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ORIGINAL PAPER

The Use of Poly(Sodium *N*-Undecanoyl-L-Leucylvalinate), Poly(Sodium *N*-Undecanoyl-L-Leucinate) and Poly(Sodium *N*-Undecanoyl-L-Valinate) Surfactants as Chiral Selectors for Determination of Enantiomeric Composition of Samples by Multivariate Regression Modeling of Fluorescence Spectral Data

Sayo O. Fakayode · Alicia A. Williams · Marianna A. Busch · Kenneth W. Busch · Isiah M. Warner

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Abstract Steady-state fluorescence spectroscopy was employed to investigate the use of chiral polymeric surfactants as chiral selectors in chiral analysis by multivariate regression modeling of spectral data. Partial-least-squares regression modeling (PLS-1) was used to correlate changes in the fluorescence spectral data of 1,1′-bi-2-naphthol (BOH), 1,1′-binaphthyl-2,2′-diamine (BNA), or 2,2,2-trifluoroanthrylethanol (TFA) in the presence of poly(sodium *N*-undecanoyl-L-leucylvalinate), poly(sodium *N*-undecanoyl-L-leucinate) or poly(sodium *N*-undecanoyl-L-valinate) as the enantiomeric composition of the chiral analytes was varied. The regression models produced from the spectral data were validated by determining the enantiomeric composition of independently prepared test solutions. The ability of the model to correctly predict the enantiomeric composition of future samples was evaluated using the root-mean-square percent-relative error (*RMS%RE*) of prediction. In terms of *RMS%RE*, the ability of the model to accurately predict the enantiomeric composition of future samples was dependent on the chiral analyte, the polymeric surfactant used, and the surfactant medium, and ranged between 1.57 and 6.10%. Chiral analyte concentrations as low as 5×10^{-6} M were found to give regression models with good predictability.

Keywords Chiral analysis · Fluorescence spectroscopy · Multivariate regression analysis · Chiral polymeric surfactant · Chiral selector-guest complexation

S. O. Fakayode · A. A. Williams · I. M. Warner (✉)
Department of Chemistry, Louisiana State University,
Baton Rouge, Louisiana 70803, USA
e-mail: iwarner@lsu.edu

K. W. Busch
e-mail: kenneth_busch@baylor.edu

M. A. Busch · K. W. Busch
Department of Chemistry and Biochemistry, Baylor University,
One Bear Place # 97348, Waco, Texas 76798, USA

Introduction

Chiral analysis continues to be a topic of keen interest in the pharmaceutical industry because of the differences in the pharmacological and physiological properties of enantiomers. While one enantiomer of a chiral drug may have therapeutic effects, the other enantiomer may be ineffective or toxic, leading to serious health problems for humans [1–3]. These potentially harmful effects of different enantiomers have prompted serious health concerns from government- and regulatory agencies. This is particularly true for drugs that were initially approved as racemates, but are now being submitted for approval by the pharmaceutical industry as single-enantiomer drugs. Because of these concerns, the pharmaceutical industry is required to document the pharmacological and physiological properties of all single-enantiomer drugs.

Chiral analysis is often performed by use of chromatography or capillary electrophoresis [4–8], and nuclear magnetic resonance (NMR) using chiral solvents [9]. Chiral stationary phases used in chiral chromatographic separations frequently employ chiral cavitands that involve the formation of transient non-covalent guest-host complexes between the chiral guest analyte and a chiral selector. Several chiral cavitands such as cyclodextrins [10–12], protein antibiotics [13, 14] and crown ethers [15, 16] have been widely used for

chiral discrimination and for enantio-differentiation of chiral molecules. Chiroptical methods such as polarimetry, optical rotatory dispersion, circular dichroism, and vibrational circular dichroism have also been used for chiral analysis [4, 17, 18].

While most of these techniques are unquestionably effective, some of the current analytical techniques of chiral analyses have several major drawbacks. For example, chromatography and capillary electrophoresis are slow and not particularly attractive for high-throughput- or fast screening of chiral compounds. Moreover, in the case of chiral chromatography, chiral columns are frequently expensive and can have relatively short lifetimes. Chiroptical methods, such as the polarimetric method of chiral analysis, require a relatively large sample size and the measured optical rotation by the polarimeter can be solvent dependent. In addition, the sensitivity of some techniques like circular dichroism is relatively low, while techniques like NMR and mass-spectrometric methods are very expensive in terms of instrumentation. A rapid, relatively inexpensive spectroscopic method, like fluorescence spectroscopy, is therefore highly desirable in the pharmaceutical industry today, where accurate and fast screening of chiral molecules is of considerable interest as the marketing of drugs switches from racemic mixtures to single-enantiomer formulations.

Busch and co-workers recently reported a new rapid, accurate, and robust method for determining the enantiomeric composition of chiral molecules that combines ordinary ultraviolet/visible absorption- or fluorescence spectroscopy, cyclodextrin (CD) guest-host chemistry, and multivariate regression modeling [19–23]. In these studies, chiral analysis by the regression modeling of spectral data was shown to be a reliable method for determining the enantiomeric composition of chiral samples using ordinary spectroscopic methods. Subsequently, Tran and co-workers used a similar approach with near-infrared spectroscopy for determination of the enantiomeric composition of molecules of pharmaceutical interest [24, 25].

Poor solubility of native CDs as well as highly hydrophobic guests are major problems encountered in previous studies, and different strategies, such as the use of modified CDs [21] or the use of an achiral monomeric sodium dodecyl sulfate surfactant in combination with organic solvents [20] have been employed in an attempt to ameliorate these problems.

In this paper, we report the use of three chiral polymeric surfactants as chiral selectors for the determination of the enantiomeric composition of three chiral molecules (Fig. 1) using steady-state fluorescence spectroscopy and multivariate regression modeling of the spectral data. The three chiral surfactants used were poly(sodium *N*-undecanoyl-L-leucylvalinate) [poly-L-SULV], poly(sodium *N*-undecanoyl-L-leucinate) [poly-L-SUL] and poly(sodium *N*-undecanoyl-L-valinate) [poly-L-SUV]. The two chiral binaphthyl analyte molecules [1,1′-bi-2-naphthol (BOH) and 1,1′-binaphthyl-2,2′-diamine (BNA), see Fig. 1], as well as a chiral anthracene derivative, 2,2,2-trifluoroanthrylethanol (TFA), were selected for their fluorescence properties. BOH and BNA, although they do not possess typical chiral centers, are, nevertheless, chiral because they have axial chirality. Both compounds are stable to racemization.

Molecular micellar agents, also known as surfactants, are amphiphilic in nature, containing an apolar long-chain hydrocarbon tail and polar head groups. Chiral surfactants may function as nearly ideal chiral selectors for analytes such as the binaphthyl and anthracene derivatives used in this study by providing a chiral micellar environment for the highly hydrophobic analytes. Both chiral monomeric and polymeric surfactants have been used as selectors for chiral discrimination [26–31]. However, the use of polymeric surfactants is desirable because they are more stable and more rigid than monomeric surfactants. In addition, because polymeric surfactants have controllable sizes and have no critical micelle concentration, the use of polymeric surfactants eliminates the dynamic equilibrium between the micelles and the monomer. Compared to other chiral selectors such as cyclodextrins, crown ethers or protein antibiotics, polymeric surfactants are relatively more soluble in aqueous and organic solvents. Additionally, the polar head group as well as the number of stereogenic centers in the polymeric surfactant can easily be controlled and modified. Polymeric surfactants, therefore, have potentially wider applications and can be used for chiral analytes of various molecular size and polarity.

Poly-L-SULV, a negatively charged dipetide polymeric surfactant with two chiral centers, has a low aggregation number and is highly soluble in water. Furthermore, poly-L-SULV has good chiral discriminating capability and has been used successfully for the chiral separation of various analytes with widely different molecular structures in micellar electrokinetic chromatography (MEKC) [32]. In addition, the chiral recognition ability of poly-L-SULV, using fluorescence anisotropy, has been demonstrated [33]. Poly-L-SUL and poly-L-SUV are single amino-acid-based polymeric surfactants, each with one chiral center, that have been used for enantiomeric separation of several analytes in MEKC [31].

Materials and methods

Materials

Enantiomerically pure (*R*)-1,1′-bi-2-naphthol (*R*-BOH), (*S*)-1,1′-bi-2-naphthol (*S*-BOH), (*R*)-1,1′-binaphthyl-2,2′-diamine (*R*-BNA), (*S*)-1,1′-binaphthyl-2,2′-diamine (*S*-BNA), (*R*)-2,2,2,-trifluoroanthrylethanol (*R*-TFA), and (*S*)-2,2,2,-trifluoroanthrylethanol (*S*-TFA), sodium borate, and tris(hydroxymethyl)aminomethane (TRIS) were purchased

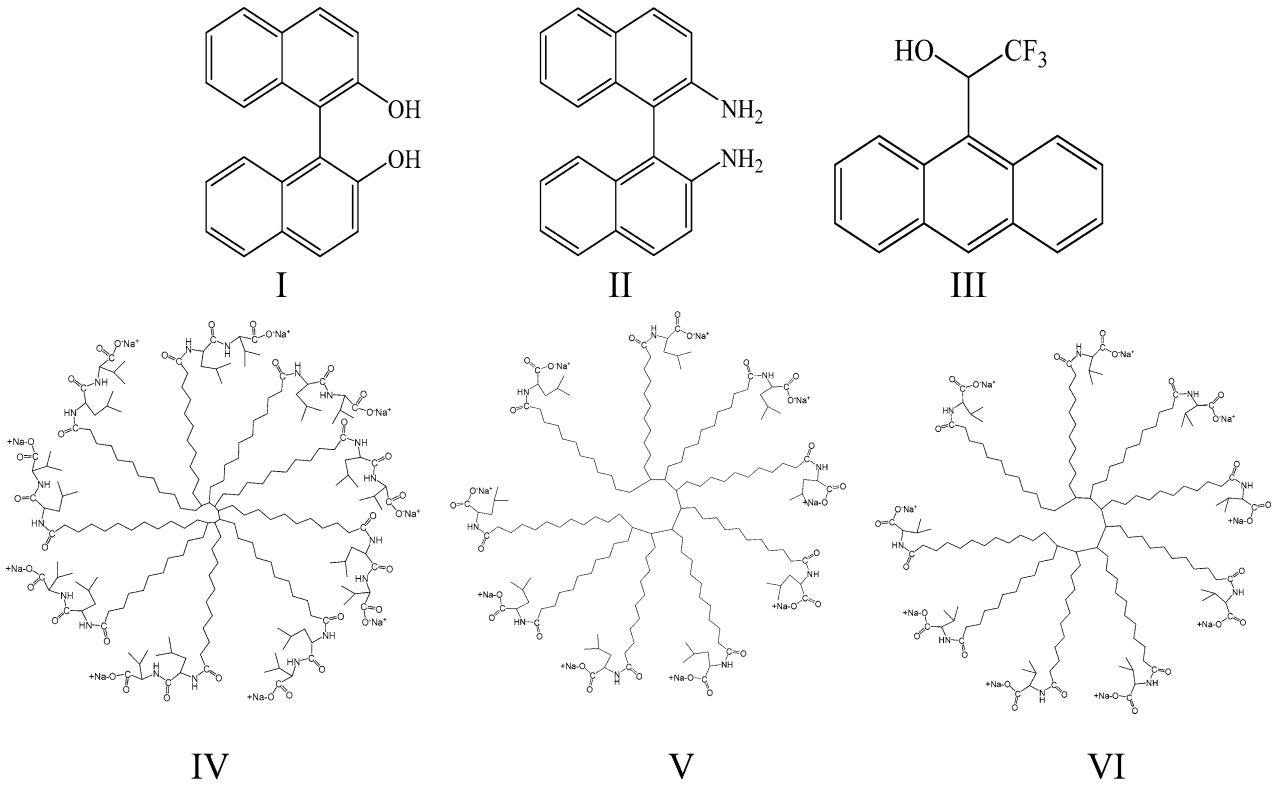

Fig. 1 Molecular structures of: (**I**) 1,1′-bi-2-naphthol; (**II**) 1,1′-binaphthyl-2,2′-diamine; (**III**) 2,2,2,-trifluoroanthrylethanol; (**IV**) poly(sodium *N*-undecanoyl-L-leucylvalinate). (**V**) poly(sodium *N*-undecanoyl-L-leucinate); (**VI**) poly(sodium *N*-undecanoyl-L-valinate)

from Aldrich Chemical Company (Milwaukee, WI) and used as received. The methanol used in the study (ACS certified) was also obtained from Aldrich. Doubly-deionized water, used throughout in the study, was obtained from a PURELAB Ultra Genetic water polishing system (US Filter).

Surfactant synthesis

The synthesis, purification and characterization of poly-L-SULV, poly-L-SUL, and poly-L-SUV surfactant have been previously described [33].

Sample preparation

A solution containing 1.5% w/v of polymeric surfactant was made by dissolving 1.500 g of polymeric surfactant in 100 mL of doubly-deionized water or in a solution containing 100 mM Tris and 10 mM borate buffer at pH 10.0. Stock solutions of each enantiomer were made by accurately weighing appropriate amounts of each enantiomer and dissolving them in methanol. From the stock solution, appropriate concentrations (1×10^{-4} or 5×10^{-6} M) of the enantiomer solutions were made by transferring appropriate aliquots of the stock solution to a dry volumetric flask. After transfer, the methanol was then gently evaporated under a stream of ultra-high-purity nitrogen gas. The solution was then made up to the mark with 1.5% polymeric surfactant solution and sonicated for at least 20 min to ensure complete dissolution of analyte. Following dissolution, the samples were allowed to equilibrate for 15 min.

Training-set samples and calibration-set samples were made for each chiral analyte so that for a given experiment, all solutions contained a fixed polymeric surfactant concentration and a fixed concentration of chiral analyte. The enantiomeric composition of the calibration samples was varied from 0.1 to 0.9 mol fraction. The samples were allowed to equilibrate for at least 30 min before the fluorescence emission spectra of the samples were recorded.

Instrumentation

The fluorescence emission of each sample was recorded using a spectrofluorometer (SPEX Fluorolog-3) equipped with double excitation and emission monochromators. A 400 W Xe-arc lamp was used for excitation and a thermoelectrically cooled Hamamatsu R-928 photomultiplier tube, operating in the photon-counting mode, was used for detection. All data were collected using a 0.4-cm path length quartz cuvet.

Data analysis

The mean-centered spectral data were subjected to multivariate regression analysis using commercial chemometric software (The Unscrambler™ vers. 9.1; CAMO, Inc., Corvalis, OR). Partial-least-square-regression models (PLS-1) were developed from the spectral data and initially evaluated with full-cross validation. Full-cross validation (also known as leave-one-out validation) is a computer-generated form of validation that makes as many sub-models as there are samples, with each sub-model leaving out one of the samples for testing. Full-cross validation is not ideal, however, and it should be stressed that all regression models presented here were subsequently validated with new independently-prepared test sets of validation samples.

Results and discussion

Study with poly-L-SULV polymeric surfactant

Figure 2a shows fluorescence emission spectra ($\lambda_{ex} =$ 380 nm) of 1×10^{-4} M (*R*)- and (*S*)-BOH enantiomers in 1.5% w/v poly-L-SULV. Although the concentrations of both enantiomers are the same, they have notably different emission spectra in the presence of poly-L-SULV. The differences in the spectra of the enantiomers shown in Fig. 2a can be attributed to different noncovalent enantiomeric interactions within the micellar environment of the chiral polymeric surfactant. Such differences in enantiomeric interactions with the chiral poly-L-SULV surfactant will ultimately produce diastereomeric effects that influence the spectra. As expected, there was no apparent difference in the spectra of the two enantiomers in the presence of achiral poly-(sodium *N*-undecylenic sulphate) surfactant.

Figure 2b shows the fluorescence emission spectra obtained for a set of eight solutions containing a fixed BOH analyte concentration (1×10^{-4} M) of various enantiomeric composition in the presence of chiral poly-L-SULV surfactant. The samples have maximum emission at 445 nm. Although the BOH concentration was fixed, the fluorescence emission intensity of the spectra varied with the enantiomeric composition of the BOH samples. Samples containing different ratios of the (*R*)- and (*S*)-enantiomers will produce different diastereomeric effects, resulting in spectra that vary with enantiomeric composition.

Better insight into the spectral variations that occur with different enantiomeric compositions can be gained from a plot of the mean-centered spectra (Fig. 2c). The mean-centered plot was obtained by averaging the spectra of the eight solutions and then subtracting this average spectrum from the spectrum of each individual sample on a wavelength-by-wavelength basis. Figure 2c is interesting because the spectra of samples containing enantiomeric mol fractions of (*R*)-BOH less than 0.5 are above the origin of the graph while those containing mol fractions of (*R*)-BOH greater than 0.5 are below.

Fluorescence emission spectra ($\lambda_{ex} = 365$ nm) of 1×10^{-4} M (*R*)- and (*S*)-BNA in the presence of poly-L-SULV are shown in Fig. 3a. As observed for BOH, the (*R*)- and (*S*)-BNA enantiomers have different spectra in the presence of poly-L-SULV. With this analyte, the two enantiomers have the same general spectral profile, but the fluorescent intensities observed for the two enantiomers are different. In addition, while the (*S*)-BOH isomer produced the most fluorescence intensity in the study with poly-L-SULV (Fig. 2a), the opposite was observed for BNA in the presence of poly-L-SULV.

Figure 3b shows the fluorescence emission spectra of the eight solutions containing 1×10^{-4} M of BNA of various enantiomeric composition in the presence of poly-L-SULV. The samples have a maximum fluorescence emission at 412 nm. Once again, a variation of emission spectral intensity with enantiomeric composition of BNA analyte is observed. The mean-centered spectral plot for the BNA samples is shown in Fig. 3c. In contrast to the mean-centered spectral plot obtained for the BOH samples, samples containing enantiomeric compositions of (*R*)-BNA less than 0.5 mol fraction are below the origin, while those containing less than 0.5 mole fraction of (*R*)-BNA are above.

Figure 4a shows the fluorescence emission spectra of (*R*)- and (*S*)-TFA enantiomers in the presence of poly-L-SULV ($\lambda_{ex} = 380$ nm). In this case, only a slight change in emission intensity is observed for the two enantiomers. The emission spectra of seven solutions containing a fixed concentration of TFA analyte (1×10^{-4} M) of various enantiomeric compositions in the presence of poly-L-SULV is shown in Fig. 4b. The maximum fluorescence emission for the TFA samples was at 414 nm. Again, the spectra of the various TFA samples depend on the enantiomeric composition of the samples. Compared to the variations observed in the emission spectra of BOH and BNA (Figs. 2b and 3b), however, changes in the emission intensity observed with the TFA samples are somewhat smaller. As with BOH, the mean-centered emission spectra of samples containing mol fractions of (*R*)-TFA less than 0.5 are above the origin of the graph, while those containing more than 0.5 mol fraction of (*R*)-TFA are below.

Multivariate regression methods have been widely used for correlating small spectral changes with known compositional changes, and the methods are well established in analytical chemistry [34–37]. Multivariate regression modeling is a two-phase process. In stage one, or the calibration phase, spectra of a training set of known composition (i.e. the enantiomeric composition of the analyte in this study) are collected over a given wavelength range. Then a regression model is developed to correlate the changes in the

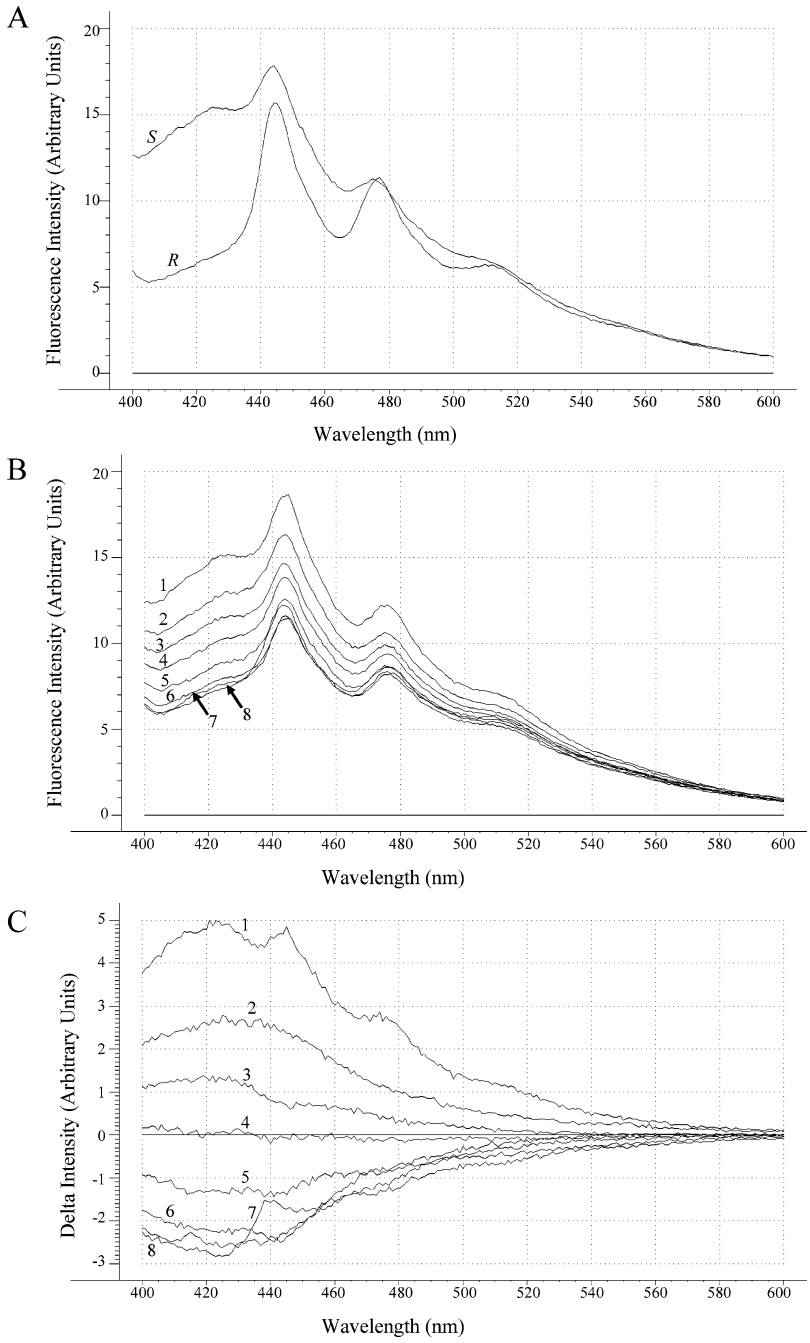

Fig. 2 **A** Fluorescence emission spectra ($\lambda_{ex} = 380$ nm) of 1×10^{-4} M BOH enantiomers in the presence of 1.5% w/v poly-L-SULV. **B** Fluorescence emission spectra of solutions containing 1.5% w/v poly-L-SULV and 1×10^{-4} M BOH of various enantiomeric compositions. Mol fraction of (*R*)-BOH: (1) 0.1; (2) 0.3; (3) 0.4; (4) 0.6; (5) 0.7; (6) 0.8; (7) 0.9; (8) 0.95. **C** Mean-centered spectral plot of solutions containing 1.5% w/v poly-L-SULV and 1×10^{-4} M BOH of various enantiomeric compositions. Mol fraction of (*R*)-BOH: (1) 0.1; (2) 0.3; (3) 0.4; (4) 0.6; (5) 0.7; (6) 0.8; (7) 0.9; (8) 0.95

fluorescence emission spectral data with the known compositions of the training-set samples. In the second stage, or validation phase, the regression model developed in the calibration phase is validated with a new, independently prepared test- or validation-set of samples of known enantiomeric composition. It must be stressed that while the analyte concentration in the validation- and calibration-sample sets must be the same (i.e., 1×10^{-4} M in this study), the two sets must contain samples with different enantiomeric compositions. In the validation phase, the spectra of the validation samples are taken over the same wavelength range that was used to prepare the model in the calibration phase. The enantiomeric compositions of the validation samples are then predicted from the spectral data using the model developed in the calibration phase. The performance of the model in predicting future samples is evaluated by how well the predicted enantiomeric compositions compare with their actual values.

The correlation coefficient, the slope, and the offset obtained from the PLS-1 regression modeling of the BOH samples in the presence of poly-L-SULV surfactant were 0.9986, 0.9972 and 1.69×10^{-3}, respectively. In the study

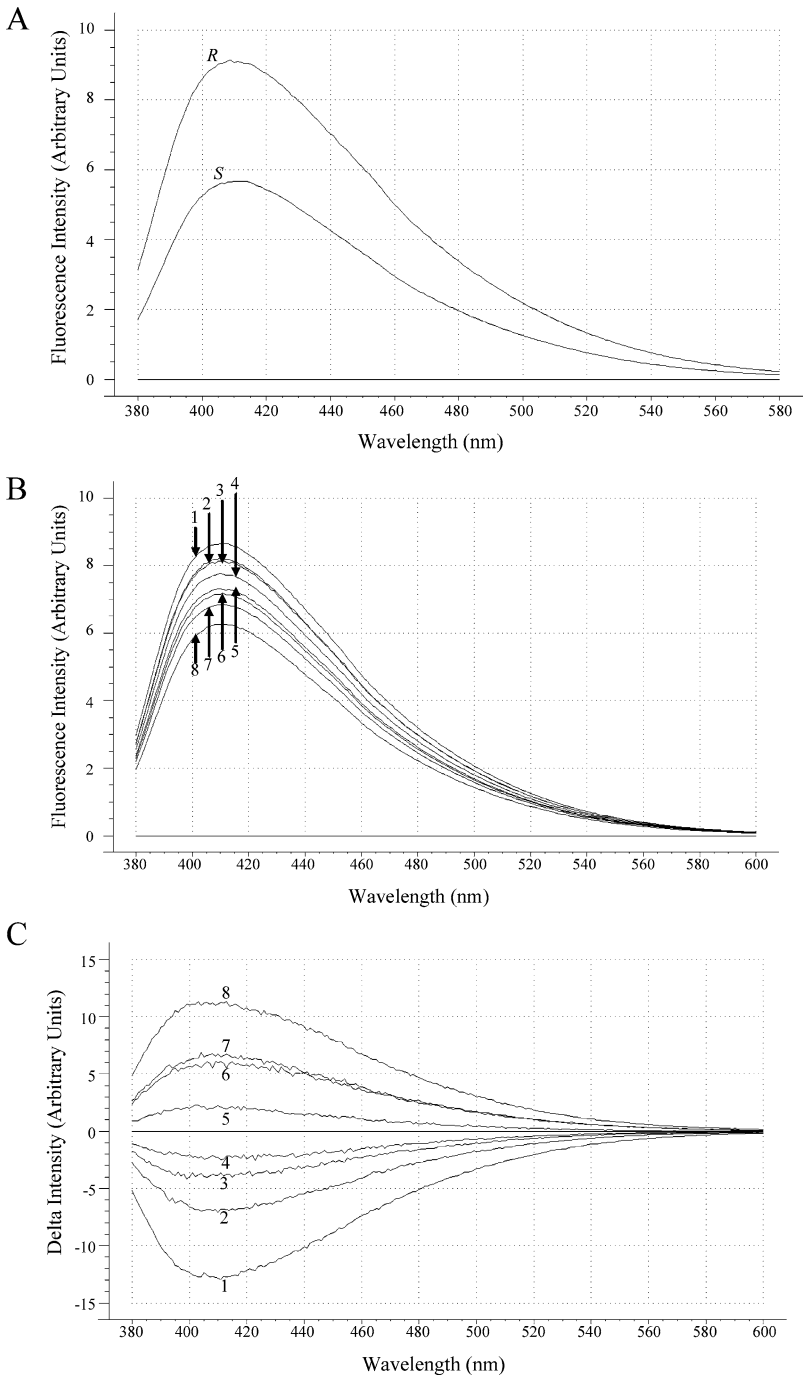

Fig. 3 **A** Fluorescence emission spectra ($\lambda_{ex} = 365$ nm) of 1×10^{-4} M BNA enantiomers in the presence of 1.5% w/v poly-L-SULV. **B** Fluorescence emission spectra of solutions containing 1.5% w/v poly-L-SULV and 1×10^{-4} M BNA of various enantiomeric compositions. Mol fraction of (*R*)-BNA: (1) 0.115; (2) 0.350; (3) 0.450; (4) 0.500; (5) 0.650; (6) 0.750; (7) 0.820; (8) 0.980. **C** Mean-centered spectral plot of solutions containing 1.5% w/v poly-L-SULV and 1×10^{-4} M BNA of various enantiomeric compositions. Mol fraction of (*R*)-BNA: (1) 0.115; (2) 0.350; (3) 0.450; (4) 0.500; (5) 0.650; (6) 0.750; (7) 0.820; (8) 0.980

with BNA, a correlation coefficient, a slope, and an offset of 0.9979, 0.9959 and 2.37×10^{-3}, respectively, were obtained. In the study with the TFA samples in the presence of poly-L-SULV, a correlation coefficient of 0.9989, a slope of 0.9979 and an offset of 1.15×10^{-3} were obtained. A perfect model would have a correlation coefficient of 1, a slope of 1, and an offset of 0. As expected, better correlations of the spectral data with the enatiomeric composition of the analytes were obtained in the wavelength regions that showed the most variation in the spectral data obtained with the training set of samples.

While the regression parameters for the different models look quite good, the real test of any regression model is its ability to correctly predict the composition of future samples. To test the prediction ability of the models, the models were validated with sets of independently prepared validation samples of known enantiomeric composition. For this purpose, new sets of sample solutions containing 1×10^{-4} M of each analyte were prepared in 1.5% w/v poly-L-SULV,

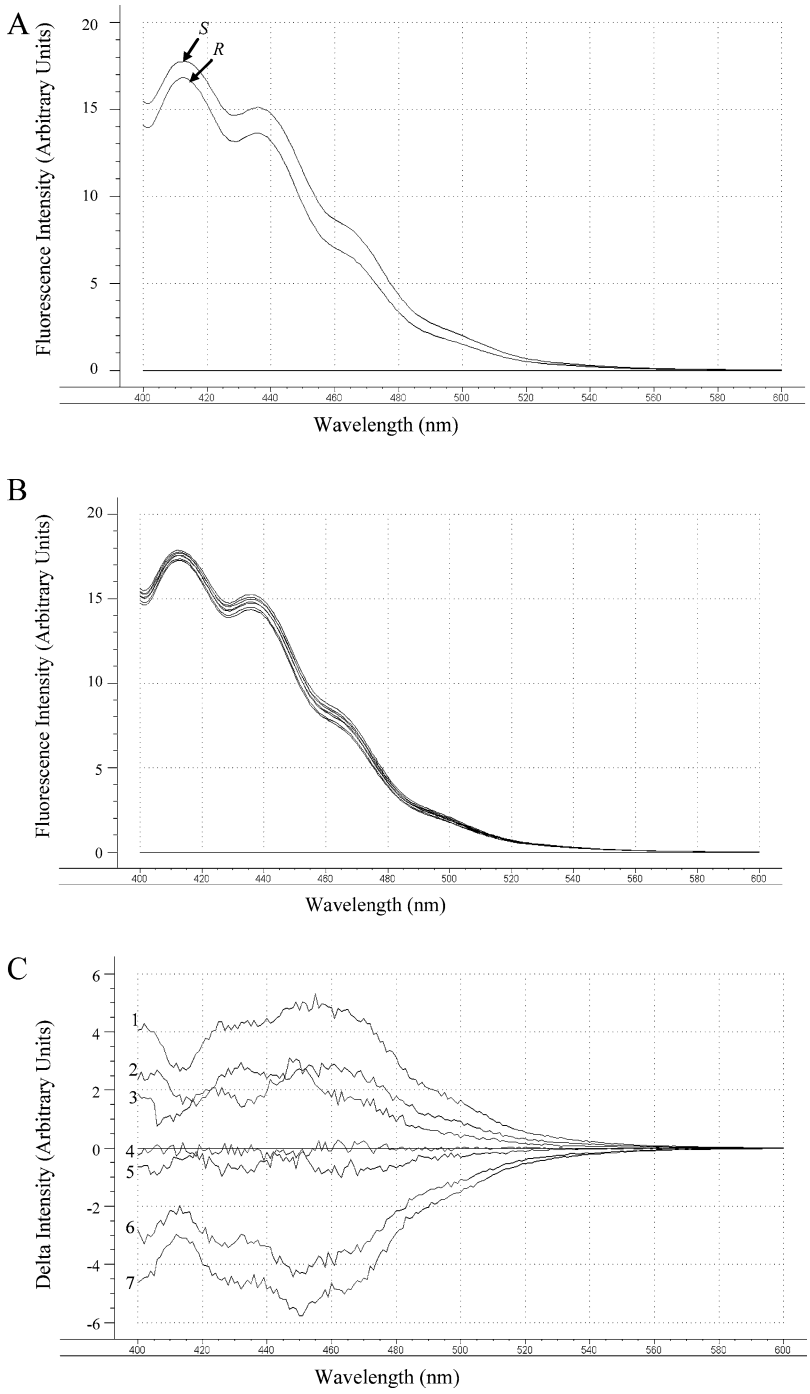

Fig. 4 **A** Fluorescence emission spectra ($\lambda_{ex} = 380$ nm) of 1×10^{-4} M TFA enantiomers in the presence of 1.5% w/v poly-L-SULV. **B** Fluorescence emission spectra of solutions containing 1.5% w/v poly-L-SULV and 1×10^{-4} M TFA of various enantiomeric compositions. Spectra too close to label individually. **C** Mean-centered spectral plot of solutions containing 1.5% w/v poly-L-SULV and 1×10^{-4} M TFA of various enantiomeric compositions. Mol fraction of (*R*)-TFA: (1) 0.20; (2) 0.35; (3) 0.40; (4) 0.55; (5) 0.60; (6) 0.80; (7) 0.90

having different enantiomeric compositions from those used to prepare the regression models. The spectra of these samples were then recorded over the same wavelength region as used to develop the regression models. The results of the validation study for each guest are shown in Tables 1–3. The ability of the model to correctly predict enantiomeric composition of the validation samples was evaluated by use of the root-mean-square percent relative error (*RMS%RE*) given by

$$RMS\%RE = \sqrt{\frac{\sum (\%RE_i)^2}{n}}, \quad (1)$$

where $\%RE_i$ is the percent relative error calculated from the known and predicted values for the *i*th validation sample, and *n* is the number of validation samples in the set.

In the study with BOH, the *RMS%RE* for the ten validation samples was 2.78%. For the validation study with BNA,

Table 1 Actual and predicted mol fraction of (*R*)-1,1′-bi-2-naphthol for solutions containing 1×10^{-4} M 1,1′-bi-2-naphthol in 1.5% w/v Poly-L-SULV chiral selector

Actual mol fraction	Predicted mol fraction	Relative error (%)
0.283	0.290	2.47
0.318	0.320	0.63
0.465	0.444	− 4.52
0.501	0.487	− 2.79
0.628	0.637	1.43
0.792	0.763	− 3.66
0.846	0.876	3.55
0.957	0.978	2.19
0.978	1.008	3.07
0.989	0.982	− 0.71
RMS%RE		*2.78*

the *RMS%RE* was 3.81%, and for the validation study with TFA, the *RMS%RE* was 5.21%. While the results of the validation studies for the three analytes are quite good, they do depend somewhat on the analyte. In terms of *RMS%RE*, the validation result obtained for BOH was slightly better than those obtained for BNA and TFA. The analyte dependence of the *RMS%RE* observed in this study will ultimately depend on the extent of the interaction between the chiral analyte and the chiral selector. In this study, for example, BOH and TFA are partially anionic whereas BNA is neutral. Analyte differences like this will ultimately influence the interactions with the negatively charged poly-L-SULV, resulting in models with different predictive capabilities.

The ability of the model to accurately predict the enantiomeric composition of the validation samples depends on the extent of the spectral variation obtained with the test set of samples in the calibration phase. For example, comparing the spectral data in Figs. 2b, 3b and 4b for the calibration sets, the spectral variation with BOH and BNA in the presence of poly-L-SULV is much greater than that observed with the TFA samples.

Table 2 Actual and predicted mol fraction of (*R*)-1,1′-binaphthyl-2,2′-diamine for solutions containing 1×10^{-4} M 1,1′-binaphthyl-2,2′-diamine in 1.5% w/v Poly-L-SULV chiral selector

Actual mol fraction	Predicted mol fraction	Relative error (%)
0.125	0.131	4.80
0.347	0.337	− 2.88
0.465	0.439	− 5.59
0.511	0.493	− 3.52
0.634	0.647	2.05
0.792	0.787	− 0.63
0.846	0.807	− 4.61
0.978	1.027	5.01
0.995	0.974	− 2.11
RMS%RE		*3.81*

Table 3 Actual and predicted mol fraction of (*R*)-2,2,2,-trifluoroanthrylethanol for solutions containing 1×10^{-4} M 2,2,2,-trifluoroanthrylethanol in 1.5% w/v Poly-L-SULV chiral selector

Actual mol fraction	Predicted mol fraction	Relative error (%)
0.101	0.093	− 7.60
0.265	0.278	4.91
0.310	0.315	1.61
0.400	0.376	− 6.00
0.531	0.555	− 5.80
0.603	0.568	4.52
0.798	0.831	4.13
0.955	0.932	− 2.41
0.985	0.998	1.32
0.995	1.080	8.54
RMS%RE		*5.21*

The spectral differences observed with the calibration samples as the enantiomeric composition of the samples is varied will ultimately depend on the diastereomeric interactions that occur between the analyte and the chiral selector. While the exact details of these diastereomeric interactions are not known at this time, factors like the hydrophobicity of the analyte, the solubility of the analyte in the chiral poly-L-SULV micellar environment, the possibility of multiple analyte/surfactant interactions, the polarity, charge, and size of the analyte may all play a role in producing subtle spectral variations that depend on the enantiomeric composition of the analyte.

In the case of BOH and TFA, the hydrophilic hydroxyl groups can form hydrogen bonds with the carbonyl groups of poly-L-SULV. In the case of BNA, strong electrostatic interactions can also occur between the amine groups on BNA and the carbonyl groups of the poly-L-SULV surfactant. Unlike TFA, which has only one hydroxyl group, the two hydroxyl groups of BOH and two amine groups of BNA can simultaneously interact with the two carbonyl groups of poly-L-SULV, which may result in stronger diastereomeric interactions (because two sites are involved) for BOH and BNA.

Comparative study of single amino acid and dipetide based polymeric surfactants

The dipeptide poly-L-SULV surfactant used in the previous study has two chiral centers associated with the dipeptide composed of valine and leucine. To study the influence of the chiral surfactant on the diastereomeric micellar interactions with the analyte, two single-amino-based polymeric surfactants (poly-L-SUL and poly-L-SUV) were selected. By contrast with poly-L-SULV, the two single-amino-based polymeric surfactants (poly-L-SUL and poly-L-SUV) each have only one chiral center associated with the single amino acids on the respective polymeric surfactants.

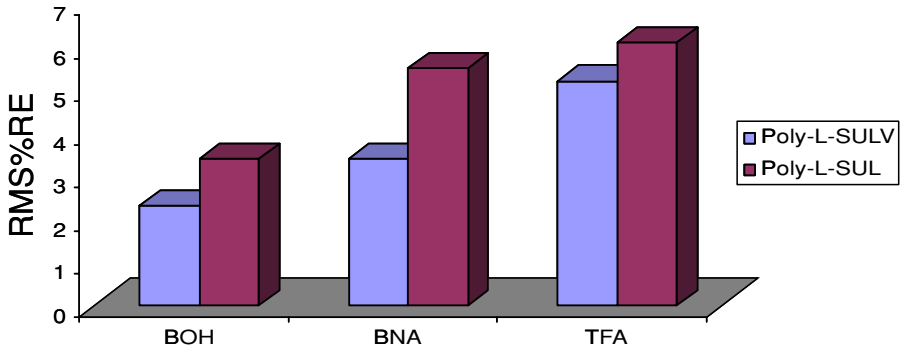

Fig. 5 *RMS%RE* for BOH, BNA, and TFA using poly-L-SULV and poly-L-SUL surfactants

In the studies with the single-amino-acid-based polymeric surfactants, the sample preparation and multivariate regression modeling were performed as described in the study with poly-L-SULV. In these studies, spectral variations were observed with test sets of BOH, BNA, and TFA of varying enantiomeric composition for samples containing poly-L-SUL. By contrast, no notable spectral variations were observed with test sets of BOH and BNA when poly-L-SUV was used as chiral selector. As a result, it was not surprising that no reasonable model could be developed from the spectral data obtained with BOH and BNA in the presence of poly-L-SUV. Similar poor enantiomeric resolution was observed for BOH and BNA in MEKC studies [31] when poly-L-SUV was used as a chiral selector. By contrast, when poly-L-SUV was used as a chiral selector with TFA, reasonable spectral variations were observed for samples with various enantiomeric compositions.

The effectiveness of a given chiral selector to discriminate a given chiral analyte ultimately depends on the extent of the diastereomeric interactions that occur with the pair. This is a highly complex phenomenon and simple rationalizations may be inadequate to account for the results. However, having said that, it is interesting to speculate on the results obtained with the different chiral selectors.

The results observed with poly-L-SUV may be due to the difference in the chirality of BOH and BNA compared with TFA. BOH and BNA are both axially chiral and do not possess asymmetric carbons. In contrast, TFA does possess an asymmetric carbon that can interact with the single chiral center of poly-L-SUV. Thus, the difference with chiral molecules having an asymmetric carbon may be the result of the chirality of molecules with axial chirality extending over a greater portion of the molecule and is not confined to a particular molecular location or stereogenic center. As a result, molecules such as BOH and BNA with axial chirality will be undoubtedly more difficult to differentiate enantiomerically with a chiral selector since in order to induce diastereomeric interactions with the chiral selector, more than one interaction point (e.g. with poly-L-SULV, which has two chiral centers) may be required. Therefore, poly-L-SUV, with only one chiral center, may not be able to induce diastereomeric interactions of sufficient magnitude with BOH and BNA, but it can induce them in TFA. These observations are consistent with the chiral separation using this set of molecules in MEKC.

Figure 5 shows a bar graph that compares the *RMS%RE* values obtained for the regression models for BOH, BNA, and TFA in the presence of single-amino acid poly-L-SUL and dipetide poly-L-SULV. In all cases, better predictions of the enantiomeric composition of samples were obtained when dipetide poly-L-SULV was used as a chiral selector. While the two polymeric surfactants have slightly different hydrophobicities and molecular sizes, the major difference in the prediction ability of the regression models made with poly-L-SULV may be due to the fact that poly-L-SULV has two chiral centers. As discussed above, it would be expected that better chiral discrimination would result with a chiral selector that could simultaneously interact at two chiral centers with a chiral analyte, particularly one with axial chirality.

Guest-host complexation in tris/borate buffer medium

To study the influence of the solvent medium on the chiral discrimination with polymeric surfactants, a series of experiments was performed in Tris/borate buffered solutions. It is known, for example, that, in MEKC, the medium and pH can play a prominent role in the chiral discrimination capability of polymeric surfactants. Once again, the sample preparation and multivariate regression modeling were performed as described previously. However, in this study, the polymeric surfactants chiral selectors were prepared in a 100 mM Tris and 10 mM borate buffer solution at pH 10.0. This buffer condition was chosen for the study because Tris/borate buffer at pH 10.0 has been previously shown to be the optimum buffer condition for the separation of BOH and BNA in MEKC [32].

Figure 6 shows the summary of the *RMS%RE* values obtained from the validation studies when buffered and unbuffered solutions of the polymeric surfactants were used as chiral selectors. As shown in the figure, in all cases better

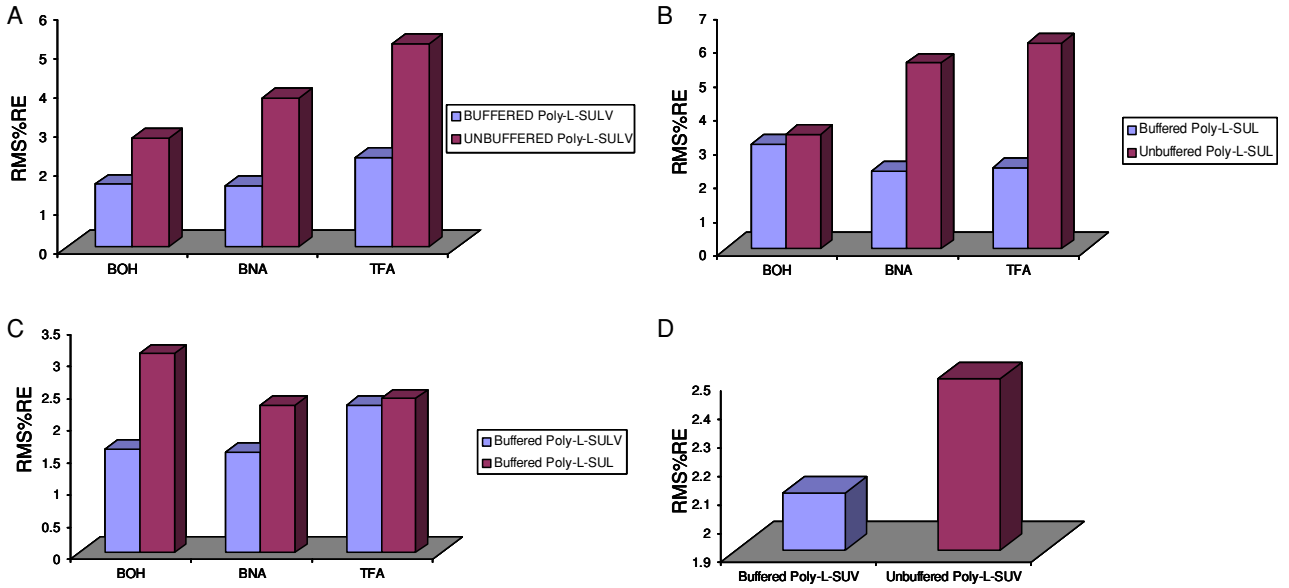

Fig. 6 *RMS%RE* for BOH, BNA, and TFA with various surfactants in buffered and unbuffered solutions. **A** Using poly-L-SULV. **B** Using poly-L-SUL. **C** Using poly-L-SULV and poly-L-SUL. **D** Study with TFA using poly-L-SUV

predictions were obtained when Tris/borate buffered solutions were used. In agreement with the results of the comparative study of poly-L-SULV and poly-L-SUL for BOH and BNA in ordinary aqueous solution, better predictions were obtained for BOH and BNA when poly-L-SULV was used as a chiral selector in a Tris/borate buffered solution (Fig. 6c). Fig. 6d shows the results of the validation study conducted for TFA when poly-L-SUV was used as a chiral selector in both aqueous and Tris/borate buffered solutions. In keeping with the results obtained for BOH and BNA, better results were obtained for TFA in the buffered solutions. The better regression models obtained in the buffered solutions may be attributed to differences in the charge of the analytes in the buffered solutions.

Effect of surfactant-to-analyte ratio

To determine the effect of surfactant concentration on the analytical results obtained by regression modeling, a series of experiments was carried out with BNA and TFA where the chiral analyte concentration was reduced to 5×10^{-6} M while keeping the concentration of poly-L-SULV constant at 1.5% w/v as in the previous studies. This was possible because of the high sensitivity afforded by using fluorescence spectroscopy with the highly fluorescent polynuclear aromatic chiral analytes. Once again, the sample preparation and multivariate regression modeling were performed as described previously. By lowering the chiral analyte concentration while keeping the concentration of the polymeric surfactant constant at 1.5 w/v%, the ratio of the surfactant concentration to the chiral analyte concentration is increased. In other words, there is more poly-L-SULV per mol of chiral analyte.

Figure 7 shows the predicted versus actual plots obtained from the regression models prepared with BNA and TFA. Validation studies conducted with these models for BNA and TFA gave *RMS%RE* values of 2.1 and 2.3%, respectively. Compared with the previous studies where higher analyte concentrations were used (1×10^{-4} M), the regression models made with lower chiral analyte concentrations (5×10^{-6} M) actually had better prediction capabilites (lower *RMS%RE* values). This result might be attributed to having a higher surfactant-to-analyte ratio, particularly if more surfactant leads to more micellar interactions with the analyte. Indeed, better selectivity and enatiomeric discrimination of chiral analytes have also been reported in MEKC at high surfactant concentration in background electrolytes [38].

Admittedly, further study of the diastereomeric micellar interactions is needed to fully understand how chiral polymeric surfactants serve as chiral selectors. The use of fluorescence anisotropy and fluorescence lifetime studies to investigate these micellar interactions might give additional information and a clearer understanding of their nature. We are currently conducting studies in the laboratory along these lines, and the results of our findings will be reported in future manuscripts. Additionally, we are currently developing fluorescent chiral polymeric surfactants that may be used as universal chiral selectors for non-flourescent chiral analytes.

Potentially, with further development, this technique may be useful for the rapid- and high-throughput screening of hundreds of potential drug candidates by the pharmaceutical

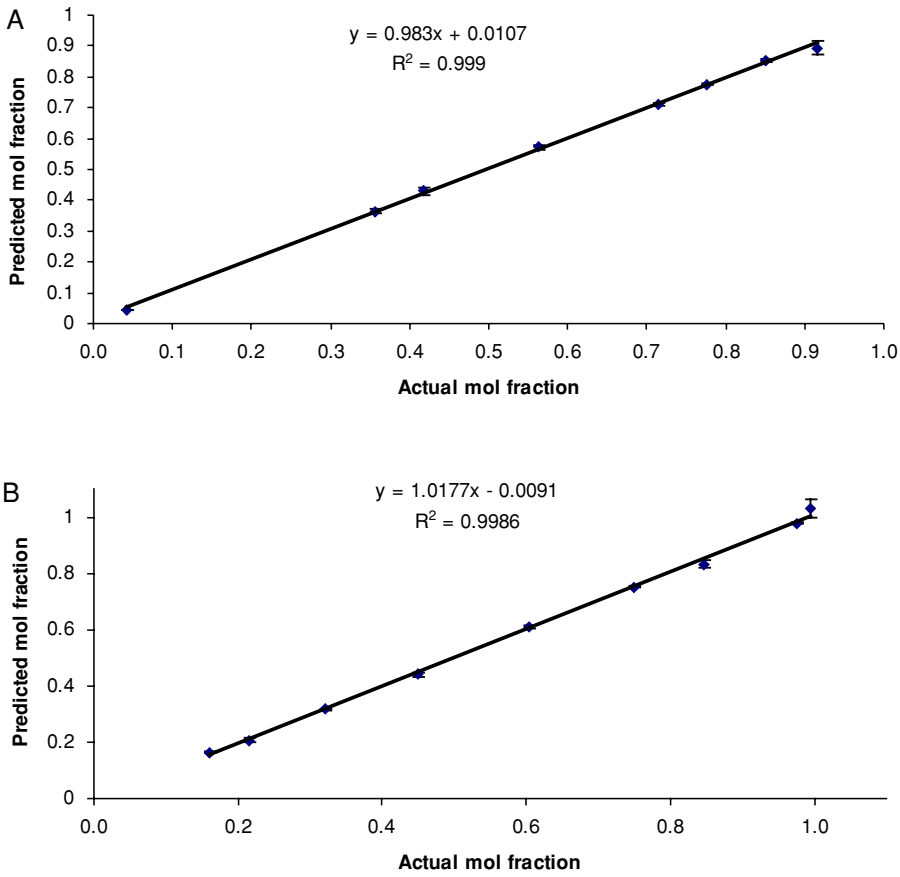

Fig. 7 Predicted mol fraction composition versus actual known composition for 5×10^{-6} M of (*R*)-enantiomer in 1.5% w/v poly-L-SULV chiral selector. **A** (*R*)-BNA. **B** (*R*)-TFA

industry and for routine analysis of racemates, pure enantiomers, and any intermediates in the manufacturing process. In addition, this technique is expected to be useful for the determination of enantiomeric composition of chiral pesticides and herbicides in the environment.

Conclusions

Chemometric modeling by PLS-1 regression analysis of steady-state fluorescence spectral data obtained for chiral analytes in the presence of chiral polymeric surfactants has been shown to produce regression models with good predictive abilities. The ability of the model to correctly predict the enantiomeric composition of future samples was found to depend on the chiral analyte being analyzed, the chiral polymeric surfactant used, and the solvent medium used. Generally, better predictions were obtained when the dipetide polymeric surfactant (poly-L-SULV) was used as a chiral selector and when the samples were prepared in Tris/borate buffered solutions. Better predictions were also obtained when the concentration ratio of polymeric surfactant to chiral analyte was increased. Compared with chiral selectors like cyclodextrin, the use of chiral polymeric surfactants facilitated easy solubility of highly hydrophobic analytes that would not have been possible with cyclodextrin alone.

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