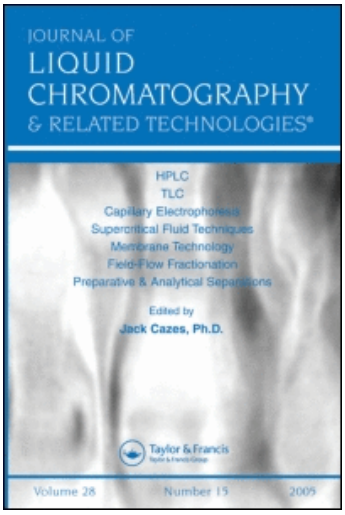

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CE RESOLUTION OF NEUTRAL AND ANIONIC RACEMATES WITH GLYCOPEPTIDE ANTIBIOTICS AND MICELLES

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ABSTRACT

The glycopeptide antibiotics vancomycin, ristocetin A and teicoplanin have been shown to be effective chiral selectors in capillary electrophoresis (CE). However, most of the resolved enantiomers were anionic or acidic compounds that contained carboxylic acid, phosphate, or sulfonate groups. Conversely, a large number of neutral racemates are resolved using these same chiral selectors attached to the stationary phase in HPLC. The problem in resolving neutral analytes in CE is outlined and discussed. Addition of sodium dodecyl sulfate (SDS) micelles to the run buffer greatly increases the available elution window, thereby facilitating the separation of neutral racemates. Neutral analytes resolved in this CE system include bromacil, bendroflumethiazide and 5-(4-hydroxyphenyl)-5-

phenylhydantion. The separations of coumachlor and warfarin are also demonstrated. In addition, the effect of surfactant concentration and organic co-solvents on elution time and enantioresolution are discussed.

INTRODUCTION

Macrocyclic antibiotics are the newest class of chiral selectors for capillary electrophoresis (CE) and liquid chromatography (1-9). The primary associative interaction between a chiral analyte and the enantioselective antibiotic appears to be electrostatic in nature (2,3,5). For example, rifamycin B (an anionic ansa compound) best resolves compounds containing amine functional groups (2). Conversely, glycopeptide antibiotics are most useful for resolving a variety of acidic or anionic analytes when used below their isoionic point (3,5). The additional secondary interactions needed for chiral recognition (depending on the solvent system used and the nature of the analyte) include one or more of the following: hydrogen bonding, dipolar interactions, hydrophobic interactions, π-π interactions and steric repulsion (1-4).

The use of micelles in CE separations also can be useful (8,10-12). The correct pseudophase model for the use of micelles plus a chiral selector (or any other pseudophase) in CE was elucidated recently (8). Sodium dodecyl sulfate (SDS) micelles tend to enhance the efficiency of many glycopeptide antibiotic CE separations by an order of magnitude (8). Also they change the elution order of all components including enantiomers (8). In this work we extend the use of the antibiotic, vancomycin, as a CE chiral selector to neutral molecules. This is done using an anionic micelle composed of sodium dodecyl sulfate surfactants. The theory and use of micelles in separations has been reviewed previously (12).

EXPERIMENTAL

Materials

Sodium dihydrogen phosphate, sodium hydroxide and potassium hydroxide were purchased from Sigma Chemical Company (St. Louis, MO). Electrophoresis grade sodium dodecyl sulfate was obtained from Bio-Rad (Richmond, CA). Warfarin, coumachlor, bendroflumethiazide and 5-(4-hydroxyphenyl)-5-phenylhydantoin were purchased from Aldrich Chemical Company (Milwaukee, WI). Bromacil was purchased from Chem Service (West Chester, PA). Vancomycin was generously provided by ASTEC (Whippany, NJ). Solvents were purchased from Fisher (St. Louis, MO). Deionized water was used to prepare all buffer solutions.

Methods

Separations were performed using a Beckman P/ACE 2000 capillary electrophoresis unit (Palo Alto, CA) or a Waters Quanta 4000 (Millford, MA). The P/ACE unit was used unless otherwise indicated. The P/ACE was equipped with a 50 mm x 37 cm (30 cm to the detector) fused silica capillary thermostated at 25°C. A 50 mm x 32.5 cm (25 cm to the detector) fused silica capillary at ambient temperature was used with the Quanta 4000. All separations were monitored at 254 nm. Phosphate buffers were prepared by adjusting the pH of sodium dihydrogen phosphate solutions with sodium hydroxide or hydrochloric acid. All phosphate buffers were prepared as 50 mM, pH 7.0 unless otherwise indicated. Samples were prepared at about 0.1 mg/mL in 50/50 methanol/buffer and injected using the pressure mode (0.5 psi) or the hydrostatic mode for 1 second. Capillaries were rinsed daily with 0.5 N potassium hydroxide for 15 minutes followed by water for 10

minutes and run buffer for 15 minutes. Between runs, the capillary was rinsed with 0.1 potassium hydroxide, water and run buffer for 2 minutes each. The run voltage for all separations was +5 kV. Organic solvent/phosphate buffer mixtures were prepared by volume. It should be noted that mixtures of SDS and vancomycin used in this study tend to precipitate in organic solvent/phosphate buffer solutions. These additives can be dissolved initially by sonication but will precipitate from solution within a few hours as evidenced by a deterioration in the electropherogram.

RESULTS AND DISCUSSION

The structure of vancomycin is shown in Figure 1. The properties of this macrocyclic antibiotic which make it a useful chiral selector in LC and CE have been described in detail previously (1,3,4). When used as an HPLC chiral stationary phase, vancomycin resolves a large number of neutral organic racemates as well as negatively charged analytes. However, in CE, only the enantioresolution of negatively charged racemates have been reported thus far.

The isoelectric point of vancomycin is ~ 7.2 (1). Consequently at pH 7.0, it elutes at nearly the same time as a neutral solute carried by the electroosmotic flow. This is illustrated in Figure 2A. Note that a neutral racemate would have to elute and separate in the very small window between the vancomycin peak and eof trough (Fig. 2). This expected elution profile assumes that there is no interaction between the solute-selector complex and the capillary wall. Lowering the pH to 5.0 increases the elution window and therefore the possibility of obtaining a separation to some extent (Fig. 2B). However, this still represents a very limited area in which to achieve a separation. The somewhat greater distance between the vancomycin peak and the eof trough at this pH results from the fact that electrophoretic mobility

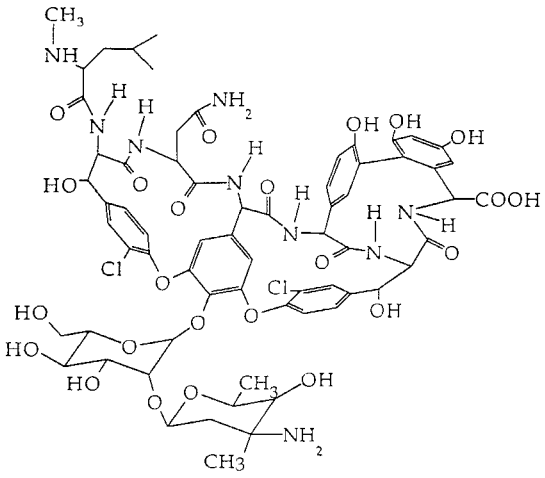

Figure 1. Structure of vancomycin (MW 1449). Note the semirigid aglycone basket composed of 3 fused macrocyclic rings as well as the pendant, freely rotating, disaccharide moiety.

of vancomycin becomes more positive as it acquires a greater positive charge. The addition of sodium dodecyl sulfate (above the CMC) to the run buffer has a more profound effect on the elution window for neutral solutes. This is shown in Figure 2C. Neutral racemates can elute and potentially resolve anywhere between the eof trough and the SDS peak. The elution profile shown in Figure 2C results from the fact that vancomycin binds to the oppositely charged micelle, thereby reversing its electrophoretic mobility. This is illustrated in Figure 3. Previous studies have shown that vancomycin is ~ 90% bound to the micelle under these experimental conditions (8). The analyte can now partition between three pseudophases (i.e., the vancomycin-SDS mixed micelle, the free vancomycin, and the bulk aqueous solution, Figure 3C). A complete theoretical and mathematical treatment of this pseudophase system was published recently (8). The same theory applies to micelle

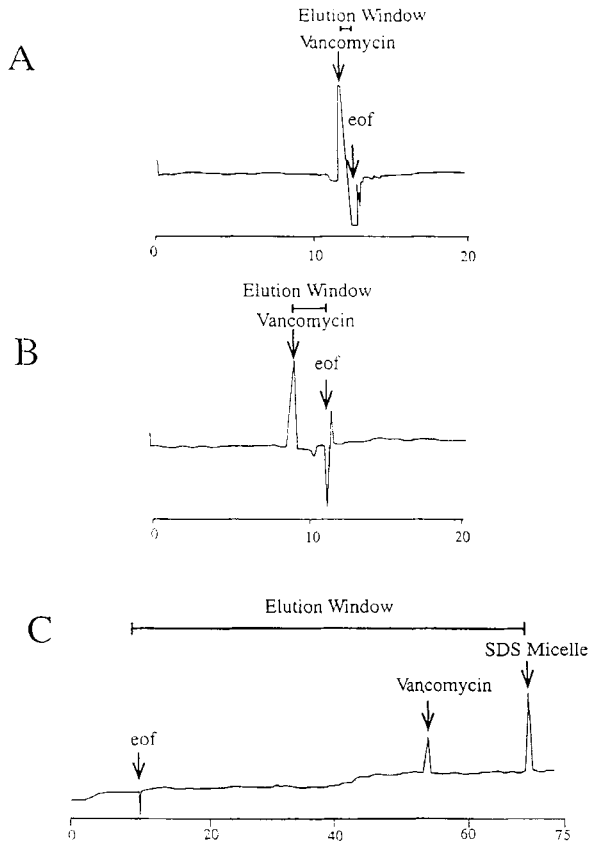

Figure 2. Capillary electropherograms showing the variation in the size of the elution window for neutral solutes in the presence and absence of SDS micelles. These examples do not consider the binding of vancomycin to the capillary wall. Run conditions are as follows: 2 mM vancomycin in phosphate buffer, voltage: +5 kV, capillary: 50 mm x 37 cm (30 cm to detector), detector wavelength: 254 nm.

(A) Demonstration of the small elution window that is available at pH 7.

(B) Example of the slightly extended elution window available for neutral solutes at pH 5.0. The run buffer was prepared from 0.1 M, pH 5.0 phosphate buffer.

(C) Demonstration of the greatly extended elution window brought about by using SDS micelles in the run buffer. The run buffer was prepared from 0.05 M pH 7.0 phosphate buffer containing 100 mM SDS.

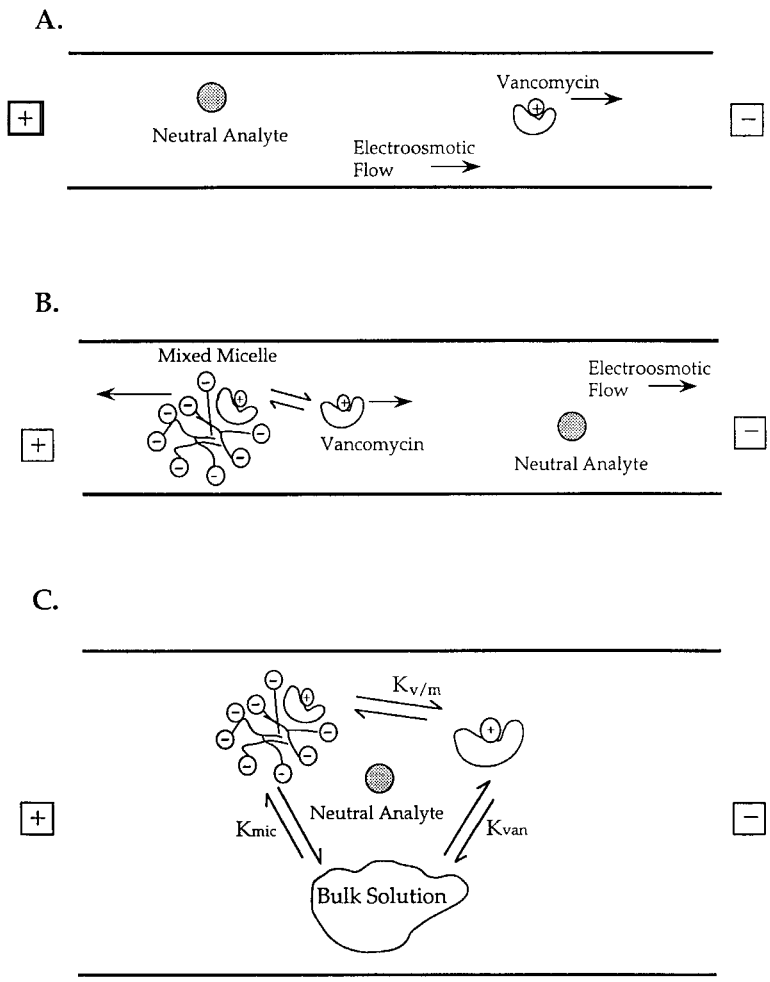

Figure 3. Representation of the electrophoretic mobilities of the neutral analytes, chiral selector and mixed micelles in: (A) buffer containing vancomycin, and (B) buffer containing vancomycin and SDS. Note that the migration time of vancomycin changes from about 8.8 min in phosphate buffer to 33.8 min in 21 mM SDS. (C) shows the pseudophase equilibria of neutral analytes between the bulk solution, the free vancomycin, and the mixed micelle.

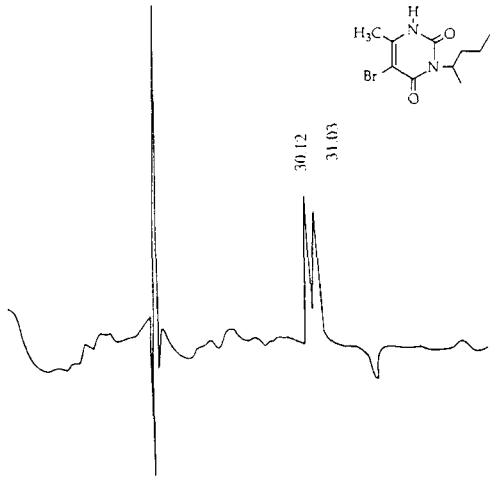

Figure 4. CE separation of the enantiomers of bromacil. Experimental conditions: 2 mM vancomycin, 25 mM SDS, 5% methanol, 95% 50 mM pH 7.0 phosphate buffer. See the experimental section for further details.

cyclodextrin systems, mixed cyclodextrin systems and CE systems that utilize a stationary phase or where wall interactions occur (8). Clearly the micellar association of the chiral selector causes a large change in the electrophoretic mobility of vancomycin. When higher SDS concentrations are used in the run buffer, the vancomycin peak in Fig. 2C elutes even closer to the SDS peak.

Figures 4-8 show the CE resolution of three neutral racemates (bromacil, bendroflumethiazide, 5-(4-hydroxyphenyl)-5-phenylhydantoin) and two anionic racemates (warfarin, and coumachlor) with the vancomycin-SDS system. With the exception of coumachlor, these compounds are not resolved in CE at pH 7.0 without the SDS. Figures 9 and 10 show the effect of SDS concentrations on retention and enantioresolution. In general, higher SDS concentrations increase retention but do not appreciably enhance enantioresolution.

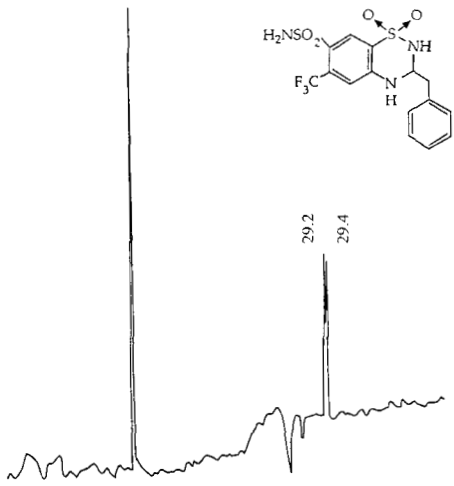

Figure 5. CE separation of the enantiomers of bendroflumethiazide. Experimental conditions: 2 mM vancomycin and 25 mM SDS in 50 mM pH 7.0 phosphate buffer. See the experimental section for further details.

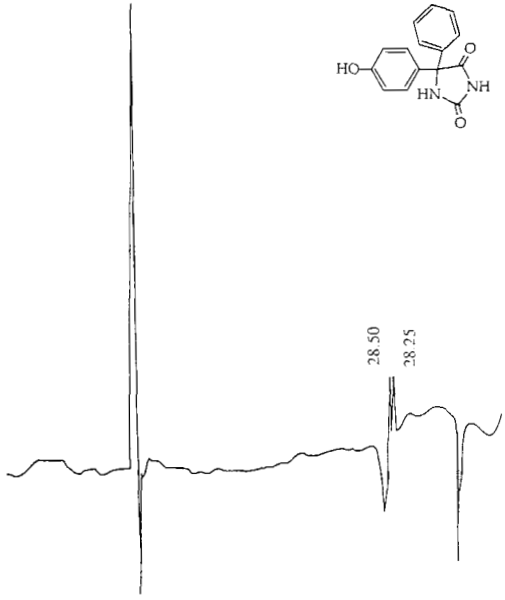

Figure 6. CE separation of the enantiomers of 5-(4-hydroxyphenyl)-5-phenylhydantoin. Experimental conditions: 2 mM vancomycin, 50 mM SDS in 50 mM pH 7.0 phosphate buffer. See the experimental section for further details.

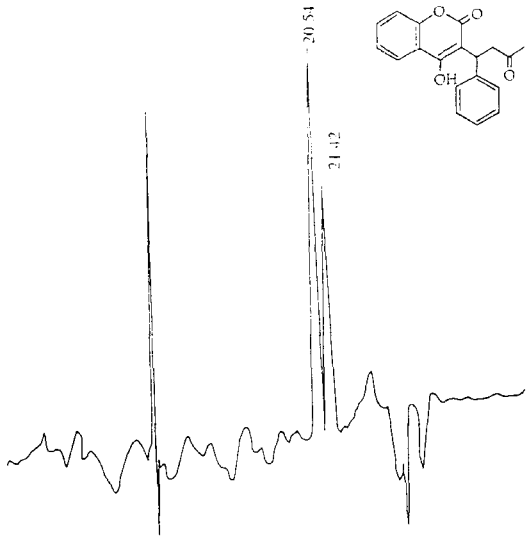

Figure 7. CE separation of the enantiomers of warfarin. Experimental conditions: 2 mM vancomycin, 25 mM SDS in 50 mM pH 7.0 phosphate buffer. See the experimental section for further details.

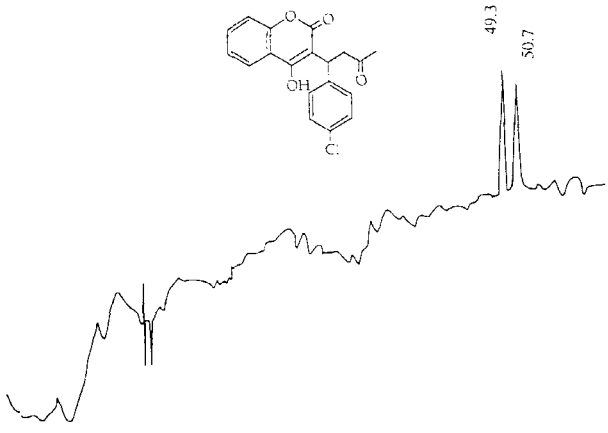

Figure 8. CE separation of the enantiomers of coumachlor. Experimental conditions: 2 mM vancomycin, 50 mM SDS, 10% acetonitrile, 90% 50 mM pH 7.0 phosphate buffer. See the experimental section for further details.

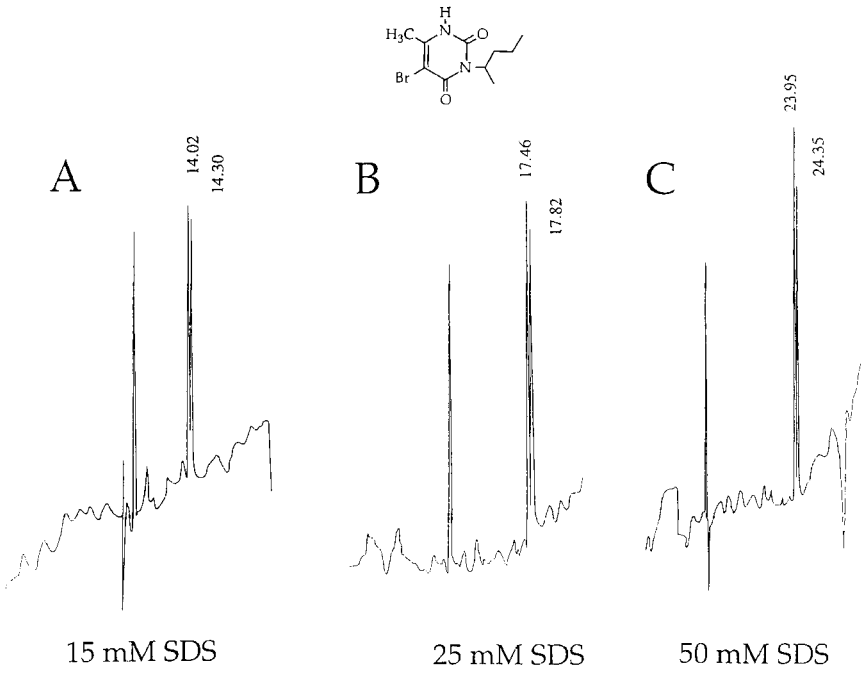

Figure 9. Effect of SDS concentration on the CE separation of the enantiomers of bromacil. Experimental conditions: 2 mM vancomycin with the indicated SDS concentration in 50 mM pH 7.0 phosphate buffer. See the experimental section for further details.

Figure 11 shows the effect of adding miscible organic co-solvents to the run buffer. Previous results have shown that the organic modifiers can sometime enhance enantioresolution in CE systems that utilize certain antibiotic chiral selectors (1,2). As was found previously, organic modifiers increase migration times by decreasing electroosmotic flow velocities. However, in every case with the SDS system, the enantioresolution was either unchanged or significantly impaired when

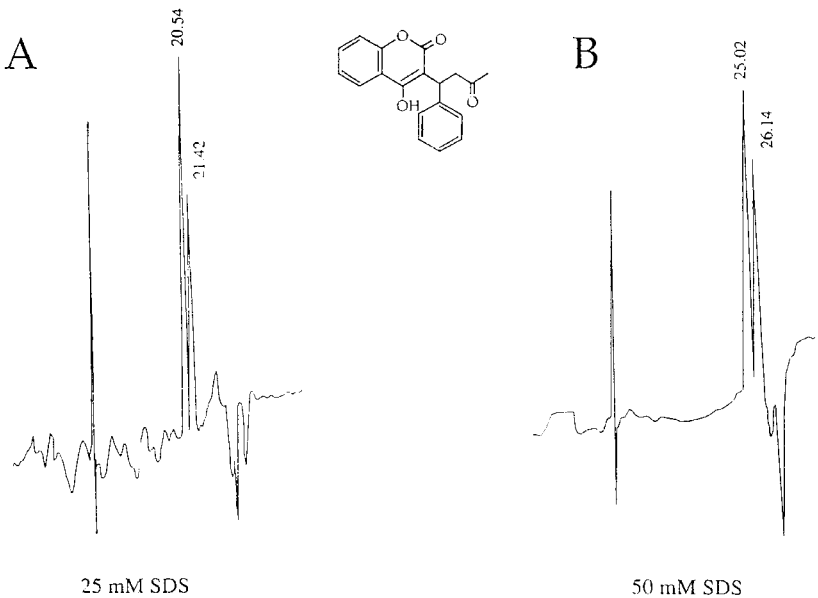

Figure 10. Effect of SDS concentration on the CE separation of the enantiomers of warfarin. Experimental conditions: 2 mM vancomycin with the indicated SDS concentration in 50 mM pH 7.0 phosphate buffer. See the experimental section for further details.

organic solvents were added. Organic solvents inhibit micelle formation by shifting the micellization equilibria to the monomeric form of the surfactant. It also disrupts the solbulization equilibira of the analytes with the micelles.

Figure 12 depicts the enantioresolution of warfarin and coumachlor at pH 4.0 in the absence of SDS. These enol compounds are anionic at pH 4.0. Note that the peaks are broad indicating poor efficiency. This might be explained by the fact that vancomycin, in the absence of SDS, binds to the capillary wall thereby creating a "dynamic stationary phase". Previously it was found that the addition of SDS to

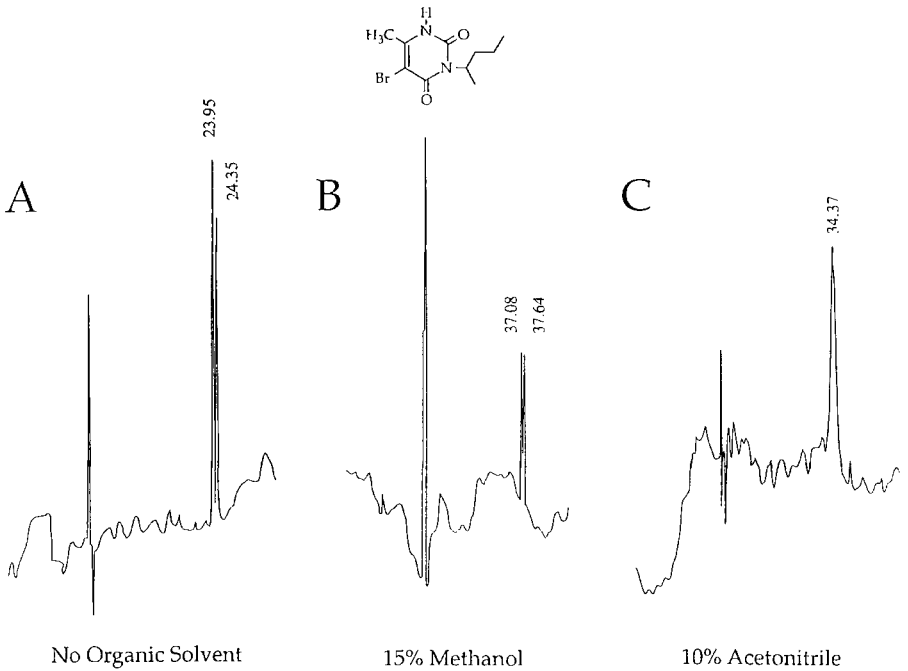

Figure 11. Effect of organic solvent on the CE separation of the enantiomers of bromacil. Experimental conditions: 2 mM vancomycin, 50 mM SDS in 50 mM pH 7.0 phosphate buffer with the indicated volume percent of organic solvent. See the experimental section for further details.

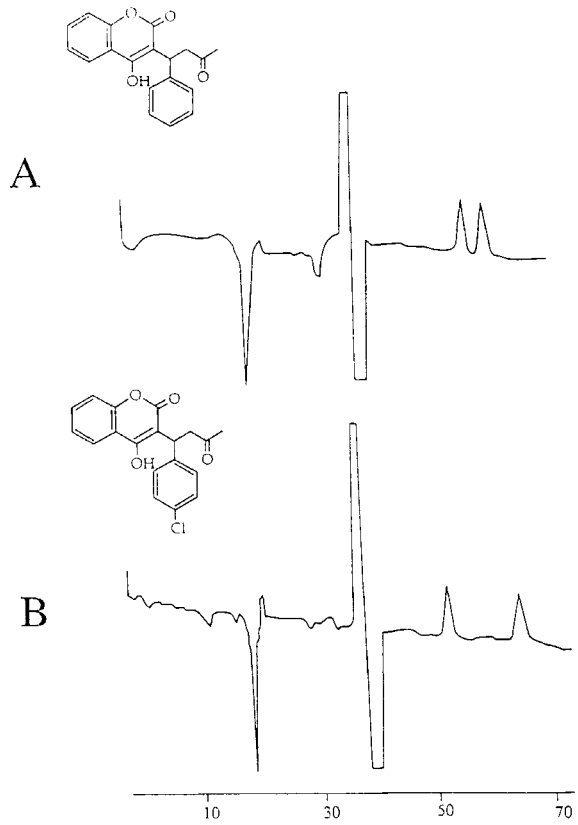

Figure 12 CE separation of the enantiomers of (A) warfarin and (B) coumachlor using vancomycin at pH 4.0 without micelles. The enantiomers elute outside the expected elution window for neutral compounds. This behavior may be due to the binding of the solute-selector complex with the capillary wall. Data was collected using a Waters Quanta 4000 capillary electrophoresis unit using a 50 mm x 32.5 cm (25 cm to the detector) at +5 kV and at ambient temperature.

vancomycin buffers resulted in a decrease in enantioresolution for dansyl-amino acids and nonsteroidal antiinflammatory compounds (8). In the case of the more hydrophobic compound, warfarin, enantioresolution was only observed at pH 7.0 when SDS micelles were added to the system.

CONCLUSION

Previous work demonstrated that the addition of SDS micelles to glycopeptide-mediated CE separations enhanced efficiency by an order of magnitude and reversed the elution order of all components (8). In this work it was further demonstrated that SDS allows the CE separation of neutral enantiomers that may not be resolved with vancomycin alone. Furthermore, the 3-phase model originally proposed for LC (13) and extended to CE (8) can be used to explain the elution behavior of these analytes as well as the expanded elution window that allows their resolution. Unlike many other antibiotic-based enantioseparations, organic modifiers do not improve enantioresolution even though they increase elution times.

ACKNOWLEDGMENT

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