POLYMERIC SURFACTANTS AS PSEUDO-STATIONARY PHASES FOR SEPARATIONS IN ELECTROKINETIC CHROMATOGRAPHY (EKC): A REVIEW

Judson L. Haynes III and Isiah M. Warner*
Department of Chemistry, Louisiana State University, Baton Rouge, LA 70803, USA
and
Shahab A. Shamsi
Department of Chemistry, Georgia State University, Atlanta, GA, 30303, USA

Nonstandard Abbreviations:

CDs = cyclodextrins; CZE = capillary zone electrophoresis; DNB-**AAs** = dinitrobenzoyl amino acids; EKC = electrokinetic chromatography; MEKC = micellar electrokinetic chromatography; CD-**MEKC** = cyclodextrin modified micellar electrokinetic; EKC-**ESI-MS** = electrokinetic chromatography-electrospray ionization mass spectrometry; **SDS** = sodium dodecyl sulfate; STDC = sodium taurodeoxycholate; TEA = triethylamine; **poly-SUA** = poly sodium-10-undecylenate; **poly-SUS** = poly sodium undecyl sulfate; **poly-D-SUV** = poly (sodium *N*-undecanoyl-D-valinate); **poly-L-SUV** = poly sodium *N*- undecanoyl -L-valinate); **poly-L-SUVV** = poly (sodium *N*-undecanoyl-L-valyl-valinate); **poly-L-SULL** = poly (sodium *N*-undecanoyl-L-leucyl-leucinate); **poly-L-SUVL** = poly (sodium *N*-undecanoyl-L-valyl-leucinate); **poly-L-SUL** = poly (sodium *N*-undecanoyl-L-leucinate); **poly-L-SULA** = poly (sodium *N*-undecanoyl-L-leucyl-alaninate); **poly-L-SUAL** = poly (sodium *N*-undecanoyl-L-alanyl-leucinate); **poly-L-SUGA** = poly (sodium *N*-undecanoyl-L-glycyl-alaninate); **poly-L-SUAG** = poly (sodium *N*-undecanoyl-L-alanyl-glycinate); **poly-L-SUAV** = poly (sodium *N*-undecanoyl-

*Address for correspondence: Professor Isiah M. Warner, Department of Chemistry, Louisiana State University, Baton Rouge, LA, 70803, USA.
Tel.: 225-388-2829; Fax: 225-388-3971:
e-mail: isiah.warner@chemgate.chem.lsu.edu

L-alanyl-valinate); **poly-L-SUVA** = poly (sodium *N*-undecanoyl-L-valyl-alaninate); **BBMA** = butyl acrylate-butyl methacrylate-methacrylic acid; **(±)-BOH** = (±)-1,1'-bi-2-naphthol; **(±)-BNP** = (±)-1,1'-binaphthyl-2,2'-diyl hydrogen phosphate; (±)-**TFAE** = (±)-trifluoro-1-(9-anthryl)ethanol; **(±)-Prop** = (±)-propranolol; **(±)-Alp** = alprenolol

Keywords: chiral separations / electrokinetic chromatography / amino acid terminated micelle polymers / dipeptide terminated micelle polymers / polymeric surfactants / chiral polymeric surfactant / pseudo-stationary phase development / PAH separations / electrokinetic chromatography – mass spectrometry

CONTENTS

1. INTRODUCTION

Micellar electrokinetic chromatography (MEKC) is a subclassification of capillary electrophoresis (CE) which allows for the separation of neutral and charged compounds using a micellar pseudo-stationary phase (PSP). In general, PSPs provide a solution-based environment in a CE capillary in which neutral or charged compounds are able to bind preferentially, based on their relative affinity for the interior and/or exterior of the PSP. Traditionally, anionic surfactants such as sodium dodecyl sulfate (SDS) have been added to function as a PSP in the running buffer in MEKC. The use of SDS above its critical micelle concentration (CMC) provides a micelle with a lipophilic interior and an ionic exterior. This mode of CE was introduced in 1984 by Terabe *et al.* /1/. Since then, SDS and a variety of other commercially

available surfactants have been shown to be useful for the separation of numerous compounds /2-5/.

Although micelles have been found to have a wide range of success in MEKC, they also face significant limitations in MEKC. One of the main problems associated with micelles in MEKC is the presence of the dynamic equilibrium between the surfactant monomers and the micelle, thus limiting the stability and operating concentration of the PSP. Extremely hydrophobic compounds such as PAHs are difficult to separate due to their co-migration with the micelle in MEKC. Methods to alleviate this difficulty involve increasing the concentration of the surfactant and/or addition of organic modifiers (such as methanol or acetonitrile). The addition of organic modifiers often disrupts micelle formation, which often leaves the option of increasing concentration of surfactant as a solution. Increasing the concentration of the surfactant may lead to longer migration times and ultimately joule heating, which is detrimental to separations in MEKC.

In addition, the on-line coupling of MEKC with mass spectrometric detection is troublesome. High concentrations of surfactant monomers create a large background signal in the low-molecular-mass region of the mass spectrum. Lastly, commercial surfactants have not been developed with chromatographic selectivity in mind, and introducing unique or desired selectivity requires either the use of additives or the synthesis of selective surfactants /6/. Thus, these limitations have fueled the need for development of new PSPs. In particular, in the field of enantiomeric separations, the search for new chiral surfactants is always of growing interest. In fact, it is now well known that enantiomeric drugs exhibit different physiological activity in both degree and nature. In some cases, the different physiological activity can have teratogenic effects, e.g., the case with Thalidomide. As a result, the Food and Drug Administration released new guidelines in the USA to regulate chiral drugs.

One potential solution to the limitations faced by commercially available micelles, is the introduction of polymeric surfactants or micelle polymers. The idea of eliminating the dynamic equilibrium between the surfactant monomers and micelles, was introduced by Lapidot, Rappoport and Wolman /7/, Larrabee and Sprague /8/, and Dagai and Elias /9/. This new type of micellar system was coined as a polymerized surfactant aggregates, surfactant oligomers, molecular micelles, and micelle polymers /7-9/. Due to the debate over the name of these systems, we herein adopt the terminology of "micelle polymers" throughout the rest of this document. Micelle

polymers provide stable PSPs for CE separations due to their zero critical micelle concentrations (CMC's), tolerance to organic solvents, and virtual formation of micelle at any concentration. Furthermore, they can be employed with CE coupled to mass spectrometric detection without serious detrimental effects.

EKC, where the micelles are replaced by covalently linked polymeric surfactant solution, provides a similar mechanism of separation to traditional micellar systems. The focus of this review article is to discuss principles, techniques, and applications of achiral and chiral separations in EKC with micelle polymers. The data presented are collected from the literature and from the authors' own laboratory. The systematic design and synthesis of ionic monomeric surfactants and ionic micelle polymers, followed by the advantages of these micelle polymers over that of monomeric surfactants will also be discussed. Moreover, the importance of chiral micelle polymers for chiral separations will be stressed. The rationale behind the focus on chiral separations extends from challenges faced by separation scientists. It will be shown that micelle polymers have provided a "user-friendly" solution to many of the challenges of chiral separations.

2. EKC CONCEPTS

2A. Surfactant and Micelles

Amphiphilic molecules (also known as surfactants or detergents) consist of polar or ionic groups called the head and a long hydrocarbon chain called the tail. In aqueous solution, the hydrophobic and hydrophilic properties of amphiphiles are in conflict /10/. One part of the molecule (the head) is very compatible with the aqueous environment. The other part of the molecule (the tail) is hydrophobic and therefore is not compatible with an aqueous environment. Thus, at low concentrations, the surfactants are dispersed in solution /11/. However, at higher concentration (above the CMC), the result of this conflict is the segregation of their hydrophobic portions from the solvent by self-aggregation. These aggregated products are known as micelles. When the hydrophobic portion of the surfactant is a hydrocarbon chain, the micelles will consist of a hydrocarbon core, with the polar groups at the surface. The average number of surfactant molecules necessary to bring about the formation of a micelle is defined as the aggregation number (N).

Small micelles typically have N values in the range of 40 – 140 /12/. Surfactants may be classified according to the type of polar head group. For example, anionic ($R\text{-}X^-M^+$), cationic ($R\text{-}N^+(CH_3)_3X^-$), zwitterionic ($R\text{-}(CH_3)_2N^+CH_2X^-$), or nonionic [$R(OCH_2)CH_2))_nOH))$], where R is a long aliphatic chain, M^+ is typically a metal ion; X^- is typically a halogen, carboxylate, sulfonate or sulfate, and n is an integer /12/. A recent addition to the traditional surfactant classification is the polyethylene oxide surfactants, C_yE_x $_y$ being the number of carbon in an n-alkyl chain and x the number of ethylene oxide groups /13/. Table 1 gives a list of some surfactants along with their properties /14/.

Micellar structure is determined by an equilibrium between repulsive forces (ionic) and short-range attractive forces (hydrocarbon). The size and shape of the micelle is somewhat uncertain and is still in many respects open to discussion (Figure 1). McBain, who was first to propose the presence of molecular aggregates in soap solutions, argued that lamellar and spherical forms coexisted in solution /15/. Hartley addressed the issue of micelle size

Table 1
Critical Micelle Concentrations (CMC) and Aggregation Numbers (AN) for Detergents

Detergent	CMC, mM	AN
Anionic		
Cholic acid, sodium salt	14	2-4
Deocycholic acid, sodium salt	5	4-10
Glycocholic acid, sodium salt	13	2
Sodium dodecyl sulfate (SDS)	8.27	62
Taurocholic acid, sodium salt	10-15	4
Cationic		
Cetyltrimethylammonium chloride	1	
Cetyltrimethylammonium bromide	1.3	78
Dodecyltrimethylammonium bromide	14	50
Hexadecyltrimethylammonium bromide	0.0026	169
Zwitterionic		
CHAPS[a]	8	10
CHAPSO[b]	8	11
Nonionic		
n-Decyl-β-D-glucopyranoside	2.2	
Triton X-100	0.24	140

[a]3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate.
[b]3-[(3-cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate.

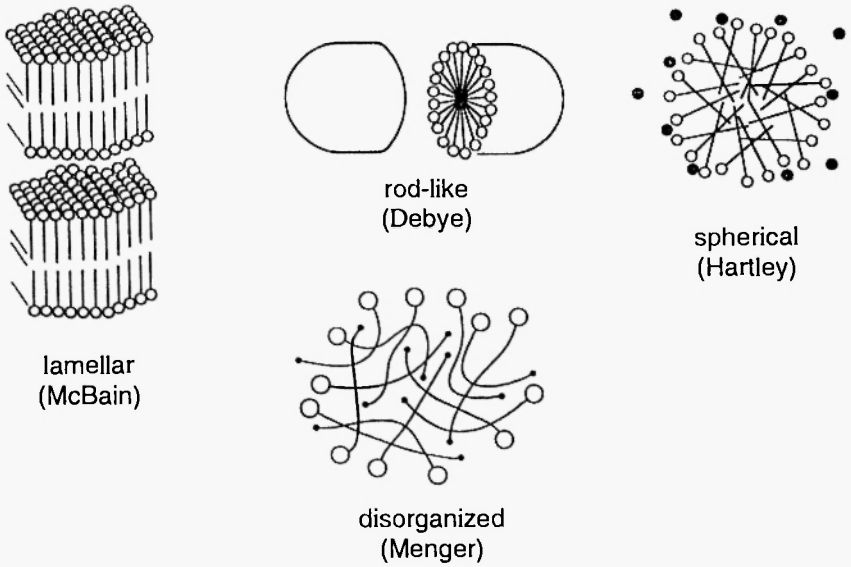


Fig. 1: Different proposed structures of the micelle. From /31/, with permission.

and shape by proposing that micelles are spherical with the charge groups situated at the micelle surface /16/. Contrasting to Hartley's model, Harkins *et al.* suggested a lamellar model similar to McBain /17,18/. Later, another model that was proposed by Debye and Anacker argued that micelles are rod-shaped rather than spherical or disklike /19/. Lastly, Menger proposed a radically different micellar model. In his model, Menger envisioned micelles having a rough surface with water-filled pockets, chain looping, non-radial distribution of chains, and contact of terminal methyl group with water /20/. Despite the different views of micelles in terms of size and shape, neutron small-angle scattering experiments on sodium dodecyl sulfate and other ionic micelles support the Hartley model of a spherical micelle /21-23/.

Since a large fraction of counterions remain associated with the micelle, the net charge of the micelle is less than the degree of micellar aggregation. These counterions form the Stern layer at the micellar surface. A significant increase in surfactant concentration, above the CMC, can lead to a pronounced difference in the shape of the ionic micelle (Figure 2). It is generally accepted that the shape of the ionic micelle changes in the order: spherical → cylindrical → hexagonal → lamella.

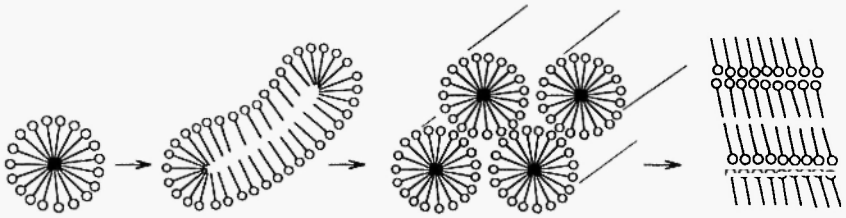


Fig. 2: Changes in micelle shape and structure with respect to change in surfactant concentration. From /11/, with permission.

Thus, the chemical structure of a given surfactant determines the size and shape of its micelles. However, it is vital to understand that in solution, micelles exist in dynamic equilibrium with the monomers from which they are formed. Therefore, micelles are generally considered to be polydispersed species due this dynamic monomer/micelle equilibrium. Polydispersity can result in a range of micelle migration velocities, resulting in band dispersion that may be detrimental to electrokinetic separations.

Electrokinetic capillary chromatography is a mode of CE based on the differential partitioning between a buffer additive and the aqueous solution phase. Buffer additives in EKC range from the use of traditional micelles (e.g., MEKC) and cyclodextrins (e.g., CD-EKC), to exotic additives such as dendrimers (e.g., DECC), crown ethers, and micelle polymers. EKC involving polymers of ionic surfactant solution has gained considerable attention for the separation of both achiral /24-28/ and chiral molecules /29,30/. EKC, where the micelles are replaced by covalently linked polymerized surfactants, provides a similar mechanism of separation to traditional micellar systems. In MEKC and EKC, neutral analytes are separated on the basis of differential interaction with the PSP, whereas charged analytes with similar charge-to-mass ratios can be resolved through electrostatic interaction at the surface of the surfactant or micelle. Since the initial reports of Terabe, Tsuda, and co-workers on MEKC, there has been an exponential growth of publications in this field /27,28/.

2B. EKC Theory with Surfactants and Micelle

MEKC involves the introduction of surfactants into the running buffer of CZE at a concentration above the CMC. The micellar run buffer serves as a

PSP for chromatographic separation. Presuming that there are no capillary wall interactions, negatively charged micelles migrate electrophoretically toward the anode and positively charged micelles, migrate electrophoretically toward the cathode. Thus, the electrophoretic migration of a negatively charged micelle is normally opposed by the EOF. The net migration of negatively charged micelles will typically be toward the cathode since the electroosmotic velocity is typically higher than the electrophoretic velocity. In the case of positively charged micelles, a reversal of the direction of EOF toward the anode (e.g., injection end in normal polarity CE) is often caused due to the interaction of the positively charged micelle with the capillary surface.

A neutral chiral solute, which is not separated by CZE, migrates at the velocity of the EOF when it does not interact with the PSP. In the case of a chiral surfactant, the chiral micellar phase usually contains a chiral polar head group and a hydrocarbon tail, which acts as a PSP or a separation carrier /31/. The surface of the chiral micelle is usually charged. This gives the chiral micelle an electrophoretic mobility in CZE, at a velocity different form the surrounding aqueous phase. A chiral solute can interact with the micelle on the surface through polar-polar interactions as well as hydrophobic interactions with the core of the micelle /12,32,33/. However, in most cases, the hydrophilic polar group of the surfactant is responsible for chiral discrimination. Consequently, separation and retention time is based on differential solubilization (interaction) as well as chiral recognition in the micellar phase.

MEKC is considered similar to micellar liquid chromatography (MLC); equations used in MLC [e.g., $t_R = (1+k')t_0$] can be used as models in MEKC with a few modifications. Neutral solutes will have a retention time between the retention time of a solute that moves with the electroosmotic velocity (elute at a time of t_0) and the retention time of a very hydrophobic solute (elute at a time of t_{mc}) [11]. To solve for the migration time of the neutral solute, x, we must determine the capacity factor, κ', which is defined by

$$\kappa' = \frac{\eta_{mc}}{\eta_{aq}} \qquad (1)$$

where η_{mc} and η_{aq} are the total number of solute molecules incorporated into the micelle and the total number of molecules dissolved in the surrounding phase, respectively. The total molecules of solute in the micelle and in the

aqueous phase, respectively, can be calculated (1) from retention times by use of the following equation:

$$\kappa' = \frac{t_R - t_o}{t_o\left(1+\left(\frac{t_R}{t_{mc}}\right)\right)} \tag{2}$$

where t_R is the retention time of the solute, and t_{mc} is the migration time of the micelle. The migration times, t_0 and t_{mc}, can be measured by using methanol or formamide as an aqueous-phase tracer and Sudan III or IV as micelle tracers, respectively. Equation 2 can be expressed in terms of t_R as

$$t_R = \frac{1+\kappa'}{1+\left(\frac{t_o}{t_{mc}}\right)\kappa'} t_o \tag{3}$$

the capacity factor is given the symbol κ' instead of the widely accepted k' in order to emphasize the difference in the relationship of the capacity factor to retention time.

The width of the total range of elution for electrically neutral solutes can be regarded as the value of t_{mc}/t_0. When t_{mc} becomes infinite (as with micelle polymers), the term ($1- t_{mc}/t_0$) in equation (2) is negligible and reduces to the well-known relationship for retention time:

$$t_R = (1+\kappa')t_o \tag{4}$$

The capacity factor, κ', can also be related to the distribution coefficient, K, of a solute between the micellar and aqueous phases through

$$\kappa' = K\,\frac{V_{mc}}{V_{aq}} \tag{5}$$

where V_{mc} and V_{aq} are volumes of micellar and aqueous phases, respectively. In micellar systems the phase ratio (V_{mc}/V_{aq}) can be determined by use of the following equation (6)

$$\frac{V_{mc}}{V_{aq}} = \frac{\nu\left(C_{sf} - CMC\right)}{1-\nu\left(C_{sf} - CMC\right)} \tag{6}$$

When micellar concentrations are low, the denominator on the right side of equation (6) may be approximated as unity. In addition, micelle polymer do not possess free monomer units and thus, its CMC is zero. Equation (6) can then be simplified as

$$\kappa' \cong K v C_{sf} \tag{7}$$

In the case of enantiomers, the selectivity, α, for two solutes x and y can be written as:

$$a_{xy} = \frac{\kappa'_y}{\kappa'_x} \tag{8}$$

The resolution, R_{xy}, equation of chiral MEKC is then written as:

$$R_s = \frac{\sqrt{N}}{4} * \frac{\alpha_{xy} - 1}{\alpha_{xy}} * \frac{\kappa'_y}{1+\kappa'_y} * \left(\frac{1 - \left(\frac{t_o}{t_{mc}} \right)}{1 + \left(\frac{t_o}{t_{mc}} \right) \kappa'_x} \right) \tag{9}$$

where N is the theoretical plate number and α is the selectivity factor (separation factor) defined as κ'_y/κ'_x (< 1) and κ'_x and κ'_y are the capacity factors for solutes x and y, respectively.

In the case of micelle polymers, t_{mc} is assumed infinite. Thus, equation (9) reduces to the resolution equation used in conventional chromatography:

$$R_s = \frac{\sqrt{N}}{4} * \frac{\alpha_{xy} - 1}{\alpha_{xy}} * \frac{\kappa'_y}{1+\kappa'_y} \tag{10}$$

3. MICELLE POLYMERS

3A. Concept of Surfactant/Micelle Equilibria

As discussed earlier, micelles have been successfully implemented in CE for separation of charged and neutral analytes. However, micelles are somewhat problematic in EKC due to the dynamic equilibrium that exists

between the surfactant monomer and micelle (Figure 3) and the high current generated by some charged micelles. Both of these effects lead to band broadening and separation deterioration in MEKC. Methods such as increasing concentration of surfactant monomers (to drive the dynamic equilibrium to the far right) is often useful. However, in most cases, this leads to excessive joule heating and ultimately separation deterioration. In an effort to circumvent problems associated with dynamic equilibrium, Larrabee and Sprague developed micelle polymers /34/. Larrabee and Sprague proposed enhanced stability and rigidity due to elimination of the dynamic equilibrium by the formation of covalent bonds between the surfactant aggregates. Based on Larrabee and Sprague's discovery, Hara and Dobashi /88/ as well as Wang and Warner /35,89/ speculated that the advantages of micelle polymers would be advantageous for CE as well as their use as PSP will offer improved separations in MEKC.

3A.1. Kinetics of Micelle formation

Micelles of low molecular weight surfactants are involved in a highly dynamic equilibrium as demonstrated in kinetic studies /36/. It should be noted that Aniasson and Wall have proposed a kinetic model for micelle formation, which is now generally accepted and has been adopted as the basis for the relaxation process of micelles /36-37/. According to the Aniasson and Wall model, the following equilibria are observed

$$\begin{aligned} S_1 + S_1 &\underset{K_{-2}}{\overset{K_2}{\leftrightarrow}} S_2 \\ S_2 + S_2 &\underset{K_{-2}}{\overset{K_2}{\leftrightarrow}} S_3 \\ S_{n-1} + S_1 &\underset{K_{-n}}{\overset{K_n}{\leftrightarrow}} S_n \end{aligned} \quad , \qquad (11)$$

where S_1, S_2 and S_n are the surfactant monomer, dimer and n-mer, respectively. From this stepwise formation process, it can be deduced that a micellar solution will contain aggregates with values of n from 2 to far above the average n value. Thus, normal micelles are polydispersed aggregates. Polydispersity is detrimental to chromatography and results in poor chromatographic resolution.

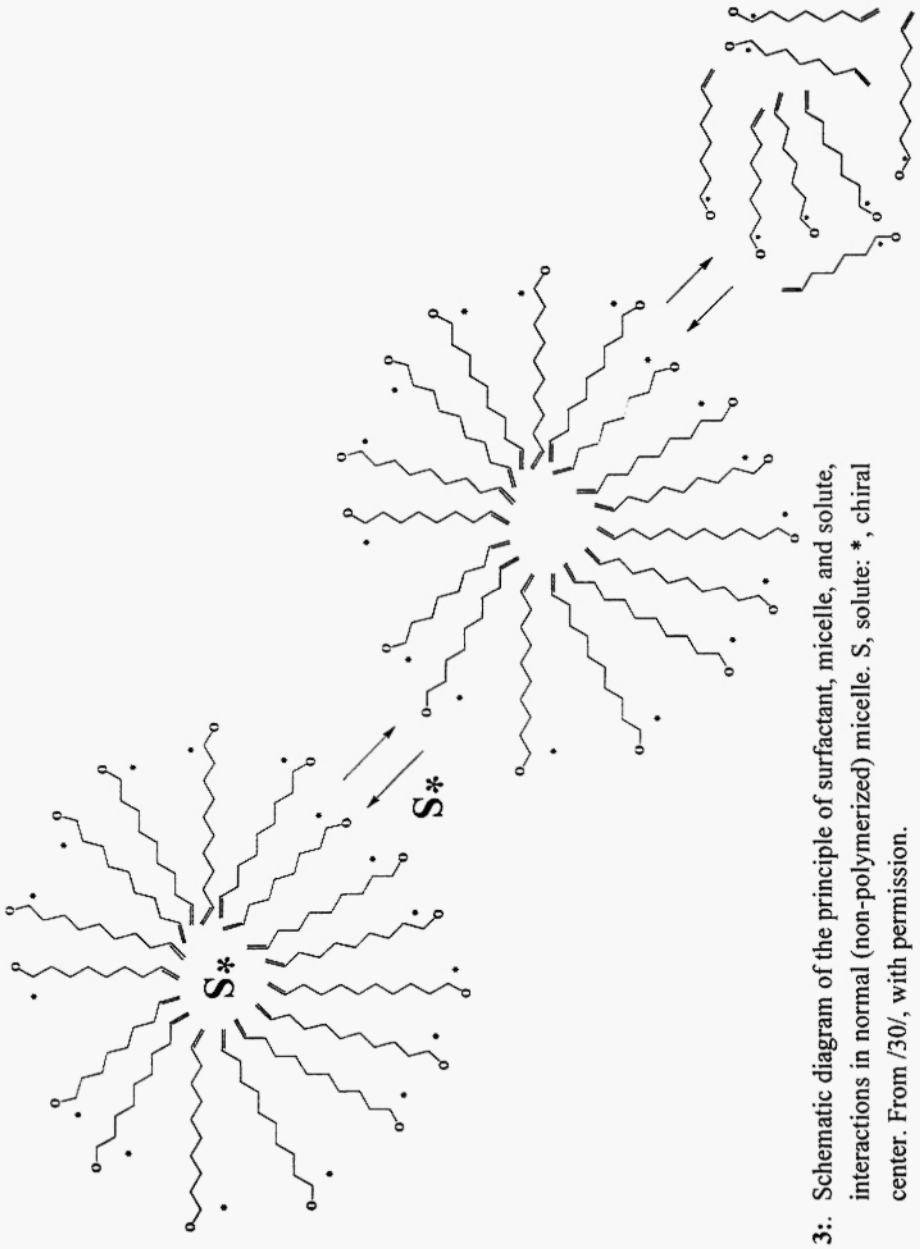


Fig. 3:. Schematic diagram of the principle of surfactant, micelle, and solute, interactions in normal (non-polymerized) micelle. S, solute: *, chiral center. From /30/, with permission.

3A.2. Thermodynamic of micelle formation

Equilibrium thermodynamic requires that in a system of surfactant monomers (S) which form micelles (M_n) of aggregation number η /38/, the association equilibrium can be approximated by the mass action law, i.e.

$$nS \overset{K}{\leftrightarrow} M_n . \tag{12}$$

The micellization constant K is therefore, written as

$$K = \frac{[M_n]}{[S]^n}, \tag{13}$$

where $[M_n]$, $[S]$ are the molar concentrations of monomers and N-mers, respectively.

The total concentration of surfactant (C_i) then becomes

$$C_i = [S] + nK[S]^n . \tag{14}$$

The free energy of micellization for a nonionic surfactant is expressed as:

$$-\Delta G = RT \ln K \tag{15}$$

where K is the micellization constant defined in Eq. (13). The value of K in Eq. (13) can be substituted in Eq. (15) to obtain

$$-\Delta G = RT\left(\ln[M_n] - \eta \ln[S]\right). \tag{16}$$

The free energy for inserting one monomer unit into the micelle can be expressed by

dividing the above equation by the number of monomers, i.e.

$$-\Delta G^o = \frac{\Delta G}{n} = \frac{RT}{\eta} \ln[M_n] - RT \ln[S] \tag{17}$$

and for a large value of η

$$\Delta G^\circ = RT \ln[S] \tag{18}$$

If the added surfactant monomers form new micelles above the CMC, then the concentration of free surfactant monomers will be constant at the CMC. Therefore, [S] = CMC, and Eq. (18) can be written as

$$\Delta G^\circ = RT \ln CMC \tag{19}$$

Plots of ΔG° *vs.* T can be used to determine ΔS° (from the negative of the slopes). The value of ΔH° can then be calculated using

$$\Delta G = \Delta H^\circ - T\Delta S^\circ \tag{20}$$

Similar to Eq. (19), the energetics of micellization for ionic surfactants can be written as

$$-\Delta G = RT\left[2 - \frac{p}{\eta} \ln CMC\right] \tag{21}$$

where p is the effective charge on the micelle /32/.

3A.3. Micelle-Substrate Interaction

As discussed previously, a process of fundamental importance to this review is the interaction of ligands with micelles. For the purpose of our discussion here, we will define the partition coefficient of an arbitrary ligand with a micelle. Thus, the partition coefficient, K, is defined as:

$$K = \frac{C_m}{C_w} \tag{22}$$

where C_m represents the concentration of the substrate, which has partitioned into the micelle and C_w represents the concentration of the substrate dissolved in the aqueous phase. Therefore, under standard state conditions, the free energy of association is given by

$$\Delta G^\circ = -RT \ln K \tag{23}$$

where R is the gas constant and T is the temperature on the Kelvin scale. Partitioning into and interacting with the micelle must also depend on the micellization process since interaction with micelles cannot occur if micelles are not present. Thus, the entire process of ligand interaction with the micelle is less complicated if the micellization process is eliminated. We will return to this concept in our later discussions on chiral interactions and separations with chiral micelles. The observation and hypothesis cited above suggests that control of dynamic equilibria may provide enhanced discrimination for molecule with small difference in ΔG of interaction, e.g. racemic mixtures. This hypothesis serves as the focus of this review; the use of non-chiral and chiral micelle polymers for improved separations.

3B. Achiral Micelle Polymers for EKC Separations

3B.1. Anionic Micelle Polymers

The first successful use of micelle polymers in EKC was demonstrated by Palmer *et al.* /25,26/. Poly sodium-10-undecylenate (poly-SUA) was employed as a PSP to achieve separation of alkyl phthalates and polynuclear aromatic hydrocarbons (PAHs) in buffers as high as 50% methanol or 45% acetonitrile. In addition to the high performance separation of these neutral compounds, poly-SUA was also observed to have a unique selectivity relative to micelles of sodium dodecyl sulfate (SDS). Although poly SUA provided high efficiency separation, its application was limited by the carboxylated headgroups, whose ionization influences the electrophoretic mobility and solubility of the polymer at low pH values. Furthermore, problems such as erratic migration times and cloudiness of the anodic buffer vial after several runs have been reported /25,26/. To overcome these difficulties with poly-SUA, Palmer and Terabe /28,29,39/ synthesized a micelle polymer with a sulfate head group, poly (sodium undecyl sulfate) (poly-SUS). Palmer and Terabe reported poly-SUS provided selective and efficient separations of a variety of substituted benzene and naphthalene compounds in aqueous and modified aqueous buffers (Figure 4A). Similar to its carboxylate analog, poly-SUS modified with high concentrations of organic solvents were found superior to those of conventional SDS micelles (Figure 4B). As noted by Palmer /28,29,39/ the two polymer (poly-SUA and poly-SUS) have essentially the same chemical selectivity for amine and hydroxyl aromatic compounds, despite the difference in the chemistry of their ionic head groups. However, these studies used potassium persulfate as a free

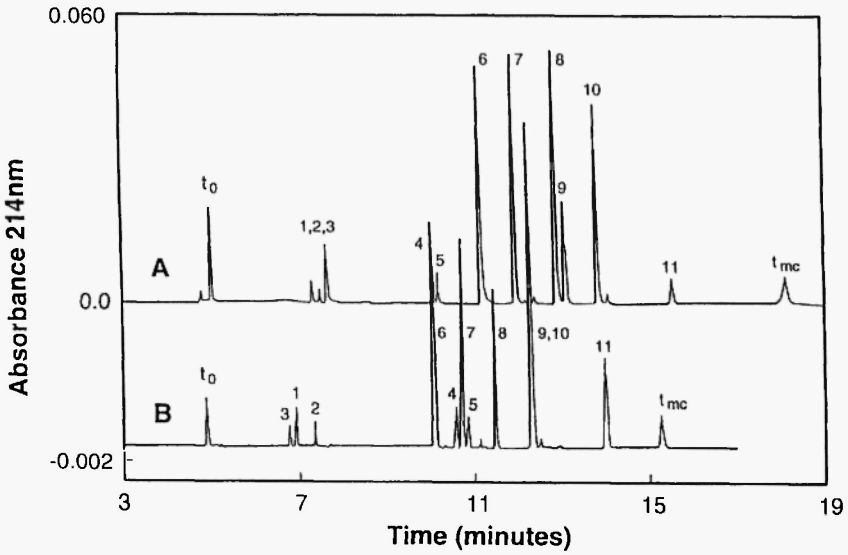


Fig. 4: Separation of substituted benzene and naphthalene compounds. (A) 0.83% SUS polymer, (B) 30 m*M* SDS; capillary is 50 cm effective length and 57 cm total length, 16.1 kV applied potential. A phosphate-borate buffer at pH 7.3 was employed; 1 = nitrobenzene, 2 = anisole, 3 = *p*-nitroaniline, 4 = *o*-xylene, 5 = *m*-xylene, 6 = naphthylamine, 7 = naphthalenemethanol, 8 = acenaphthenol, 9 = naphthalene, 10 = naphthaleneethanol, 11 = diphenyl ether. From /25/ with permission.

radical initiator for the polymerization of SUS. Two major limitations reported by Palmer with the chemical method of polymerization were (1) low synthetic yields and (2) contamination of the product with sodium sulfate /25,26/.

Shamsi *et al.* /40/ synthesized the same micelle (poly SUS) using γ-irradiation to initiate polymerization and employed this polymer for separations of PAHs. In addition, a combination of poly-SUS with β- or γ-cyclodextrin provided improved chiral separations /40/. This polymer provided efficient and selective separations of a variety of substituted benzene, naphthalene compounds, and the structurally selective PAHs /40/. The advantage of polymer initiation using γ-irradiation was previously indicated by Wang and Warner /30/ in EKC studies with poly (sodium

N-undecylenyl-L-valinate). Shamsi and coworkers /41/ demonstrated that the limitations reported by Palmer could be avoided if γ-irradiation were used to initiate polymerization. A good example of the usefulness of poly-SUS over conventional micelles is the separation of 2 – 6 ring polycyclic aromatic hydrocarbons (PAHs). Figure 5 (A,B,C) shows the selectivity differences for monomeric (SDS and SUS) and polymeric (poly SUS) surfactants for the separation of 16 polyaromatic hydrocarbons (EPA priority pollutants). Some improvements in separation with SUS over SDS were attributed to π-π interaction between the PAHs and the terminal double bond of the SUS surfactant. Enhanced separations of PAHs with excellent peak shapes using poly-SUS are clear. Through a careful optimization of the concentration of the poly-SUS, pH and acetonitrile concentration, baseline separation of all 16 PAHs in about 30 minutes was possible for the first time in EKC by a single-surfactant system /40,41/ [Figure 6].

More recently, Moy *et al.* reported separation of PAHs using micelle polymers of sodium undecylenic acid in the presence of organic additives of acetonitrile, acetone, and tetrahydrofuran /42/. Moy and coworkers /42/ refer to these micelle polymers as molecular micelles and oligomers of sodium undecylenic acid. However, these authors utilize the same synthetic procedure as Palmer *et al.* /25,26/, i.e. polymerization via chemical initiation. In about 48 minutes, Moy *et al.* reported separation of 16 PAHs with tetrahydrofuran as a co-additive, which was previously unattainable by Palmer. However, no indication of the migration order or explanation for the jagged baseline was discussed. In addition, not all 16 PAHs were baseline resolved. Shamsi *et al.* /40/ reported elution orders of most PAHs generally followed an increase in length-to-breath ratio /43/, with anthracene and indenopyrene as the only two exceptions. Furthermore, Shamsi and coworkers reported even more rapid separation (15 minutes) of the 16-PAHs by increasing the acetonitrile concentration to 65 % (v/v). Under such conditions, the peaks for the first eight PAHs were a little compressed. However, the last three PAHs (with high k' values) showed higher efficiencies with improved resolutions. It is evident that γ-irradiation as an initiation of polymerization is far superior to chemical initiation. This conclusion has been verified earlier /8/.

3B.2. Cationic Micelle Polymers

Despite the abundant volume of published research on anionic polymeric micelles, the implementation of cationic micelle polymers in EKC is

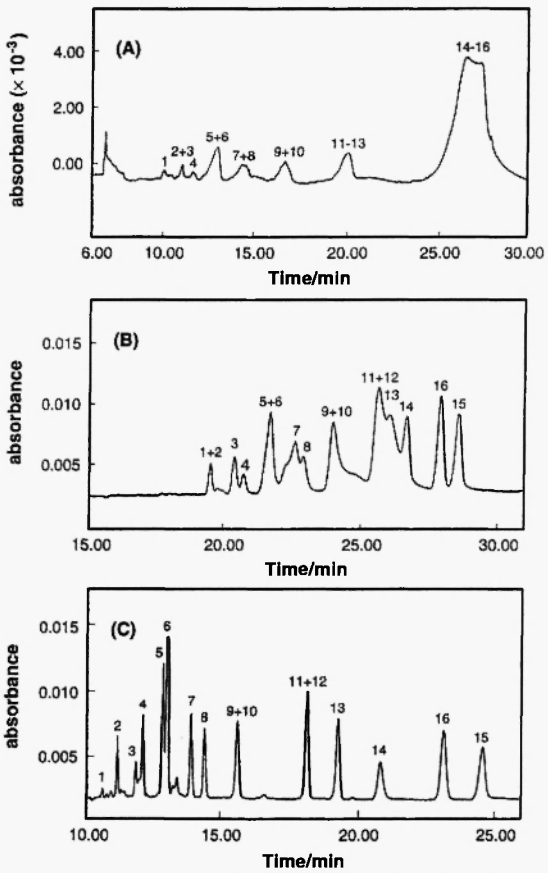


Fig. 5: Comparison between (A) sodium dodecyl sulfate (SDS), (B) nonpolymerized sodium undecyl sulfate (SUS), and (C) poly-SUS for the separation of PAHs. EKC conditions: 1.5 % (w/v) each of SDS and SUS; 1.0 % (w/v) of poly-SUS in 12.5 m*M* each of Na_2HPO_4 and $Na_2B_4O_7$ buffered at pH 9.2 with 57 % v/v of ACN; pressure injection for 3s; + 30 kV applied for separation; current, 55 μA for SDS, 50 μA for SUS, and 38 μA for poly-SUS; UV detection at 254 nm. Peak identification 1 = naphthalene (NAPH), 2 = acenaphthylene (ACY), 3 = acenaphthene (ACE), 4 = fluorene (FLU), 5 = phenanthrene (PHEN), 6 = anthracene (ANTH), 7 = fluoranthene (FLT), 8 = pyrene (PYR), 9 = benz[a]anthracene (BaA), 10 = chrysene (CHRY), 11 = benzo[b]fluoranthene (BbF), 12 = benzo[k]fluoranthene (BkF), 13 = benzo[a]pyrene (BaP), 14 = dibenzo[ah]anthracene (DIBAhA), 15 = benzo[gh]perylene (BghiP), and 16 = indeno[123cd]pyrene (INPY). From /41/ with permission.

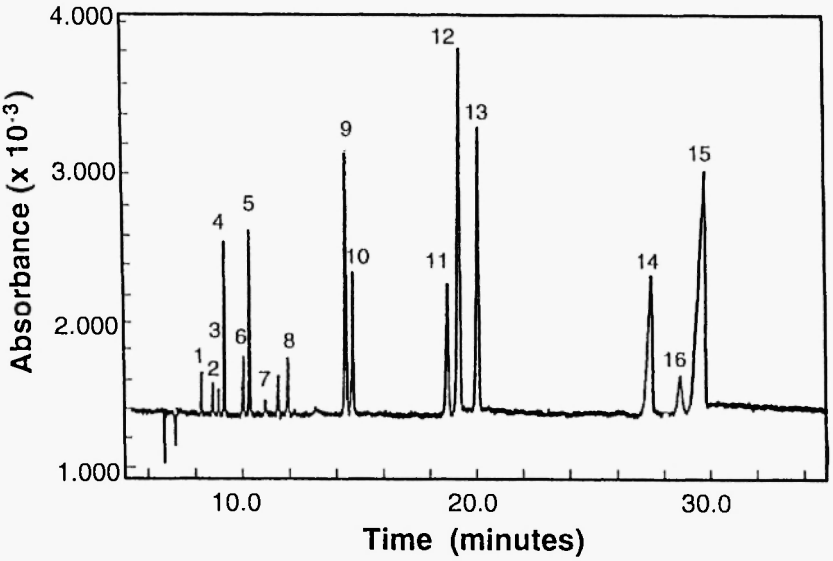


Fig. 6:. Optimized electrokinetic chromatograpm for the separation of 16 PAHs. EKC conditions: 0.5 % (w/v) of Poly-SUS in 40 % ACN; Separation voltage, + 30 kV; current, 42 μA. 12.5 m*M* each of Na_2HPO_4 and $Na_2B_4O_7$ buffered at pH 9.2. From /41/ with permission.

somewhat obscure. For the most part, acrylate copolymers such as butyl methacrylate-methacryloxyethyl-trimethyl ammonium chloride copolymer – BMAC /44/, butylacrylate-butyl methacrylate-methacrylic acid – BBMA /45/, and polyallylamine – PAA /46,47/, have served as the only cationic polymer-like supported PSP used in EKC. Since acrylate copolymers don't fall under the title of cationic micelle polymers, we refer the reader to a detailed discussion on this topic by Palmer and Tanaka /48/.

The chemical synthesis of cationic surfactants and micelle polymers are feasible. However there have been no reported chiral separations involving such systems. Cationic micelle polymers could offer substantial differences in EKC enantioselectivity as per predictions of the Wren and Rowe Theory /49/. This theory predicts that cationic PSP's should provide enhanced resolution of anionic analytes, due to the counter migration of the PSP and analyte.

3C. Chiral Micelle Polymers for EKC Separation of Enantiomers

Enantiomeric separations in MEKC tend to be affected by the concentration of the micelle within the running buffer as well as the interaction of solutes with the aggregates (Figure 3). Conventional micelles are polydispersed species usually composed of an equilibrium mixture of monomers, and species containing monomers in numbers well below the average aggregation number. Since the differential binding between the micelle and a pair of enantiomers is generally small, such dynamic equilibrium between the surfactant monomers and the micelle may deteriorate the enantioselectivity of the micelle. In contrast, polymerized surfactants are void of such problems since the covalent bonds formed between monomers eliminate the dynamic equilibrium between the surfactant monomers and the micelles, thus simplifying and enhancing the process of binding between the micelle and the solute (Figure 7). Furthermore, an inherent disadvantage of micellar liquid chromatography and also of MEKC is peak broadening associated with slow mass transfer of the solute between the micelle and the bulk solvent. Since micelle polymers have more compact structures than normal micelles /30/, the solute does not penetrate as deeply into the core of the polymeric surfactant as it does in the case of normal micelles. Thus, enhancement at the rate of mass transfer of the solute with the micelle polymer should be observed.

3C.1. Synthesis and characterization of poly (sodium-N-undecanonoyl-L-valinate)

The structures of L-SUV and poly-L-SUV are depicted in Figure 8. L-SUV was prepared from a procedure reported by Lapidot *et al.* /8/. The

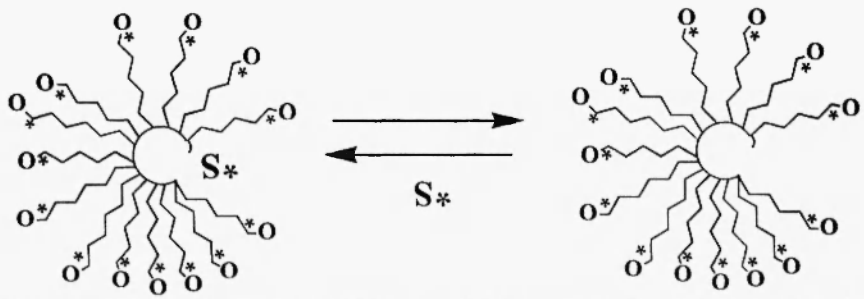


Fig. 7: Schematic diagram of the principle of polymerized surfactant and solute interactions, S, solute; *, chiral center. From /30/ with permission.

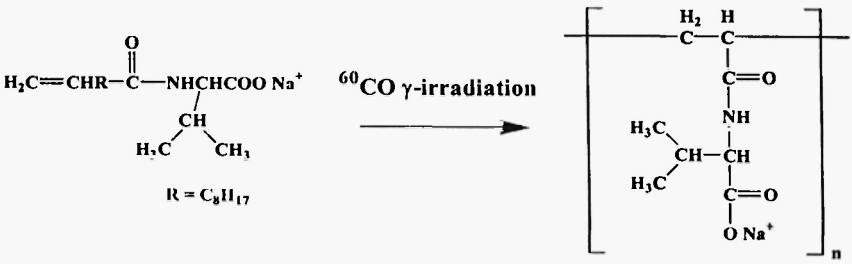


Fig. 8: Chemical structure of sodium *N*-undecenoyl-L-valinate (monomer), (L-SUV) and its corresponding polymerized from poly-L-SUV.

N-hydroxysuccinimide ester of undecylenic acid was reacted with L-valine followed by its conversion to the sodium salt. Proton NMR was used to indicate complete reaction by use of via the multiplets at approximately 5.8 and 5.0 ppm, for the terminal vinyl group (Figure 9A). The CMC of L-SUV was determined to be about 21 mM using a DuNouy Tensiometer. The radical polymerization was initiated by ^{60}Co γ-irradiation of a 50 mM surfactant solution. After 36 hours of irradiation (8.9 Mrad/hr), the poly L-SUV solution was lyophilized and recrystallized with hot ethanol (to extract unreacted monomers). Dialysis was also used as a means for purification. A regenerated cellulose membrane with a 2000 Da cutoff was found to be better for purification. Proton NMR spectroscopy was used to determine success of polymerization of poly L-SUV. For example, NMR spectroscopy indicated the disappearance of the vinylic double bond with resonance broadening of the remaining peaks (Figure 9B). Polymerization was further confirmed by Fourier transform infrared spectroscopy, which showed the loss of C-H stretching vibration at approximately 3100 cm^{-1}. Optical rotation measurements ($[\alpha]^{25}_{589}D$) were taken before and after polymerization. The measurements were ($[\alpha]^{25}_{589}D$) equal to –2.9° and –8.9° (c = 1.00 methanol, and c = 1.00 water) for the monomer and polymer respectively, demonstrating that the chirality of the polar head group had not been destroyed /30/.

3C.2. Comparison of micelle polymers vs. unpolymerized micelles

There are numerous publications on the characterization and the utility of polymeric achiral and chiral surfactants /50-52/. However, these publications often highlight the similarity between the association of a ligand with a micelle polymer and the association of the same ligand with the

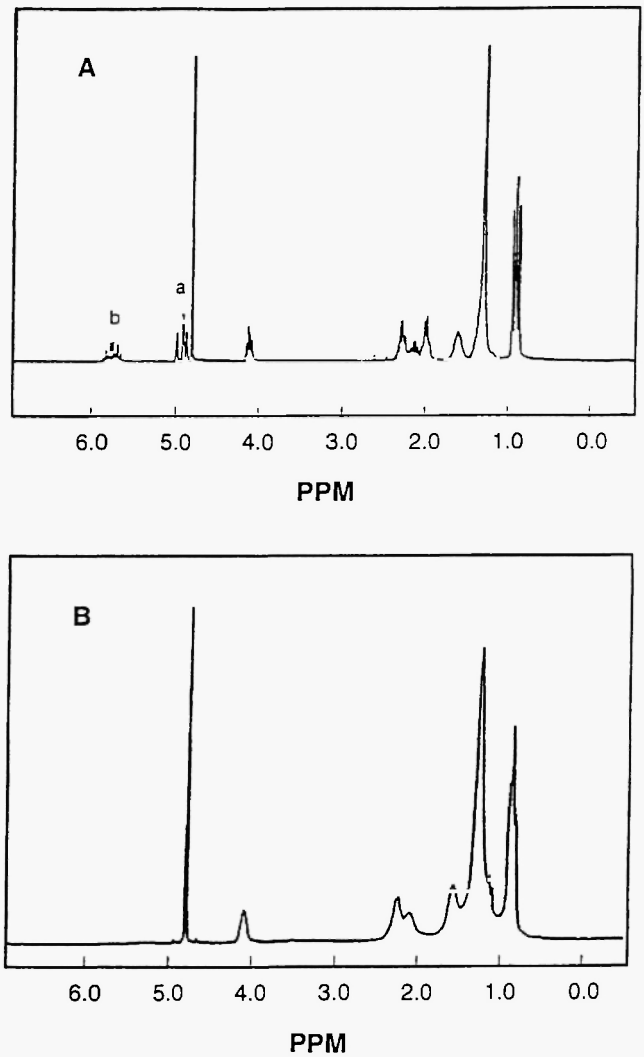


Fig. 9: Proton NMR of (a) L-SUV and (b) poly-L-SUV

unpolymerized form of the same micelle. Wang and Warner /30/ bridged the gap between the advantages of micelle polymers and their utility in EKC for chiral separations. Figure 10 displays the chiral recognition ability of the micelle polymer poly-L-SUV as compared to that of L-SUV. In Figure 10A,

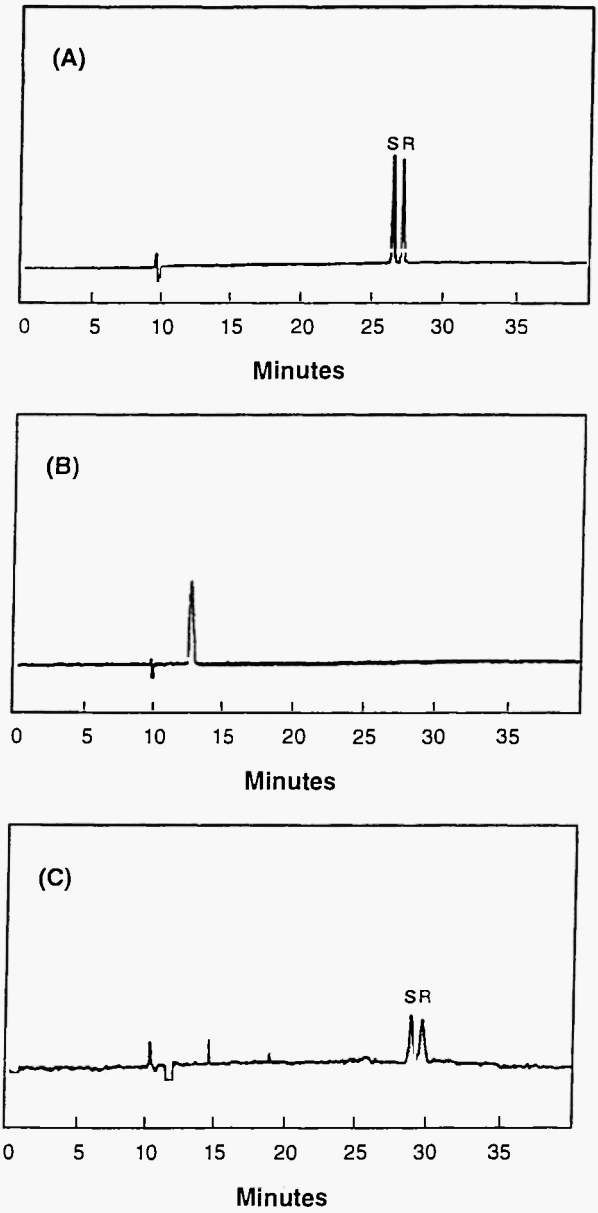


Fig. 10: Comparison between polymerized surfactant and nonpolymerized micelle for separation of (±)-1,1'-bi-2-naphthol, (a) 0.5 % w/v poly-L-SUV; (b) 0.5 % (w/v) L-SUV; (c) 1 % w/v L-SUV, Buffer : 25 m*M* borate (pH 9.0), applied voltage, 12 kV; current, (a) 39 μA, (b) 40 μA, (c) 51 μA; UV detection at 290 nm. From /30/ with permission.

baseline enantiomeric separation of the atropisomers of (±)-BOH is illustrated using 0.5 % w/v poly-L-SUV (25 mM sodium borate buffer, pH 9.0). The (S)-(-)BOH enantiomer eluted before the corresponding (R) form. This indicates that (R)-(+)-BOH has a higher affinity with the (S)-(-)-BOH form of the chiral polymer. In addition, as expected, a reversal in the migration order of (±)-BOH was observed when the (R)-(D)-form of the polymer (i.e., poly-D-SUV) was used (electropherogram not shown). Figure 10 (B,C) illustrates the comparison of 0.5 % w/v and 1 % w/v for the separation of (±)-BOH under the same conditions as for Figure 10A. No chiral separation was obtained when 0.5% w/v was employed, which is below the CMC of L-SUV micelle. Upon increasing the L-SUV concentration to 1 % w/v, chiral separation was obtained. However, it is clear that the micelle polymer provided better discrimination and hence better chiral separation. Furthermore, note that the much higher efficiency (N = 102,240) by use of the polymeric surfactant system (Figure 10C) as compared to unpolymerized micelle system (N = 28,070). These results are consistent with the spectroscopic data reported by Paleos *et al.* /53/ and our model of micelle solute interaction (see Fig. 3 *vs.* 7). All things considered, the solute does not incorporate as deeply into the cavity of the micelle polymer as it does in the case of normal micelles. For this reason, an increase in the rate of mass transfer of solute in and out of the polymeric pseudo-stationary phase is expected.

In order to get a better understanding of enantiomeric separations with poly-L-SUV, Wang and Warner investigated the effect of concentration of poly-L-SUV and pH on migration time, resolution, and selectivity. The use of organic solvents and buffer conditions can also influence resolution and efficiency in EKC. However, we have chosen to discuss this in another section. A unique advantage of micelle polymers is that such surfactants can form a micelle with one molecule. In addition, the CMC of the polymeric surfactant is essentially zero. Thus, the micelle polymer is not concentration-dependent and can be used at any concentration. Figure 11 illustrates the effect of changing the concentration of poly-L-SUV in the chiral separation of (±)-BOH. Although there is an optimum concentration of poly-L-SUV which is necessary to achieve the highest possible resolution of (±)-BOH, baseline resolution of the (±)-BOH was nevertheless possible when the poly-L-SUV concentration was as low as 0.05% w/v. However, as shown earlier in Figure 10B and Figure 10C, the concentration of the non-polymerized micellar surfactant has to be higher than the CMC in order

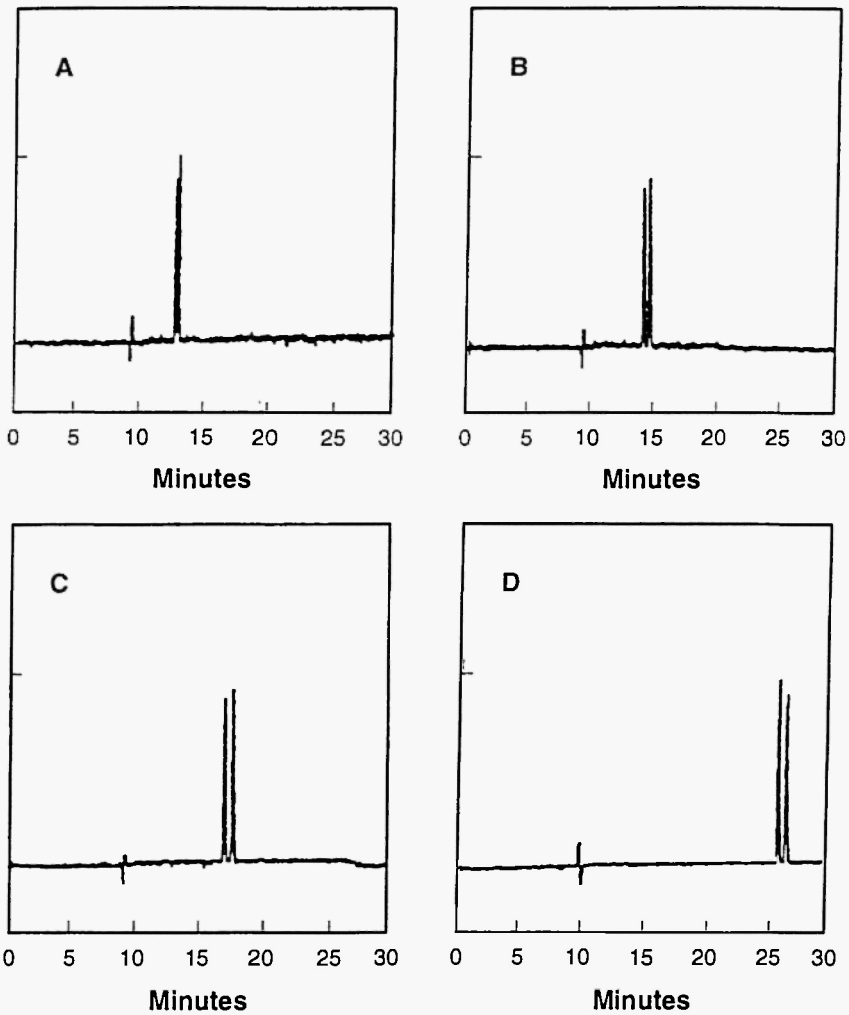


Fig. 11: Influence of poly-L-SUV concentration on the resolution of (±)-1,1'-bi-2-naphthol, (a) 0.02 % w/v; (b) 0.05 % w/v; (c) 0.10 % w/v; and (d) 0.5 % w/v of poly-L-SUV. Other conditions as in Figure 19. From /30/ with permission.

to function as a PSP. This is because concentrations below the CMC will not allow micelle formation for unpolymerized surfactants. One limitation of using high concentrations of charged surfactants within MEKC run buffer is the result of Joule heating, which is deleterious to separations in CE. Thus, the use of micelle polymers as a PSP even at low concentrations is another advantage of such micelle polymers over normal micelles.

3C.3. Enantioseparation of anionic, cationic, and neutral racemates with an amino-acid terminated micelle polymer (poly-L-SUV)

3C.3a. Neutral enantiomers

Angew-Heard *et al.* /54/ extended the utility of poly-L-SUV to anionic, cationic, and neutral racemates. These authors studied three atropisomers such as racemic mixtures of Troger's base, (R,S)-1,1'- bi-naphthyl-2,2'-diamine ((±)-BNA) along with (±)-BOH /54/. Figure 12 shows the effect of pH on the migration order of (±)-BOH and (±)-BNA. Under neutral pH conditions, both of these compounds are electrically neutral. However, (±)-BOH is partially ionic at pH 10 and 11. Interestingly, the elution order obtained over this pH range for (±)-BOH shows a gradual decrease in migration time relative to (±)-BNA. Apparently, an increase in the anionic character of (±)-BOH caused a decrease in binding with anionic poly-L-SUV micelle polymer. This electrostatic repulsion results in faster migration of (±)-BOH at pH 11. These data indicate that the ionization of this analyte inhibits binding with the anionic micelle polymer but does not necessarily decrease the enantiomeric resolution. Under all pH conditions, the (S)-(-)-enantiomers of both BOH and BNA eluted earlier than its corresponding (R)-(+)-form. This suggests that the migration times and order of enantiomer elution are a direct indication of the analyte/PSP association.

3C.3b. Cationic enantiomers

Laudanosine, a cationic biosynthetic precursor of morphine, and its two analogs, laudanosoline and norlaudanosoline, have been separated with poly-L-SUV /54/. Under neutral and alkaline conditions, laudanosoline and norlaudanosoline were partially resolved whereas laudanosine was only resolved (R_s = 1.2) at pH 11. Figure 13 depicts a comparison of the paveroline analogs. The electropherograms obtained under optimized conditions (0.5 % w/v poly-L-SUV, 25 mM dibasic phosphate) were compared at pH 5.6 and 6.0 with a coated and uncoated capillary, respectively. Interestingly, the selectivity and resolution was enhanced using a coated capillary for norlaudanosoline, whereas enantioseparation could not be achieved with laudanosine. However, laudanosine was reported to be almost baseline-resolved at pH 10 (electropherogram not shown) /30/. The order of elution for the three analogs was laudanosoline > norlaudanosoline > laudanosine.

Agnew-Heard and coworkers speculated that the amine functional group of the paveroline derivative appeared to be important in interactions with the

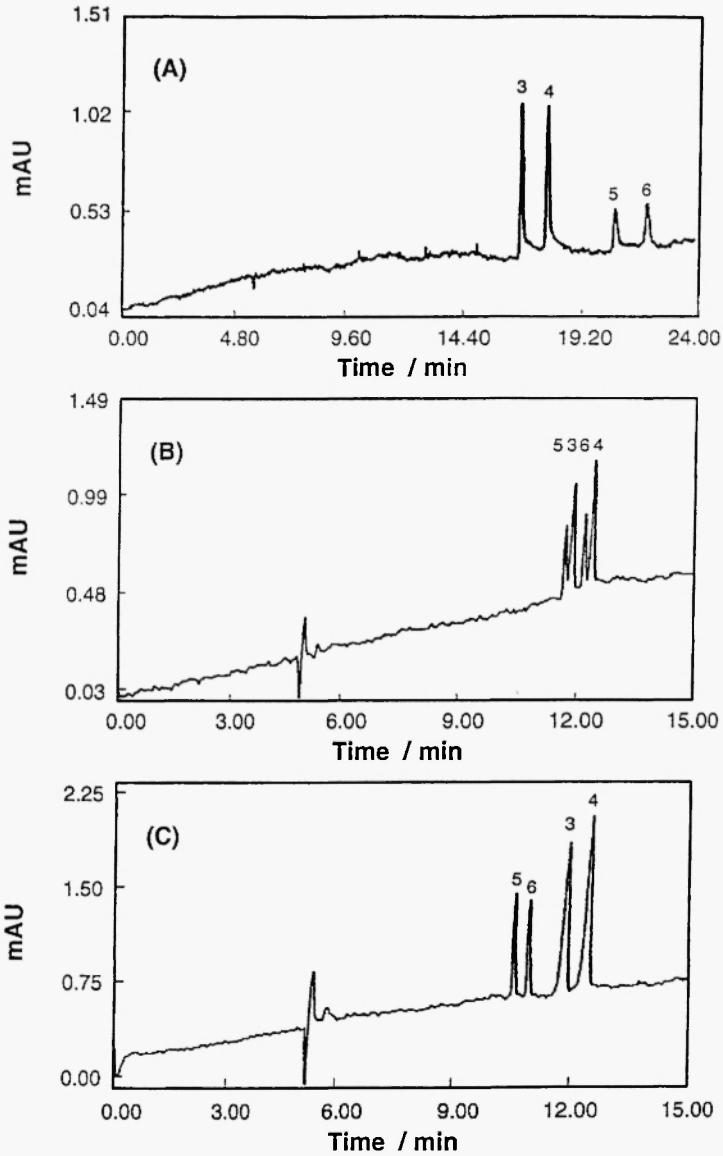


Fig. 12: Influence of pH on the separation of atropisomer at (a) pH 9.0, (b) pH 10, (c) pH 11, Peak Identification: 3,4 = (±)-1,1'-bi-2-naphthol, 5,6 = (R,S)-1,1'-binaphthyl-2,2'-diamine. The running buffers composed of 0.25 % w/v of Poly-L-SUV with 25 mM dibasic phosphate: capillary 50 mm x 60 cm (55.5 cm effective length); applied voltage, + 20 kV, detection 280 nm. From /54/ with permission.

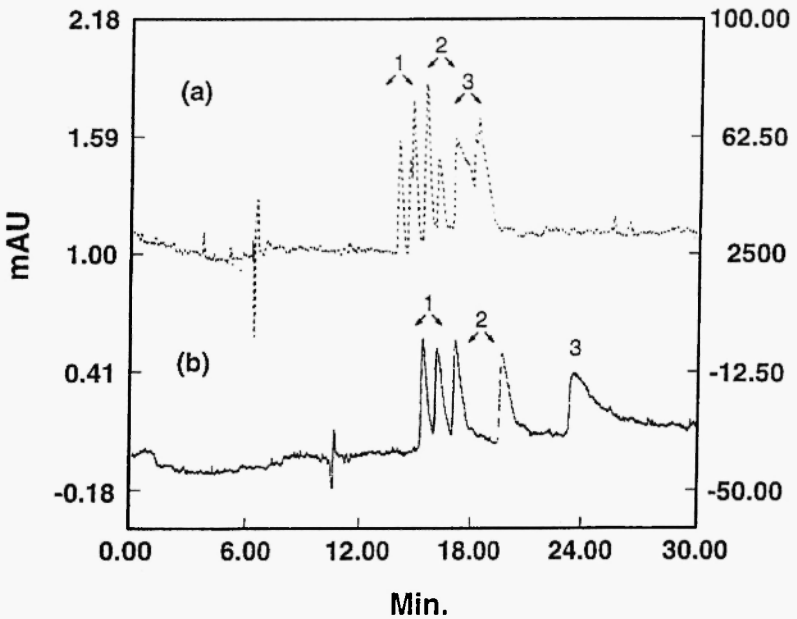


Fig. 13: Enantiomeric separation of paveroline derivatives using (a) uncoated silica capillary at pH 6 and (b) polyvinyl alcohol (PVA) coated capillary at pH 5.6. Peak identification: 1 = laudoanosoline, 2 = norlaudanosoline, 3 = laudanosine. Other conditions as in Figure 21. From /54/ with permission.

micelle polymer. Since laudanosoline is a larger cationic species, it was expected to migrate faster than its derivatives. However, the opposite trend was observed, probably because the molecule is more hydrophobic. This higher hydrophobicity enables laudanosine to interact more with the inner core of the micelle. However, it should be noted that this longer retention time with the micelle did not result in better resolution or selectivity. Lastly, although all of the paveroline analogs are cationic, they do not elute before the EOF. This indicates that the EKC mode is operative in which the cationic species binding to the anionic micelle polymer migrates toward the anode.

3C.3c. Anionic enantiomers

Warfarin is a coumarinic anti-coagulant drug, which is a derivative of coumarin. It is used in the treatment of thromboemolic diseases. Coumachlor, an analog of warfarin, has been used in high performance liquid

chromatography (HPLC) as an internal standard. Both warfarin and coumachlor are structurally related acidic drugs. They are both electronegative due to their keto-enol groups. The phenolic group on warfarin has a pK_a of 5.1 /55/. Theoretically, anionic analytes are not expected to complex strongly to the anionic surfactant under neutral and basic pH conditions. Ideal conditions for this model would employ very acidic buffer solutions in order to increase the elution window. Acidic conditions would also increase the positive charge of the amide functional group and decrease the negative charge on the valinate functional group. However, at pH values below 5.5, the micelle was found to precipitate out of solution due to a decrease in the ionization of the carboxylate functionality of poly-L-SUV.

To optimize the chiral resolution of coumachlor and warfarin, 50 mM phosphate and 50 mM acetate buffer in the pH range (5.5 - 6.5) were evaluated with poly-L-SUV. The optimum weight fraction of poly-L-SUV required to achieve optimum resolution of these two drugs was determined to be about 0.5 % w/v. Figure 14 (A,B) shows the effect of pH on chiral

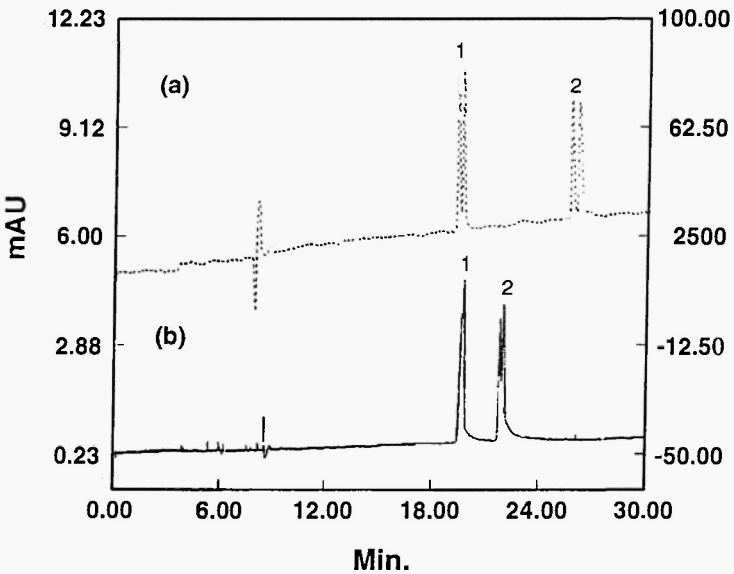


Fig. 14: Influence of pH on the chiral separation of anionic racemates of warfarin (1) and coumachlor (2). CE conditions: buffer 0.5 % w/v Poly-L-SUV with 25 m*M* monobasic phosphate at (a) pH 5.6 and (b) pH 6.5. Other conditions as in Figure 21. From /54/ with permission.

separation of coumachlor and warfarin for this study. As observed in Figure 14 (A,B), an increase in pH from 5.6 to 6.5 resulted in deterioration in the quality of the separation. Above pH 6.5, no chiral resolution was observed for either of the two drugs. Overall, higher resolutions were achieved with coumachlor, except with the monobasic phosphate buffer at pH 5.9, where warfarin was better resolved. Coumachlor displayed a lower electrophoretic mobility toward the cathode (detection end). This can be attributed to the larger electronegative charge in the chlorine group of this molecule. Agnew-Heard and coworkers speculated that the better enantioseparation of coumachlor is probably due to the importance of the chiral center being located between the aromatic moiety and the negative charge on the racemates /54,56/.

Recently, Dobashi *et al.* /57/ used poly (sodium (10-undecenoyl)-L-valinate) which they refer to as poly-SUVal for the separation of DNB-AA isopropyl ester. Note that this poly-SUVal, is the same micelle polymer introduced by Wang and Warner /30/. One of the few distinctions between these two works lies in the procedure used for the polymerization process. Dobashi and coworkers utilized ultraviolet lamps for the polymerization process, whereas Wang and Warner initiated polymerization using ^{60}Co γ-irradiation. Figure 15 shows enantiomeric separation of DNB-AAs. Peak tailing and drifting solute migration times were reported. The authors reported that the addition of 2 M urea improved the reproducibility of migration times. However, it did not improve peak tailing. Ultimately, SDS had to be added to the micelle polymer in order to reduce the peak tailing.

3C.4. Comparison of amino acid terminated micelle polymers vs. dipeptide terminated micelle polymers for EKC separation of enantiomers

The successful outcome of the results obtained by use of poly-L- and poly-D-SUV has encouraged continued studies of the synthesis, and applications of many different kinds of alkyl amino acid terminated micelle polymers. Recent studies in this area center on the introduction of multiple chiral centers on to the polar head group (utilizing dipeptides instead of single amino acids) of a micelle polymer /58,63,64/. This program of course leads to a multitude of dipeptides to study. However, Shamsi, Macossay and Warner /58/ inaugurated a systematic process toward tackling this task. Since poly-L-SUV provided such a fruitful discussion on enantiomeric separations, poly (sodium N-undecanoyl-L-valyl-valinate) (poly-L-SUVV) was an

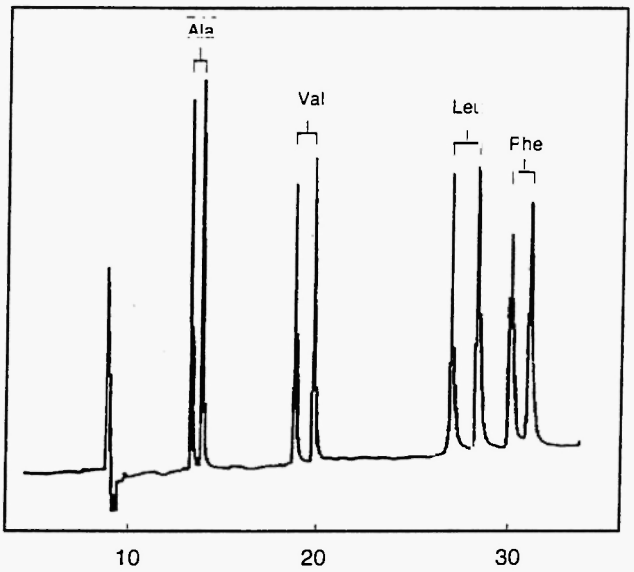
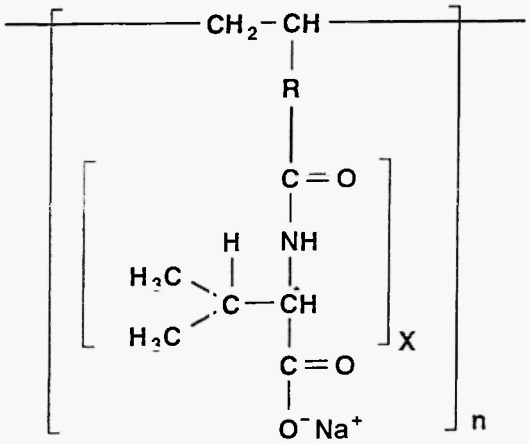


Fig. 15: Separation of a mixture of four (3,5-dinitrobenzoyl) amino acid isopropyl esters with poly(sodium 10-undecanoyl-2-valinate). Experimental conditions: fused silica capillary, 50 cm in length (50 mm i.d.); 25 m*M* poly-L-SUV in 25 m*M* borate – 25 m*M* phsosphate buffer, pH 7.0, containing 2 *M* urea and 10 m*M* SDS; total applied voltage, ca. 12.4 – 12.8 kV, detection UV at 254 nm. From /57/ with permission.

obvious choice for the first dipeptide terminated micelle polymer synthesized and employed.

Figure 16 depicts the structure of poly-L-SUVV, which differs from poly-L-SUV by one additional valine amino acid group attached to the hydrocarbon chain. Thus, poly-L-SUVV is a dipeptide terminated micelle polymer in which the α-carbonyl group of one amino acid is attached to the α-amino functional group of another amino acid by a peptide bond. The result is two amido groups and two chiral centers of the same optical configuration. Several parameters, such as type and concentration of micelle polymer, pH and injection size, as well as type and concentration of background electrolyte (BGE) were studied. These parameters were found to



R=C_8H_{17}
X = 1=poly [sodium N-undecanoyl-L-valine] (poly [L-SUV])
X = 2 = poly [sodium N-undecanoyl-L-valyl-valine] (poly [L-SUVV])

Chemical structures of the chiral polymeric surfactants

Fig. 16: Chemical structures of the chiral polymeric surfactants.

influence the migration, resolution, efficiency, and sensitivity. Shamsi and coworkers [58] compared the chiral separation behavior of two cationic, one anionic and one non-ionic racemate.

3C.4a. Enantioseparation of basic enantiomers

Propranolol (Prop) and Alprenolol (Alp) are basic drugs that are classified as β-adrenergic blockers (β-blockers) and are useful for the treatment of hypertension /59/. Prop and Alp possess similar structural features: and alkanolamine side chain terminating in a secondary amino group and an aromatic group (for their structures see Figure 17 inset). The pk_a value of the ionizable nitrogen is in the region of 9.2-9.6 /60,61/. Figure 17 (A,B)

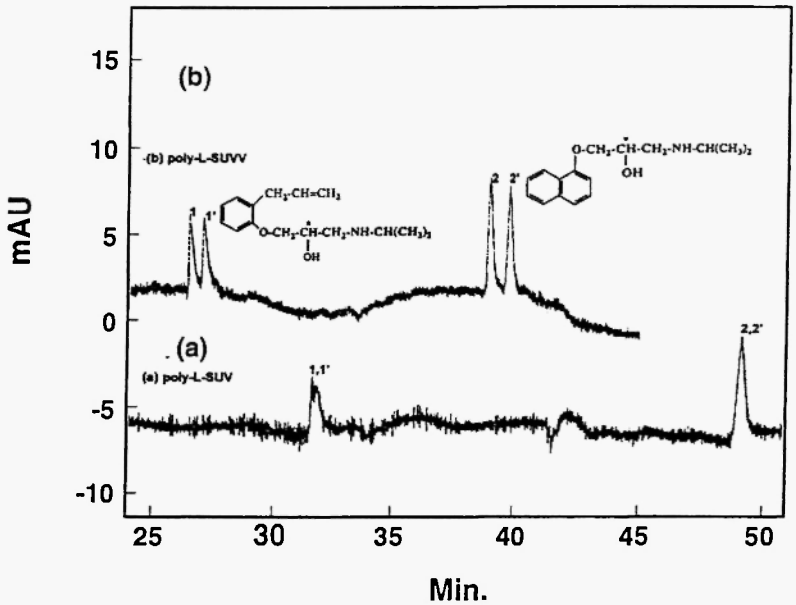


Fig. 17: Comparison of polymerized anionic surfactants for the separation of basic enantiomers. EKC conditions: 57 % and 0.50 % (w/v) of poly-L-SUV or poly-L-SUVV, respectively, in 50 m*M* $Na_2B_4O_7$ buffered at pH 9.2. Peak identification: 0.2 mg/mL each of one (1) (S)-(-)-ALP, (1') (R)-(-)-ALP; 0.1 mg/ml each of (2) (S)-(-)-PROP, (2') (R)-(+)-PROP. Pressure injection for 2 s; +20 kV applied for separation; current, 85 µM for for poly-L-SUV and 56 µA for poly-L-SUVV. UV detection was at 214 nm. From /58/ with permission

compares the electrokinetic chromatograms for the simultaneous separations of the Alp and Prop enantiomers using optimized concentration of poly-L-SUV and poly-L-SUVV micelle polymers. The (±)-Alp and (±)-Prop analytes eluted in order of increasing hydrophobicity for both micelle polymers. However, the (S)-(-)-enantiomer of each racemate always eluted before the corresponding (R)-(+) form. Thus, the migration times and order appear to be a direct consequence of the analyte/micelle polymer binding. Furthermore, Figure 17 B clearly shows that an increase in the migration time for both (±)-Alp and (±)-Prop enantiomers using poly-L-SUV does not lead

to enhanced chiral separations. The improved chiral resolution with decreased migration time of cationic racemates using poly-L-SUVV suggests that the retention mechanism and the chiral recognition for such analytes are controlled by steric factors rather than by the hydrophobicity of the chiral pseudo-stationary phase. Shamsi and coworkers concluded that an increase in the number of stereogenic centers and hydrogen bonding sites on the ionic head group in poly-L-SUVV might have contributed to this superior chiral discrimination over poly-L-SUVV micelle polymer.

3C.4b. Enantioseparations of acidic enantiomers

In a second series of experiments, chiral separation of negatively charged (±)-1,1'-binaphthyl-2,2'-diyl hydrogen phosphate ((±)-BNP) were compared using poly-L-SUV and poly-L-SUVV micelle polymers at an optimized pH value of 7.0. Table 2 shows the influence of the type and concentration of polymeric chiral pseudo-stationary phase on the k', R_s, and N values of BNP enantiomers. Increases in k' and R_s with increasing concentration of poly-L-SUV and poly-L-SUVV were observed, indicating that the negatively charged (±)-BNP has a more or less pronounced tendency to interact with these anionic micelle polymers. At each equivalent monomer concentration, better R_s values were obtained with the dipeptide than by use of the single amino acid surfactants. Baseline resolution of (±)-BNP was achieved at 0.25 % (w/v) poly-L-SUVV. Table 2 clearly illustrates that there is an optimum concentration at which chiral resolution reaches a maximum value for each micelle polymer. The maximum resolution of 3.2 was obtained for (±)-BNP at 1.5 % (w/v) poly-L-SUVV. However, the same enantiomers were resolved (R_s = 1.2) using 1.3 % (w/v) poly-L-SUV as an optimum concentration. An increase in concentration of poly-L-SUV (i.e., ∃ 2.6 % w/v) drastically decreased the enantiomeric resolution of (±)-BNP. This is in contrast to an increase in concentration (i.e., > 3.0 % w/v) of poly-L-SUVV, which shows either very similar or no decrease in R_s values.

Typical electrokinetic chromatograms for (±)-BNP obtained using the optimized resolution values of 1.2 and 3.0 using poly-L-SUV and poly-L-SUVV micelle polymers, respectively, are displayed in Figure 18. Improved separation with higher resolution and selectivity was obtained with poly-L-SUVV than with poly-L-SUV. The successful enantioseparation of negatively charged (±)-BNP obtained with the anionic micelle polymer confirms that, although electrostatic attractive interactions can contribute in

Table 2
Comparison of Migration Factors, Resolution, and Efficiency for Binaphthol Phosphate (BNP) Enantiomers Using Various Concentrations of Poly-L-SUV and Poly-L-SUVV Surfactants[a]

concentration [% (w/v)]	equivalent monomer concn [mM]	k'	R_s	N
0.19 poly-L-SUV	6.2	1.25	0.1	101 000
0.25 poly-L-SUVV		1.46	1.5	114 000
0.38 poly-L-SUV	12.4	1.65	0.5	135 000
0.50 poly-L-SUVV		1.87	2.2	140 000
0.76 poly-L-SUV	24.8	2.46	0.7	167 000
1.00 poly-L-SUVV		2.67	3.0	172 000
1.13 poly-L-SUV	37.2	3.42	1.2	175 000
1.50 poly-L-SUVV		3.81	3.2	87 400
2.26 poly-L-SUV	74.4	10.43	0.6	69 000
3.00 poly-L-SUVV		11.30	3.1	60 000

[a] Using 50 mM phosphate (NaH_2PO_4/Na_2HPO_4) buffered at pH 7.0. Pressure injection for 4 s (0.1 mg/mL) for BNP racemate. Separation voltage, +20 kV; current, 44–78 μA. Detection was at 214 nm.

the binding of charged analytes with oppositely charged micelle polymers, these interactions do not always seem to be the major force for chiral recognition. The highly hydrophobic naphthyl moiety and the hydrogen-bonding capability of the phosphate group in (±)-BNP are probably major factors in chiral discrimination using anionic micelle polymer PSPs.

3C.4c. Enantioseparation of neutral enantiomers

In order to compare the effect of poly-L-SUV and poly-L-SUVV micelle polymers on chiral separations, (±)-trifluoro-1-(9-anthryl)ethanol ((±)-TFAE) (see Figure 19 inset) was selected as an example of a neutral racemate. The enantiomers of TFAE have been used as chiral NMR resolving agents for the discrimination of enantiomeric purity of optically active compounds [62]. In general, both k' and N for (±)-TFAE increase gradually with an increase in concentrations of both polymers (Table not shown). Figure 19 compares the separation of (±)-TFAE at optimized R_s values of 2.5 and 2.1 using poly-L-SUV and poly-L-SUVV micelle polymers, respectively. The higher R_s of (±)-TFAE obtained using poly-L-SUV, as compared to that obtained

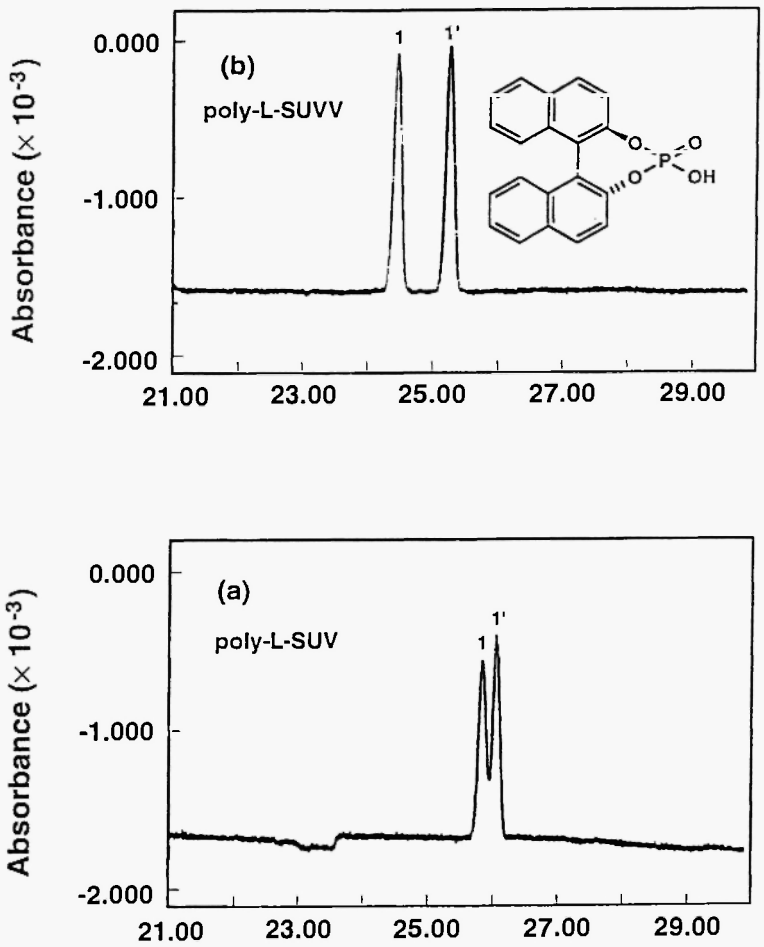


Fig. 18: Comparison of polymerized anionic surfactants for the separation of acidic enantiomers. EKC conditions: 1.13 % and 1.00 % (w/v) of poly-L-SUV or poly-L-SUVV, respectively, in 50 m*M* phosphate (Na_2HPO_4/NaH_2PO_4) buffered at pH 7.0. Peak identification: 0.1 mg/mL each of (1) (S)-(-)-BNP, (1') (R)-(+)-BNP. Pressure injection for 4s; + 20 kV applied for separation; current, 64 μA for Poly-L-SUV and 50 μA for poly-L-SUVV. UV detection was at 214 nm. From /58/ with permission.

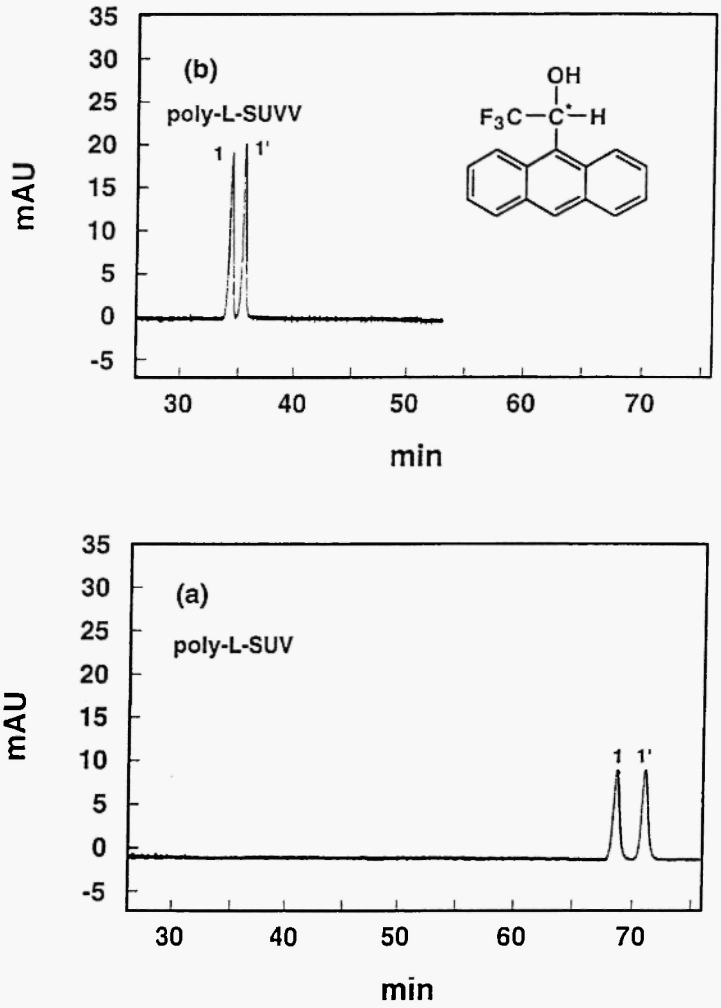


Fig. 19: Comparison of polymerized anionic surfactants for the separation of neutral enantiomers. EKC conditions: 0.38 % and 0.10 % (w/v) of poly-L-SUV and poly-L-SUVV in 50 m*M* $Na_2B_4O_7$ buffered at pH 10.2. Peak identification: 0.1 mg/mL each of (1) (-)-TFAE, (1') (+)-TFAE. Pressure injection for 4 s; +20 kV applied for separation, current 82 µA for poly-L-SUV and 78 µA for poly-L-SUVV. UV detection was at 254 nm. From /58/ with permission.

using poly-L-SUV, occurred only at the expense of longer analysis times and lower electrokinetic efficiencies.

3C.5. Effect of Amino Acid Order in Dipeptide terminated micelle polymers for EKC separation of enantiomers

Up until our initial report, no publications on dipeptide terminated micelle polymers for chiral separations with EKC have appeared in the literature. That study was followed up by Billiot *et al.* /63/, who set out to further understand chiral separations. Billiot and co-workers studied in detail the effect of the order of the amino acids in the dipeptide terminated micelle polymers on chiral separations. This step was only logical due to the well known fact that a chiral selector's size and shapes as geometric arrangement of its functional group help to determine its enantioselectivity /64/. The two main dipeptide terminated polymeric surfactants involved were poly (sodium *N*-undecanoyl-L-valyl-L-leucinate) [poly-L-SUVL] and poly (sodium *N*-undecanoyl-L-leucyl-L-valinate) [poly-L-SULV]. In addition, four other micelle polymers such as sodium *N*-undecanoyl-L-leucyl-L-leucinate [L-SULL], sodium *N*-undecanoyl-L-leucinate [L-SUL], poly-L-SUVV, and poly-L-SUV, were also used to compare their enantioselectivity with poly-L-SULV.

3C.5a. Comparison of poly-L-SUVL vs. poly-L-SULV

Initially, (±)-BNP and (±)-BOH were compared using poly-L-SUL and poly-L-SUVL for the EKC study. Figure 20 illustrates the variation in the enantiomeric resolution of (±)-BNP and (±)-BOH as a function of poly-L-SUV and poly-L-SUVL concentrations. The chiral recognition of (±)-BNP between poly-L-SUVL and poly-L-SULV was found to be very different. The maximum resolution achieved with poly-L-SUVL was less than 1. In contrast, poly-L-SULV was able to resolve (±)-BNP with a resolution of almost 8 under the same conditions. The difference in chiral selectivity observed for (±)-BOH was not as dramatic, R_s = 2.5 for L-SUVL and R_s = 6 for L-SULV. However, this resolution was still significant. Billiot and coworkers showed that the optimum concentration of the polymer was to be analyte dependent, i.e., analyte resolution is dependent on polymer [63]. In contrast, the optimum concentration of polymer for a given analyte appeared to be independent of the polymer.

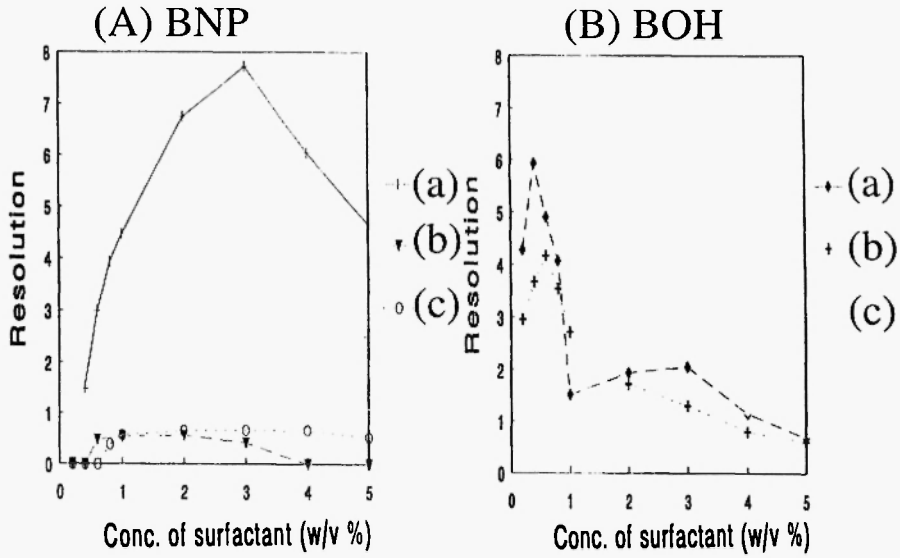


Fig. 20: Comparison of poly (L-SUV) and poly (L-SUL) to poly (L-SULV). (A) For BNP; (a) poly (L-SULV), (b) poly (L-SUL), and (c) poly (L-SUV). (B) for BOH: (a) poly (L-SULV), (b) poly (L-SUL), and (c) poly (L-SUV). From /63/ with permission.

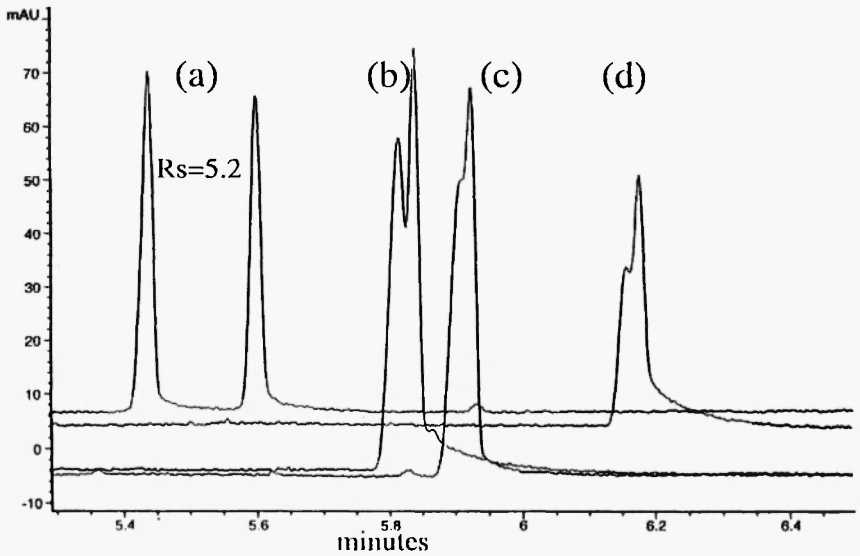


Fig. 21: Separation of (±)-BNP with 1 % (w/v) of various polymerized surfactants. (a) Poly (L-SULV), (b) poly (L-SUL), (c) poly (L-SUVL), and (d) poly (L-SUV). From /63/ with permission.

3C.5b. Comparison of poly-L-SULV to poly-L-SUL and poly-L-SUV

To better understand why poly-L-SULV provided better separation for (±)-BOH and (±)-BNP, poly-L-SUVL, poly-L-SUV, and poly-L-SUL were studied. The hypothesis that valine or leucine was responsible for the observed improvement in chiral resolution, depending on how far the analyte penetrates into the core of the micelle polymer was evaluated. If either of these two micelle polymers showed separation which is comparable to that of poly-L-SULV, then the differences in chiral separations may be due to analyte interaction with one of the chiral centers rather than some type of synergism of the two chiral centers. Figure 21 is a display of differences in resolving power of the various micelle polymers. The micelle polymer poly-L-SULV was able to separate (±)-BNP in less than 6 minutes with a resolution of 5.2 with polymer concentration at 1 % (w/v). In contrast, poly-L-SUVL, poly-L-SUL, and poly-L-SUV were unable to adequately separate (±)-BNP under the conditions used. Billiot and coworkers concluded that either the observed improved chiral separation was due to a form of synergism between the two chiral centers or steric effects of the dipeptide as compared to the single amino acid terminated micelle polymer /63/.

3C.5c. Comparison of poly-L-SULV to poly-L-SULL and poly-L-SUVV

Billiot *et al.* /63/ further explained their results by investigating their hypothesis that the enhanced chiral recognition observed with poly-L-SULV was due to a combined effect of two chiral centers or steric configuration of the dipeptide terminated micelle polymers, compared to single amino acid terminated polymers. Two dipeptide terminated micelle polymers with the same amino acids, i.e., poly-L-SULL and poly-L-SUVV, were compared to poly-L-SULV for enantiomeric resolution of (±)-BNP and (±)-BOH. The polymer of L-SULV was again observed to perform better than either poly-L-SULL or poly-L-SUVV in the enantioseparation of (±)-BNP and (±)-BOH (Figure 22). Poly-L-SULV was able to resolve (±)-BOH with a resolution of about 6, while poly-L-SUVV and poly-L-SULL had resolutions of 3 and 2.2, respectively. The resolution achieved for (±)-BNP with the polymers of L-SULV, L-SUVV, and L-SULL were approximately, 8, 2, and 4, respectively. Interestingly, the opposite trend was observed with (±)-BNP. The bulkier micelle polymers poly-L-SULL and poly-L-SUVV separated (±)-BNP better than the less bulky, less sterically hindered, single amino acid terminated micelle polymers. The single amino acid micelle polymers, (poly-L-SUL and poly-L-SUV) however, separated (±)-BOH better than the

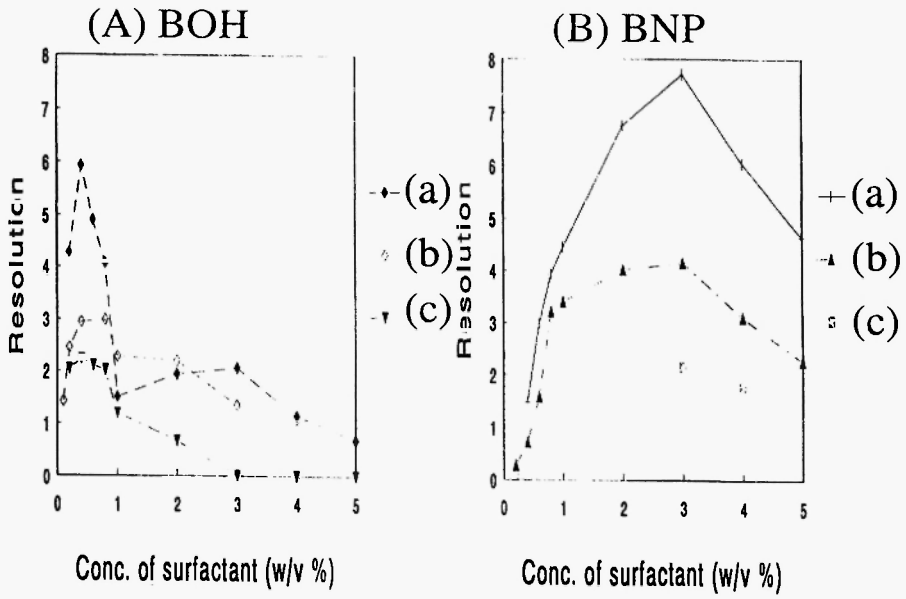


Fig. 22: Comparison of poly (L-SUVV) and poly (L-SULL) to poly (L-SULV). (A) for BOH: (a) poly (L-SULV), (b) poly (L-SUVV), and (c) poly (L-SULL). (B) for BNP: (a) poly (L-SULV), (b) poly (L-SULL), and (c) poly (L-SUVV). From /63/ with permission.

dipeptide terminated micelle polymers (poly-L-SULL and poly-L-SUVV). Billiot and coworkers concluded that the enantioseparation of (±)-BNP was favored by an increase in steric factors, while this same increase in steric factors decreased the enantiomeric resolution of (±)-BOH.

Due to the high enantioselectivity of poly-L-SULV, it was possible to achieve baseline separation of (±)-BNP and (±)-BOH in less than 1 minute (Figure 23). This ultrafast separation was achieved using 1 % (w/v) polymer with reverse polarity and injecting the sample at the detector end (making the effective length of the capillary only 8.5 cm). This separation was done with enantiomeric excess of the R-form of (±)-BOH and (±)-BNP in order to determine the elution order of the enantiomers. Hence, this ultrafast separation would provide higher sample throughput and, thus, increased laboratory efficiency.

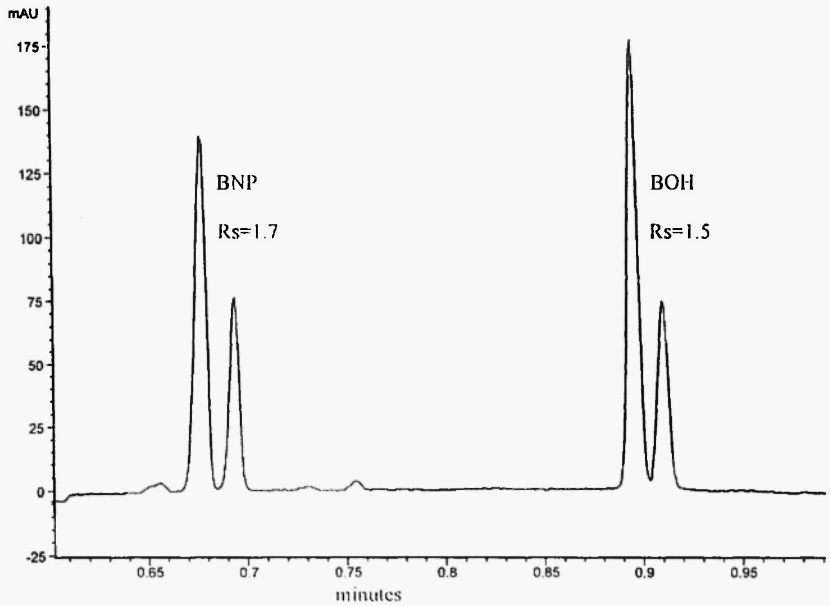


Fig. 23: Ultrafast Separation of (±)-BOH and (±)-BNP with 1% (w/v) poly (L-SULV) using short method. Same conditions as in Figure 22 except negative polariy (-30 kV) was used and injection was done at the detector end, making the effective capillary length 8.5 cm. From /63/ with permission.

3C.6. Amino acid order in dipeptide terminated polymeric micelles: Effect on physical properties and enantioselectivity

As discussed above, the amino acid order significantly affects the chiral recognition ability of the dipeptide terminated polymeric micelle. In further studies to understand this phenomena, Billiot *et al.* /64/ examined a larger group of homologue of amino acid terminated polymeric micelles. The authors investigated all possible dipeptide combinations of the L-form of alanine, valine, leucine, and the achiral glycine (except glycine-glycine) surfactants. In addition, single amino acid terminated micelle polymers of alanine, valine, and leucine were also studied to understand the role of one versus two chiral centers. Fluorescent probe studies were employed to better understand the interactions of the analytes with these synthetic chiral micelle polymers. The characterization of these dipeptide terminated micelle polymers by fluorescence spectroscopy, lead Billiot and coworkers /64/ to propose a structure of the micelle polymer in solution.

3C.6a. Proposed structure

Billiot and coworkers /64/ proposed that the lowest energy configuration of dipeptide terminated polymeric micelle in solution was when the larger of the R-groups, i.e. the most hydrophobic group, is inside facing the core of the micelle polymer. The proposed structure of the dipeptide terminated micelle polymer is shown in Figure 24. The backbone of these dipeptide terminated micelle polymers is the same, with the R-groups providing the only structural difference. The final conformation of the dipeptide in the micelle polymer is governed by two major effects that are involved in determining the minimum energy configuration, the first of which would involve the two hydrophobic groups, R_1 and R_2 to face the inner core of the micelle polymer rather than be exposed to the bulk aqueous phase. However, due to the packed configuration of the micelle polymer, this preferred confirmation is unlikely to occur due to steric hindrance. Secondly, another structural implication occurs if the larger amino acid is in the second position. In such a case, the large bulky R-group could limit access of a large bulky analyte to interact with the first chiral center attached to R_1.

The implications of the proposed structure was illustrated by a comparison of the dipeptide terminated micelle polymer, poly L-SUAL and poly L-SULA, interacting with (±)-BOH (Figure 25). Billiot and coworkers speculated that when leucine (the larger of the two amino acids) was in the first position (Figure 25A), the R-group of alanine is directed away from the hydrophobic core and more towards the aqueous phase. This configuration allows (±)-BOH to interact more with the heteroatom on the alanine, restricting the movement of (±)-BOH and thus, enhancing the chiral selectivity of this micelle polymer. Conversely, if leucine is in the second position, its bulky R group (Figure 25B) could block access to the first chiral center attached to R_1, thereby reducing the chiral selectivity of the dipeptide terminated micelle polymer.

In the case of poly L-SUGV and poly L-SUAV, the larger of the two R-groups would be the R-group on valine and it would be facing the core of the micelle polymer. With poly L-SUGV, there is no competition between the two R-groups since the R-group on glycine is a hydrogen. Therefore, the carbonyl adjacent to R_1 in poly-SUGV is free to rotate and face the aqueous phase without the steric hindrance associated with the competing R-group (R_1). Thus, when the carbonyl adjacent to R_1 faces the aqueous phase, the conformation of this micelle polymer increases the hydrophobicity of the core.

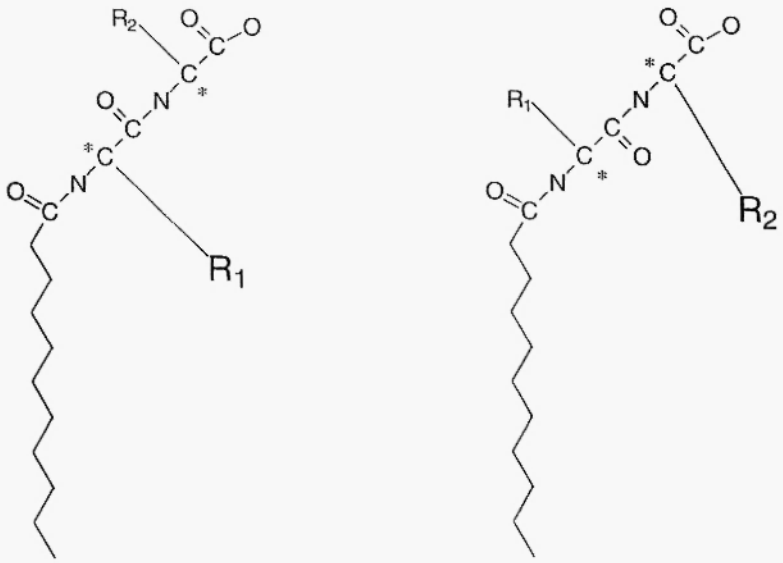


Fig. 24: Proposed structure of dipeptide surfactants

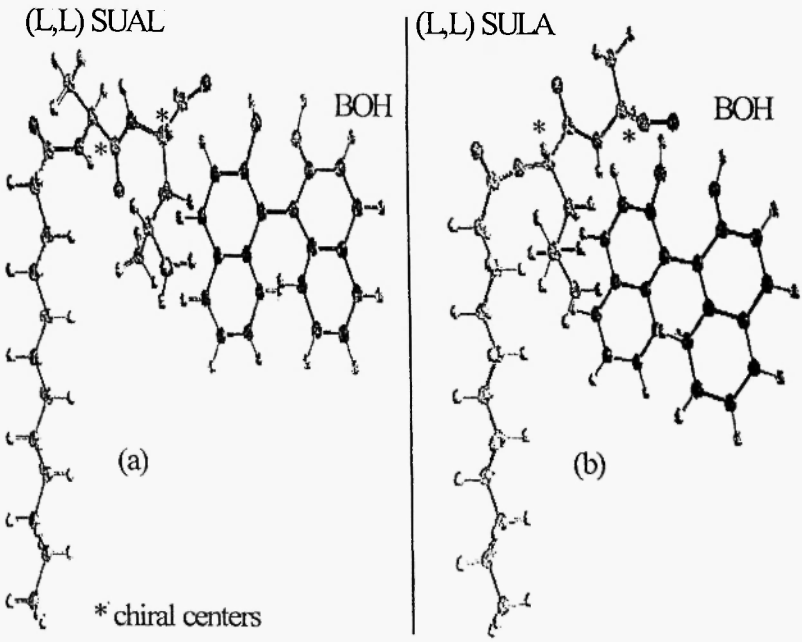


Fig. 25: Proposed interaction between (±)-BOH with poly-L-SUAL and poly-L-SULA. From /64/ with permission.

3C.6b Fluorescent probe study

In order to better understand the role of the R-groups and the carbonyl functional groups in the dipeptide terminated polymeric micelles, Billiot and coworkers employed fluorescent probes: Prodan (6-propionyl-2-(dimethylamino)naphthalene) and pyrene. These probes serve to measure polarity/hydrophobicity of the micro-environment of the micelle polymer.

The experimental hydrophobicity data for the various micelle polymers is shown in Figure 26. As observed in Figure 26a, the hydrophobicity trend suggests that the core of poly L-SUA is the least hydrophobic of the three single amino acid terminated micelle polymers, followed by poly L-SUV, then poly L-SUL. A similarly the trend is observed when glycine is held constant in the first (N-terminal) position of the dipeptide terminated micelle polymer and the size of the amino acid in the second position is increased, Figure 26b. Moreover, when valine or leucine are kept constant in the second position of the dipeptide terminated micelle polymer and the amino acid in the first position is increased in size from left to right (i.e., alanine to valine to leucine), the hydrophobicity again follows expected trends, [Figure 26(c,d)].

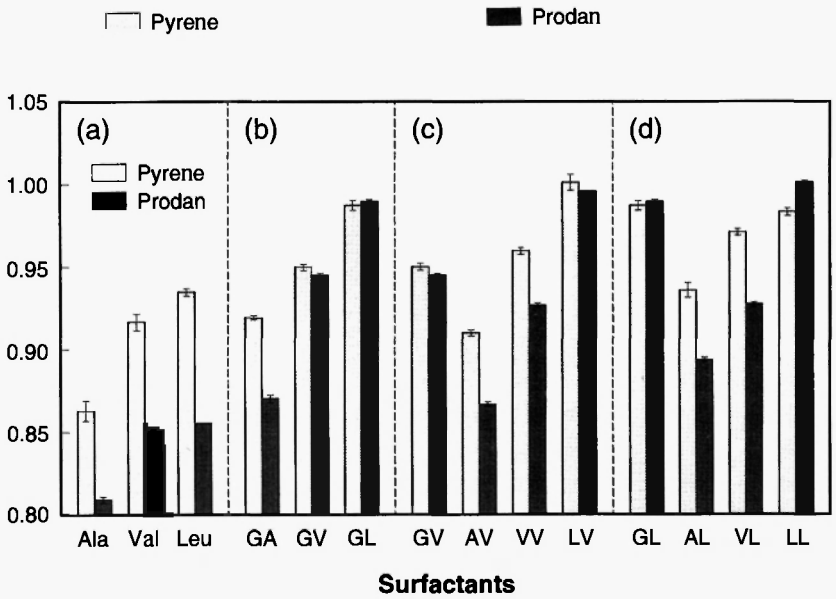


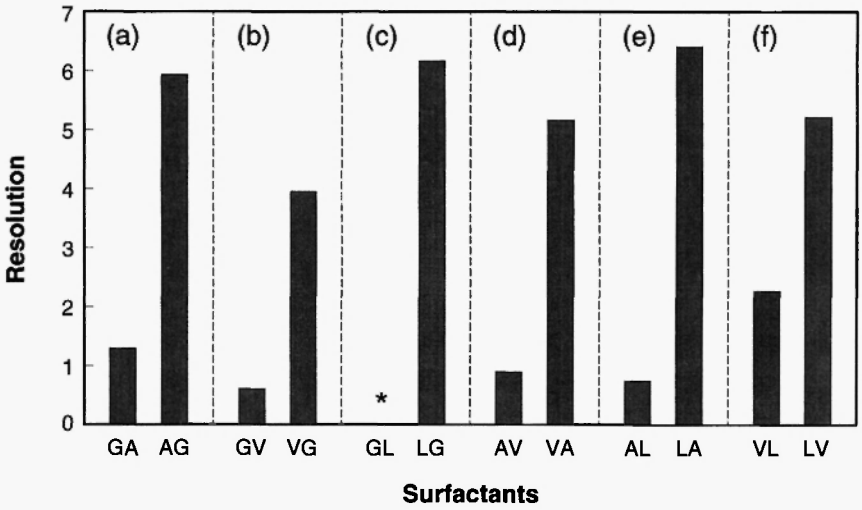
Fig. 26: Bar graph comparing the hydrophobicity of various dipeptide terminate micelle polymers. From /64/ with permission.

Billiot and coworkers /64/ further noted that if glycine is present in the first position of the dipeptide terminated micelle polymer, an increase in hydrophobicity is observed as compared to the intuitive decrease that one would expect in going from a less hydrophobic surfactant poly L-SUGV to a more hydrophobic surfactant poly L-SUAV, Figure 26c. The same unexpected behavior is observed in Figure 26d when comparing poly L-SUGL to poly-L-SUAL. Furthermore, the hydrophobicity of the micro-environment of the core of poly L-SUGL is greater than poly L-SUAL and poly L-SUVL and about the same as poly L-SULL. The reason for this apparent anomaly is that the probe experiences only the microenvironment inside the core of the polymeric surfactant.

3C.6c Capillary electrophoresis study

Billiot and coworkers' hypothesis was that if the more hydrophobic bulky R-group of the dipeptide terminated micelle polymer is in the second position, it could limit access of a bulky analyte to the first chiral center attached to R_1 /64/. In order to test this hypothesis, Billiot *et al.* investigated the chiral separation of two large bulky chiral compounds (BNP and BOH). A comparison of the enantiomeric resolution of (±)-BOH with the various dipeptide terminated micelle polymers is shown in Figure 27. They speculated that the preferred order of the amino acids in the dipeptide for chiral recognition of large bulky analytes would be with the larger of the two amino acids in the first position (i.e., the second dipeptide terminated polymeric micelle in the paired grouping). As seen in Figure 27, the prediction held true for the separation of (±)-BOH. In fact, baseline resolution was not achieved for any of the surfactants when the smaller of the two amino acids is in the first position, except poly L-SUVL. In contrast, when the larger of the two amino acids is in the first position, resolution values of 4 or better were achieved.

Similar trends were also observed in comparing the resolution of (±)-BNP (Figure 28). When the smaller amino acid is in the first position, baseline separation was only obtained for two of the dipeptide terminated polymeric micelle (i.e., poly L-SUGV and poly L-SUGL). However, with the larger amino acid in the first position, nearly twice the value of baseline separation was achieved in the other cases. The only exception to this trend was found with the first pair of micelle polymers, poly L-SUGA and poly L-SUAG, [Figure 28A]. Both poly L-SUGA and poly L-SUAG were not able to separate the enantiomers of (±)-BNP.

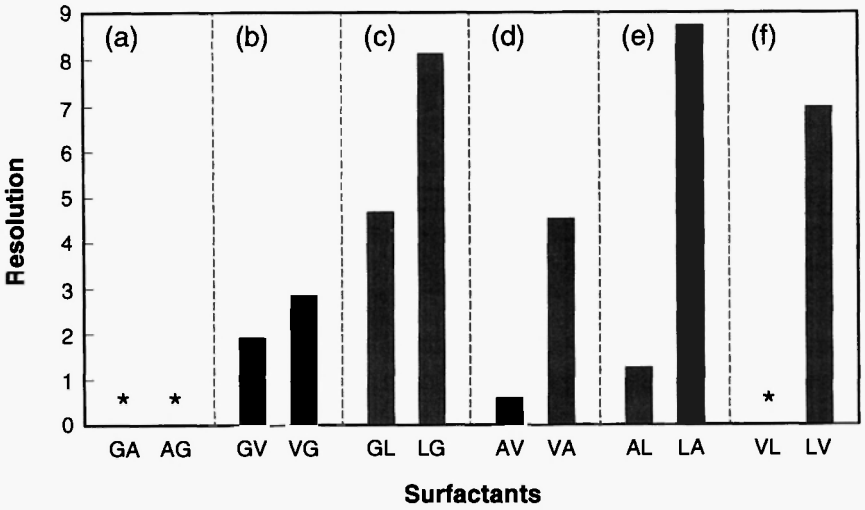


* no resolution

Fig. 27: Bar graphs illustrating the effect of amino acid order in dipeptide terminated micelle polymers on the chiral resolution of (±)-BOH. EKC conditions: applied voltage + 30 kV, buffer solution prepared with 100 mM TRIS and 10 mM sodium borate at pH 10.0, 25°C, 5 mM each of the micelle polymers at equivalent monomer concentrations. From /64/ with permission.

3C.7 Combination of a chiral micelle polymer with γ-CD (A synergistic approach)

In addition to the successful implementation of micelle polymers for enantioseparations, similar success has been found in the presence of other chiral selectors. One example of this is cyclodextrins (CDs), which is widely used as chiral selectors either in CZE or EKC combined with CZE (commonly referred to as CD-EKC) /65/. In CD-MEKC, CDs and micelles are incorporated into the same run buffer. This combined use of CD and micelles was initially used to separate highly hydrophobic compounds /65/. However, this approach is now commonly used in chiral separations /66-69/. When normal micelles are used, there is an inherent disadvantage with the CD-MEKC approach for chiral separations. The surfactant monomers are partly associated in a complex with the CDs /70, 71/. For this reason,



* no resolution

Fig. 28: Bar graphs illustrating the effect of amino acid order in dipeptide terminated micelle polymers on the chiral resolution of BNP. EKC conditions same as Figure 27. From /64/ with permission.

complexation of surfactant monomers with CDs will interfere with interactions between the individual enantiomers and the CDs. This interference will directly influence the enantioselectivity of the system and may reduce the observed chiral resolution. Moreover, surfactant monomers with high CMC values give increased conductivity for the running buffer, thus leading to joule heating, which ultimately will degrade a separation. The advantages of micelle polymers provide greater potential for EKC. For example, the use of micelle polymers eliminates the normal inclusion phenomena between surfactant monomers or unpolymerized micelles with CDs.

Using a synergistic approach, Wang and Warner have reported the first chiral separation by use of a combination of a micelle polymer and γ-CD /35/. Poly-L-SUV and its antipode poly-D-SUV were the chiral micelle polymers used in this study. Since, the CD-modified chiral micelle polymer combination is analogous to a normal CD-MEKC system. A similar theoretical approach of CD-MEKC /72/ with some alterations can be used.

Thus, the micelle polymer acts as a pseudo-stationary phase and the neutral γ-CD is a part of the aqueous phase. Moreover, it is assumed that the

enantiomers interact independently with the micelle polymer and the γ-CD. As discussed earlier, the capacity factor, κ', can be defined by Equation (24) where η_{mp} and η_{aq} are the moles of analyte molecules associated with the micelle polymer and aqueous phase (containing CD), respectively. Furthermore, the moles of the analyte in the aqueous phase included not only the moles of free solution, η_f, but also the analyte molecules incorporated into the CD, η_{CD}. Thus, we can write:

$$\eta_{aq} = \eta_f + \eta_{CD} \tag{24}$$

Based on a partitioning mechanism in this chiral CD-MEKC system, there are two important partitions for an enantiomer A

$$\begin{aligned} K_{mp,A} &= \frac{[A]_{sp}}{[A]_f} \\ [A]_f &= [A]_{sp} \end{aligned} \qquad \begin{aligned} K_{CD,A} &= \frac{[A]_{CD}}{[A]_f} \\ [A]_f &= [A]_{CD} \end{aligned} \tag{25}$$

where $[A]_f$, $[A]_{sp}$ and $[A]_{CD}$ are the concentrations of the enantiomer, A, in the aqueous phase, micelle polymer, and CD, respectively. Thus, we can obtain

$$\frac{\eta_{CD,A}}{\eta_{f,A}} = K_{sp,A}\frac{V_{sp}}{V_f} \qquad \frac{\eta_{CD,A}}{\eta_{f,A}} - K_{CD,A}\frac{V_{CD}}{V_f} \tag{26}$$

where K_{sp} and K_{CD} are partition coefficients between the micelle polymer and the aqueous phase, and between the CD and aqueous phase, respectively, The parameters V_f, V_{sp}, V_{CD} are the respective volumes of the aqueous, the micelle polymer phase and the CD phase. Combining equations (25 and 26), we obtain the following equation for capacity factor:

$$k' = \frac{K_{sp} V_{sp}}{V_f + V_{CD} K_{CD}} \tag{27}$$

The selectivity or separation factor for an enantiomeric pair can be defined as:

$$\alpha = \frac{k'_{app,B}}{k'_{app,A}} \tag{28}$$

where $k'_{app\,A}$ and $k'_{app\,B}$ are the respective apparent capacity factors for the enantiomeric pair, A and B. Since selectivity (α) is directly related to resolution (R_s), selectivity for the polymer CD-MEKC system can be derived as follows:

$$\alpha = \frac{1 + \Phi_{CD} K_{CD}, K_{sp,B}}{1 + \Phi_{CD} K_{CD}, K_{sp,A}} \tag{29}$$

where Φ_{CD} is the phase ratio of the volume of CD (V_{CD}) to that of the aqueous phase (V_f). According to Equation (29), for a given theoretical plate number (N) and capacity factor (k'), a higher value of α results in higher resolution. In the case when the selectivity >1; it can be shown that there are three possible combinations of these parameters: (I) if $K_{CD.A} > K_{CD\,B}$ and $K_{sp\,B} > K_{sp,A}$ (enantiomer resolution will be superior to that obtained using either CD or the micelle polymer alone), (II) if $K_{CD,A} > K_{CD,B}$ and $K_{sp,A} > K_{sp,B}$ (enantiomeric resolution will be poorer than by using γ-CD alone), or (III) if $K_{CD,A} < K_{CD,B}$ and $K_{sp,B} > K_{sp\,A}$ (then the resolution will be poorer than when using the micelle polymer alone).

Figure 29a provides the validation of equation 29, where a combination of γ-CD and poly-D-SUV was used. When using only γ-CD as a chiral selector, (R)-BOH has a high affinity for γ-CD and will migrate faster than the (S)-form. Using only poly-D-SUV as a chiral selector, the (S)-BOH more strongly with the polymer than the (R)-form. Thus, (R)-BOH will migrate through the capillary faster than the (R)-form (Figure 29b). The combination of γ-CD and poly-D-SUV presents the following conditions $K_{CD,R} > K_{CD,S}$ and $K_{sp,S} > K_{sp,R}$. Therefore, chiral resolution will be enhanced resolution obtained by use of either chiral selector alone. The synergistic effect of γ-CD and poly-D-SUV on the separation of (R)-BOH is illustrated in Figure 29c.

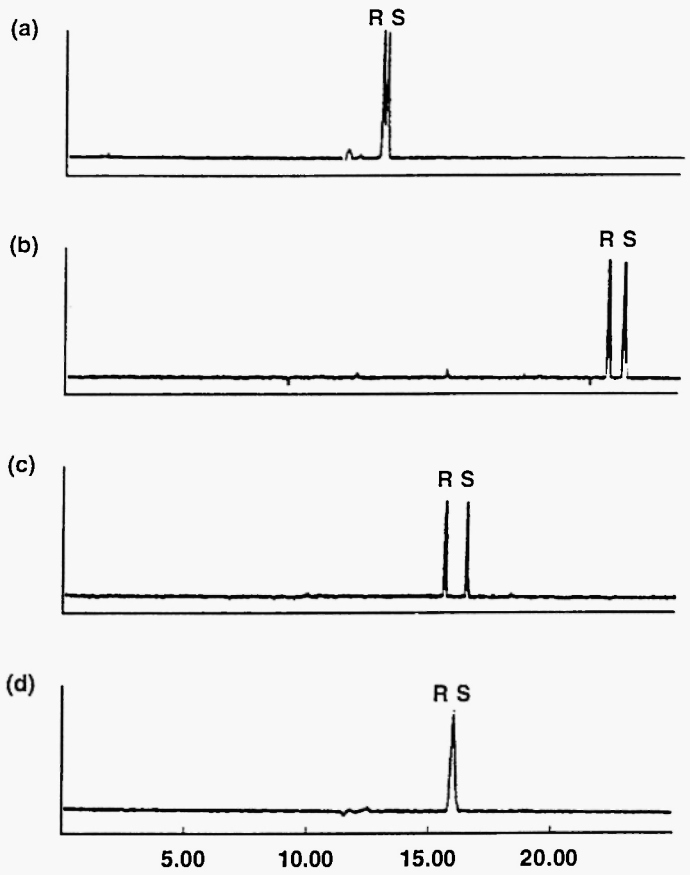


Fig. 29: Chiral separations of (±)-1,1'-bi-2-naphthol by use of poly (D-SUV) and γ-CD. CE conditions: 25 m*M* borate buffer (pH 9); applied voltage, 12 kV; UV detection 280 nm. (a) 10 mM γ-CD, (b) 0.5 % poly (D-SUV), (c) 10 m*M* γ-CD and 0.5 % poly (D-SUV), (d) 10 mM γ-CD and 0.5 % poly (L-SUV). From /35/ with permission.

However, the chiral resolution is diminished when γ-CD and poly-L-SUV were combined (Figure 29d) corresponding to the conditions where $K_{CD,R} > K_{CD,S}$ and $K_{sp,R} > K_{sp,S}$.

Wang and Warner observed that the combination of γ-CD and poly-D-SUV not only improved the enantioselectivity but also extends the elution window (since the neutral CD travels with the EOF). Figure 30 shows

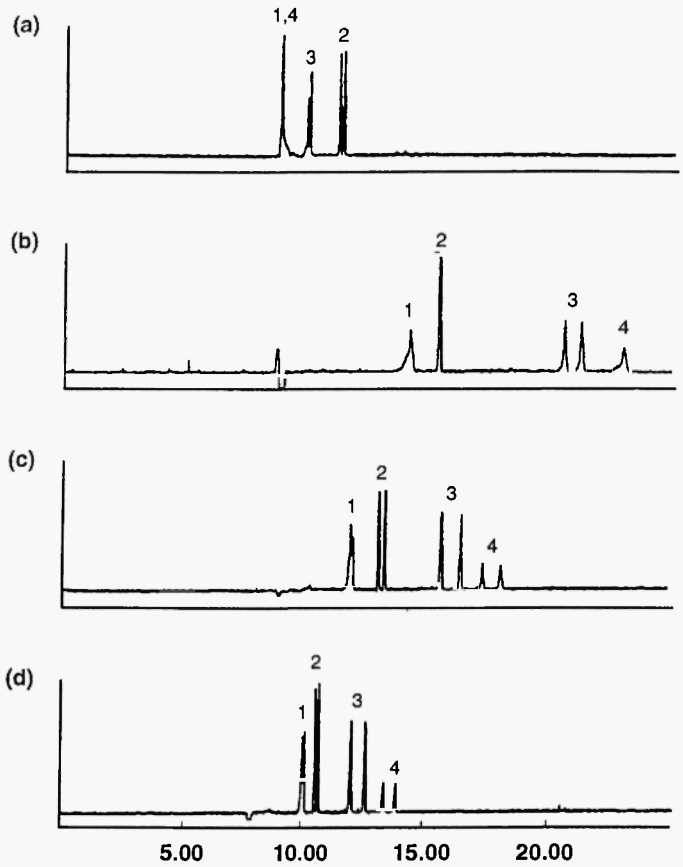


Fig. 30: Separation of a mixture of four enantiomeric pairs. Corresponding peaks: (1) DL-Laudonosine, (2) (±)-1,1'-binaphthyl-2,2'-diyl hydrogen phosphate, (3) (±)-1,1'-bi-2-naphthol, (4) (+)-verapamil. Experimental conditions: (a) 10 m*M* γ-CD, (b) 0.5 % w/v poly-D-SUV, (c) 10 m*M* γ-CD and 0.5 % w/v poly-D-SUV, buffer for (a), (b), and (c) is 25 m*M* borate (pH 9.0), (d) 10 m*M* γ-CD and 0.5 % w/v poly-D-SUV, 5 m*M* borate (pH 9.0); applied voltage 12 kV; UV detection, 280 nm. From /35/ with permission.

the enantioseparation of the four racemates, (±)-BOH, (±)-verapamil, (±)-BNP, and DL-laudonosine. Using either γ-CD or poly-D-SUV alone, no satisfactory resolution was obtained (Figure 30(a,b)). However, when both CD and poly D-SUV was used at the same concentrations, three of the racemates were baseline resolved (Figure 30c). The optimization of various

separation parameters (buffer concentration, γ-CD concentration, and organic solvent) provided enantioseparation for all four racemates.

In a detailed study of the separation parameters (concentration of γ-CD, concentration of background electrolyte, and organic solvents) it was observed that increasing the concentration of γ-CD up to 15 mM, increased the migration time and improved the resolution. Above 15 mM, the resolution degraded for most of the racemates. As expected, the migration window for each of the enantiomeric compounds was lengthened with increasing borate buffer concentration (5 mM to 45 mM). The resolution, for most of the enantiomeric pairs was increased except for DL-laudonosine, where the resolution decreased. Wang and Warner /35/ concluded that the charged chiral micelle polymer is more flexible at lower buffer concentrations than at higher buffer concentrations. At low buffer concentrations, the anionic micelle polymer has fewer closely associated counterions. Thus, the polymer can extend more at lower concentrations, which may have induced a large difference in affinities between the individual D- and L- enantiomers.

In this system, the addition of organic solvents (methanol and acetonitrile) enhances the enantiomeric resolution for some analytes and decreased enantiomeric resolution in other cases. Thus, this parameter seems to be more analyte- than organic modifier-dependent. For traditional MEKC or CZE system, the migration time of all the analytes will increase upon raising the fraction of organic solvent for the buffer. In traditional MEKC or CD-MEKC, the micelle will decompose into surfactant monomer if the concentration of organic solvent is too high, thus inhibiting analyte-micelle interaction. However, with micelle polymers, very high concentration of organic solvents are still effective. Figure 31 shows that the resolution of the enantiomers are good even when the methanol concentration was as high as 40 % v/v.

3D. Applications of micelle polymer in EKC-MS

The advantages of micelle polymers for achiral and chiral separations in EKC have been demonstrated with data from the literature. These advantages also extend into other fields in analytical chemistry such as EKC with mass spectrometric (MS) detection. On-line CE-MS has been recognized as a powerful method since it has the capability for separating and identifying components in a complex unknown mixtures. However, there are only a few

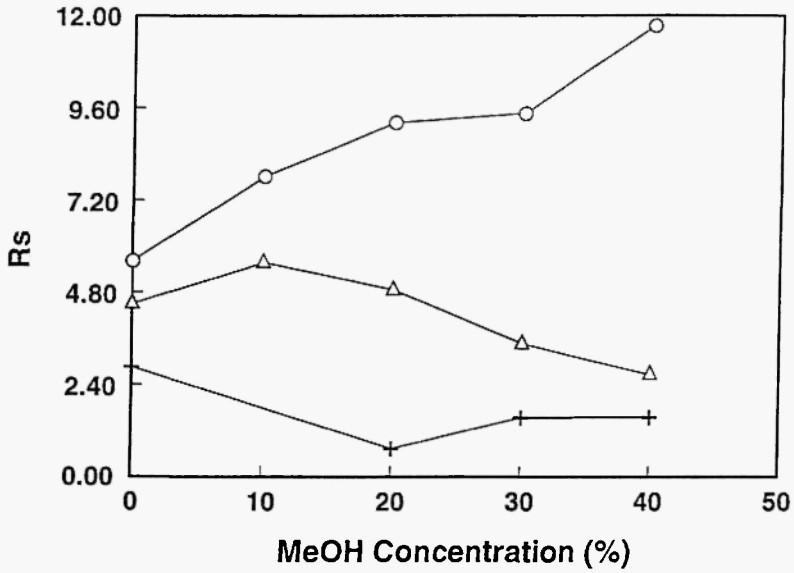


Fig. 31: Effect of methanol concentration on the resolution of the enantiomeric mixture. Experimental conditions: 0.5 % w/v poly-D-SUV, 25 mM borate (pH 9.0), 10 mM γ-CD, 0 – 40 % v/v methanol; applied voltage, 12 kV; UV detection, 280 nm. ° = (±)-1,1'-bi-2-naphthol, Δ = (±) – verapamil, + = (±)-1,1'-binaphthyl-2,2'diyl hydrogen phosphate. From /35/ with permission.

reports on the use of achiral surfactants with CE coupled to electrospray ionization (ESI)-MS /72-78/.

3D.1 Problems of MEKC-MS with surfactants

The main problem associated with the use of surfactants for on-line MEKC-ESI-MS systems is that accumulation of surfactants can cause fouling of the ion source, and suppression of analyte signals in ESI-MS /79,80/. The use of high concentration of nonvolatile surfactants such as SDS gives rise to strong background signals in both positive and negative ion modes. Therefore, at SDS concentrations required for micellar-mediated CE separations, SDS-analyte adducts formation suppresses the ionization efficiency of the source resulting in poor S/N ratios for the analytes /80/. For these reasons, several approaches such as use of high molecular mass micelle

polymers /81,82/, introduction of electrospray chemical ionization interface /83,84/, partial filling MEKC /85,86/, and anodically migrating micelles /87/ have been reported in the literature. However, in this section of this review, we will evaluate the use of micelle polymers as one possible solution to the aforementioned problems of EKC-MS.

3D.2 EKC-MS with acrylate copolymers

Ozaki *et al.* were the first to demonstrate the utility of EKC-MS using polymers /45/. Ozaki and coworkers employed butyl acrylate-butyl methacrylate-methacrylic acid copolymer sodium salt (BBMA) as a pseudo-stationary phase for an on-line EKC-MS of sulfur drugs (sulfamethazine, sulfisomidine, sulfadiazine, and sulfisoxazole), as well as for hydrophobic cationic analytes (phenyltrimethyl ammonium chloride, naphthylamine, quinine sulfate, tetraphenylphosphonium chloride). Figure 32A shows the single-ion chromatogram obtained by MEKC-ESI-MS of the mixture of these hydrophobic cationic analytes. The solutes were separated and detected using 2% BBMA. Under the MS conditions (2% BBMA), abundant intact molecular ions of phenyltrimethyl ammonium chloride, naphthylamine, quinine sulfate, and tetraphenylphosphonium chloride was observed. The migration times of all solutes increased with increasing concentration of BBMA. Thus, the separation was based on the differential partitioning of the solutes between the migrating BBMA micelle and the surrounding aqueous phase.

Ozaki and co-workers employed studies investigating the dependence of intensities on concentration of BBMA, for the sulfur drugs and a standard mixture of analytes. Based on the trend of solute intensity decreasing with increasing concentration of BBMA, Ozaki and co-workers concluded that a separation solution containing low concentration BBMA should be employed, especially for the detection of analytes for which the ionization efficiency is low.

Figure 33 shows (A) MEKC separation with a UV detector and (B) MEKC-ESI-MS of sulfamides using 1 % BBMA. By viewing the separation between peaks 1 and 2, it can be observed that the separation efficiency in MEKC-ESI-MS was far less than in conventional MEKC. Ozaki and co-workers noted that the deterioration of separation efficiency was probably caused by the ESI interface. However, the separation and sensitivity were reproducible.

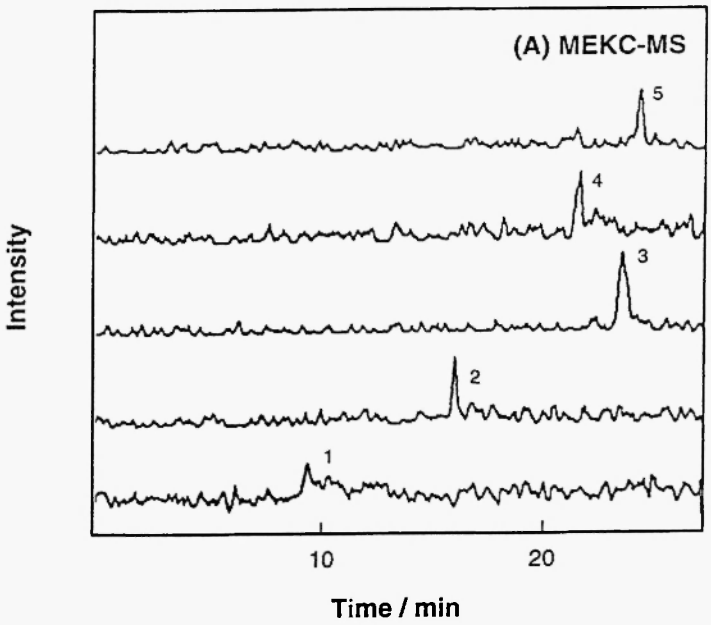


Fig. 32: Single-ion chromatograms obtained by MEKC-ESI-MS. Solutes: 1 = phenyltrimethylammonium chloride; 2 = 1-naphthylamine; 3 = quinine sulfate; 4 = tetraphenylphosphonium chloride; 5 = oxta-oxyethylenedodecanol. Conditions: electrospray voltage, 3 kV; MS scanning, from m/z 1 to 1000 at 4 per scan; drift voltage, 70 V; focusing voltage, 140 V; resolution, 55; sheath liquid flow, water-methanol-formic acid (50:50:1, v/v/v) at ca. 5 µl/min; sample solutions were introduced by syringe injection. From /81/ with permission.

3D.3 EKC-MS with poly-(sodium 10-undecylsulfate)

Lu and coworkers /85/ reported the application of on-line EKC-ESI-MS in the analysis of three tricyclic antidepressant drugs (i.e. imipramine, doxepin, and amitriptyline) and two β-blockers (i.e., propranolol and alprenolol) using poly-SUS. These cationic drugs have similar electrophoretic mobilities. The use of an aqueous/methanol-based CZE buffer could not separate these drugs. In order to improve the CE separation, poly-SUS was employed. This sulfated micelle polymer was extremely suitable because of the zero CMC. Moreover, separation with this micelle polymer was proven to provide separation at low concentrations /26,41,86/.

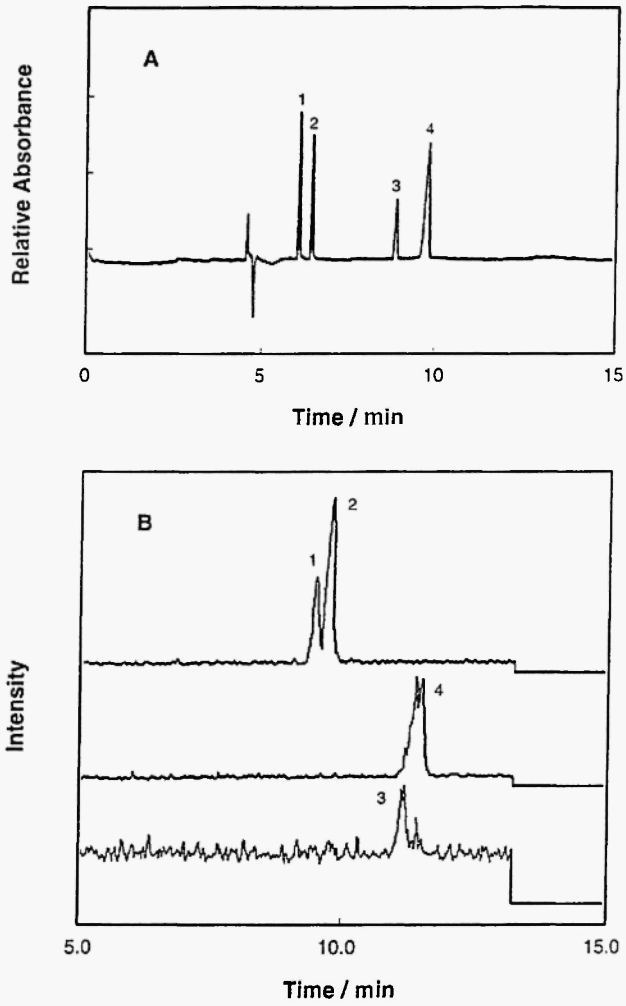


Fig. 33: (A) MEKC with a UV detector and (B) MEKC-ESI-MS of sulfamides and (C) their molecular structures. Solutes: 1 = sulfamethazine; 2 = sulfisomidine; 3 = sulfadiazine; 4 = sulfisoxazole. Conditions: (A) separation solution, 1 % BBMA in 10 % methanol and 100 mM borate-50 mM phosphate buffer (pH 7); capillary, 48 cm (40 cm to the detector) x 50 µm I.D. fused silica; applied voltage, 20 kV; detection wavelength, 210 nm. (B) Other conditions as in Figure 32 except for the interface; the MS instrument was operated in the SIM mode. From /81/ with permission.

Figure 34 (a,b) illustrates the results of the EKC-ESI-MS determination of the three antidepressant drugs in buffer containing 0.1 % poly-SUS, 10 mM ammonium acetate, pH 9, in water/methanol (80/20 %). At this low concentration of poly-SUS, impramine was baseline separated from doxepin, and the latter was partially resolved form amitriptyline. Moreover, it was observed that higher concentrations (> 0.1 % w/v) of poly-SUS did not help further resolve these analytes, but decreased the analyte signal /85/. Lower concentrations of poly-SUS deteriorated the resolution of these drugs although detection sensitivity of the analytes increased. A 0.1 % w/v poly-SUS CE buffer was also tested for the EKC-ESI-MS of propranolol and alprenolol. Figure 35 (a,b) shows the SIM electropherogram for determination of these two β-blockers using the same buffer as that for the

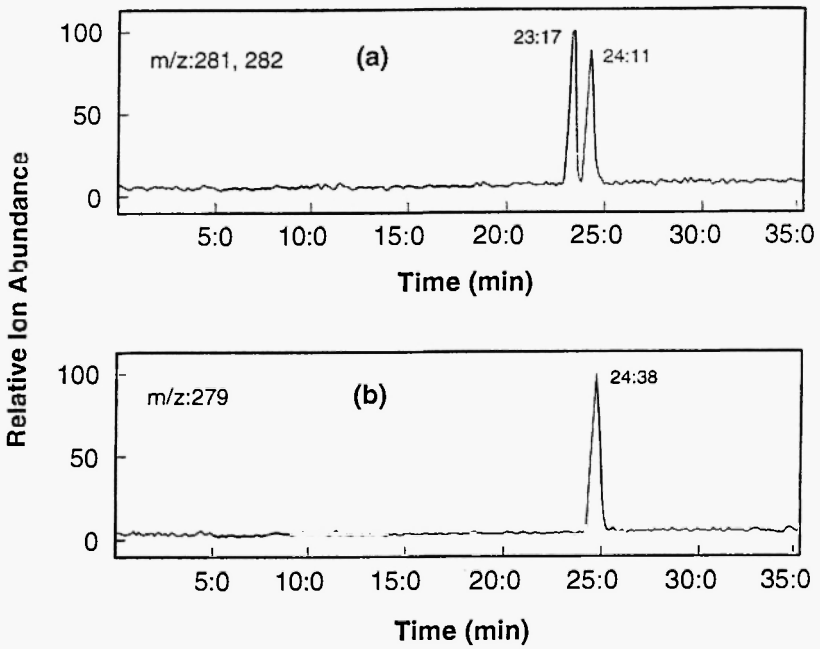


Fig. 34: Selected-ion electropherogram from on-line EKC-ESI-MS analysis of the tricyclic anti-depressant drugs: (a) impramine (MH^+, *m/z* 281) and doxepin (MH^+, *m/z* 282), (b) amitriptyline (MH^+, *m/z* 279). Experimental conditions are defined as for Figure 34., except 0.1 % w/v poly-SUS was added to the BGE. From /82/ with permission.

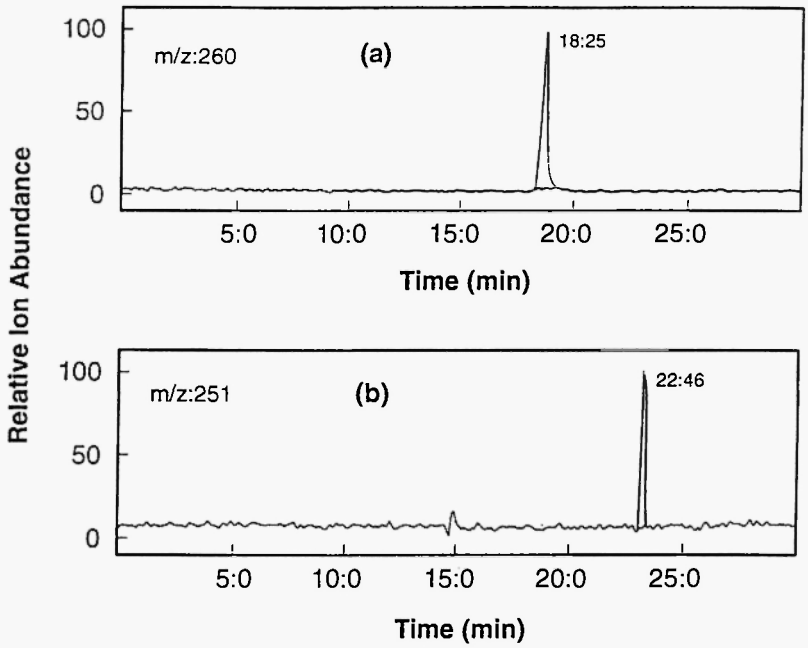


Fig. 35: Selected-ion electropherogram from on-line EKC-ESI-MS analysis of the β-adrenegic blockers: (a) propranolol (MH^+, *m/z* 260) and (b) alprenolol (MH^+, *m/z* 251). Experimental conditions are as defined in for Figure 34. From /82/ with permission.

tricyclic antidepressant drugs. Compared to CZE-ESI-MS, a large improvement in the separation for EKC-ESI-MS of propranolol and alprenolol was reported with poly-SUS /82/. However, improved resolution of both classes of these cationic drugs occurred at the expense of longer migration times. This is because the anionic micelle polymer migrates to the injection side (i.e., against the EOF directions). In addition, strong electrostatic interactions between the polymerized anionic micelle polymer and the cationic analytes are possible. It can be estimated from Figure 35a that the limit of detection of propranolol is about 10^{-5} M based on the signal-to-noise ratio of 3.

4. FUTURE PROSPECTS

Micelle polymers have added a new dimension to separations in EKC. Advantages such as structural rigidity, zero CMC, lowered joule heating and in particular, tolerance to larger concentrations of organic solvents have made micelle polymers extremely amendable to separations of highly hydrophobic compounds. Our studies have clearly demonstrated that the separation characteristics using achiral and chiral micelle polymers are usually superior to separations by monomeric ones. Viewing these distinct advantages, more applications for micelle polymers need to be developed, and there is still room for growth.

The interesting data previously generated using chiral micelle polymers has encouraged us to devote considerable time and effort to the design and synthesis of other novel achiral and chiral micelle polymers for EKC. Based on the previously reported synthesis of poly-L-SUV /30/, we can develop a variety of novel ionic chiral micelle polymers (i.e., multi-chiral center, zwitterionic, cationic and peptide terminated). Studies of this type may enable us to understand the fundamentals of chiral separations in micellar media, which may be useful for a variety of applications in the pharmaceutical and agricultural industries.

The development of new PSPs continues to blossom, and micelle polymers certainly will play a major role in advancing EKC in the future. For example, dendrimers are a new type of PSP which have found utility in EKC /90-95/. Dendrimers are well defined, highly branched macromolecules that emanate from a central core /96/. They have been described as "unimolecular micelles" /97/ and studies have demonstrated similar topological distinction of a micelle inside and outside /98/. Furthermore, dendrimers are readily synthesized and functionalized. We believe that these molecules will provide separation scientists with the next generation of "tools" aiding in solving the complex science of enantiomeric separations.

5. ACKNOWLEDGEMENTS

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