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Chiral Separation of Pharmaceutical Compounds using Electrochemically Modulated Liquid Chromatography (EMLC)

by

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DISCLAIMER

DEDICATION

This thesis is dedicated to my father, who passed away three years ago, and my mother, for their love, support, and sacrifices. I will forever be indebted to them.

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CHAPTER 1. GENERAL INTRODUCTION

Thesis Organization

The work in this thesis explores the application of a new technique, termed electrochemically modulated liquid chromatography (EMLC), to the chiral separations of pharmaceutical compounds. The introduction section, which precedes two papers, provides a literature review of the technique and its applications, as well as a brief overview of the research described in each of the next two chapters. Chapter 2 investigates the EMLC-based enantiomeric separation of a group of chiral benzodiazepines with β-cyclodextrin as a chiral mobile phase additive. Chapter 3 demonstrates the effects of several experimental parameters on the separation efficiency of drug enantiomers. The thesis concludes with a general summary and possible directions for future studies. References are compiled in a separate section at the end of each chapter.

Literature Review

The chiral separation of enantiomers has become increasingly important in a wide variety of areas, particularly in the pharmaceutical and medicinal fields [1]. The majority of the newly approved drugs and the most often prescribed drugs in the United States have at least one asymmetric center [2]. Approximately half of

these chiral drugs are of a racemic composition. Usually, one of the enantiomers is physiologically active while the other is inactive or may have toxic effects [3]. Most of the recent technological advances in chiral separations have been in the area of high performance liquid chromatography (HPLC) [4]. In HPLC, the enantiomers can be separated chromatographically by various methods; however, it is always necessary to use a chiral selector or discriminator [5, 6]. There are at least three general approaches for the HPLC separation of enantiomers. The first involves the use of chiral stationary phases. The second employs the use of chiral mobile phase additives in conjunction with achiral stationary phases. The third entails the derivatization of the analytes with chiral reagents to produce diastereomeric complexes that can then be separated by achiral chromatographic methods.

Between the early 1980s and the early 1990s, the rapid and routine separation of enantiomers went from infancy to where these techniques are now common-place [1]. The expansion of enantiomeric separations was fueled by the commercialization of useful LC chiral stationary phases. Although these phases have demonstrated effectiveness in the separation of different groups of enantiomers, none are universally applicable to all types of chiral separations or even partially approach that idealization [7]. This lack of universality is understandable when recognizing that the simultaneous, preferential, and distinct three-point interactions between a chiral stationary phase and a chiral analyte

must be realized to achieve a successful enantiomeric separation [8]. Additional limitation is imposed by the inability to alter the composition of a stationary phase. For a given column, the separation can be optimized only by altering the organic fraction of the mobile phase or by adding a modifier to the mobile phase. The stationary phase therefore plays significant but "fixed" role in the optimization process. Consequently, the analyst is often faced with time-consuming searches for optimum separation conditions, as well as the generation of a large amount of mixed waste, an ever increasing portion of operational costs.

To address this obstacle, several alternatives, such as chemically transformable stationary phases and dynamic coating techniques [9, 10], have been investigated. EMLC is one of the new alternative techniques, and involves the combination of electrochemistry and LC. The basis of this approach derives from the conversion of a chromatographic column into a three-electrode electrochemical cell, with the stationary phase being utilized as the working electrode. This conversion results in the ability to alter the retention characteristics of conductive stationary phases through changes in the voltage applied (E_{appl}) to such phases, which can be exploited to manipulate separation efficiency.

Like many techniques, EMLC has an interesting historical evolution. In early 1960's, Fujinaga et al. [11], as well as Strohl [12] and Roe [13], proposed the union of a thin-layer electrochemical cell and a LC column to manipulate

separations. With this design, the composition of the stationary phase could be controlled electrochemically. The stationary phase, which also severed as the working electrode in a thin-layer cell, consisted of either glass carbon [12], amalgamated nickel [13], or amalgamated platinum [14] particles that were packed into a porous vycor tube. The design, in fact, paralleled that in fluidized bed electrochemical reactors [15]. The conductive nature of the stationary phase allowed alterations in E_{appl} to be used as a convenient means for changing the surface charge of the packing. These early studies focused primarily on metal ion mixtures whereby metal ions were reductively deposited onto a stationary phase and then stripped in a stepwise manner to complete the separation. Fujinaga further demonstrated that a voltage gradient could be applied throughout the stationary phase to enhance these separations using an alternate column design [16, 17].

Strohl [18, 19] extended the investigation of this technique by showing that changes in E_{appl} to a packing of carbonaceous particles could be used to modify the adsorption of organic species (i.e., quinones), illustrating the ability of this new technology to separate compounds without changing their chemical form. In 1978, Strohl [20] further demonstrated that compositional changes (i.e., pH) in the mobile phase could be generated through E_{appl} to manipulate separations of inorganic cations.

A significant advance in this area was reported by Yacynych et al. [21, 22] in 1984 through the development of an new EMLC column design. The major modification was to employ stainless steel as opposed to glass as the container for the stationary phase which allowed the column to withstand high operational pressures (~3000 psi). Using this new design, these researchers showed that the capacity factors for the analytes could be modulated through applications of various fixed voltages to the carbonaceous column.

As a means for tailoring the selectivity of the stationary phase for various analytes, efforts have focused on the functionalization of carbon surface material with electroactive ionomers [23-26] and electroactive polymers [27-34]. After modification, the stationary phase could be converted electrochemically between its oxidized and reduced forms, with the concomitant uptake or expulsion of analyte (or electrolyte) ions. For example, Wallace et al. [28, 29] reported on the use of a polypyrrole stationary phase that was coated onto reticulated vitreous carbon, whereby alterations in E_{appl} could significantly modify the ion-exchange capacity of the column, as well as hydrophobicity, dipole, donor-acceptor properties of the polymeric coating.

More recently, Nagoka et al. have investigated the use of changes in E_{appl} to separate of a variety of organic and inorganic species at different column packings, including microporous glassy carbon [35] and nonporous glassy carbon coated with crown ethers [30], polyaniline [33, 34], and polypyrrole [34]. Similarly,

Przybycien [36] has studied the retention behavior of β-lactoglobulin on a heme-agarose bed with the redox states of the stationary phase manipulated by additives in the mobile phase. In each of the works detailed above, however, the difficulty in constructing an EMLC column that functioned effectively both as a electrochemical cell and as a chromatographic column resulted in low and generally unusable separation efficiencies.

Our group started exploring EMLC in the early 1990's. The focus of our efforts has been on improvements in column design, and on enhancing the chromatographic performance of this technique. Deinhammer, Shimazu, and Porter reported an improvement in the preparation of a polypyrrole coating onto glassy carbon, applying such coatings to the separation of adenosine phosphates [37] and dansyl amino acids [38]. These studies demonstrated, for the first time, the ability to manipulate retention by changing electrochemically the composition of the stationary phase during analyte elution. Although this first generation of column proved effective in proof-of-concept demonstrations, improvements in chromatographic performance were still needed in order to become competitive with conventional HPLC technique.

A new column design was then developed [39, 40] in which a porous stainless steel column was used to support physically the Nafion tube, as well as to make electrical contact to the particulate phase. The new column was able to withstand pressures up to 6000 psi, enabling the use of conductive packing

materials with particle diameters less than 10 μm. With this new design, chromatographic efficiencies were dramatically increased, at last becoming competitive with conventional HPLC techniques that used porous graphitic carbon (PGC) as a stationary phase [41].

However, there were still some drawbacks with this column design, especially the limitation in the electrochemical performance at negative values of E_{appl} [42]. This drawback was illustrated by the failure in the attempts to induce the transformation of redox species with large cathodic formal reduction potentials. After re-examining the column design, the research team found that the poor electrochemical performance originated from the use of the stainless steel tubing as part of the working electrode, and the resulting potential drop from the reduction of the molecular oxygen at the stainless steel component of the working electrode. Upon redesign, whereby the stainless steel cylinder was 1) insulated electrically from the PGC packing, and 2) configured to function as the auxiliary electrode in the electrochemical cell, this limitation was greatly reduced. This newly designed EMLC column has been successfully applied to manipulate the efficiency of separations of a variety of different analytes, including corticosteroids [43], benzodiazepines [44], aromatic amino acids and polycyclic aromatic hydrocarbons [45], as well as pharmaceutical chiral compounds [46]. This is the column design used in the research described in this thesis.

Thesis Overview

Two research projects are presented in this thesis. Both projects address novel applications of EMLC to enantiomeric separations of pharmaceutical compounds. The basis of the approach derives from the potential-induced electrosorption of an organic additive onto the conductive stationary phases, resulting in a new dimension in the ability to manipulate the chirality and selectivity of such stationary phases. Chapter 2 demonstrates the chiral separation of a group of closely related enantiomeric benzodiazepines (i.e., oxazepam, lorazepam, and temazepam) using a PGC stationary phase and β-cyclodextrin (β-CD) as a chiral mobile phase additive. All three analytes were enantiomerically resolved for the first time under isocratic elution conditions. The results indicate that both retention and enantioselectivity of the separation can be altered by the manipulation of extent of the electrosorbed β-CD through the changes in E_{appl} to the stationary phase. A separation mechanism that qualitatively explains the basis of the observed separations is then proposed and discussed.

Chapter 3 extends our investigation of EMLC as an effective and facile means for chiral separations by studying the effects of several experimental parameters on our earlier enantiomeric separation [45] of two therapeutically important compounds mephenytoin and hexobarbital. This study also advanced our understanding of the mechanism of these EMLC-based separations. While changes in the E_{appl} to the stationary phase play a major role in the alteration of

efficiency and elution order of the enantiomers, it is also found that the enantioselectivity and retention were influenced by the identity and concentration of the chiral organic additive, and the pH and flow rate of the mobile phase. These observations are discussed in terms of the differences of chemical structures of the analytes, and the resulting impact on retention behavior.

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CHAPTER 4. GENERAL CONCLUSIONS AND PROSPECTUS

This thesis has explored the feasibility and versatility of EMLC for the chiral separation of drug enantiomers. The results demonstrated that the retention and the enantioselectivity of chiral separation in EMLC can be manipulated through the potential-induced electrosorption of a chiral additive onto the conductive stationary phase (i.e., PGC).

In Chapter 2, the application of EMLC to the chiral separation of a group of enantiomeric benzodiazepines was investigated at a PGC stationary phase using β-CD as a chiral mobile phase additive. Results indicated that both the retention and enantioselectivity of the analytes could be markedly and effectively manipulated through alterations in E_{appl}. This capability reflects the potential dependence of the extent of immobilization of β-CD onto the stationary phase. Additionally, results also show that changes in E_{appl} affect the overall retention of three analytes differently. From a brief mechanistic analysis, the observed differences are attributed to the differences in the strength of the inclusion complexation formed between the analytes and β-CD. More importantly, we have shown for the first time that mixtures of each of the three racemates can be effectively resolved using the same isocratic elution conditions.

Chapter 3 described the effects of operational parameters on the resolution of drug enantiomers using EMLC, extending insights into the chiral separation

mechanism of this new technique. While changes in E_{appl} to the stationary phase play a primary role in the alteration of efficiency and elution order of the enantiomers, the results also show that experimental parameters such as the identity and the concentration of the chiral additive in the mobile phase, and the pH and the flow rate of the mobile phase can be modified to optimize the separation efficiency. Interestingly, no chiral separation was realized with sulfated β-CD as the chiral additive, probably owing to the high degree of sulfonation and the ensuing enhancement solubility.

This work also indicated that by carefully selecting the mobile phase additive, EMLC offers the possibility of requiring only one column for the separation of a wide range of racemic mixtures.

Future work directed toward the examination of different types of chiral mobile phase additives, and studies of separation of different classes of chiral compounds will be fruitful. Both neutral and ionic derivatives of cyclodextrins (e.g., hydroxypropyl-β-CD, dimethyl-β-CD, 2,3,6-tri-O-methyl-β-CD, carboxymethyl-β-CD, carboxyethyl-β-CD, sulfated-β-CD, and aminated-β-CD) are of great importance as chiral selectors. By derivatization, the solubility of the chiral additives in the mobile phase could be improved, and the three-point interaction between enantiomers and additive could also be enhanced. Besides cyclodextrins, investigations of other types of chiral selectors such as bile salts and surfactant amino acids would also be of clear interest. The potential

applications of EMLC on chiral separations are reviewed as numerous. For example, the study of enantiomeric separations of amino acids, peptides and β-blockers, along with other pharmaceutical compounds would appear promising. Some of this work is currently underway in our laboratory. Finally, the development of conductive chiral stationary phases (e.g., β-CD trapped sol gel) should also be valuable as a simple and direct means for altering retention and enantioselectivity of the separation in EMLC. The interaction between the analyte and the chiral selector could be enhanced by having more analyte electrosorbed onto the stationary with E_{appl}. Together, the above studies have proven valuable in the expanding the range and scope of EMLC as a novel separation technique.

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