serum samples, aliquots of deionized water containing various concentrations of drugs were injected to the vial containing lyophilized bovine serum to the specified volume. These spiked serum samples were then diluted by the same volume of 25 mM acetate buffer (pH 4.5) to favor the extraction. Each extraction was conducted by immersing a SPME probe into a 125 μL sample solution for 15 minutes, followed by rinsing the probe with D. I. water to remove any serum adsorbed on the probe

surface. The probe was then transferred into a Teflon tube (inside diameter 1.2 mm, Small Parts, Miami Lakes, FL) containing 10 μl of 20 mM NaOH and allowed to sit for 30 minutes. At the end of the back extraction, the probe was removed from the Teflon tube and a 1 ml syringe was used to push out the back extraction solution into a sample vial for analysis.

3.4 RESULTS AND DISCUSSION

3.4.1 Separation of the drug mixture by CZE and MEKC

Eight analytes are separated by CZE using pH 8.00 Tris-TAPSO buffer, and by MEKC with the same buffer containing 24 mM SDS. The advantage of using Tris-TAPSO buffers is that they generate little heat with high concentration, thus can improve the CE separation[30] by applying relatively high voltage. With the Tris concentration at 90 mM, the current is only 19 μA in CZE and 28 μA in MEKC when a 27 kV voltage is applied.

Both CZE and MEKC give satisfactory separation of the eight compounds, as shown in Figure 3.2. In CZE, we note that the migration time of these compounds is related to their pK_a values: the ones with higher pK_a values are less ionized at pH 8.00 and have migration velocity closer to EOF. PH (5,5-diphenylhydantoin) is the only exception. This is probably because of its bulky size compared to other compounds. The peaks of two compounds: EPS (pK_a 9.17), and EPH (pK_a 8.85) are so close to the initial void peak, that their quantification is affected by the wide initial void peak in stacking mode in which a large volume of samples is injected (Figure 3.3A).

In MEKC, the stacking effect is not satisfactory at this circumstance, as shown in Figure 3.3B, since the sample is prepared in water. Later work shows greatly increased stacking performance by preparing the sample in NaOH. The peak order is different from that in CZE. Five compounds, PH, EPS, SB, MB, and EPH have longer migration time than in CZE. The other three compounds, AB, PB, and EPO have similar migration time as in CZE. The shift of the migration time of a solute is related to its retention factor (k') that defines the affinity of a compound for the micelle pseudostationary phase. The retention factor of an anionic solute in

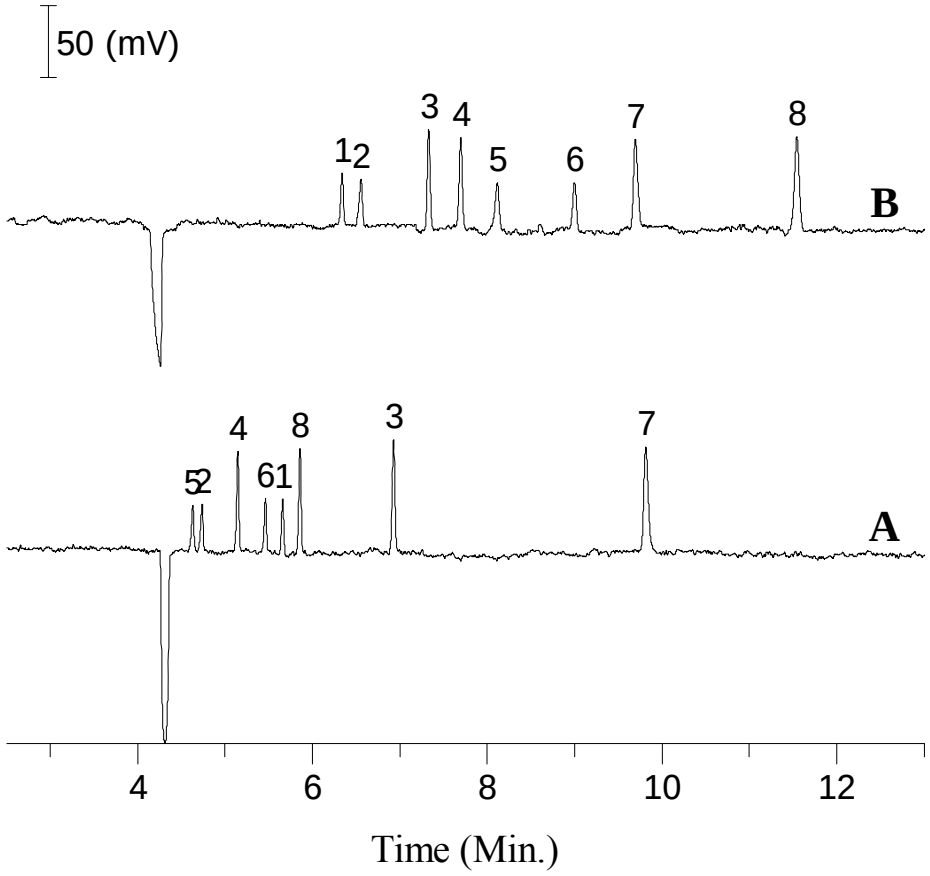


Figure 3.2 *Separation of barbiturates and their analogues by CEZ and MEKC.*

Separation was carried out on a 70 cm fused silica capillary applied with 27 kV voltage. A: CZE, buffer: 90 mM Tris-TAPSO solution (pH 8.00). B: MEKC, buffer: 90 mM Tris-TAPSO solution (pH 8.00) containing 24 mM SDS. Injection: 5 s at 0.5 psi vacuum. Detection: UV detection at 210 nm. Analytes: 100 μM for each (dissolved in water). Peaks: 1 (AB), 2 (EPH), 3 (PB), 4 (MB), 5 (EPS), 6 (SB), 7 (EPO), and 8 (PH).

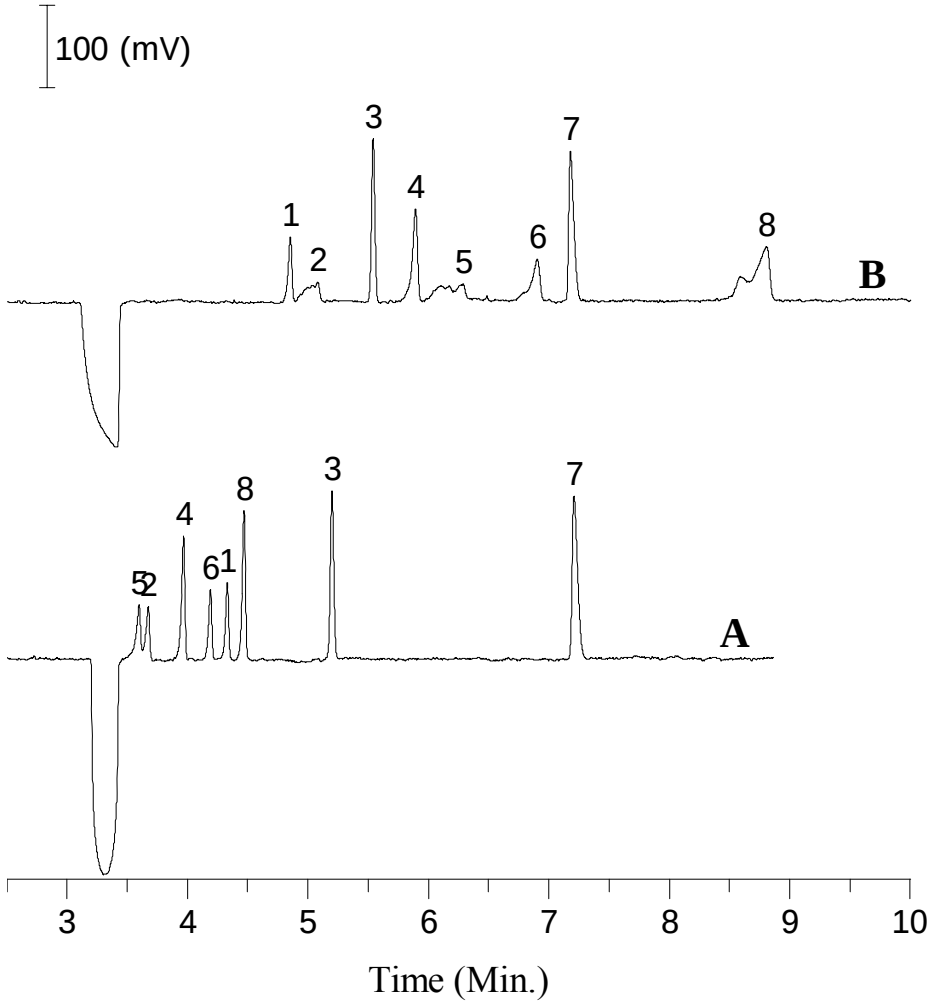


Figure 3.3 *Sample stacking in CZE and MEKC.*

The conditions were the same as that in Figure 3.2, except that the concentration for each analyte was 50 μM (dissolved in water), and the injection time was 30 s. A: CZE; B: MEKC. Peaks: 1 (AB), 2 (EPH), 3 (PB), 4 (MB), 5 (EPS), 6 (SB), 7 (EPO), and 8 (PH).

micellar solution, k_i', can be calculated by comparing the separation in CZE and in MEKC according to Equation 3.1.[8]

$$k_i' = \frac{\mu_i - \mu_{EP(i)}}{\mu_{mc} - \mu_i} \qquad \text{(Equation 3.1)}$$

where μ_i is the observed electrophoretic mobility of an anion in a micellar solution, $\mu_{EP(i)}$ is the observed mobility of the anion in the absence of micelles, and μ_{mc} is the mobility of the micellar phase that can be measured by Sudan III. As shown in Table 3.1, the retention factors of these compounds are in the range of 0.1 to 2.5 at the selected MEKC conditions.

3.4.2 Optimum MEKC sample stacking condition

The effect of SDS concentration, sample buffer pH and concentration, sample injection time, etc., on the MEKC sample stacking was studied by Hong Zhao in our lab. The optimum condition is listed as follow: The run buffer is 90 mM Tris-Tapso buffer at pH 8.0, containing 24 mM SDS. The sample buffer is 20 mM NaOH and injection time 30 seconds at 0.5 psi vacuum.

3.4.3 Determination of drugs in bovine serum

To apply the established method to serum samples, pretreatment is found necessary. A solid phase microextraction-CE (SPME-CE) procedure has been developed in our lab[30, 35]. In SPME, plasticizers function as nonvolatile solvents. The forward extraction is carried out in an acidic buffer (pH 4.50), and the back extraction is in a basic solution (20 mM NaOH), which is also the optimized sample preparation condition for MEKC stacking. Various PVC plasticizers: tributylphosphate (TBP), dioctyl sebacate (DOS), and Santicizer 141 (90% octyl diphenyl phosphate) have been investigated. Santicizer 141-doped PVC films are stable in the experimental conditions and provide satisfactory extraction for all the eight compounds. Figure 3.4 shows the electropherograms of a direct injection of a serum sample and an injection of serum sample treated by SPME. The SPME procedure is very effective to remove the background of the serum matrix. Comparing with the SPME treatment of aqueous standard solutions, the spiked serum samples have lower preconcentration factors for all the eight

Table 3.1 *The retention factors of the analytes for SDS micelles.*

Analyte	AB	EPH	PB	MB	EPS	SB	EPO	PH
Peak No.	1	2	3	4	5	6	7	8
pK_a [39]	7.83	8.85	7.63	7.97	9.17	7.81	5.55	8.33
	±0.20	±0.50	±0.20	±0.60	±0.50	±0.20	±0.70	±0.50
Retention factor (k_i')[a]	0.24	0.64	0.17	0.89	1.4	1.3	0.10	2.5

[a]The retention factors were measured under the same conditions as in Figure 3.2B.

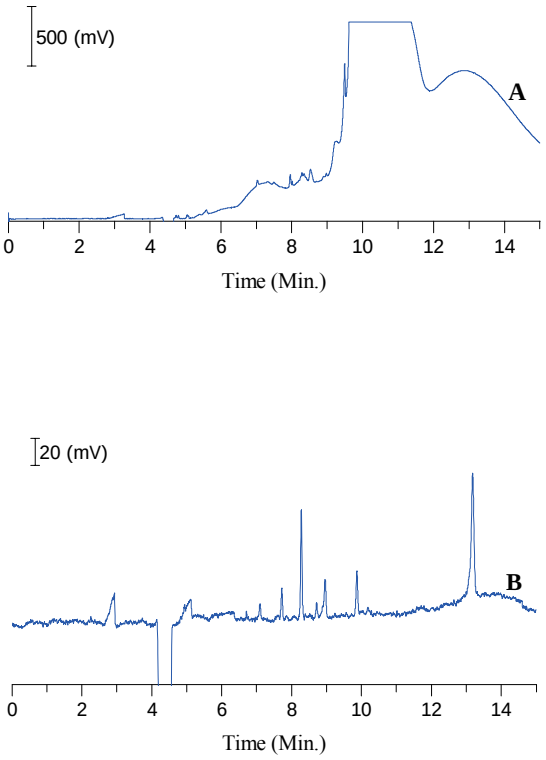


Figure 3.4 *Determination of anticonvulsants in serum with stacking-MEKC.*

A: direct injection of a serum sample spiked with 50 μM drug standards. B: injection of a serum sample pretreated with SPME: extraction with a SPME rod coated with PVC-santicizer film for 15 minutes, back-extraction with 10 μl 20 mM NaOH solution for 30 minutes. CE injection: 30 s.

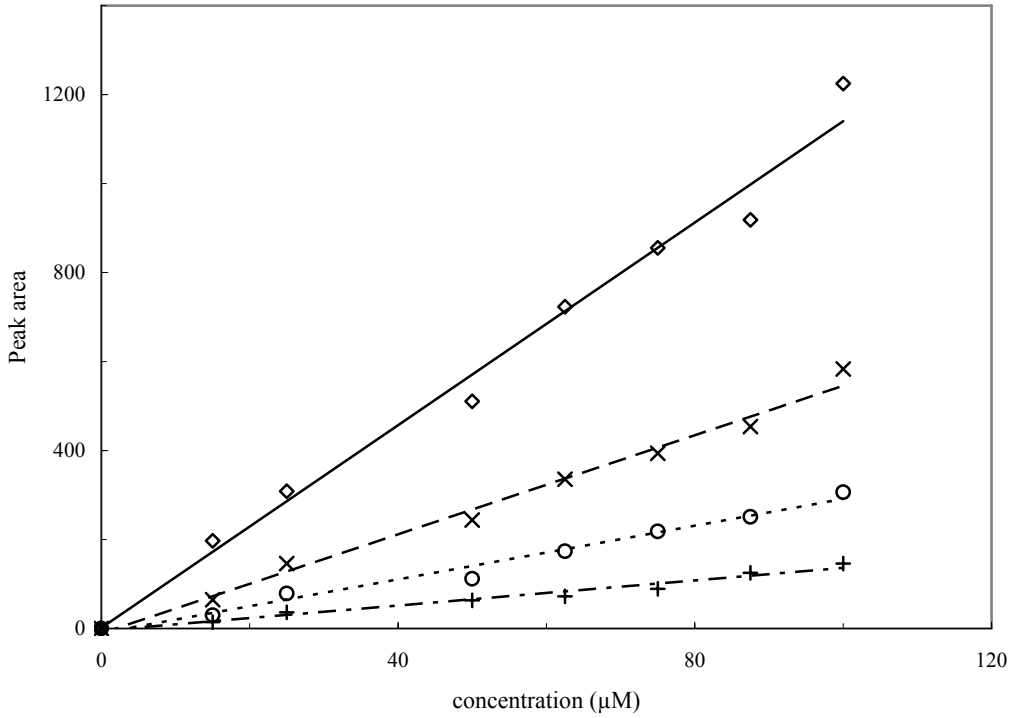


Figure 3.5 *Standard curves for analytes spiked in bovine serum. The analysis procedures were the same as in Figure 3.4B. Analytes: PH (◊), SB (O), MB (+), and PB (×).*

compounds. This might due to the binding of these compounds to the serum proteins.[30] The preconcentration factor of EPO is too low to get its quantitative measurement. Calibration curves of serum samples with concentration range from 15 μM to 100 μM give good linearity for the rest seven investigated compounds, with correlation coefficients from 0.990 to 0.997. Figure 3.5 shows the calibration curves for four anticonvulsants that might be prescribed for patients: 5,5-diphenylhydantoin (PH), phenobarbital (PB), secobarbital (SB), and mephobarbital (MB).

3.5 REFERENCE

(1) Boone, C. M.; Franke, J. P.; de Zeeuw, R. A.; Ensing, K. *Journal of Chromatography. A* **1999**, *838*, 259-272.

(2) Couderc, F.; Causse, E.; Bayle, C. *Electrophoresis* **1998**, *19*, 2777-2790.

(3) Jiang, J.; Lucy, C. A. *Journal of Chromatography, A* **2002**, *966*, 239-244.

(4) Qian, J.; Wu, Y.; Yang, H.; Michael, A. C. *Analytical Chemistry* **1999**, *71*, 4486-4492.

(5) Baldwin, R. P. *Electrophoresis* **2000**, *21*, 4017-4028.

(6) Schmitt-kopplin, P.; Frommberger, M. *Electrophoresis* **2003**, *24*, 3837-3867.

(7) Chen, Y.-R.; Tseng, M.-C.; Chang, Y.-Z.; Her, G.-R. *Analytical Chemistry* **2003**, *75*, 503-508.

(8) Baker, D. R. *Capillary Electrophoresis*, 1995.

(9) Burgi, D. S.; Chien, R. L. *Analytical Chemistry* **1991**, *63*, 2042-2047.

(10) Terabe, S.; Otsuka, K.; Ichikawa, K.; Tsuchiya, A.; Ando, T. *Analytical Chemistry* **1984**, *56*, 111-113.

(11) Issaq, H. J. *Electrophoresis* **1999**, *20*, 3190-3202.

(12) Molina, M.; Silva, M. *Electrophoresis* **2002**, *23*, 3907-3921.

(13) Quirino, J. P.; Terabe, S. *Journal of Capillary Electrophoresis* **1997**, *4*, 233-245.

(14) Pyell, U. *Fresenius' Journal of Analytical Chemistry* **2001**, *371*, 691-703.

(15) Kim, J.-B.; Terabe, S. *Journal of Pharmaceutical and Biomedical Analysis* **2003**, *30*, 1625-1643.

(16) Chien, R.-L. *Electrophoresis* **2003**, *24*, 486-497.

(17) Urbanek, M.; Krivankova, L.; Bocek, P. *Electrophoresis* **2003**, *24*, 466-485.

(18) Liu, Z.; Sam, P.; Sirimanne, S. R.; McClure, P. C.; Grainger, J.; Patterson, D. G., Jr. *Journal of Chromatography, A* **1994**, *673*, 125-132.

(19) Kruaysawat, J.; Marriott, P. J.; Hughes, J.; Trenerry, C. *Electrophoresis* **2003**, *24*, 2180-2187.

(20) Quirino, J. P.; Terabe, S. *Journal of Chromatography, A* **1997**, *781*, 119-128.

(21) Aguete, E. C.; Gago-Martinez, A.; Leao, J. M.; Rodriguez-Vazquez, J. A.; Menard, C.; Lawrence, J. F. *Talanta* **2003**, *59*, 697-705.

(22) Palmer, J.; Munro, N. J.; Landers, J. P. *Analytical Chemistry* **1999**, *71*, 1679-1687.

(23) Carabias-Martinez, R.; Rodriguez-Gonzalo, E.; Revilla-Ruiz, P.; Dominguez-Alvarez, J. *Journal of Chromatography, A* **2003**, *990*, 291-302.

(24) Choy, T. M. H.; Chan, W.-H.; Lee, A. W. M.; Huie, C. W. *Electrophoresis* **2003**, *24*, 3116-3123.

(25) Quirino, J. P.; Terabe, S.; Bocek, P. *Analytical Chemistry* **2000**, *72*, 1934-1940.

(26) Quirino, J. P.; Terabe, S. *Science (Washington, D. C.)* **1998**, *282*, 465-468.

(27) Quirino, J. P.; Terabe, S. *Analytical Chemistry* **2000**, *72*, 1023-1030.

(28) Eadie, M. J.; Vajda, F. J. E.; (Editors) *Antiepileptic Drugs: Pharmacology and Therapeutics*; Springer, Berlin, Germany, 1999.

(29) Valenta, J. N.; Weber, S. G. *Journal of Chromatography, A* **1996**, *722*, 47-57.

(30) Li, S.; Weber, S. G. *Analytical Chemistry* **1997**, *69*, 1217-1222.

(31) Meier, P.; Thormann, W. *Journal of Chromatography* **1991**, *559*, 505-513.

(32) Thormann, W.; Meier, P.; Marcolli, C.; Binder, F. *Journal of Chromatography* **1991**, *545*, 445-460.

(33) Zaugg, S.; Caslavska, J.; Theurillat, R.; Thormann, W. *Journal of Chromatography. A* **1999**, *838*, 237-249.

(34) Srinivasan, K.; Bartlett, M. G. *Rapid Communications in Mass Spectrometry* **2000**, *14*, 624-632.

(35) Zhang, X.; Zhao, H.; Chen, Z.; Nims, R.; Weber, S. G. *Analytical Chemistry* **2003**, *75*, 4257-4264.

4.0 SCREENING OF PEPTIDE MIMICS AS POTENTIAL CHIRAL STATIONARY PHASES IN LIQUID CHROMATOGRAPHY

4.1 ABSTRACT

Peptide mimics have the potential to be useful as chiral stationary phases (CSPs). For fast evaluation of their enantioselectivity, a screening method is proposed based on molecular recognition in plasticized PVC membrane. The advantage of this method is that it does not require the covalent immobilization of either the analyte or the selector, and the potential selector usage is at the microgram level. The method needs to be verified prior to application to libraries of peptide mimics. R/S-N-(3,5-dinitrobenzoyl)-phenylglycine bonded to silica gel is a commercial available brush-type CSP which can resolve various racemic mixtures, such as Troger's base, 2,2,2-trifluoro-1-(9-anthryl)-1-ethanol, 1-phenyl-butanol, etc. We have used freely diffusing selectors (R or S-N-(3,5-dinitrobenzoyl)-phenylglycine and its methyl ester). The selectors are doped into plasticized PVC membranes. The test analytes are the three mentioned above. Distribution difference of the two enantiomers of the Troger's base and 1-phenyl-butanol could be detected by the extraction of plasticized PVC membrane containing the test chiral selector. The difference in the selectivities is significant ($p<0.01$) by a t-test. Thus, we have demonstrated that a partitioning experiment with a selector-doped membrane can be used to determine the efficacy of a potential chiral selector.

4.2 INTRODUCTION

Chiral separation of racemate compounds is getting increased interests with the development of pharmaceutical industry.

How to resolve racemic mixtures into two enantiomers? It necessarily requires involvement of a chiral resolving agent – a chiral selector, to form a diastereomeric complex with one of the enantiomers, or both, but with different formation constants. Enantioselectivity, α, the ratio of the binding constants in the interaction of the chiral selector with the two enantiomers, is the key leading to the resolving of racemates. Depending on different values of α, either "single step" or "multi-step" procedure can be chosen to fulfill the goal of chiral separation. In the case of "single step" procedure, in order to obtain a product with an optical purity of 98-99%, the discrimination of the enantiomers by a factor of $\alpha = 100$ is desired.[1] While in most applications, it is almost impossible to find such a chiral selector with that extremely high enantioselectivity. As a result, chromatography, standing as a "multi-step" separation method, becomes the most used technique to effectively employ a large number of chiral selectors with the value of α as low as 1.01. Today, the most applicable technique for chiral separation remains either liquid or gas chromatography on chiral stationary phases.

Giving a library of enantiopure compounds, how to evaluate their abilities to be useful as chiral selectors? Or, in our case, if the synthetic peptide mimics can be employed as CSPs in LC? A screening method is required for the rapid evaluation of potential chiral selectors.

Reciprocal principle was proposed by Pirkle[2] and employed widely[3-5] for the fast screening of brush-type chiral selectors. This indirect method though sometimes effective, is limited to a tethered version of analytes, and the analyte attachment to the solid phase can not be prevented. Evaluation of chiral selectors by NMR or by CE[6, 7] is also not straightforward. Welch[8, 9] provided a direct evaluation of the selector-analyte interaction, but the immobilization of every potential selector to the solid phase is burdensome.

Do we have to covalently immobilize each of the peptide mimics to silica gel and pack them into a column? Do we have to measure optical resolution of every racemic analyte resolved by the column? Not really. The proposed method in this chapter provides an efficient and direct way to measure α with a relatively low value hence tells the possibility of a peptide mimic as a chiral selector.

In our previous work,[10] we have proved that doping of artificial receptor to a plasticized PVC membrane can greatly increase the solute extraction from the aqueous phase to the membrane phase. The receptor forms complex with the solute in the membrane by hydrogen bonds thus increases the distribution coefficient of the solute significantly. According to Pirkle's

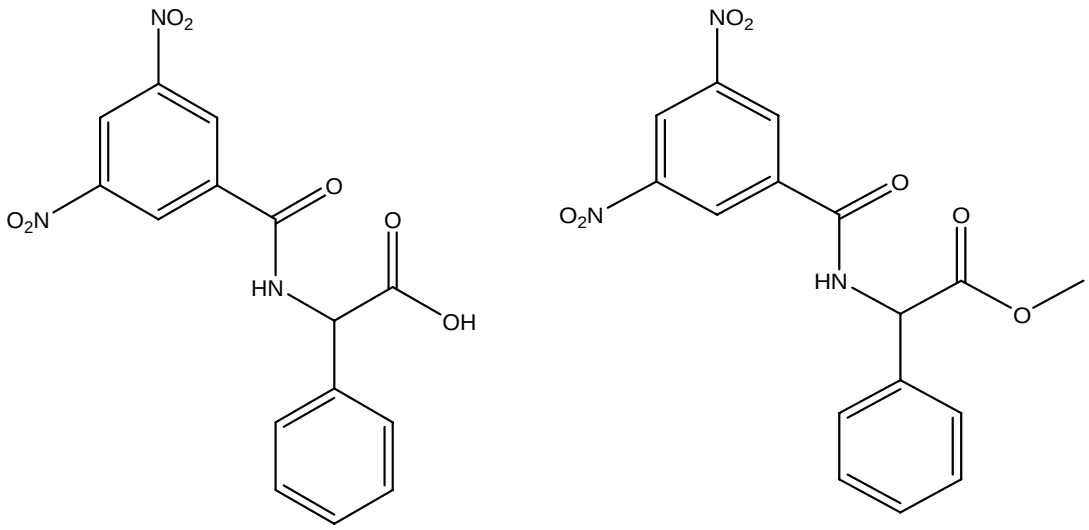
"three-point interaction model",[11] hydrogen bonding is among the most important interactions between the chiral selector and the analyte, as well as π - π bonding, dipole – dipole interaction, etc. Therefore, in principle, by doping of the chiral selector into a plasticized PVC membrane, one of the analyte enantiomers should also have an increased distribution into the membrane. This actually is a "single step" process. Not mentioning achiral partitioning, with a relatively low enantioselectivity, this process can not obtain 100% optical pure product definitely. Actually, both enantiomers should have a greater distribution coefficient based on the formation of the selector – analyte complex, but there should be still a difference if α is greater than 1. In other words, different amount of enantiomers will be extracted into the chiral selector doped membrane. The concentration of enantiomers remaining in the liquid phase will also be different as well.

Though the above mentioned concentration difference is pretty small, it provides us a possibility to measure it and in return calculate α. The first step of this research is to verify this method. A commercial available CSP[12] was chosen and its analogue was prepared as R/S-N-(3,5-dinitrobenzoyl)-phenylglycine methyl ester. We doped the test selector into plasticized PVC membranes. Test analytes were started from what Felix refered to as the "classical racemates".[13]

4.3 EXPERIMENTAL SECTION

Reagents and Solutions Racemic Troger's Base, R/S-2,2,2-trifluoro-1-(9-anthryl)-1-ethanol (TFAE), R/S-1-phenyl-butanol, R/S-N-(3,5-dinitrobenzoyl)-phenylglycine (DNBPG), tributyl phosphate (TBP), dioctyl phthalate (DOP), HPLC grade tetrahydrofuran (THF) were purchased from Aldrich. Secobarbital is purchased from Sigma. PVC (high molecular weight, Selectophore), dioctyl sebacate (DOS, Selectophore) and chloroparaffin (CLP) were purchased from Fluka. TRI-n-butyl citrate (TBC) was purchased from ICN Biomedicals, Inc. (Aurora, Ohio). Water used in all the experiments was deionized water purified with Milli-Q A10 System (Millipore, Bedford, MA). All other compounds and solvents were AR grade or better and purchased from commercial sources.

Synthesis of (S)- N-(3,5-dinitrobenzoyl)-phenylglyciyl methyl ester (shown as *2* in Figure 4.1) (S)- N-(3,5-dinitrobenzoyl)-phenylglycine (177 mg) was dissolved in 1 mL methanol and



1 ***2***

Figure 4.1 *N-(3,5-dinitrobenzoyl)-phenylglycine (DNBPG) (**1**) and its methyl ester (**2**).*

3.5 mL hexane, cooled to 0 ºC. 0.75 mL TMSCHN2 (2M in hexane) was added, the solution was then warmed to room temp and stirred for 1h. After washed by $NaHCO_3$, extracted by ethyl acetate, then washed by saturated NaCl solution and water, dried by MgSO4, light red solid was obtained. The yield is 180 mg. ^{1}H NMR (300 MHz, DMSO): δ 9.98 (d, 1H, NH), 9.13 (s, 2H, ArH), 8.98 (s, 1H, ArH), 7.49-7.42 (m, 5H, ArH), 5.73 (d, 1H, CH), 3.69 (s, 3H, OCH_3).

Analyte solution preparation Racemic Troger's base was dissolved in 1-10 mM HCl solution. R- and S- 2,2,2-trifluoro-1-(9-anthryl)-1-ethanol enantiomers were firstly dissolved in ethanol then diluted by water, respectively. R- and S- 1-phenyl-butanol were prepared in 25 mM HCl / ethanol (90/10) solution. 1 mM secobarbital was prepared in water.

Determination of analyte concentration Capillary electrophoresis was used to perform chiral resolution of Troger's base as well as quantitive analysis. The concentration of secobarbital and R/S-1-phenyl-butanol was determined by micellar electrokinetic chromatography (MEKC). UV spectrophotometry was used to determine the concentration of R- and S- 2,2,2-trifluoro-1-(9-anthryl)-1-ethanol enantiomers.

Equipments Chiral separations of Troger's base were performed on an ISCO 3850 Capillary Electropherograph (ISCO Inc., Lincoln, NE). Data acquisition and integration were operated through Peak Simple (SRI Instruments, Torrance CA). MEKC and other CE separations were performed on a BioFocus 3000 capillary electrophoresis system. UV spectra were acquired with an HP 8453 spectrometer.

Membrane preparation Plasticized PVC membranes were made in mass ratios PVC: DOS = 50:50 and PVC: TBP =50:50. The ratios were kept the same in plasticized PVC membranes containing chiral selectors. The selector concentration is calculated as below:

$$C_s = \frac{n_m}{V_m} = \frac{W_s / M_s}{W_m / d_m} \qquad \text{(Equation 4.1)}$$

Where C_s is the concentration of selector in plasticized PVC membrane, n_m is the moles of the selector. V_m is the membrane volume, W_s is the weight of selector contained in the membrane, M_s is the molecular weight of selector, W_m is the weight of membrane piece, and d_m is the density of membrane which is estimated as 1g/cm^3.

For Troger's base, secobarbital and R/S-1-phenyl-butanol distribution experiments The PVC/plasticizer mixture (1 g total in mass) was dissolved in 20 mL THF. The solution was then transferred to a dish (diameter 7.5 cm) with an optically flat bottom. The solvent was

allowed to evaporate overnight. The membrane was removed from the glass surface and cut into small pieces to facilitate partitioning.

For TFAE distribution experiments The PVC/plasticizer mixture was dissolved by THF to make a 50 mg/ml solution. Aliquots (0.3-1 ml) of the solution were transferred to autosampler vials (Agilent). After solvent evaporation, membranes were formed at the bottom of these vials.

Troger's base, secobarbital and R/S-1-phenyl-butanol distribution experiments Small pieces of membrane were weighed then transferred to scintillation vials. Membrane volume was calculated as W_m / d_m (refer to Equation 4.1). Analyte solutions were added to these vials with certain volume ratios. With stirring, the extraction was performed for a certain period of time. The concentration of analytes remained in the solution was determined by CE. For Troger's base, the membrane pieces were taken out after the extraction, dip washed by D.I. water then transferred to 100 μL of 100 mM HCl solution for back extraction. By carrying chiral CE separation, the concentration of the two enantiomers of Troger's base could be determined.

TFAE partitioning experiments R- and S- 2,2,2-trifluoro-1-(9-anthryl)-1-ethanol solutions, respectively, with fixed volume, were transferred to autosampler vials containing membranes. After a long enough time sitting for the reaching of partition equilibrium (usually more than 10 hours), the solution was one by one transferred to quartz cuvettes for UV analysis to determine the TFAE amount remaining in the solutions.

Chiral separation of Troger's Base Chiral separation of Troger's Base was performed on an ISCO 3850 Capillary Electropherograph (ISCO Inc., Lincoln, NE). An uncoated fused silica capillary (Polymicro Technologies, Inc., Phoenix, AZ) with 50 μm I.D. and a length of 70 cm in total/ 45 cm to the detection window was employed. A detection window of 0.5 cm wide was created by stripping the polyimide coating of the capillary. UV absorbance was monitored at the wavelength of 210 nm for maximum detection sensitivity. A new CE capillary was conditioned with 1.0 M sodium hydroxide for 1 hour followed with 0.1 M sodium hydroxide for 2 hours. Before running samples, the capillary was flushed with water and then the run buffer. The capillary was conditioned with the same procedure and stored in 0.1 M sodium hydroxide everyday after experiments.

Cyclodextrins (CDs) and their derivatives are the most commonly used chiral selectors in CE at the present time. CDs offer multiple forces for efficient interaction with guest molecules,

combining with the different cavity dimensions of α-, β- and γ-CDs and their derivatives, hence provide the most widespread applications in chiral CE separation. Troger's base could get optical resolution run in a sulfated-β-CD containing buffer[14, 15]. The separation is optimized here by tuning the run buffer pH and sulfated-β-CD concentration.

By applying sulfated-β-cyclodextrin (3% w/v) containing 10 mM sodium phosphate solution as run buffer, optical resolution of Troger's base could be achieved at various pH values: 4.5, 6.8 and 8.0. The retention times of the two enantiomers decrease respectively with the increase of buffer pH due to the greater EOF at more basic environment. However, by suppressing the EOF, analytes could react with the selector for a longer time, thus increase the plate number as well as the separation efficiency. Another way to help the separation is to increase the selector concentration in run buffer. Experiments showed that at pH 8.0, 3% w/v sulfated-β-cyclodextrin was needed while at pH 4.5, 1.5% w/v was sufficient to provide a nice separation. For uncoated fused silica capillary, electrophoresis separation run at basic environment gives better reproducibility. Hence we chose sulfated-β-cyclodextrin (3% w/v) in 10 mM sodium phosphate buffer (pH 8.0) as run buffer, for the chiral separation and concentration determination of Troger's base. In some other cases, when the complex formation of sulfated-β-cyclodextrin with the analyte enantiomers is too weak, run buffer at low pH might be required, since high concentration sulfated-β-cyclodextrin significantly increases the joule heating resulted from the greatly increased operation current. Organic modifiers such as methanol may also help to decrease the conductivity of the run buffer as well as the current.

MEKC Concentration determination of secobarbital and R/S-1-phenyl-butanol enantiomers was performed on a BioFocus 3000 capillary electrophoresis system. Same uncoated fused silica capillary was used as above, except for length of 50 cm in total/ 46 cm to the detection window. The new CE capillary was flushed at 100 psi with 1.0 M sodium hydroxide for 20 min followed with 0.1 M sodium hydroxide for 30 min. Before running each sample, the capillary was flushed with water and then the run buffer for 2 minutes, respectively. The capillary was stored in 0.1 M sodium hydroxide everyday after experiments, and after the same condition process. The separation buffer was 25 mM sodium dodecyl sulfate (SDS) in 115 mM Tris solution adjusted to pH 8.01 with TAPSO. Samples were injected at the inlet of the capillary for 5 psi·s. The separation was performed at 20 ºC and the voltage applied was 25.0 kV. The current was ~43 μA. The wavelength set for detection was 215 nm.

4.4 RESULTS AND DISCUSSION

4.4.1 Simple model

By assuming that the complex formation constants of the chiral selector with the two enantiomers of the analyte remain the same at various selector concentrations in the receiving phase, we can establish a simple model to predict the optimum parameters we should follow, for the purpose of maximizing the concentration difference of the two enantiomers remaining in the starting phase after reaching the distribution equilibrium and thus determine the enantioselectivity more accurately.

In order to illustrate the model, we have the following notations.

***V_1*:** volume of starting phase

***V_2*:** volume of receiving phase

***C_0*:** initial concentration of analyte in the starting phase

***C_{11}*:** analyte concentration in the starting phase after reaching the partition equilibrium, in the case of with no chiral selector in the receiving phase

***C_{12}*:** analyte concentration in the receiving phase after reaching the partition equilibrium, in the case of with no chiral selector in the receiving phase

***Kp*:** partition coefficient of analyte from the starting phase to the receiving phase, $Kp = \frac{C_{12}}{C_{11}}$

***C_{21}*:** analyte concentration in the starting phase after reaching the distribution equilibrium, in the case of with chiral selector in the receiving phase

***C_{22}*:** analyte (both free and complex state) concentration in the receiving phase after reaching the distribution equilibrium, in the case of with chiral selector in the receiving phase.

***Cs*:** selector concentration in the receiving phase

***Cx*:** complex concentration in the receiving phase after equilibrium

***Kf*:** complex formation constant, $Kf = \frac{Cx}{(C_{22} - Cx)(Cs - Cx)}$

In the case of with no selector in the receiving phase

$$C_0 = \frac{C_{11}V_1 + C_{12}V_2}{V_1} \qquad \text{(Equation 4.2)}$$

we can solve the equation and get

$$C_{11} = \frac{C_0}{1 + \frac{KpV_2}{V_1}} \qquad \text{(Equation 4.3)}$$

In the case of with selector in the receiving phase

$$C_0 = \frac{C_{21}V_1 + C_{22}V_2}{V_1} \qquad \text{(Equation 4.4)}$$

together with

$$Kf = \frac{Cx}{(C_{22} - Cx)(Cs - Cx)} \qquad \text{(Equation 4.5)}$$

we can get

$$C_{21} = \frac{-b + \sqrt{b^2 - 4ac}}{2a} \qquad \text{(Equation 4.6)}$$

where

$$a \equiv V_1 KpKf + V_2 Kp^2 Kf$$

$$b \equiv V_1 + V_2 Kp + CsV_2 KpKf - C_0 V_1 KpKf$$

$$c \equiv -C_0 V_1$$

By applying Equation 4.3 and 4.6 to a Matlab program, with $\boldsymbol{V_1}$, $\boldsymbol{V_2}$, $\boldsymbol{C_0}$, $\boldsymbol{Cs}$, $\boldsymbol{Kp}$, $\boldsymbol{Kf}$ as variables, we can simulate the analyte partition and distribution processes.

The first concern for the screening purpose is that, if the potential selector combines with the analyte to form complexes in the receiving phase? If yes, what phenomena we are expecting to observe?

It is obvious that, with the chiral selector in the receiving phase, more analyte should be extracted from the starting phase, if the selector has the ability to form complexes with the analyte. Figure 4.2 shows the concentration of analyte remaining the starting phase after extraction, with or without selector. The relative concentration difference is ranging from near zero to more than 30%, as shown in Figure 4.3. When the partition coefficient is small, low $\boldsymbol{V_1}$ to $\boldsymbol{V_2}$ ratio favors the relative concentration difference. While with the increase of $\boldsymbol{Kp}$, higher $\boldsymbol{V_1}$ to $\boldsymbol{V_2}$ ratio is required to maximize the concentration difference. In return, as long as $\boldsymbol{C_{11}}$ is

measured, ***Kp*** is calculable. Together with the detection of the concentration difference, ***Kf*** is also educible. In general, accurate calculation for ***Kf*** requires precise measurement of the relative concentration difference, which could be maximized by controlling C_0, ***Cs***, V_1 to V_2 ratio and sometimes ***Kp***. In all cases, the result is favored by low initial analyte concentration and high selector amount.

Binding of the analyte to the selector is not enough; a difference to the two enantiomers is required to apply the potential selector as a CSP. In principle, by the process discussed above, if the selector has different formation constants with the two enantiomers of the analyte, different values of ***Kf*** should be educed. However, is this difference significant enough to be detectable?

Supposing the selector combines with the two enantiomers with a formation constant difference of 10, Figure 4.4 shows concentration and relative concentration difference of the analyte remaining in the starting phase. The relative difference ranges from 1.05 to 1.30, depending on ***Kp*** and ***Kf***. The worst case, when ***Kf*** = 100, indicating an enantioselectivity of 1.1, the relative difference of the analyte concentration is around 1.05, and varies little with the increase of ***Kp***. The concentration is around 1×10^{-5} M (10 μM) at this circumstance. For lots of compounds containing phenyl groups, UV spectrophotometry can determine their concentration at this level with a less than 1% error.

From this model, we know that high selector concentration in the receiving phase, low solutes concentration in the starting phase, can help to increase the gap of concentration we want to observe. However, from previous experiences, after increasing the selector amount in the plasticized PVC membrane above a certain level, the distribution would not accordingly increase, but in some cases, decrease. As a result, we have to control the selector concentration in the receiving phase too. Though the above mentioned gap benefits from low solute concentration in the starting phase, the detection limit is a challenge. For different test analytes, we have to carefully control their concentration in favor of the above two factors. Phase ratio, partitioning coefficient are also tunable parameters to optimize the distribution result.

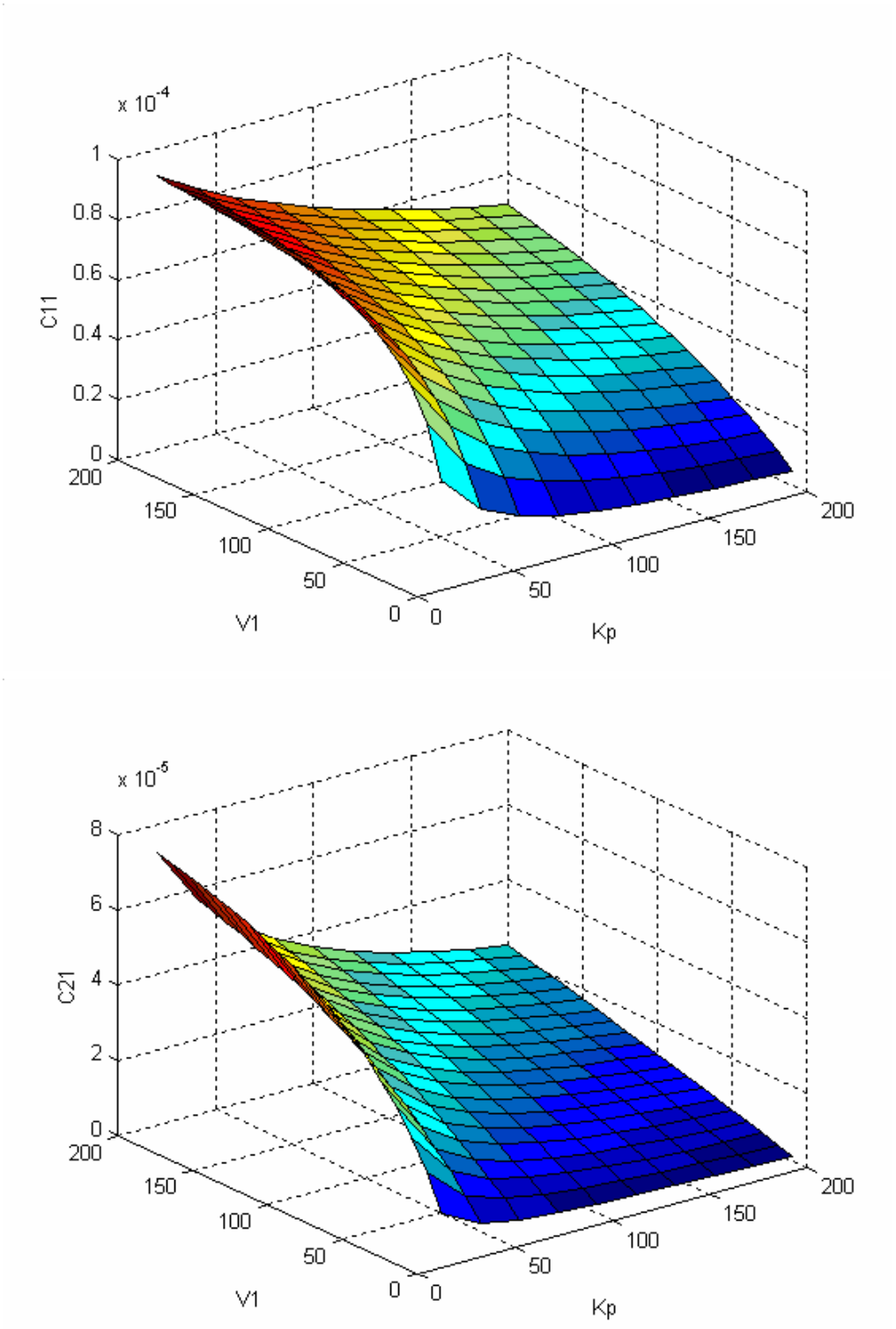


Figure 4.2 *Concentration of analyte remaining in the starting phase: (top) without, (bottom) with selector in the receiving phase.*
(C_0 = 0.0001 M, Cs = 0.060 M, V_2 = 1, Kf = 50)

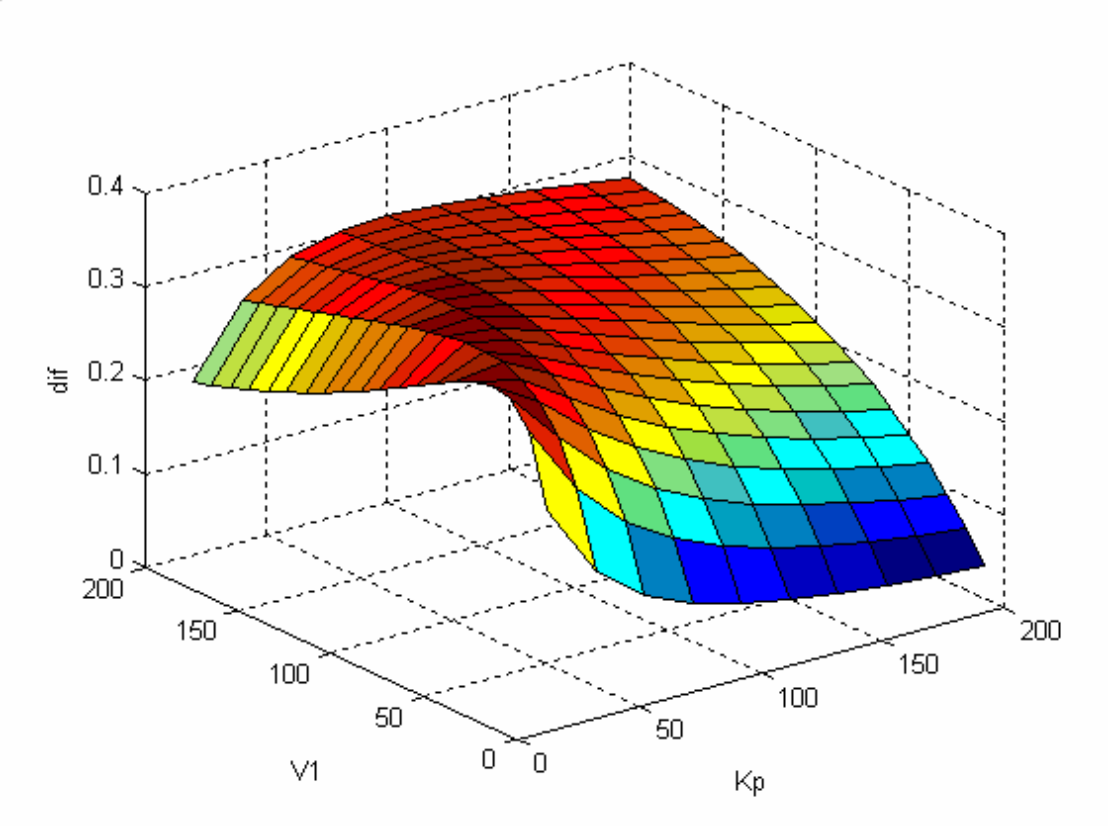


Figure 4.3 *Relative concentration difference.*

$$dif = \frac{C_{11} - C_{21}}{C_0}$$

*(**C_0** = 0.0001 M, **Cs** = 0.060 M, **V_2** = 1, **Kf** = 50)*

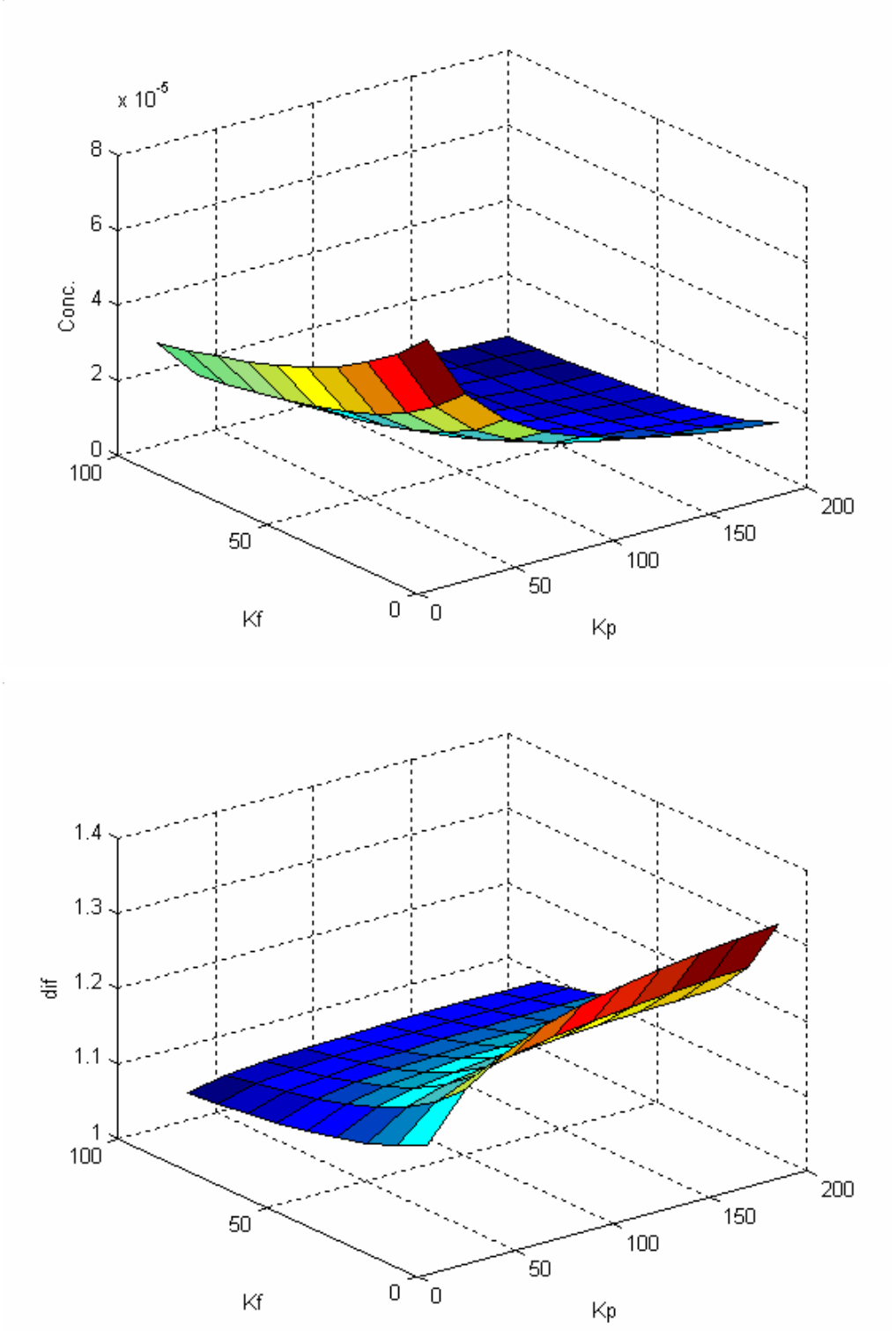


Figure 4.4 *Concentration (top) and relative concentration difference (bottom) of analyte remaining in the starting phase after extraction with the chiral selector in the receiving phase. (C_0 = 0.0001, Cs = 0.060, V_1 / V_2 = 50)*

4.4.2 Troger's Base distribution experiments

The partition coefficient plays an important role in the distribution experiments. For plasticized PVC membranes, plasticizer species, PVC to plasticizer ratio are tunable factors to control the partitioning. Analyte in different solutions will also give different partitioning behaviors. Troger's base, with its pKa at 2.95, does not show good solubility in neutral water but is more soluble in acidic solution. To observe the complex formation of Troger's base and the chiral selector in the plasticized membrane, the precondition is the partitioning of Troger's base from the forward solution to the receiving phase. After extraction, we expect to detect that the Troger's base has decreased concentration in the starting solution but increased amount in the membrane. Moreover, without the doping of chiral selector in the membrane, the partitioning should have no enantioselectivity. In the distribution experiments, Troger's base was prepared in solution with different HCl concentrations. Figure 4.5 shows the effect of HCl concentration on partitioning. With lower HCl concentration in the forward phase, the partition coefficient of Troger's base to the membrane phase is greater; hence more Troger's base is extracted to the PVC/DOS membrane, resulting in the higher analyte concentration in the back-extraction solution since the back-extraction conditions are identical. The area ratio of peak2 to peak 1 remains at 1.36(±0.04), which is not apparently different from the value obtained from the Troger's base stock solution. Therefore, PVC/DOS membrane itself shows no enantioselectivity.

By doping of the chiral selector into the PVC/DOS membrane, the extraction of Troger's base to the membrane phase should have enantioselectivity to the two enantiomers. The area ratio of the two peaks in CE chromatogram is expected to increase or decrease at a detectable level. Figure 4.6 shows the effect of S-DNBPG(OMe) as chiral selector on Troger's base distribution from 10 mM HCl solution to PVC/DOS membrane. The methyl ester was used rather than S-DNBPG to prevent from chiral selector in the membrane being extracted to the liquid phase. The results of four consecutive CE separations are shown in this figure: samples 1 and 4 are 1 mM Troger's base in 10 mM HCl, while samples 2 and 3 are Troger's base after extraction by PVC/DOS membrane containing 60 mM S-DNBPG(OMe) and back-extraction by 100 mM HCl. The results of the two sets of parallel experiments show little variation and the peak area ratio decreases from ~1.37 to ~1.33, indicating more enantiomer of Troger's base represented by the 1st peak being extracted. Figure 4.7 compares the relative enantiomer

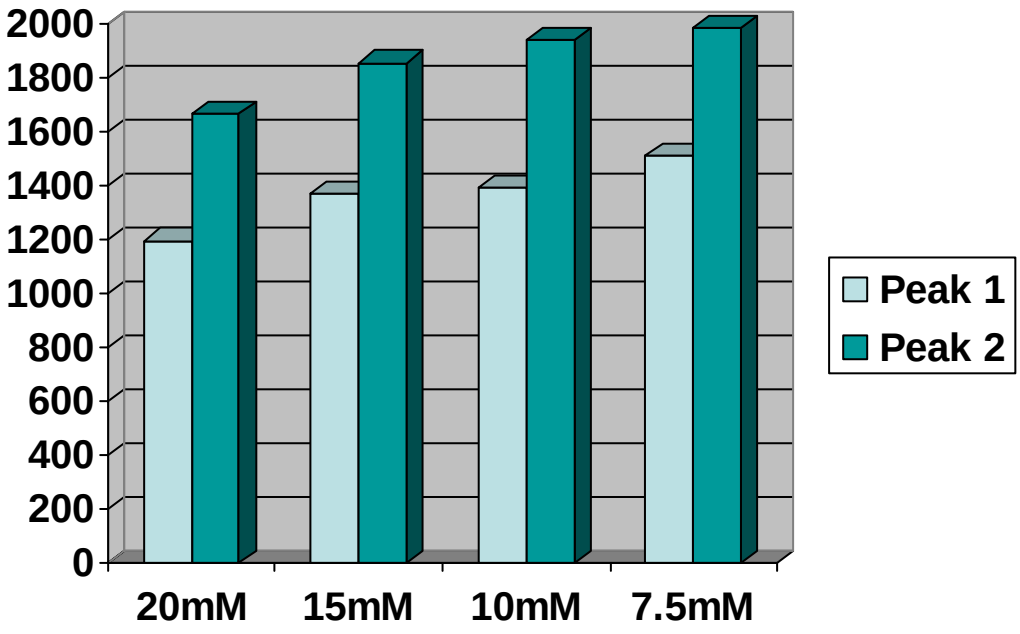


Figure 4.5 *Quantitative analysis by chiral CE of the two enantiomers of 550 µM Troger's base prepared in different concentration HCl solutions, after 250 minutes extraction by PVC/DOS =1:1 membrane and 80 minutes back-extraction by 100 mM HCl.*

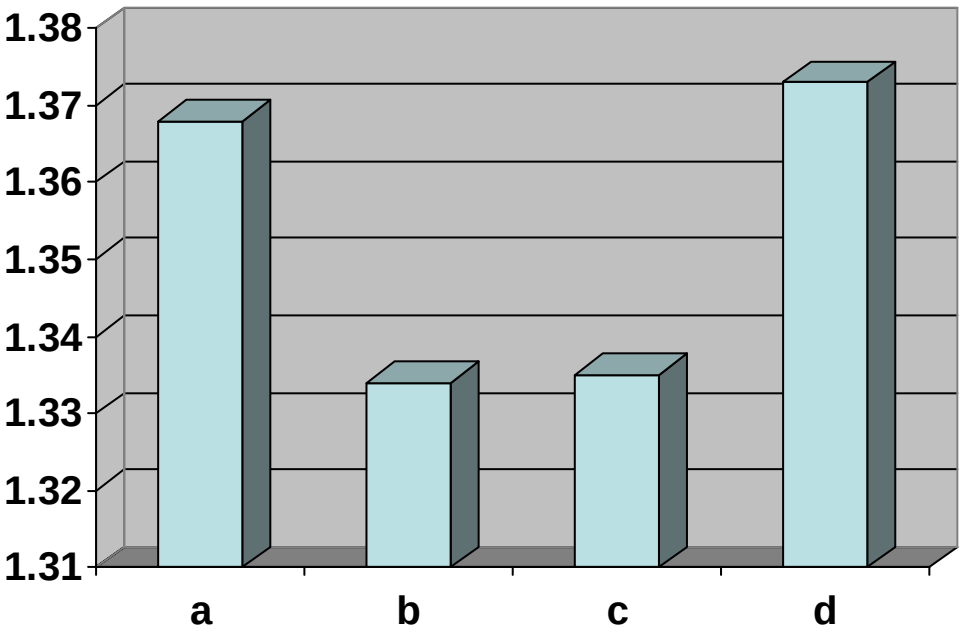


Figure 4.6 *Quantitative analysis by chiral CE of the two enantiomers of Troger's base: a and d: 1mM Troger's base in 10 mM HCl; b and c: 1mM TB in 10 mM HCl, after 20 minutes extraction by PVC/DOS = 1:1 membrane containing 60 mM S-DNBPG(OMe) and 30 minutes back-extraction by 100 mM HCl. 10mM Sodium Phosphate (pH=8) containing 3% sulfated-beta-CD as CE run buffer. Applied voltage = 15 kV, current = 42 µA, 10 s sample injection.*

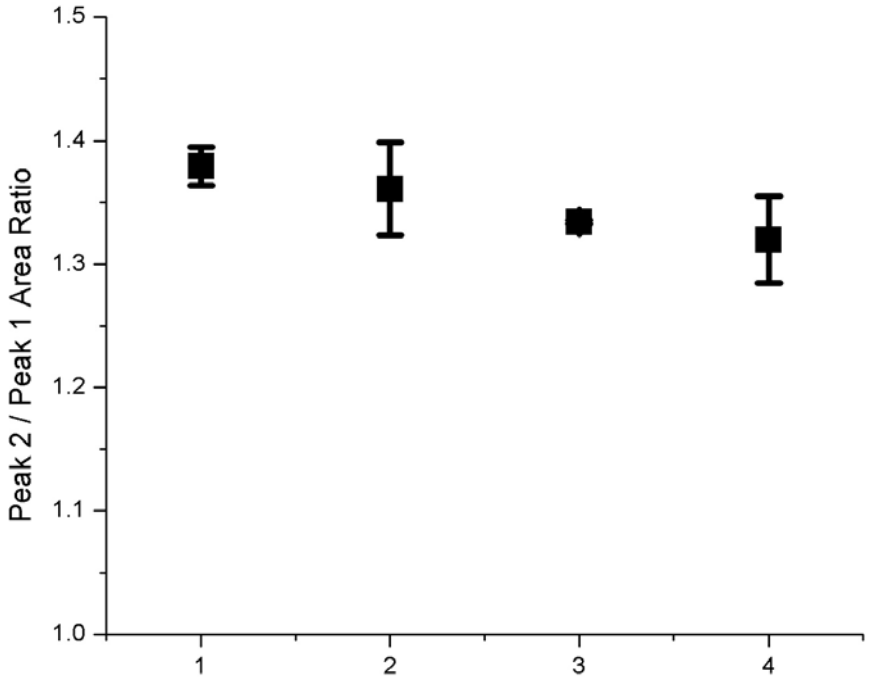


Figure 4.7 *Quantitative analysis by chiral CE of the two enantiomers of Troger's base: (1) Troger's base in 10 mM HCl; (2) Troger's base extracted by PVC/DOS = 1:1 membrane for 250 minutes, back-extracted by 100 mM HCl for 80 minutes; (3) Troger's base extracted by PVC/DOS = 1:1 membrane containing 60 mM S-DNBPG(OMe) for 20 minutes, back extracted by 100 mM HCl for 30 min; (4) Troger's base extracted by PVC/DOS = 1:1 membrane containing 60 mM S-DNBPG(OMe) for 20 minutes, back extracted by 100 mM HCl for 80 min.*

abundance of Troger's base at four different conditions. Obviously, with the forward extraction by PVC/DOS membrane containing the chiral selector, the relative abundance of the two enantiomers of Troger's base is different from its original value. While without the chiral selector in the membrane, the relative abundance shows no big difference before and after extraction. Table 4.1 shows the result of t-test data analysis and proves the above conclusion. Though we did not spike the sample with R or S enantiopure Troger's base to assign the two peaks, the experimental results are sufficient to support the hypothesis that the distribution difference of the two enantiomers of an analyte is detectable at this circumstance. It also has to be pointed out that, with the doping of the chiral selector, the amount of Troger's base being extracted does not have a significant difference, which inhibiting the calculation of ***Kf*** as well as enantioselectivity. This might be due to the limited solubility of the chiral selector in DOS. More discussion will be shown in Chapter 5.

4.4.3 R/S-1-phenyl-butanol and secobarbital distribution experiments

Is the enantiomer discriminative partitioning a special case to Troger's base or general to all analytes? Is the case still applicable for analytes which are not water soluble? By hypothesis, the stock solution should have some effect on the analyte partitioning but not the enantioselectivity, since the selector-analyte complex formation takes place only in the membrane phase. Figure 4.8 shows the effect of S-DNBPG as chiral selector on 1-phenyl-butanol distribution from 25 mM HCl / ethanol (90/10) solution to PVC/DOS membrane. 1-phenyl-butanol is hard to dissolve in water so ethanol was added to enhance the solubility. HCl was used to prevent the acid selector in membrane from being extracted to the liquid phase. From this figure, more R enantiomer than S-1-phenyl-butanol is extracted when the S-DNBPG is doped in the membrane, indicating a greater complex formation constant for R-1-phenyl-butanol with S-DNBPG. The partition coefficients calculated are 29.4 and 28.0 for the extraction of R and S analyte enantiomers, respectively, which shows little difference. The complex formation constant between the selector and the R enantiomer is only about 6 based on the proposed model. The S enantiomer has an even smaller formation constant, resulting in no change on the extraction with the doping of the selector. Figure 4.9 shows the hypothesis model of the complex formation between the R-1-phenyl-butanol and S-DNBPG, according to Pirkle's "three-point

Table 4.1 *T-test for the significance of the difference between the means of two independent samples.*

count	Xa	Xb
1	1.368	1.333
2	1.373	1.335
3	1.397	1.345
4	1.389	1.295
mean	1.382	1.327
st. dev.	0.014	0.022
$Mean_A$ - $Mean_B$	0.0548	
t	+4.2422	
df	6	
p	**one-tailed**	0.002713
	two-tailed	0.005426

Xa 1-3: Troger's base in 10 mM HCl; Xa 4: Troger's base in 10 mM HCl, extracted by PVC/DOS = 1:1 membrane for 250 minutes, back-extracted by 100 mM HCl for 80 minutes; Xb 1-2: Troger's base extracted by PVC/DOS = 1:1 membrane containing 60 mM S-DNBPG(OMe) for 20 minutes, back extracted by 100 mM HCl for 30 min; Xb 3-4: Troger's base extracted by PVC/DOS = 1:1 membrane containing 60 mM S-DNBPG(OMe) for 20 minutes, back extracted by 100 mM HCl for 80 min.

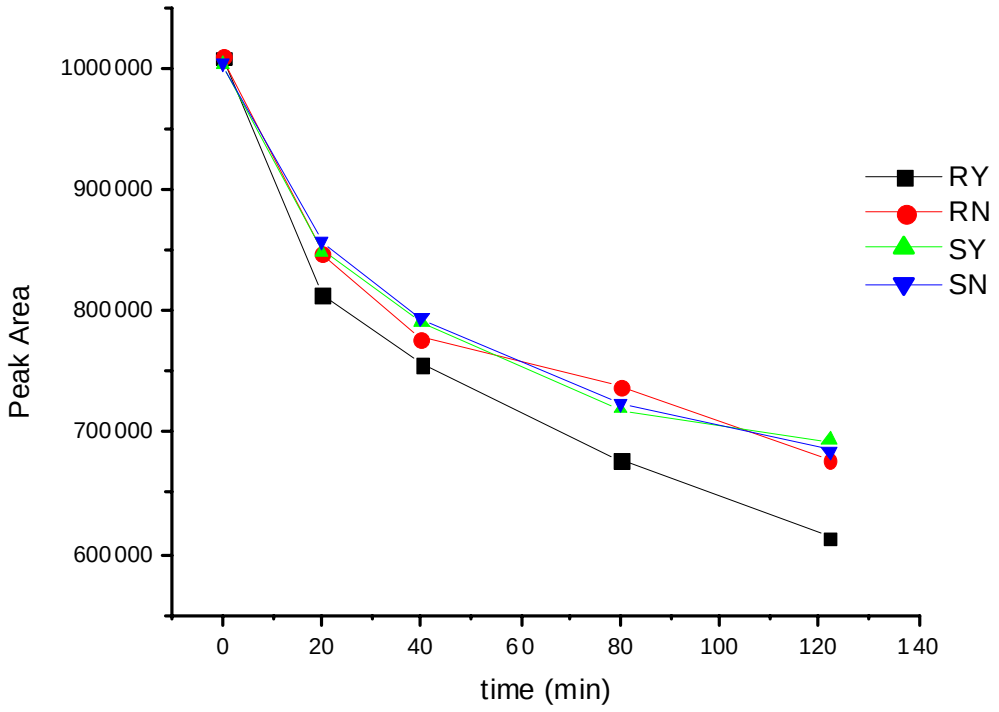


Figure 4.8 *1 mM R and S enantiomers of 1-phenyl-butanol, respectively, remained in 25 mM HCl / ethanol (90/10) solution vs. time with the extraction of (N): PVC/TBP = 1:1 membrane, (Y) PVC/TBP =1:1 membrane containing 60 mM S-DNBPG.*

Phase ratio: membrane / liquid = 1 / 60.

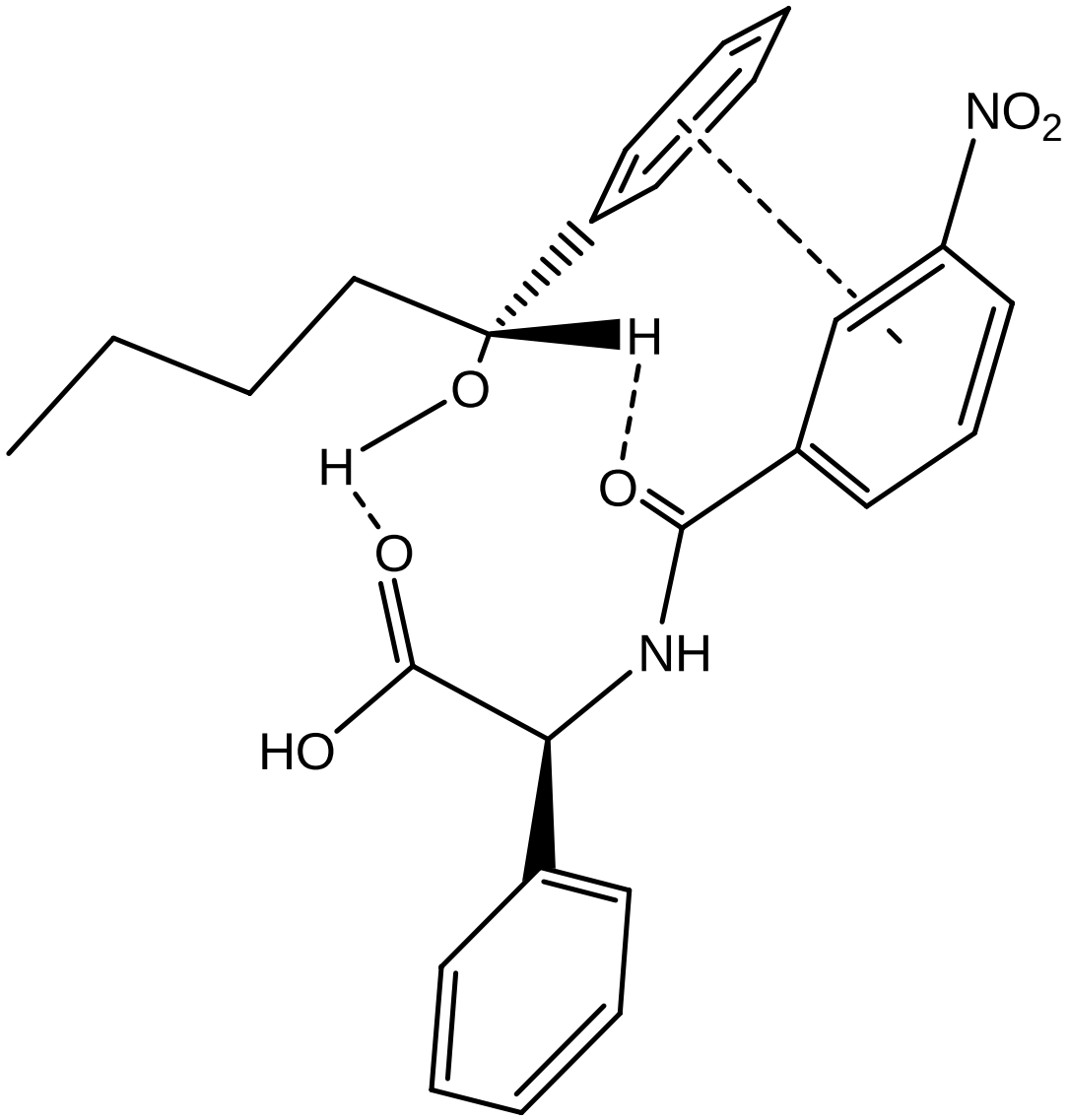


Figure 4.9 *Hypothesis of complex formation between (R)-1-phenyl-butanol and (S)-DNBPG.*

interaction" theory.[11] The dinitrophenyl group is a π-acceptor while the phenyl group of 1-phenyl-butanol is a π-donor. The hydroxyl group hydrogen is the most acidic site of the alcohol, which will form hydrogen bond with the most basic site, the carboxyl group oxygen, of the selector. The second acidic hydrogen of the analyte and the second basic oxygen of S-DNBPG form the other hydrogen bond.

The partition coefficient could be controlled not only by analyte stock solution but also by membrane composition. As discussed in chapter 2, analytes partitioning decreases with the increase of PVC to DOS ratio. Replacing the plasticizer by another one sometimes is also an efficient way to control partitioning.[16] However, in cases of organic solvents being used, applications of some plasticizers are limited since their good solubility in organic solvents makes the plasticized PVC membrane instable. Table 4.2 illustrated the stability of various plasticized PVC membranes in some organic solvents. Among them, TBP is a good choice to make plasticized PVC membrane and keep stable in alcohols and hexanes. However, is the selector-analyte recognition still applicable in PVC/TBP circumstance?

Secobarbital has good solubility in both aqueous and organic phases. It also provides sufficient sites to form hydrogen bonds with the selector S-DNBPG. Figure 4.10 shows the effect of S-DNBPG on secobarbital distribution from water to the PVC/TBP membrane. Supposing the partition and distribution processes have reached their equilibrium, the partition coefficient is 33 from calculation based on the proposed model. The selector-secobarbital complex formation constant is 155. Though we can not tell the distribution difference of the R and S enantiomers respectively at present, the relatively strong binding gives us confidence that the complex does form in the PVC/TBP membrane which could increase the distribution of analyte to the membrane phase greatly. However, extraction by PVC/DOS membrane, with or without the selector, shows little difference. This is contradicted to the fact that TBP is a more polar plasticizer than DOS, thus the solvent would compete with the selector to bind with the potential hydrogen bond sites of the analyte hence decreases the selector-analyte complex formation constant. Our hypothesis is that, the selector, as an amino acid derivative, has a better solubility in TBP rather than in DOS. Chiral selectors dissolved in DOS may exhibit an oversaturated status, the free selector which can provide binding sites to the analytes is actually less than in the TBP.

Table 4.2 *PVC and plasticized PVC membrane resistance in organic solvents. Membranes with different components were weighed then transferred to organic solvents. After sitting for 20 minutes, membranes were taken out, dried, and weighed again.*

Weight (mg)	MEMBRANE Components (1:1 except PVC)					
	PVC	DOS/PVC	DOP/PVC	TBP/PVC	TBC/PVC	CLP/PVC
Acetonitrile	9.5	70.9	55.0	44.0	46.8	60.8
	9.2	46.2	32.0	25.1	30.5	33.0
Isopropyl Alcohol	6.0	23.8	48.4	44	57.7	54.5
	6.0	13.9	44.3	43.3	55.5	32.7
Ethyl Alcohol	18.8	20.0	41.1	53.3	60.3	41.2
	18.8	12.2	27.4	53.3	57.6	25.9
Methyl Alcohol	7.3	19.4	43.8	48.7	55.0	57.2
	7.1	15.3	25.8	48.6	54.1	34.0
Hexanes	6.0	20.4	38.4	55.0	57.5	48.8
	6.0	11.6	34.9	54.7	46.0	31.5
Ethyl Acetate	7.1					
	6.1					
THF	8.2					
	0					
DMF	9.8					
	0					
DMSO	7.4					
	0					
Chloroform	8.0					
	0					

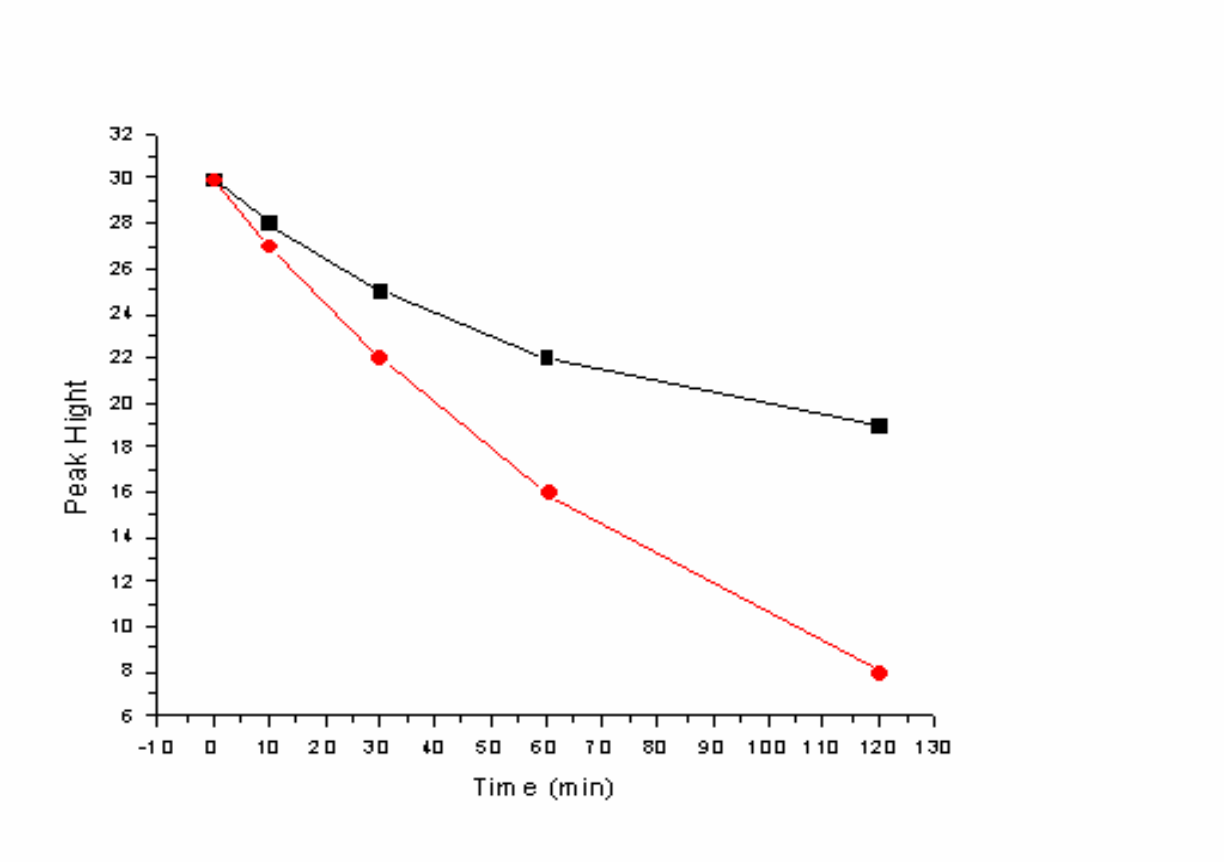


Figure 4.10 *1mM secobarbital remaining in water vs. time with the extraction of (black) PVC/TBP = 1:1 membrane, (red) PVC/TBP = 1:1 membrane containing 56 mM R-DNBPG. Phase ratio: membrane/water = 1/56.*

In order to increase the selector solubility, DNBPG derivative of methyl ester was prepared, as has been shown in the Troger's base distribution experiments. However, the solubility result is still not satisfactory. The synthesis of DNBPG ester of 3,3-dimethylbutan-1-ol is discussed in Chapter 5.

4.4.4 TFAE distribution experiments

2,2,2-trifluoro-1-(9-anthryl)-1-ethanol is among the first successful Pirkle-type CSPs.[17] By applying the "reciprocal principle", various racemate compounds were tested by this CSP and N-(3,5-dinitrobenzoyl)-phenylglycine was found an excellent candidate for a new generation of CSP.[12, 18]

TFAE can hardly get optical resolution by chiral CE with sulfated-β-CD as the chiral selector (Figure 4.11). Do we have any idea to deal with this situation? Luckily, both R and S enantiomers of TFAE are commercially available and we may study their distributions individually under the effect of chiral selector. The concentration of each enantiomer of the TFAE remaining in the membrane phase could be determined by UV spectrophotometry.

Figure 4.12 shows the UV absorbance of 2 μM TFAE before and after extraction, indicating a ~ 250 partition coefficient from the aqueous to the membrane phase. Since TFAE is not water soluble, 2% ethanol was added to increase the solubility. As shown in Table 4.2, PVC/DOS has little stability in ethanol. Though small amount of ethanol will not destroy the membrane, the background due to the back-extraction of DOS still brings trouble to the concentration determination of the analyte. There are two ways to prevent the background interference: 1.When performing the analyte extraction, a PVC/DOS membrane, as a control, is back-extracted in 2% ethanol solution without the analyte. UV spectrophotometry of this solution is applied as a background for deduction (refer to Figure 4.9). 2. As shown in Figure 4.13, the UV spectrum of the TFAE solution after extraction (and back-extraction of the plasticizer), except from wavelength 235 nm to 264 nm, can be fit as a polynomial curve. The difference of the raw data and the fit curve gives the spectrum of the TFAE without background disturbance. Further distribution experiment requires the chiral selector to have good solubility in DOS. This selector, DNBPG ester of 3,3-dimethylbutan-1-ol, is still in preparation.

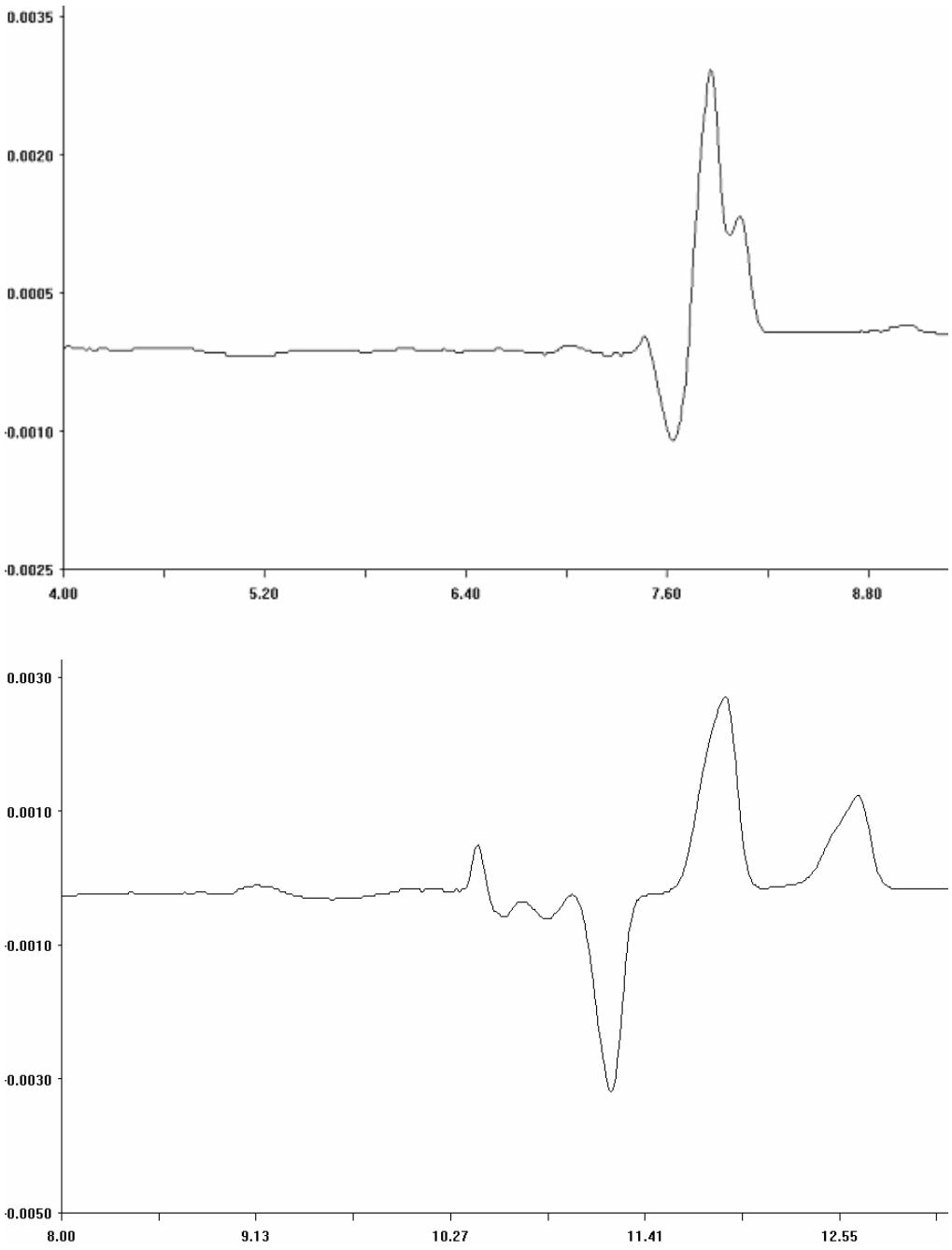


Figure 4.11 *Chromatogram of 200 µM 2,2,2-trifluoro-1-(9-anthryl)-1-ethanol (R:S = 2:1) in water/ethanol (96:4).*

10mM Sodium Phosphate (pH=6.8) containing 2% (top) or 5% (bottom) sulfated-beta-CD as CE run buffer. Applied voltage = 15 kV, current = 36 µA (top) / 74 µA (bottom). Sample injection for 5 psi·s.

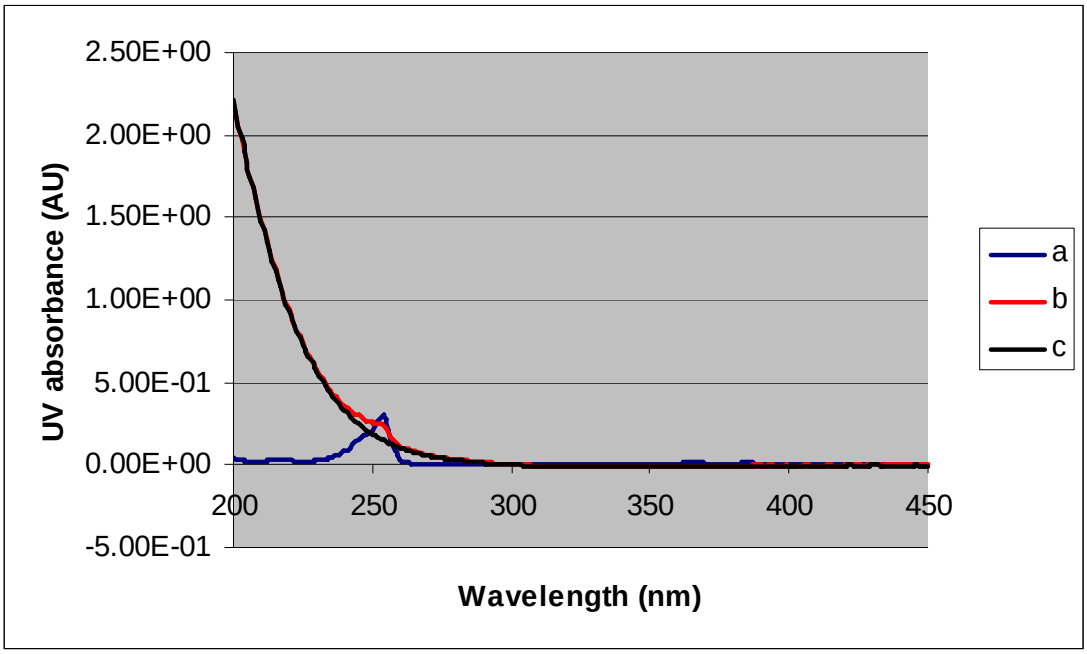


Figure 4.12 *UV absorbance of 2 µM TFAE in 2% ethanol, before (blue) and after (red) extraction by PVC/DOS membrane for 12 hours, phase ratio 1 / 200. (black) PVC/DOS membrane sitting in 2% ethanol for 12 hours as background.*

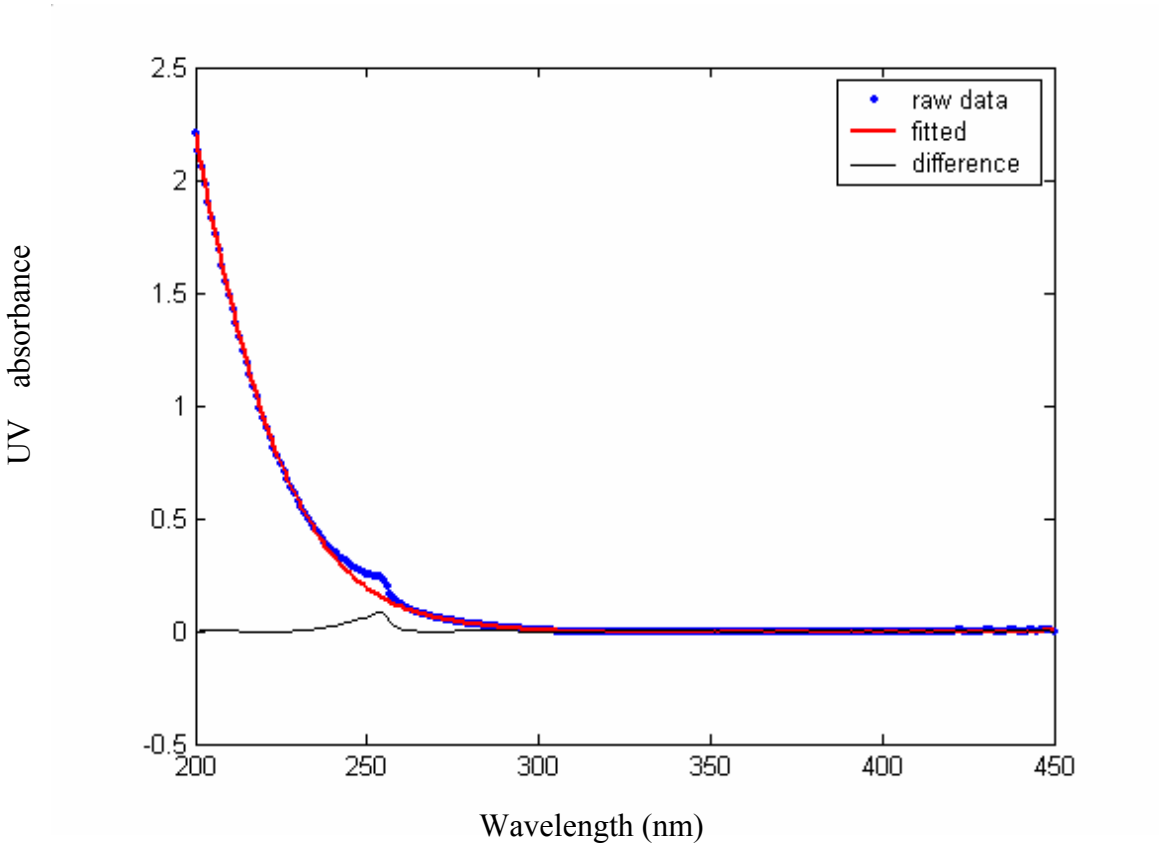


Figure 4.13 *UV absorbance of 2 µM TFAE in 2% ethanol after 12 h extraction by PVC/DOS membrane.*

Phase ratio: 1 / 200.

4.5 CONCLUSION

A screening method is proposed for fast evaluation of potential chiral selectors. This method is based on molecular recognition in plasticized PVC membrane. The advantage of the method is that it does not require the covalent immobilization of either the analyte or the selector to the solid phase. In order to verify this method, R/S-N-(3,5-dinitrobenzoyl)-phenylglycine and its methyl ester are chosen as test selectors. Test analytes were started from what Felix referred to as the "classical racemates".[13] Capillary electrophoresis and UV spectrophotometry are applied for quantitative analysis of analyte enantiomers remaining in the starting phase after membrane extraction. Comparing these two detection methods, UV gives lower detection limit and is more accurate due to longer path-length, but chiral CE prevents error from two separate extractions and also provides the possibility to run several analytes at the same time.

4.6 REFERENCE

(1) Davankov, V. A. *Advances in Chromatography (New York, NY, United States)* **1980**, *18*, 139-195.

(2) Pirkle, W. H.; Welch, C. J.; Lamm, B. *Journal of Organic Chemistry* **1992**, *57*, 3854-3860.

(3) Welch, C. J. *Journal of Chromatography, A* **1994**, *666*, 3-26.

(4) Wu, Y.; Wang, Y.; Yang, A.; Li, T. *Analytical Chemistry* **1999**, *71*, 1688-1691.

(5) Bluhm, L. H.; Wang, Y.; Li, T. *Analytical Chemistry* **2000**, *72*, 5201-5205.

(6) Armstrong, D. W.; Tang, Y.; Chen, S.; Zhou, Y.; Bagwill, C.; Chen, J.-R. *Analytical Chemistry* **1994**, *66*, 1473-1484.

(7) Armstrong, D. W.; Rundlett, K. L.; Chen, J.-R. *Chirality* **1994**, *6*, 496-509.

(8) Welch, C. J.; Protopopova, M. N.; Bhat, G. *Enantiomer* **1998**, *3*, 471-476.

(9) Welch, C. J.; Pollard, S. D.; Mathre, D. J.; Reider, P. J. *Organic Letters* **2001**, *3*, 95-98.

(10) Zhang, X.; Zhao, H.; Chen, Z.; Nims, R.; Weber, S. G. *Analytical Chemistry* **2003**, *75*, 4257-4264.

(11) Pirkle, W. H.; Pochapsky, T. C. *Chemical Reviews (Washington, DC, United States)* **1989**, *89*, 347-362.

(12) Pirkle, W. H.; Finn, J. M.; Schreiner, J. L.; Hamper, B. C. *Journal of the American Chemical Society* **1981**, *103*, 3964-3966.

(13) Felix, G. *Journal of Chromatography A* **2001**, *906*, 171-184.

(14) Wu, W.; Stalcup, A. M. *Journal of Liquid Chromatography* **1995**, *18*, 1289-1315.

(15) Stalcup, A. M.; Gahm, K. H. *Analytical Chemistry* **1996**, *68*, 1360-1368.

(16) Valenta, J. N.; Weber, S. G. *Journal of Chromatography, A* **1996**, *722*, 47-57.

(17) Pirkle, W. H.; House, D. W.; Finn, J. M. *Journal of Chromatography* **1980**, *192*, 143-158.

(18) Pirkle, W. H.; Finn, J. M. *Journal of Organic Chemistry* **1981**, *46*, 2935-2938.

5.0 FUTURE PLANS

5.1 REQUIREMENT OF THE CHIRAL SELECTOR WITH GOOD SOLUBILITY IN PLASTICIZERS

For Troger's base, the extracted amount of analyte shows little difference, with or without the doping of the selector (S-methyl N-(3,5-dinitrobenzoyl)-phenylglycinate). This is not expected based on the proposed model. Same situation occurs in the case of 1-phenyl-butanol. The complex formation constant of the selector (S-N-(3,5-dinitrobenzoyl)-phenylglycine) with the analyte (R-1-phenyl-butanol) is only 6, and even less with the S enantiomer. There are two reasons to explain the observed phenomena: 1. The selectors only have very weak bindings with the analytes in the plasticizer; 2. The selectors do not have good solubility in the plasticizer, hence limit the available binding sites to the analytes, which is not expected by the proposed model.

The secobarbital distribution experiment confirmed the first hypothesis. Though TBP is thought of not a good plasticizer to provide the molecular recognition environment, the complex formation constant is still about 155 for the N-(3,5-dinitrobenzoyl)-phenylglycine with the secobarbital. Experiments of molecular recognition in TBP with other analytes have also been tested, but the results are not satisfactory, due to the hydrogen bonding competition of TBP to the analytes over the selector.[1] However, by comparing these experimental results, to employ the proposed model to application, the good solubility of the chiral selector in DOS is preferred.

5.2 SYNTHESIS OF R/S-N-(3,5-DINITROBENZOYL)-PHENYLGLYCINE ESTER DERIVATIVES

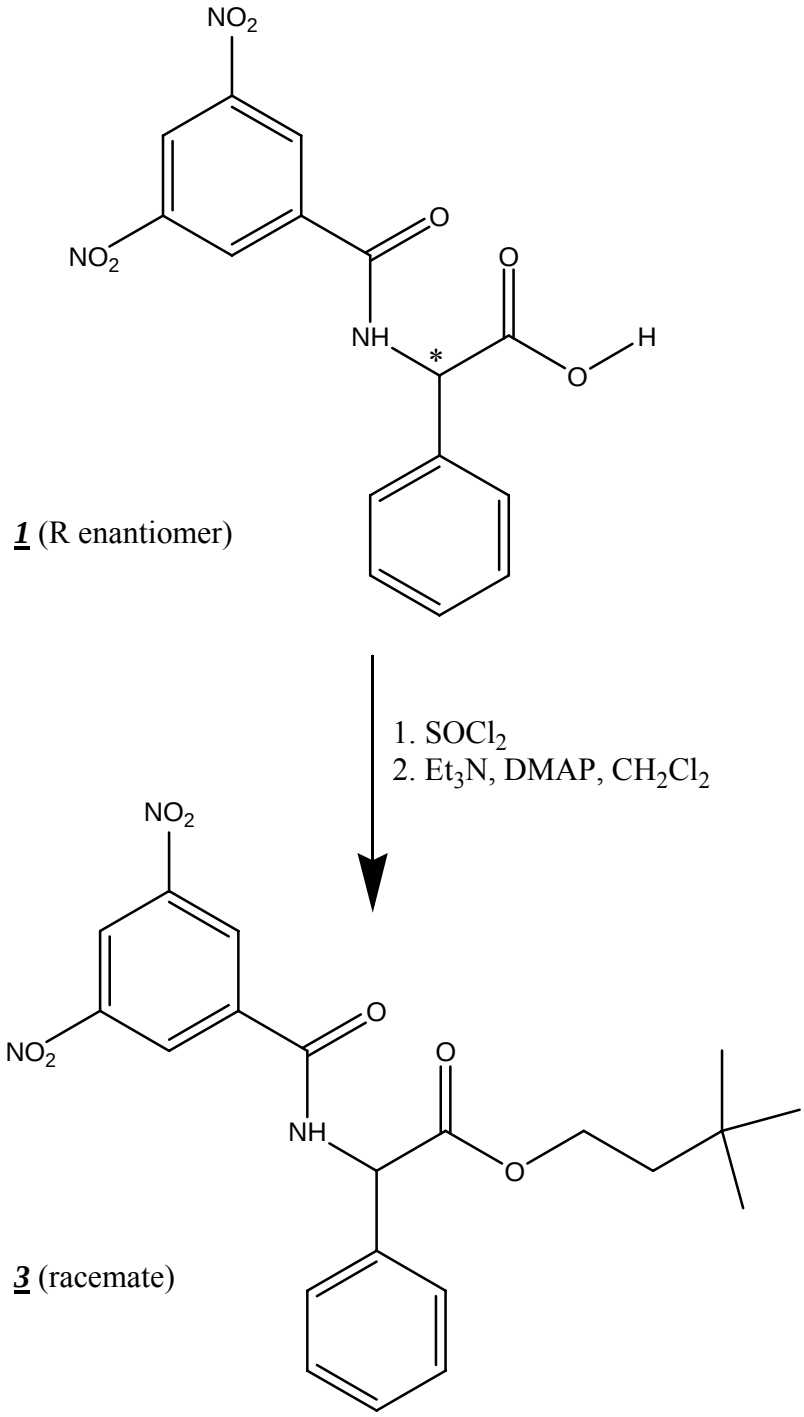
In order to have a chiral selector with a better solubility in DOS, effort toward the synthesis of R-3,3-dimethyl-1-butanyl N-(3,5-dinitrobenzoyl)-phenylglycinate was made but not successful. Complete racemization occurred during the preparation process.

Synthesis of *3* (refer to Figure 5.1) Sulfonyl chloride (10 mL) was added to (R)- N-(3,5-dinitrobenzoyl)-phenylglycine (600 mg). The solution was stirred for 6h at room temp. Sulfonyl chloride was removed under vacuum and yellow solid was obtained. Anhydrous dichloromethane was added to dissolve the solid then triethylamine (0.18 mL) and small amount of DMAP were added. Finally the 3,3-dimethyl-butanol was added. The solution was stirred overnight, quenched with water, extracted with ether, dried over $MgSO_4$. After flash chromatography (hexane : ethyl acetate = 10:1), white solid was obtained. The yield is 200 mg. ^{1}H NMR (300 MHz, DMSO): δ 10.17 (s, 1H, NH), 9.14 (d, *J*=2.07 Hz, 2H, ArH), 9.00 (t, *J*=2.07 Hz, 1H, ArH), 7.66-7.64 (m, 2H, ArH), 7.48-7.40 (m, 3H, ArH), 4.03-3.97 (m, 2H, CH), 3.65-3.62 (m, 1H, CH), 3.48-3.31 (m, 1H, CH), 1.57-1.53 (m, 2H, CH), 1.37-1.32 (m, 2H, CH), 1.22 (s, 1H), 0.93-0.84 (m, 9H, CH), 0.78-0.74 (m, 9H, CH). EI-MS (*m/z*): 428 $[M-H]^+$. EI-HRMS: 428.1458, found 428.1448. $[\alpha]_D^{20}$=0.

The racemization is induced by a tautomeric equilibrium at basic condition. In order to prevent the product epimerization, a highly hindered base is required.[2] An synthetic route to obtain 3,3-dimethyl-1-propanyl N-(3,5-dinitrobenzoyl)-phenylglycinate (***4***) without racemization is proposed in Figure 5.2.

5.3 FURTHER UNDERSTANDING OF MOLECULAR/CHIRAL RECOGNITION IN PLASTICIZED PVC

The successful derivation of N-(3,5-dinitrobenzoyl)-phenylglycine provides a basis to further understand the molecular/chiral recognition mechanism in plasticized PVC, if the chiral selector has good solubility in relatively non-polar solvents. According to the proposed model,



1 (R enantiomer)

3 (racemate)

Figure 5.1 *Synthetic route to obtain 3,3-dimethyl-1-butanyl N-(3,5-dinitrobenzoyl)-phenylglycinate*

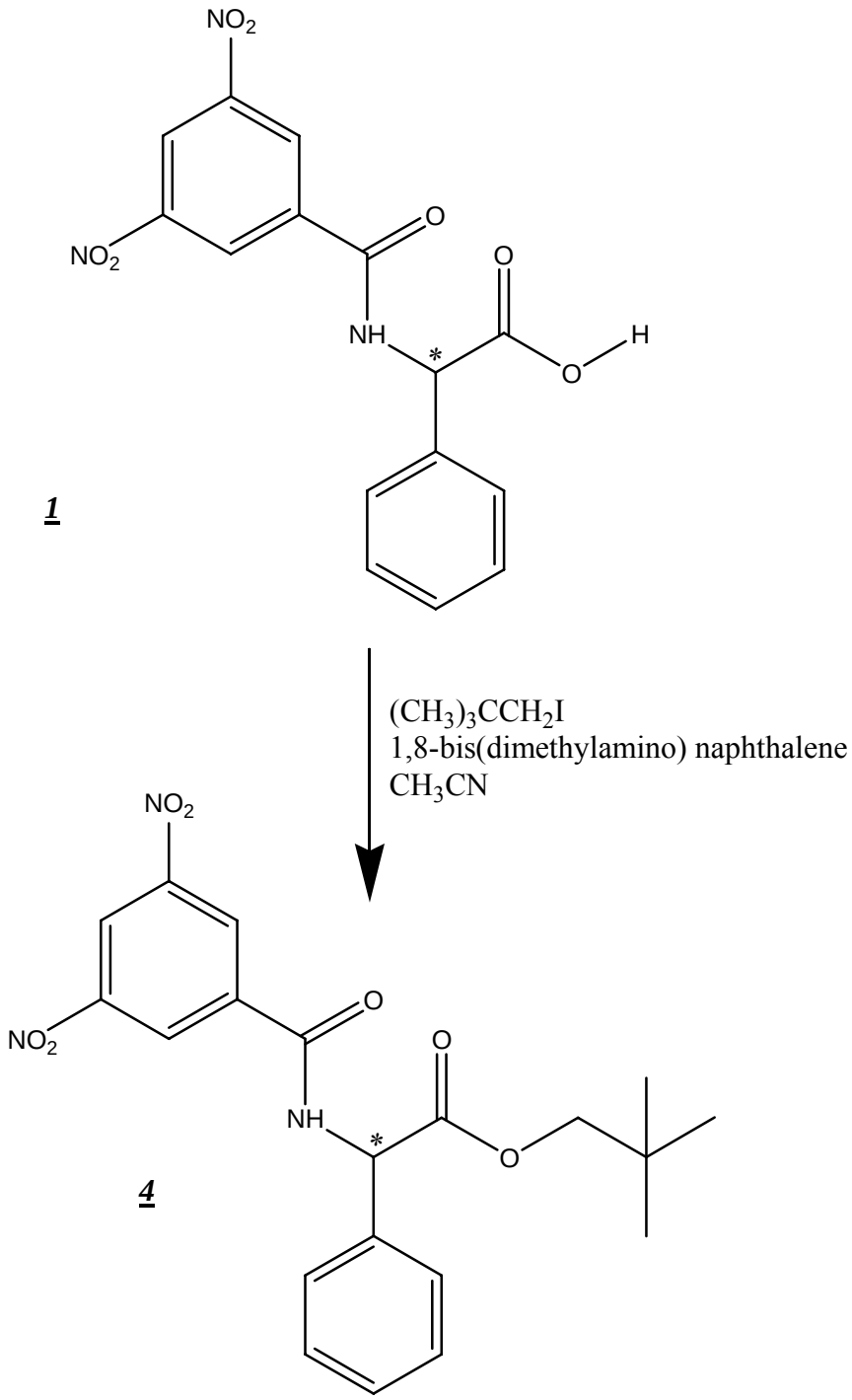


Figure 5.2 *Synthetic route to obtain 3,3-dimethyl-1-propanyl N-(3,5-dinitrobenzoyl)-phenylglycinate*

the first step is to determine the partition coefficient, which can be calculated based on the extraction experiment without the chiral selector in the membrane phase. Thereafter, extraction with the selector in the receiving phase provides the distribution coefficient as well as the selector-analyte complex formation constant. However, these preliminary results are not accurate and precise enough if some factors are not tuned to get the best extraction results required for the calculation of enantioselectivity. These factors include the analyte initial concentration, the chiral selector concentration, receiving to starting phase ratio, and even the partition coefficient. Future work will carefully study the effect of these factors and correlate them to the proposed model.

5.4 ADVANCED DETECTION METHOD

For the concentration determination of analyte enantiomers in the aqueous/organic phase, chiral CE and UV spectrophotometry were applied, both of them have their own advantages and shortcomings. Chiral CE is ideal for samples prepared in aqueous solution, and is able to monitor two enantiomers simultaneously; it also has the potential to separate several analyte enantiomers for only one run. However, the relatively poor quantitation ability and the water-solubility problem of most analytes limit a wider usage of CE. Chiral micro-HPLC has all the advantages of CE and is better for the quantitation purpose. Moreover, most analytes and peptide mimics do not have the solubility problem in normal-phase HPLC solvents. The screening procedure with the chiral micro-HPLC as the detection technique is developing.

For the purpose of fast screening, microplate readers have found their wide usage. A SpectroMax M2 microplate reader is recently purchased in our lab. It provides a scanning for 96 samples in less than 20 seconds to determine their UV absorbance or fluorescence (excitation and emission). Plasticized PVC membranes are to be prepared in the 96 wells of a microplate (polypropylene). Analyte solutions after extraction in these wells are to be transferred to a quartz plate for concentration determination.

5.5 APPLICATION OF THE CHIRAL SELECTOR SCREENING METHOD TO THE LIBRARY OF PEPTIDE MIMICS

The synthesized peptide mimics generally have good solubility in relatively non-polar solvents, such as DOS, thus are able to be doped into PVC/DOS membranes with no challenge. Depending on the screening process, more analytes are to be introduced for comprehensive understanding of the molecular/chiral recognition mechanism. Combinational methodology will also be brought in for faster and more systematic evaluation. Peptide mimics as good chiral selector will be immobilized to silica gel and packed into column for determination of actual separation efficiency. Later work will also include the design of tandem columns for more general usage of the new CSPs.

5.6 REFERENCE

(1) Valenta, J. N.; Weber, S. G. *Journal of Chromatography, A* **1996**, *722*, 47-57.

(2) Ribeiro, A. S.; Kanazawa, A.; Navarro, D. M. A. F.; Moutet, J.-C.; Navarro, M. *Tetrahedron: Asymmetry* **1999**, *10*, 3735-3745.