Project of Excellence:

Investigating the Chiral Selectivity of the Leucine-Valine Surfactant in the Presence of Ethylene

and Propylene Diamine Counterions

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I. Abstract

In this study, the effects of alkyl diamine counterion selection and pH on the chiral separation of several binaphthyl derivatives with L,L- undecanoyl leucine-valinate (und-LV) was examined by use of Micellar Electrokinetic Chromatography (MEKC). The objective of this research seeks to expand an understanding of the parameters required for optimization of the chiral selectivity of the und-LV surfactant in order to advance its use in enantioseparation. The chiral separations of [1,1'-bi-2-naphthyl-2,2'-diyl hydrogen (BNP) 1,1′-bi-2-naphthyl-2,2′-diamine (BNA) and 1,1′-bi-2-naphthol (BOH)] by und-LV varied widely in the presence of three counterions, 1,2 diaminoethane, 1,2 diaminopropane, and 1,3 diaminopropane, at pH 8 and pH 11. The results of this study have shown that separation of BNP enantiomers at pH 8 produces similarly optimal resolutions with use of 1,2 diaminoethane (10.9) and 1,2 diaminopropane (10.12), while the remaining counterion confers poor chiral selectivity of und-LV, evidenced by a weak resolution of 1.6. Interestingly, use of 1,2 diaminopropane in the separation of BNP enantiomers resulted in the lowest (1.61) retention factor value of all separations conducted under the same conditions, while use of 1,3 diaminopropane resulted in a separation with the highest retention factor value (2.38). With all counterions, conducting separations of each binaphthyl derivative at pH 11 decreased the observed retention factor values. Resolution values of 1,2 dimaminoethane and 1,2 diaminopropane also decreased under this pH, though use of 1,3 diaminopropane instead demonstrated a slight increase in its resolution value. Similar trends are observed when separating enantiomers of BOH at pH 8, while separation of BNA enantiomers in an equitable pH instead demonstrated that use 1,3 diaminopropane provides a high resolution value (6.12) in comparison to its use with the other binaphthyl derivatives. Unique structural

characteristics of each counterion, their varying protonation states under high and low pH conditions, and pH-dependent charges of each analyte likely contribute to these findings.

II. Introduction

a. Background

Surface-active agents, termed surfactants, are versatile molecules suitable for near-ubiquitous use across a diverse range of industries, including pharmaceutical, agricultural, cosmetic, and detergent-associated applications. Contributing to their broad use are unique structural characteristics, which confer an amphipathic profile to the molecule; regions of hydrophobicity are conferred by long alkyl chain tails, while its hydrophilic counterpart is supplied by the molecule's polar head group. When exposed to aqueous solution above a specified surfactant concentration known as the critical micelle concentration (CMC), the amphiphilic nature of surfactants promotes their assembly into the micellar form, with polar head groups exposed to the medium, while hydrocarbon tails are shielded in the micellar core. Such conformation minimizes entropically unfavorable interactions with water, providing intermolecular stability between surfactants.

Surfactant types are variable, though the numerous advantages affiliated with use of amino acid-based surfactants, such as their cost-effectiveness, industrial prevalence, and biodegradability, promote their utilization. This research narrows the intrinsic diversity of amino acid-based surfactants to the realm of chiral selectivity, where micellization is studied as a mechanism of enantiomeric separation.

Separation of racemic mixtures into their respective enantiomers, chiral molecules that are non-superimposable mirror-images of one another and are alike in both physical and chemical properties, is an integral component of the synthetic purification process. This is

especially prevalent in the pharmaceutical industry; enantiomers are frequently made distinct by their biochemical implications, as each form is likely to produce different physiological effects. Thus, chiral separation of each enantiomeric form is vital to ensuring human health and safety.

The chirality of amino acid-based surfactants makes them suitable chiral selectors, a necessity in conducting enantioseparation. An efficient means of implementing and quantifying the success of enantioseparation regards the use of Micellar Electrokinetic Chromatography (MEKC), a modification of capillary electrophoresis (CE). Including micelles of chiral surfactants in solution allows interactions with a chiral analyte, providing a means interpreting column elution as a function of the analyte's incorporation with the micelle; a prolonged retention in the chromatographic column is indicative of increased binding. Analyte-micelle interactions are governed by the differential interactions of enantiomers with the chiral atoms of the surfactant head groups, leading to a separation that can be characterized by differing elution times.

Previous studies have progressed the investigation of both single amino acid and dipeptide surfactants, though the latter is associated with more pronounced extents enantiomeric separation. The presence of additional chiral centers in dipeptide surfactants confers an advantage of greater chiral selectivity and is likely the explication for their success. Moreover, other studies have identified increasingly efficient parameters that define the conditions for pronounced enantioseparation, such as those noting the influential capacities of counterion selection, analyte selection, and pH. Counterion selection has been shown to alter the shapes, sizes, and CMC of surfactant aggregates, such as in a prior study demonstrating increased chiral separation of binaphthyl compounds from the selection of amino acid counterions in comparison to a sodium counterion standard. Similarly, pH has been explored as a means of understanding

what effect the charge and subsequent ionization of functional groups in the surfactant's polar head region might maintain on separation. The documented success of a particular dipeptide, undecyl leucine-valine, promotes its continued exploration in continuing endeavors.

b. Problem Statement and Objective

This study seeks to explore optimization of the chiral selectivity of L,L- undecanoyl leucine-valinate (und-LV) by examining the influential characteristics of alkyl diamine counterion selection and pH in the chiral separations of the binaphthyl derivatives: binaphthyl phosphate (BNP), binaphthol (BOH), and binaphthyl amine (BNA). These endeavors aim to expand and inevitably simplify the process of inducing compound purity by enantiomeric separation, an industrial convention that is currently both time and resource intensive.

III. Methodology

In this study, the chiral selectivity of several binaphthyl derivatives with L,L- undecanoyl leucine-valinate will be examined by use of Micellar Electrokinetic Chromatography (MEKC), a chromatographic technique undertaken by use of Capillary Electrophoresis (C.E.) instrumentation. Variables such as: counterion type, analyte type, and pH of the solution were assessed in their roles in influencing the chiral selectivity of und-LV. The three counterions intended for examination include 1,2 ethylenediamine, 1,2 diaminopropane, and 1,3 diaminopropane, observed comparatively with that of sodium for persistent, enhanced enantiomeric separation of each analyte [1,1'-bi-2-naphthyl-2,2'-diyl hydrogen (BNP) 1,1′-bi-2-naphthyl-2,2′-diamine (BNA) and 1,1′-bi-2-naphthol (BOH)]. By the examining individual and cross-referenced electropherogram data produced by previously completed trials of chiral separations under conditions that vary according to the above parameters, patterns are discernable in the chemical behavior of each variable in inducing separation; this information

serves as a valuable asset in understanding optimization for these specifications, which may then be extended to other parameters in subsequent studies. All work was completed under the direction of Dr. Fereshteh Billiot, advising in the surfactant/solution production, chromatographic trial experimentation, and data interpretation processes.

IV. Results

Firstly analyzed are the resolution values of BNP enantiomers, separated with und-LV surfactant in the presence of three counterions, 1,2 ethylenediamine, 1,2 diaminpropane, and 1,3 diaminopropane at pH values of 8 and 11. At a pH of 8, 1,2 ethylenediamine exhibits a resolution of 1.11 at a concentration of 8 mM and a resolution of 10.9 at a concentration of 20 mM, which is the highest resolution achieved of the studied counterions. Counterion 1,2 diaminopropane provided similar, though slightly slower resolution values under the same conditions, with resolution values ranging from 1.01 at a low concentration of 8 mM to 10.12 at the higher 20 mM concentration. Conversely, use of counterion 1,3 diaminopropane exhibited poor separation of BNP enantiomers under equivalent conditions, with resolution values of 0 and 1.6 at concentrations of 8 mM and 20 mM, respectively. At a higher pH value of 11, resolutions with use of 1,2 ethylenediamine and 1,2 diaminopropane were significantly lower than under lower pH conditions. 1,2 ethylenediamine provided resolution values ranging from 0.1 and 5.59 at 8 mM and 20 mM, respectively, while 1,2 diaminopropane provided resolution values ranging from 0.1 and 4.06 at the same concentrations and under equivalent pH conditions. Interestingly, this decrease in resolution was not observed in the presence of 1,3 diaminopropane. Resolution values instead increased at a higher concentration, ranging from 0 at 8 mM to 3.5 at 20 mM. This analysis is extended by illustrating the variability in retention factor, according to counterion selection and pH conditions. At a pH of 8, 1,2 ethylene diamine exhibits a retention factor value

of 1.71 at 8 mM and 2.35 at 20 mM. In comparison, use of 1,2 diaminopropane expresses lower resolution factors, with values between 0.99 and 1.61 at the same low and high pH values and concentrations. Surprisingly, counterion 1,3 diaminopropane offers the highest retention factor values of all three counterions, despite previously demonstrating the lowest resolutions across varying concentration and pH conditions. Separation of BNP enantiomers at in the presence of 1,3 diaminopropane at a pH of 8 provides retention factor values that range from 1.73 at 8 mM to 2.38 at 20 mM, results that are markedly similar to that of 1,2 ethylenediamine. When studied at a higher pH value of 11, retention factor values for each counterion decreased, with 1,2 ethylenediamine and 1,3 diaminopropane once again expressing similar values that are greater than that 1,2 diaminopropane. Use of 1,2 ethylenediamine in this higher pH condition produces retention factor values ranging from 0.54 to 1.1 at concentrations of 8 mM and 20 mM, respectively, while 1,3 diaminopropane produces values of 0.45 and 1.22 at equivalent concentrations. In the presence of 1,2 diaminopropane, retention factor values are lowest, ranging from 0.35 to 0.75 at the designated low and high concentration levels. The electropherograms produced from MEKC contribute to these observations by demonstrating separation of BNP enantiomers with use of the three studied counterions under low pH 8 conditions. Of all the counterions, use of 1,2 ethylenediamine produces the greatest resolution, with a value of 7.85. Separation in the presence of 1,2 diaminopropane exhibits the next highest resolution, with a value of 7.59, while use of 1,3 diaminopropane produces the least resolution, with a value of 1.5. Interesting to note from each diagram is the retention time of each enantiomer of BNP analyte. Despite 1,2 ethylenediamine and 1,2 diaminopropane performing the separation with similar resolutions, use of the latter counterion results in a more rapid separation and elution of enantiomers, noted by the lesser retention times of each chromatographic peak.

Retention times of each enantiomer are 4.178 minutes and 14.465 minutes in the presence of 1,2 diaminopropane, while use of 1,2 ethyelendiamine produces retention times of 17.253 minutes and 18.312 minutes. Conversely, 1,3 diaminopropane offers the slowest separation characterized by the highest retention times, with chromatographic peaks occurring at 26.604 minutes and 27.136 minutes. Also included are critical micelle concentrations of the 1:1 surfactant-counterion complexes formed from each of the three counterions under low pH 8 and high pH 11 conditions. At a pH of 8, CMCs in the presence of 1,2 ethylenediamine, 1,2 diaminopropane, and 1,3 diaminopropane are 7.4, 7.8, and 7.3, respectively. The similarities that exist between CMCs for each counterion are also observed at higher pH conditions, where the values are each higher, but still approximately equivalent to one another. At a pH of 11, CMC values for 1,2 ethylenediamine, 1,2 diaminopropane, and 1,3 diaminopropane are 10.5, 10.5, and 11.4, respectively. Integrating pKa1 and pKa2 values for each counterion contributes an additional facet to the study. pKa1 values for 1,2 ethylendiamine and 1,2 diaminopropane are similar, with the former counterion exhibiting a value of 9.92 and the latter exhibiting a value of 9.82. These similarities are also observed with comparison of pKa2 values between the two counterions. 1,2 ethylenediamine maintains a pKa2 value of 6.86, while 1,2 diaminopropane has a pKa2 value of 6.61. In comparison, the pKa values of the final counterion differ substantially; pKa1 and pKa2 for 1,3 diaminopropane are much higher than the other two counterions, with values of 10.55 and 8.88, respectively. Observations are furthered by the inclusion of diffusion coefficients for the micelle, surfactant, and counterion, mole fractions of leucine-valine monomers bound to the surfactant, mole fractions of counterion molecules bound to micelles, and finally, the micellar hydrodynamic radius. This array of data is recorded for each of the three counterions included in the study, under conditions of pH 8 and pH 11. With use of 1,2 ethylenediamine at a pH of 8, the

diffusion coefficients of the micelle, surfactant, and counterion are 0.773, 1.24, and 6.53, respectively. At a pH of 11, each of these measures increased, with diffusion coefficients of the micelle, surfactant, and counterion rising to 1.43, 1.48, and 10.7, respectively. Under each pH condition, these values are consistently higher than those observed for 1,3 diaminopropane. With use of the 1,3 diamine counterion, diffusion coefficients of the micelle, surfactant, and counterion at a pH of 8 are 0.730, 1.10, and 4.60, respectively. As shown with 1,2 ethylenediamine, adopting a pH of 11 increases each of these values. Under this higher pH condition, diffusion coefficients of the micelle, surfactant, and counterion rise to 1.06, 1.33, and 10.4. The values of fraction bound leucine-valine for 1,2 ethylenediamine and 1,3 diaminopropane are similar in each pH condition, measured at approximately 0.90; this value increases with an increase to pH 11, with fraction bound leucine-valine increasing to 0.98 for each counterion. Conversely, values of the fraction bound counterion differ across pH conditions and the identity of the counterion itself. The value of fraction bound 1,2 ethylenediamine is significantly lower under both low and high pH conditions in comparison to the fraction bound 1,3 diaminopropane. At a pH of 8, fraction bound 1,2 ethylenediamine is 0.41, and at a pH of 11, the fraction bound value is 0. With 1,3 diaminopropane, the fraction bound counterion value is 0.61 at pH 8 and 0.02 at pH 11. Finally, hydrodynamic radii of micelles in the presence of 1,2 ethylenediamine and 1,3 diaminopropane express interesting trends. Though similar, the radii of micelles with use of 1,2 ethylenediamine under both low and high pH conditions are lesser than that of 1,3 diaminopropane. At pH 8, the radius of the micelle containing 1,2 ethylenediamine is 26.8, while the value is 28.4 for the micelle containing the latter counterion. Further, each counterion experiences a decrease in micellar radius with an increase to pH 11; micelles in the

presence of 1,2 ethylenediamine produce a radius of 14.5, while those in the presence of 1,3 diaminopropane produce a radius of 16.1.

V. Analysis

The leucine-valine dipeptide surfactant utilized in the study contains a carboxylic functional group at the terminal end of the polar head region. It has a pKa value of 3.79, providing a negative charge to the molecule above the studied values of 8 and 11. The head region of the surfactant molecule also contains two chiral centers, enabling its use as a chiral selector through micellization. The process of micellization requires surfactant aggregation, which occurs above the critical micelle concentration of 7 mM at a pH of 7. Aiding the chiral selectivity of the dipeptide surfactant are the three alkyl diamine counterions included in the study, 1,2 ethylenediamine, 1,2 diaminopropane, and 1,3 diaminopropane. The structures, pKa values influencing protonation states under varying pH conditions, retention factors, binding affinities, and resolutions analyzed throughout the study make each counterion distinct in separation performance. 1,2 ethylendiamine and 1,3 diaminopropane are structurally similar, though the presence of an additional carbon lengthens the alkyl chain for the latter counterion. As an isomer of 1,3 diaminopropane, 1,2 diaminopropane maintains a three-carbon chain length, but differs in its branching structure. A methyl group at position 1 of the molecule contributes its dissimilar structure in comparison to the other two counterions. Interestingly, the position of this functional group may influence retention times of separations analyzed by MEKC. Separations completed with use of 1,2 diaminopropane and the leucine-valine surfactant consistently exhibit the least retention times, indicating more rapid micellar interactions and quicker elution of analyte enantiomers from the capillary. The branched position of the methyl group within the counterion may be extending inwards, directed towards the micellar core. Steric hindrance

between this and other surrounding groups may prevent the spatial proximity required for oppositely charged counterion and surfactant molecules to bond tightly. This, in turn, seems to promote a more open conformation of micellar aggregates, better enabling analyte molecules to interact with the micellar core in a rapid fashion and contributing to the counterion's associated rapid retention times. The retention factors of 1,2 ethylenediamine also support this consideration, given that k' values for this counterion are the lowest of all three counterions under both high and low pH conditions. Another unique structural characteristic to consider is the isomeric relationship between 1,2 diaminopropane and 1,3 diaminopropane. Despite this similarity, use of the latter counterion exhibits tendencies toward low resolutions, high retention factors, and high retention times – all trends that oppose those observed with 1,2 diaminopropane. Instead, separation performances of 1,2 ethylenediamine and 1,2 diaminopropane rival one another. An analysis of protonation states for each counterion may best explicate these differences. The pKa1 and pKa2 values of 1,2 diaminopropane and 1,2 ethylenediamine are remarkably similar, with values of approximately 9.8 and 6.7, respectively. Conversely, 1,3 diaminopropane's pKa1 and pKa2 values are 10.55 and 8.88. The differences between these values represent a deviation in counterion protonation under the low and high pH conditions selected in the study. At a pH of 8, pKa2 values of 1,2 diaminopropane and 1,2 ethylenediamine indicate a loss of positive charge. At a pH of 11, pKa1 values of these counterions indicate a further loss of positive charge. The loss of positive charge abundance in each of these counterions is associated with weaker binding to the negatively charged surfactant, reflected in counterion fraction bound values. At a pH of 8, the fraction of bound 1,2 ethylenediamine is 0.41, which becomes 0.0 at a pH of 11. 1,3 diaminopropane exhibits higher values of fraction bound counterion, with 0.61 bound at a pH of 8 and 0.02 bound at a pH of 11.

These higher counterion fraction bound values are the consequence the counterion maintaining more of its positive charge under both high and low pH conditions, enabling stronger binding to the oppositely charged surfactant in comparison to 1,2 diaminopropane and 1,2 ethylenediamine. The increased strength of interactions within the counterion-surfactant complex formed with use 1,3 diaminopropane may also be illustrated with incorporation of retention factor, an aspect analyzed for each counterion. Retention factors for 1,3 diaminopropane are highest of each of the studied counterions under both high and low pH conditions, likely reflecting the increased attraction and subsequent binding of the more positively charged counterion and negatively charged surfactant. In opposition to the micellar spatial advantage and quick retention times observed with 1,2 diaminopropane, tighter binding between the surfactant and counterion may reduce the analyte's capacity to interact with the micelle and enter its core, leading to slower retention times and higher retention factor values that are characteristic of 1,3 diaminopropane. MEKC-derived resolutions contribute an additional facet in the comparison of counterion performance. Use of 1,2 ethylenediamine and 1,2 diaminopropane achieve separations that exhibit the highest resolution values under low pH 8 conditions, while use of 1,3 diaminopropane offers the lowest resolution values under pHs of 8 and 11. This may be contributed by the negative charge associated with the BNP analyte; analyte molecules may not be binding at undLV's chiral centers, but instead at the positively-charged regions of 1,3 diaminopropane, explicating the counterion's characteristic high binding interactions, yet weak resolution.

VI. Conclusion

Separation of racemic mixtures into their respective enantiomers is an integral component of the synthetic purification process. This is especially prevalent in the pharmaceutical industry; stereospecific enantiomers are frequently made distinct by their biochemical implications, as

each form is likely to produce different physiological effects. Following the infamous discovery of these principles based upon consequences of the historically prescribed anti-nausea drug, Thalidomide, the Federal Drug Administration has since required that drug manufacturers purify each racemic mixture synthesized with pharmaceutical intent. This endeavor is not easily achieved, however. Drug manufacturing industries frequently revert to experimental determination of a method of enantiomeric purification for each compound synthesized, yet this process is often cost-inefficient, untimely, and offers variable success rates. By centering on the exploration of varying parameters that optimize the chiral selectivity of L,L- undecanoyl leucine-valinate, the knowledge obtained from this study contributes to the scientific expansion of enantioseparation. Results of this investigation provide to the literature viable recommendations for the separation of analytes similar to the binaphthyl derivatives utilized. Further, the information derived from this and other studies centering on the enhancement of chiral selectivity and enantiomeric purification may be utilized in tandem with aspects of molecular modeling, the joint efforts of which concern the development of a large, integrative database of enantio-specific optimization parameters. By equipping the database with laboratory data highlighting the particular variables useful in enhancing a chiral selector's separation performance, algorithms may be designed to examine these patterns and subsequently predict the parameters necessary in optimizing the performance of any designated chiral selector. Such a development would prove an invaluable asset to pharmaceutical and other industries reliant upon synthetic purification processes. Though the pragmatic introduction of such a scientific model is far from near, its resounding potential and current progress only exacerbate the significance of this study and others also striving in the advancement of enantioseparation.