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Electrochemically-modulated Liquid Chromatography (EMLC): Column Design, Retention Processes, and Applications

by

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GENERAL INTRODUCTION

Dissertation Organization

This work describes the continued development of a new separation technique, electrochemically-modulated liquid chromatography (EMLC), from column design, retention mechanisms to pharmaceutical applications. The introduction section provides a literature review of the technique as well as a brief overview of the research in each of the chapters. This section is followed by four chapters which investigate the issues of EMLC column design, the retention mechanism of monosubstituted aromatic compounds, and the EMLC-based applications to two important classes of pharmaceutical compounds (i.e., corticosteroids and benzodiazepines). The dissertation concludes with a general summary, a prospectus, and a list of references cited in the General Introduction.

Literature Review

High performance liquid chromatography (HPLC) has been and continues to be an important separation tool in the operation of many analytical laboratories [1-3]. A large number of columns with different stationary phases have been developed for various modes of separations, including normal-phase [4], reversed-phase [5], affinity [6], and ion chromatography [7]. Limitations and disadvantages of these columns lie in the fixed property of the stationary phases which cannot be manipulated to improve separation performance. For example, the conventional

way to solve co-elution problems is to optimize the mobile phase by changing the percentage of organic component or, for certain applications, the pH buffer or other additives. In general, however, the stationary phase plays a subordinate role in the optimization strategy. Thus, the analytical chemist is judicious in the choice of a stationary phase, and furthermore, once that choice has been made for a certain separation, the usual route pursued to achieve the necessary resolution is to vary the composition of the mobile phase. As one consequence, a large amount of waste is generated during mobile phase optimization, which is becoming an ever increasing portion of operational costs.

In order to overcome these limitations, many alternatives have been presented, such as: transformable stationary phases and dynamic coating techniques [8-9]. EMLC is one of the new alternative techniques, combining electrochemistry and chromatography. This technique originates from electrosorption and electrodesorption concepts in electrochemistry, taking advantage of the effect of changes in applied potential (E_{appl}) on the interactions between an electrode surface and analytes and, more interestingly, transforms a chromatographic column into a three-electrode electrochemical cell, with the stationary phase being the working electrode. In 1960, Blair and Murphy [10] described a process for desalting water based on periodic sorption and desorption of ions on the surface of porous carbon materials. In 1963, Fujinaga [11] first presented a design from the union of a thin-layer electrochemical cell and liquid chromatographic column to perform separations. Strohl [12] and Roe [13] in 1964

also proposed a liquid chromatographic column with a potential-controlled stationary phase. One of the similarities among these early works was the use of conductive material for stationary phases (e.g., glassy carbon and amalgamated metal particles). 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 stationary phase. Fujinaga further demonstrated through improvements in cell design the feasibility of a voltage gradient throughout the stationary phase to enhance separations [14-15]. In 1972 [16], Strohl presented the manipulation of the retention of electroactive organic species (i.e., quinones) by changing their redox states on carbonaceous particles; this was followed by an investigation of the separation of inorganic cations by changes of the pH of mobile phase with applied potential reported in 1978 [17].

These demonstrations, while suggesting the possibility of a new era in separations by on-column electrochemical reactions of analytes and mobile phase, suffered from very low chromatographic efficiencies. The low efficiencies resulted from the difficulty in constructing a column that functioned effectively both electrochemically and chromatographically. A major advance in EMLC column design was reported by Yacynych in 1984 [18] through the development of an EMLC column that could withstand operational pressures up to 3000 psi. The principle modification was to employ stainless steel as opposed to glass as the container for the stationary phase. Wallace also proposed several variations to existing column designs for EMLC [19]; however, the efficiency of these designs still

can not meet the separation efficiencies of contemporary HPLC. More recently, Nagaoka investigated the influence of retention by various polymeric coatings, such as, crown ether [20], polyaniline [20-21], and polypyrrole [21]. Przybycien also studied the retention change of β-lactoglobulin on the stationary phase of heme-agarose while the redox state of the stationary phase was manipulated by additives to mobile phase [22]. Deinhammer, Shimazu and Porter reported some improvements in the preparations of polypyrrole coating, applying these coatings to separations of adenosine phosphates [23] and amino acids [24]. The latter studies demonstrated for the first time the ability to manipulate retention by changing electrochemically the composition of a stationary phase during analyte elution.

Together, all the investigations described above hinted at the potential of EMLC as a new means for manipulating analyte retention by demonstrating its possible applications to a wide range of analytes. That is, EMLC may often serve as an alternative to conventional HPLC. To realize this possibility, however, the chromatographic efficiency of the technique needs a notable improvement before acceptance and adoption by the chromatographic community. Furthermore, additional insights into the retention mechanism are needed to place the performance of this technique on a firm fundamental footing. It is this potential that motivated the work described in this dissertation.

Dissertation Overview

In order to meet the needs aforementioned for the development of EMLC technique, we have explored the issues of column design, retention mechanisms, and possible pharmaceutical applications. In Chapter 1, a newly designed EMLC column is presented. This design is based on our previous design [25] but with important modifications to improve some limitations in performance. That is, in our previous design, the electrochemical performance is much less effective at cathodic values as opposed to anodic ones of E_{appl}. This limitation led us to re-examine the design, preparation, and material composition of the key components of our previous EMLC column in an attempt to identify and correct the sources of the limitation. The results for the performance of the new design are presented and compared to those from the earlier design. The impact from this modification to a proposed retention mechanism is also discussed.

Chapter 2 is targeted at an investigation of the retention processes for a mixture of monosubstituted benzenes using EMLC with porous graphitic carbon (PGC) as the stationary phase. An analysis on the extent of changes in retention as a function of applied potential (E_{appl}) has been performed to probe the retention mechanism of analytes on a PGC stationary phase. These findings and conclusions provide additional insight into the unique features of this new separation technique as well as the establishment of initial predictive guidelines of its applicability for future users.

Chapter 3 demonstrates an application of EMLC to the separation of a mixture of corticosteroids (i.e., prednisone, prednisolone, cortisone, and hydrocortisone) with a PGC stationary phase. As a stationary phase, PGC is selective to differences in double bond structures, but much less so to differences in functional groups. However, based on the conclusions from the fundamental study in Chapter 2, the selectivity of PGC stationary phase to differences in functional groups can be greatly enhanced by E_{appl}. A mixture of corticosteroids with the structures having a different number of double bonds and/or functional groups is employed in this chapter to demonstrate this unique attribute of EMLC.

In Chapter 4, a study continuing our investigations of the range and scope of EMLC that explores the separations of benzodiazepines is presented. The challenge in this case is posed by a mixture of benzodiazepines with only minor differences in functional groups. Other reports [26] have shown that the efficiency in the separations of benzodiazepines on PGC is limited by the poor selectivity of stationary phase to functional group differences. As found in our investigation of the separation of corticosteroids, the low selectivity of PGC toward differences in functional groups can be overcome by the effects of E_{appl} on the retention characteristics of this stationary phase. In this Chapter, we apply EMLC to the separation of a mixture of benzodiazepines at a PGC stationary phase, further demonstrating the attributes (i.e., efficiency and ease of optimization) of this new chromatographic technique.

SUMMARY AND PROSPECTUS

Summary

This dissertation has explored the development of EMLC technique from the very beginning stage (i.e., construction of column) to practical ends (i.e., pharmaceutical applications). In Chapter 1, a newly designed EMLC column has been reported. This column has a chromatographic efficiency that is comparable to contemporary HPLC and an improved electrochemical performance over our previous design. The principle modification of the design is the connection of the porous stainless steel tube as a counter electrode as opposed to part of the working electrode as figured in the previous design. The improvements from this modification have been demonstrated through an examination of the ability to control the applied potential and the resulting enhancements in the capability of EMLC to affect separations. More specifically, in an assessment of electrochemical performance, the response time of the new design (~19 min) is 2.5 times faster for a potential step from 0 V to -1 V than that of the earlier design (~49 min). Together, the success of development of the new design has provided an efficient separation tool for EMLC and triggered our continued exploration of the application of this technique.

In Chapter 2, we have presented a study of retention processes using EMLC at a PGC stationary phase. A mixture of monosubstituted aromatic compounds has been investigated for the influence of E_{appl} to the retention of analytes. Results show

a general trend of more retention of analytes at the more positive E_{appl}. Furthermore, as estimated using a π-molecular orbital calculation, the analyte possessing a larger submolecular polarity parameter and/or having a higher energy level for its highest occupied molecular orbital displays a larger sensitivity in retention to changes in E_{appl}. We attribute these findings to the differences in ability of analytes to participate in the donor-acceptor interactions between analytes and the PGC surface through electrostatic and charge transfer pathways. The conclusions of this study provide qualitative insights into the retention mechanism and have initiated the use of higher level calculations to pursue this investigation more quantitatively.

Chapter 3 has demonstrated the application of EMLC as a technique to improve the separation of a mixture of corticosteroids at a PGC stationary phase. Results indicate that the retention of these analytes can be markedly and effectively manipulated through alterations in the value of E_{appl}. Additionally, conclusions also show that the selectivity of PGC to functional groups can be enhanced significantly to achieve a fully resolved separation. This enhancement is based on the differences in the influence of donor-acceptor interactions between functional groups and PGC surface by E_{appl}. The retention of analyte with stronger donor substituent (i.e., hydroxyl group) can be manipulated by E_{appl} to a larger extent with respect to an analyte with weaker donor substituent (i.e., carbonyl group). From a brief mechanistic analysis, the observed dependencies of retention to E_{appl} are realized through the coupling of donor-acceptor interactions between the analytes

and PGC and the competitive interactions from the ionic species that make up the supporting electrolytes and PGC.

In Chapter 4, the application of EMLC to the separation of a mixture of benzodiazepines at a PGC stationary phase has been presented. The effectiveness of the separation is realized through the differences in the dependencies of the retention of the analytes on E_{appl}. That is, the retention of some of the benzodiazepines increases as E_{appl} becomes more negative, whereas that of other benzodiazepines decreases. These dependencies have the unusual effect of stretching both ends of the chromatogram as E_{appl} moves negatively, which results in the ability to resolve fully all the components of the mixture with only a small increase in the total elution time.

Prospectus

Through the above discussions, it can be realized that the success in the development of EMLC column has led to continuing studies of EMLC-based pharmaceutical separations and hints that EMLC to be a separation tool with enormous potential. To realize the potential of EMLC emerging as a main-stream technique in analytical chemistry, more developments and demonstrations are needed. The possible future developments can be discussed in two categories: column design and applications. Column design is essential for the development and commercialization of the technique. The ultimate goal for the column design is to achieve an electrochemical performance similar to the one from three-electrode

system in bulk solution. However, the performance of the current design, like the previous design but to a lesser extent, suffers from a response time to changes in E_{appl} about 19 min. This delay is attributed to the process of electrolyte double layer charging on large surface area of the porous electrode and the hindrance from the Nafion film to electrolyte transport between electrodes. One possible way to improve this electrochemical response is to use a narrow-bore column or microcolumn. The advantages through the use of smaller inner diameter column are twofold. First, the surface area of the working electrode (i.e., the stationary phase) can be decreased so the noted double layer charging process can be more rapid. Additionally, the concern about the voltage gradient along the column radius will be reduced. Second, the use of narrow-bore or micro-bore columns is a trend in contemporary HPLC that reduces the consumption of packing material, analytes, and mobile phase and easier to interface with a mass spectrometer. As such, smaller column diameters will be beneficial to EMLC in two important ways.

In addition to the modification on the column diameter, the performance limitation from the Nafion film can also be improved through advanced preparations. As mentioned in Chapter 1, the Nafion film serves as a separator between the working and counter electrodes and electrolyte ions transport through the film to complete the circuit. As a consequence, the thickness of the film is critical to the ease of electrolyte transport with a thinner film generating less hindrance to transport. Thus, the preparation of Nafion films with smaller diameters and thinner

thicknesses from Nafion solutions is likely to be an approach to further decrease the response delay.

The development of EMLC has been successful through the demonstrations of separations of corticosteroids, benzodiazepines, chiral drugs [27], and amino acids [28], which prove that EMLC is not only an universal separation tool but also highly competitive to conventional affinity, normal-phase, and reversed-phase chromatography respectively. The potential applications of EMLC are believed to be numerous. One possible future application is the EMLC-based separation of inorganic ions to compete with ion chromatography. Conventionally, separations of inorganic ions require ion exchange column and the manipulation of retention is only possible through the change of ionic strength of mobile phase. Our preliminary results show that the separation of a mixture of inorganic anions on EMLC column is much more facile than the one from ion exchange column through the application of potential to manipulate the electrostatic interactions between PGC and ions [29]. Another possible application is the separation of saccharides. The separations of saccharides are mostly performed on normal-phase chromatography because of its large hydrophilicity [30] or on anion exchange chromatography in a alkaline mobile phase [31]. A separation on PGC has been reported through the use of an aqueous mobile phase to enhance retention in reversed-phase mode [32]. However, this approach results in the difficulty in manipulation of separation through changes in mobile phase. By applying potential, EMLC may have a better opportunity to manipulate the retention of saccharides. Another EMLC-based study of geometric

isomers also seems to have great merit on the development. Compounds with different dipole moments might have different dependencies to E_{appl} to exploit in the fine-tuning of elution. Finally, a fundamental study of the separations of compounds of aliphatic chains is believed to be important and interesting. Various length and branch of aliphatic chains have different degree of adsorption to the stationary phase. This study along with the one of various functional groups should provide a better guideline of dependencies of analytes on E_{appl} for future EMLC users.

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