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Review

Chiral separations using the macrocyclic antibiotics: a review

Timothy J. Ward*, Alton B. Farris III

Department of Chemistry, Millsaps College, 1701 North State Street, Jackson, MS 39210, USA

Abstract

The macrocyclic antibiotics have recently gained popularity as chiral selectors in CE, HPLC and TLC. The macrocyclic antibiotics used for chiral separations include the ansamycins, the glycopeptides, and the polypeptide antibiotic thiostrepton. Although not strictly considered macrocyclic antibiotics, the aminoglycosides are antibiotics that have been used for chiral separations in CE. More chiral analytes have been resolved using the glycopeptides than with the other macrocyclic antibiotics combined. The glycopeptides vancomycin, ristocetin A and teicoplanin have been used extensively as chiral selectors in CE, with ristocetin A appearing to be the most useful chiral selector followed by vancomycin and teicoplanin, respectively. The macrocyclic antibiotics have also been used as chiral bonded phases in HPLC, and HPLC stationary phases based on vancomycin, ristocetin A and teicoplanin have been commercialized. Ristocetin A seems to be the most useful glycopeptide HPLC bonded phase, but its greater expense can be a drawback. The macrocyclic antibiotics have been used with micelles to improve efficiency, provide unique selectivity, and extend the range of separations to neutral solutes. Changing the macrocyclic antibiotic used in CE or HPLC can significantly alter the enantioselectivity of the separations. In fact, the glycopeptide antibiotics are complementary to one another, where if a partial enantioresolution is obtained with one glycopeptide, there is a high probability that a baseline or better separation can be obtained with another.

Keywords: Reviews; Enantiomer separation; Macrocyclic antibiotics; Ansamycins; Glycopeptides; Polypeptides; Aminoglycosides; Antibiotics

Contents

*Corresponding author. Tel.: +1-601-9741-405; fax: +1-601-9741-401.
E-mail address: wardtj@millsaps.edu (T.J. Ward).

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1. Introduction

Chirality remains an important consideration for many compounds such as pharmaceuticals, biological molecules and agrochemicals to name a few [1]. In many cases, only one isomer in a chiral compound is responsible for the desired activity, while the other isomer may exhibit no therapeutic value and may potentially cause unsuspected adverse effects [1–3]. In the early 1980s, the routine and rapid analytical resolution of stereoisomers was relatively difficult. However, by the early 1990s, significant advancement in the field of separations was made so that rapid enantioseparations of optical isomers were becoming routine and commonplace. As understanding of the biological actions of compounds with respect to stereochemistry has grown, the necessity to investigate the pharmacological and toxicological properties of individual compounds has become more apparent. These advances in the field of separations coupled with the increased awareness of biological isomers have in part contributed to recent changes in the US Food and Drug Administration policy concerning the stereochemistry of drugs and chemicals [4].

Much of the groundbreaking work in chiral separations occurred in liquid chromatography (LC); in recent years, capillary electrophoresis (CE) has gained in popularity [5]. Most CE methods for the separation of chiral compounds have their origins in LC [2]. While CE represents a relatively new technique, most CE enantioseparations have tended to use the same chiral selectors as LC, specifically cyclodextrins and ligand exchange types. There are a number of reviews which examine chiral separations using CE [1–3,5–9]. Also, the number and choice of chiral selectors for CE are limited by their solubility and detection characteristics, making fewer types of chiral selectors available for CE than for LC. However, separations achieved in CE use solution-based rather than stationary phase-based chiral selectors as in LC. The small diameter capillaries used in CE dissipate heat effectively, allowing for the use of high voltages that result in rapid and efficient separations. These differences not only allow for greater efficiency but also make it possible to resolve enantiomers with exceedingly small selectivity factors using CE [5,6]. The ease with which the separation media can be changed in CE offers another advantage, enabling one to quickly and effectively alter the run buffer to screen various separation media at a minimum cost.

Few chiral selectors offer a high degree of selectivity for numerous compounds while providing good efficiency. The macrocyclic antibiotics are the most promising in this respect and, in fact, they have already had an immediate and significant impact on the field of separations since their introduction in 1994 by Armstrong [10]. Prior to 1994, approximately 90% of all chiral CE separations were performed with cyclodextrins or a cyclodextrin derivative [11]. Since their introduction, the macrocyclic antibiotics have been used in a variety of ways, i.e., high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC), CE and CE–mass spectrometry (MS) to separate a wide number of compounds [1,2,7,8,11–16]. The macrocyclic antibiotics represent a relatively new class of chiral selectors in separation science. They are not merely an additional class of chiral selector, producing similar separations to previous chiral selectors such as the cyclodextrins, but offer unique properties that allow the resolution of many classes of compounds often with far greater selectivity than previously thought possible. In this work we will examine the use of the macrocyclic antibiotics as chiral selectors in CE, HPLC and TLC.

2. The macrocyclic antibiotics: structure and physico-chemical properties

Macrocyclic antibiotics possess several characteristics that allow them to interact with analytes and serve as chiral selectors. They have a number of stereogenic centers and functional groups, allowing them to have multiple interactions with chiral molecules. They typically have molecular masses between 600 and 2200 and often have numerous functional groups. They may be acidic, basic, or neutral, and may have little or no UV–Vis absorbance. A number of these physical and chemical properties of the macrocyclic glycopeptides are listed in Table 1. The macrocyclic antibiotics can interact by hydrophobic, dipole–dipole, π–π interactions, hydrogen bonding, as well as steric repulsion [7,8]. One of the more important interactions is an ionic or charge-to-charge interaction [11,17]. In addition to the hydrophobic moieties, these molecules possess hydrophilic groups as well as a number of ionizable groups, giving them good solubility in aqueous solution. The most successful and most extensively used macrocyclic antibiotic chiral selectors have been the glycopeptides [7,18]. The ansamycins, the polypeptide thiostrepton and the aminoglycosides, fradiomycin, kanamycin and streptomycin, also have been used as chiral selectors [1,7,9].

2.1. The ansamycins

The ansamycins, rifamycin B and rifamycin SV, are of historical significance, in that they were the first chiral selectors used exclusively in CE prior to their use in HPLC. Rifamycin B was the first of these ansa compounds used in CE to effectively resolve a variety of amine-containing analytes [19]. They have a characteristic *ansa* structure consisting of a ring structure or chromophore spanned by an aliphatic bridge. The aliphatic chain can be highly substituted, and the ansamycins differ by the type and position of substituents on their naphthohydroquinone ring as shown in Fig. 1. The aliphatic chain of both compounds has an ethyl ester on C_{21} and a methyl ether C_{23}. The ansamycins most commonly used in chiral separations are rifamycin B and rifamycin SV [17,19]. Rifamycin B has been shown to be enantioselective towards cationic com-

Table 1
Physico-chemical properties of the glycopeptide antibiotics, avoparcin, ristocetin A, teicoplanin and vancomycin and the polypeptide antibiotic, thiostrepton[a]

Property	Avoparcin[b]	Ristocetin A[c]	Teicoplanin[d]	Vancomycin	Thiostrepton
Molecular mass	α=1907.6 β=1942	2066	1877	1449	1665
No. of stereogenic centers	32	38	23	18	17
No. of macrocycles	3	4	4	3	2
No. of sugar moieties	5	6	3	2	0
No. of aromatic rings[e]	7 (α=1, β=2)	7	7 (2)	5 (2)	2
No. of hydroxyl groups[f]	16 (4)	21 (4)	15 (4)	9 (3)	5
No. of amide linkages	6	6	7	7	10
No. of amine groups	3	2	1	2	1
No. of secondary amines	1	0	0	1	1
p*I*	7.5	7.5	4.2,6.5	7.2	N/A
Relative stability	>4 weeks	3–4 weeks	2–3 weeks	1–2 weeks	
Produced by	*Streptomyces candidas*	*Norcardia lurida*	*Actinoplanes teicomyceticus*	*Streptomyces orientalis*	*Streptomyces azureus*
Separation mode(s) used	CE	HPLC, CE	HPLC, CE	HPLC, CE, TLC, EFLC	HPLC

[a] Information taken from Refs. [7,8,11,12] and references therein.
[b] Avoparcin commonly exists as a mixture of two closely related compounds approximately 67% β-avoparcin and 33% α-avoparcin.
[c] Ristocetin A exists in two forms that differ by the number of carbons attached to one of its sugar groups.
[d] Teicoplanin exists as a mixture of five similar compounds that differ by the number of carbons and substituent groups on the fatty acid side chain attached to the amino sugar.
[e] The numbers in parentheses correspond to the number of chlorinated groups attached to the aglycon basket.
[f] The numbers in parentheses denote the number of phenolic substituents.

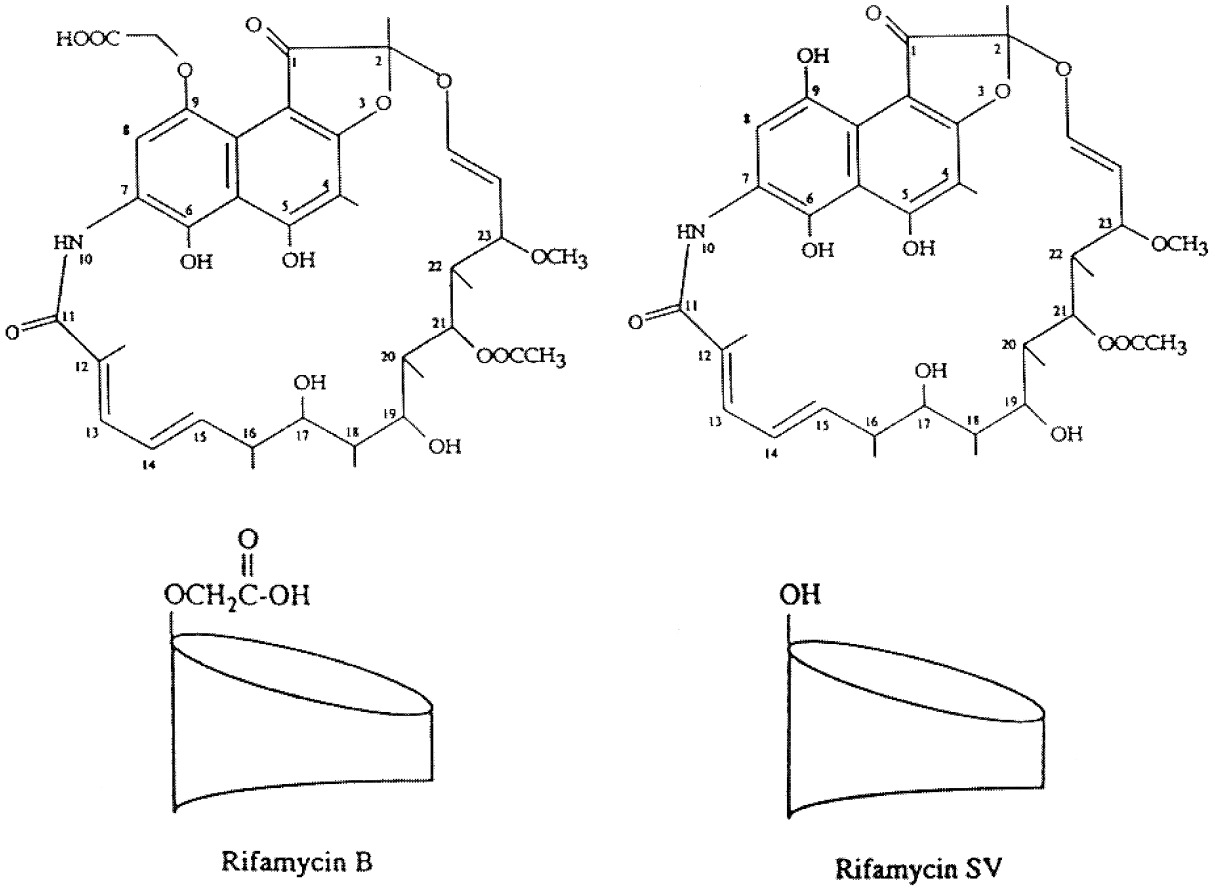

Fig. 1. Chemical structures, numbering sequence and simplified schematics for rifamycin B and rifamycin SV. Note that in the lower schematics, the narrow side on the right represents the aliphatic chain that spans the aromatic chromophore (which is represented as the longer left-hand side to which the acidic or phenolic OH group is attached) [45].

pounds, and rifamycin SV is enantioselective toward neutral and some anionic solutes [17].

The ansamycins, rifamycin B and SV, absorb strongly in the UV and visible spectral regions because of the naphthohydroquinone ring. Each compound has absorption maxima at approximately 220, 304 and 425 nm and minima at approximately 275 and 350 nm [8]. Because the rifamycins have a strong absorbance and are typically used at relatively high concentrations (20–25 m*M*), separations are usually monitored via indirect detection. Indirect detection generally results in negative peaks (a reduction in the absorbance from the high background signal) [17,19]. The physical and chemical properties of the ansamycins are listed in Table 2. It has also been shown that the ansamycins have aggregation properties, which tremendously affects their behavior in solution [17,19].

2.2. The glycopeptides

The macrocyclic glycopeptide antibiotics appear to be among the most successful chiral selectors used to date. They include avoparcin, ristocetin A, teicoplanin, vancomycin and two derivatized vancomycin analogs. All of these glycopeptides consist of an aglycon portion of fused macrocyclic rings that form a characteristic ‘‘basket’’ shape and carbohydrate

Table 2
Physico-chemical properties of the ansamycins, rifamycin B and rifamycin SV[a]

Property	Rifamycin B	Rifamycin SV
Solute types	Cationic	Anionic
Molecular mass	755	698
No. of stereogenic centers	9	9
No. of hydroxyl groups[b]	4 (2)	5 (3)
No. of aromatic rings	2	2
No. of amide linkages	1	1
No. of methoxy groups	2	2
No. of carboxylate groups	1	0
No. of methoxy-esters	1	1
Stability	1 week	1 week
Produced by	*Norcardia mediterranei*	*Norcardia mediterranei*
Separation mode(s) used	HPLC, CE	CE

[a] Information taken from Refs. [7,8].
[b] The numbers in parentheses denote the number of phenolic substituents.

moieties attached to the aglycon basket as shown in Fig. 2. The aglycon basket consists of three or four fused macrocyclic rings composed of linked amino acids and substituted phenols. The carbohydrate moieties consist of carbohydrate or saccharide groups. The glycopeptide antibiotics differ in the number and type of pendant carbohydrate groups [8]. The sugar groups attached to the aglycon basket are free to rotate and can assume a variety of orientations. Avoparcin, ristocetin A and teicoplanin are not pure compounds but exist as mixtures of known composition as shown in Table 1. Avoparcin and ristocetin A commonly exists as a mixture of two closely related compounds [20,21], while teicoplanin exists as a mixture of five similar compounds [11]. Teicoplanin is unique among the glycopeptides in that it has a hydrophobic acyl side chain attached to a 2-amino-2-deoxy-β-glucopyranosyl group. This hydrophobic acyl side chain helps to form a hydrophobic tail; therefore, teicoplanin is surface active and aggregates to form micelles unlike the other glycopeptide antibiotics [11].

The glycopeptide antibiotics like the ansamycins also have a strong absorbance in the UV region. In acidic solutions, the glycopeptide antibiotics absorb strongly below 250 nm and exhibit a small minimum around 260 nm [8,11]. Unlike the ansamycins, which require a concentration of approximately 20–25 m*M* in the run buffer, the glycopeptides effectively resolve most analytes at concentrations between 1 and 5 m*M* in the run buffer [7,18]. Although the glycopeptide antibiotics have a strong absorbance in the UV region, due to the low effective concentrations (1–5 m*M*) used in the run buffer, direct detection of most solutes is possible by operating near the absorption minimum, 260 nm [11,22]. This has proved to be quite advantageous for glycopeptide-based separations.

2.3. The polypeptides and aminoglycosides

Similar to the glycopeptides, the polypeptide antibiotic thiostrepton contains a number of aromatic ring structures and exhibits a strong absorbance in the UV region. It has a molecular mass of 1665 and contains five thiazole rings and one quinoline ring [12]. The aminoglycosidic antibiotics have low molecular masses and very low UV absorbance due to their lack of aromatic ring structures. Though these antibiotic chiral selectors are not macrocycles they will be briefly discussed. Kanamycin sulfate is produced from the bacteria *Streptomyces kanamyceticus*, streptomycin is produced from *S. griseus* [23], and fradiomycin is produced from *S. fradiae* [24]. They contain a number of glycosidic rings, three for kanamycin and streptomycin and four for fradiomycin. The apparent pK_a values of fradiomycin, kanamycin and streptomycin are reported to be 7.8, 7.2, and 8.7, respectively. They are

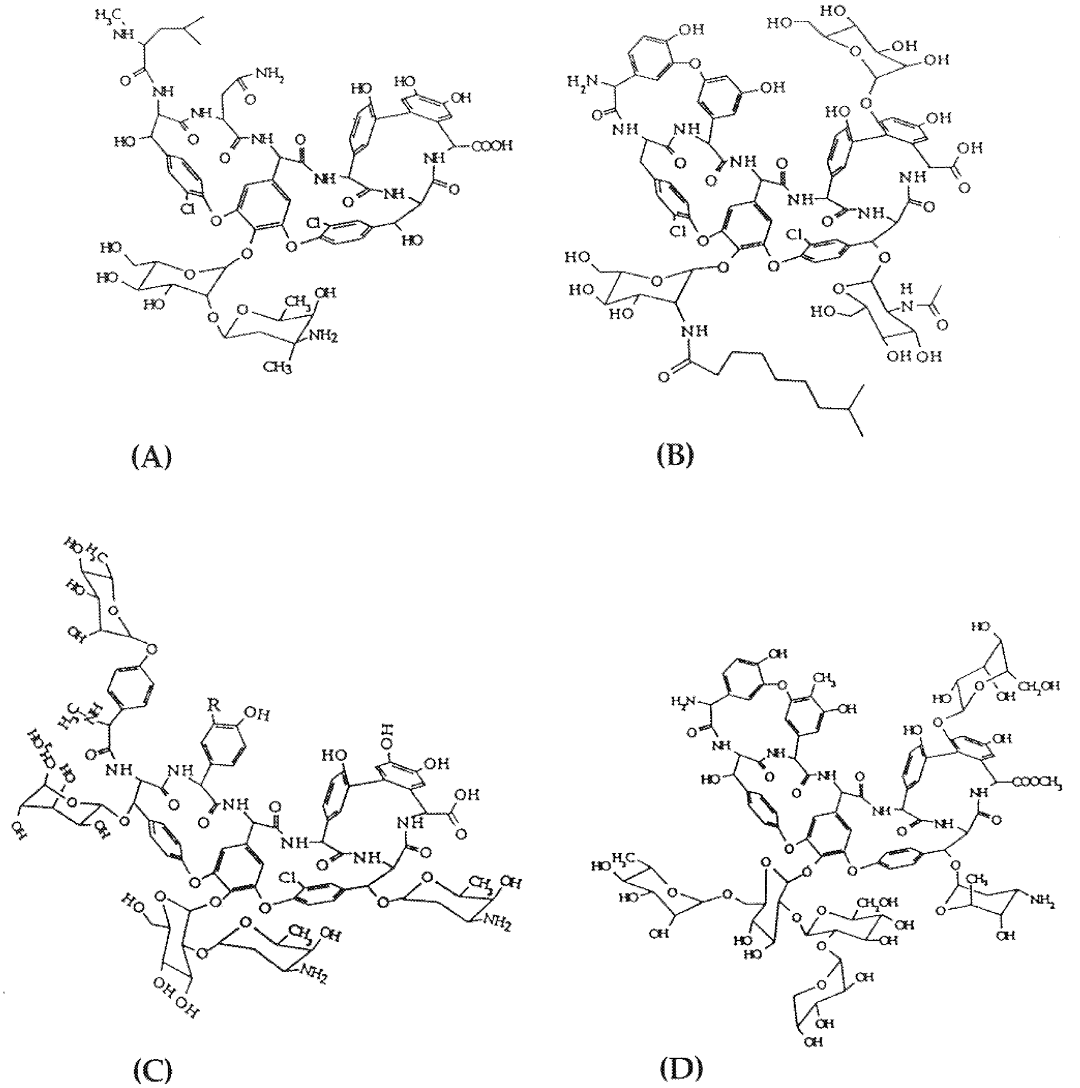

Fig. 2. Chemical structures of the macrocyclic glycopeptide antibiotics: (A) vancomycin, (B) teicoplanin, (C) avoparcin, (D) ristocetin A [7].

freely soluble in water, but practically insoluble in common alcohols and nonpolar solvents [25].

3. Capillary electrophoretic enantioseparations

3.1. The ansamycins

Although the ansamycins have not enjoyed the degree of success of the glycopeptides, two ansa compounds, rifamycin B and rifamycin SV, have been used successfully in CE [17,19]. Rifamycin B is useful for resolving hydrophilic amine-containing compounds making it complementary to the glycopeptides, which are particularly adept at separating anionic solutes. Rifamycin B is dibasic with pK_a values of 2.8 and 6.7 [19]. Thus, rifamycin B is negatively charged at the pH values commonly used and an electrostatic interaction between the anionic rifamcyin and cationic analytes is believed to be

important to achieve chiral recognition. Cationic analytes resolved using rifamycin B include vasoconstrictors, bronchodilators, vasodilators and β-adrenergic blockers. Armstrong et al. also examined other parameters such as buffer concentration, chiral selector concentration, ionic strength and organic modifiers [19]. Rifamycin SV was shown to be useful for separating some negatively charged analytes and is complementary to rifamycin B. Rifamycin SV is useful for resolving compounds containing at least two rings such as dansylaspartic acid, hexobarbital and glutethimide [17]. Interestingly as shown in Fig. 1, the only difference between these compounds is the location of a carboxylic acid group attached by a methylene moiety to the naphthohydroquinone ring.

Because the rifamycins have a strong absorbance and are typically used at relatively high concentrations (20–25 m*M*), separations are usually monitored via indirect detection. Using this detection method usually results in negative peaks (due to a decrease in absorbance from a high background signal), which places an upper limit on the concentration of chiral selector in the run buffer. At concentrations higher than 30 m*M*, background absorbance becomes excessive resulting in unacceptable signal-to-noise ratios. Ward et al. demonstrated that operating at one of the compound's UV minimum such as 275 nm, could minimize excessive background absorbance and thus significantly improve sensitivity [17].

Interestingly, no enantioselectivity was observed when organic modifiers were absent from the run buffer [19]. The ansamycins were able to enantioresolve compounds when approximately 10–40% organic modifier was added to the run buffer. This is believed to be due to the fact that rifamycin B and SV exhibit self-association behavior in solution much like the teicoplanin [7]. Like teicoplanin, it appears that organic modifier disrupts aggregation and significantly enhances enantioresolutions. One difference is that isopropanol was the most effective organic modifier with the ansamycins while acetonitrile was most effective with teicoplanin systems [7].

3.2. The glycopeptides

More chiral analytes have been resolved using the glycopeptides than the other types of macrocyclic antibiotics combined. They have proven enormously successful as chiral selectors for a number of reasons. The glycopeptides are amphoteric, containing ionizable acidic and basic groups, and they have various functionalities present that are conducive to chiral recognition. The glycopeptides have sufficiently low background absorbance at the concentrations employed in the run buffer to make direct detection feasible, and they contain hydrophilic and hydrophobic groups and are soluble in most buffers commonly used in CE, and are sufficiently stable in aqueous solutions and CE buffers [7].

All the glycopeptide antibiotics contain a number of ionizable groups, which control their charge and affect their chiral recognition of chiral analytes. The charge associated with the glycopeptides is governed by the pH of the run buffer. Fig. 3 shows how the glycopeptides' electrophoretic mobility varies as a function of pH [7]. The plots for ristocetin A, vancomycin and avoparcin are somewhat similar with isoelectric point (p*I*) values of approximately 7.2 for vancomycin, 7.5 for ristocetin A, and approximately 7.5 for avoparcin. Thus, the ionizable groups are positively charged at pH values below approximately 7.5. The curve for teicoplanin is fairly flat

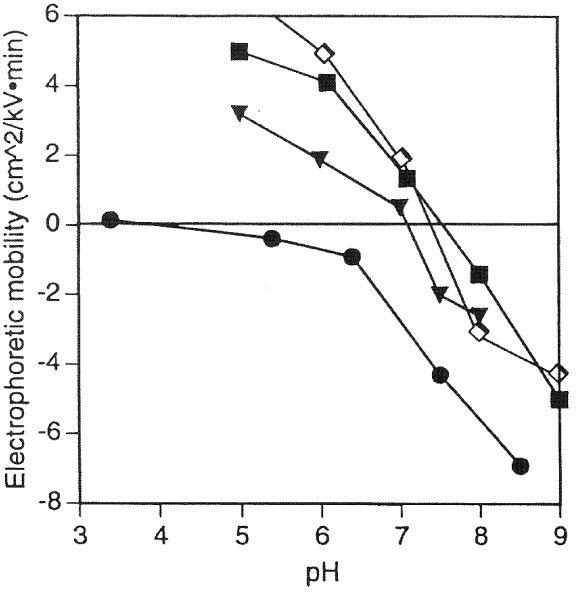

Fig. 3. Plot showing the effect of pH on the electrophoretic mobility of ristocetin A (■), vancomycin (▼), teicoplanin (●), and avoparcin (♦) [45].

between pH 6.5 and 3.0 and does not appear to begin to acquire a positive until the pH approaches 4.5. It is apparent that the electrophoretic mobility versus pH curve and the p*I* of teicoplanin is significantly different from the other glycopeptides. There are at least two factors that are believed responsible for the differences in teicoplanin's electrophoretic mobility behavior. First, teicoplanin does not possess the additional amine moiety on its pendant sugar groups, both are *N*-acylated (see Fig. 2), and second, teicoplanin is reported to self-associate and form micelles in solution [26]. It is well known that self-association affects pK_a values, p*I* values, spectral properties, solution properties, etc., for many compounds.

The macrocyclic antibiotics have been shown to be particularly selective toward molecules with anionic or acidic groups such as carboxylate, phosphate, and sulfonate moieties. Gasper et al. have demonstrated that when these groups are either α or β to the stereogenic center there appears to be an enhancement in enantiorecognition [11]. This is especially true when a chiral analyte contains a carbonyl group or aromatic ring, or an amide nitrogen in the α, β or γ position to the stereogenic center. The glycopeptide antibiotics can be used in the run buffer in CE as a chiral selector in either coated or uncoated columns and are typically used in the run buffer at concentrations of 1–5 m*M* [7,8].

3.2.1. Vancomycin

Vancomycin has been the most commonly used glycopeptide in CE enantioseparations. This is primarily attributable to its availability and low cost as compared to the other glycopeptides. Vancomycin was introduced by Armstrong et al. to resolve over 100 racemates including nonsteroidal anti-inflammatory drugs, antineoplastic drugs, pesticides, and numerous *N*-derivatized amino acids [16]. Vespalec et al. used vancomycin to resolve 6-aminoquinolyl-*N*-hydroxysuccinimdyl carbamate (AQC)-derivatized sulfur and selenium containing amino acids [27]. They found that using so-called biological good buffers, efficiencies of the order of 250 000 theoretical plates could be obtained. This increase in efficiency was believed to be due to decreasing the ionic strength of the run buffer to one tenth of the phosphate run buffer employed by Armstrong et al. Advantages and drawbacks to using vancomycin for the separation of AQC amino acids derivatives was later summarized by Vespalec et al. [28]. Wan and Blomberg evaluated vancomycin as a chiral selector for the separation of several derivatized amino acids [29]. They found that high concentrations of vancomycin gave optimum resolution, but optimum efficiency occurred at low concentrations of vancomycin. This was in agreement with previous studies by Armstrong and Rundlett which showed that at higher concentrations (2–5 m*M*) vancomycin significantly adsorbs to the capillary wall reducing efficiency and lengthening migration times [16]. Using vancomycin Wan and Blomberg also separated a number of 9-fluorenylmethylchloroformate (FMOC)-derivatized di- and tripeptides [30].

Ward et al. investigated the use of vancomycin in coated capillaries and a countercurrent process [31]. Using this countercurrent technique, of which slight variations of are also referred to as ''partial separation zone technique'' or ''partial filling method'', a number of racemic compounds including nonsteroidal anti-inflammatory drugs and several dansyl-amino acids were resolved [8,31]. In a countercurrent process, the column is first filled with vancomycin, with the negatively charged analytes being injected at the cathode or inlet of the capillary and subsequently detected at the anode or outlet as depicted in Fig. 4. This results in significant improvement in detection sensitivity and virtually eliminates wall adsorption effects resulting in excellent resolutions with a short analysis time. Vespalec et al. also used vancomycin in coated capillaries to achieve enantiorecognition [32]. Using submillimolar concentrations of vancomycin they resolved AQC-derivatized sulfur and selenium containing amino acids in the reversed polarity mode [32]. Vancomycin was used in a coated capillary employing the partial filling method to separate and quantitate the enantiomers of loxiglumide [33]. Using this method, in which the capillary is filled just to the detection window (hence the term, partial filling method) they found that sensitivity was significantly improved by removing the strong UV absorbance of vancomycin. The same authors later used vancomycin as a chiral selector in the partial filling method to resolve a number of acidic herbicides [34]. Fanali et al. also employed the use of the partial filling technique in conjunction with capillary electrophoresis–electrospray ioniza-

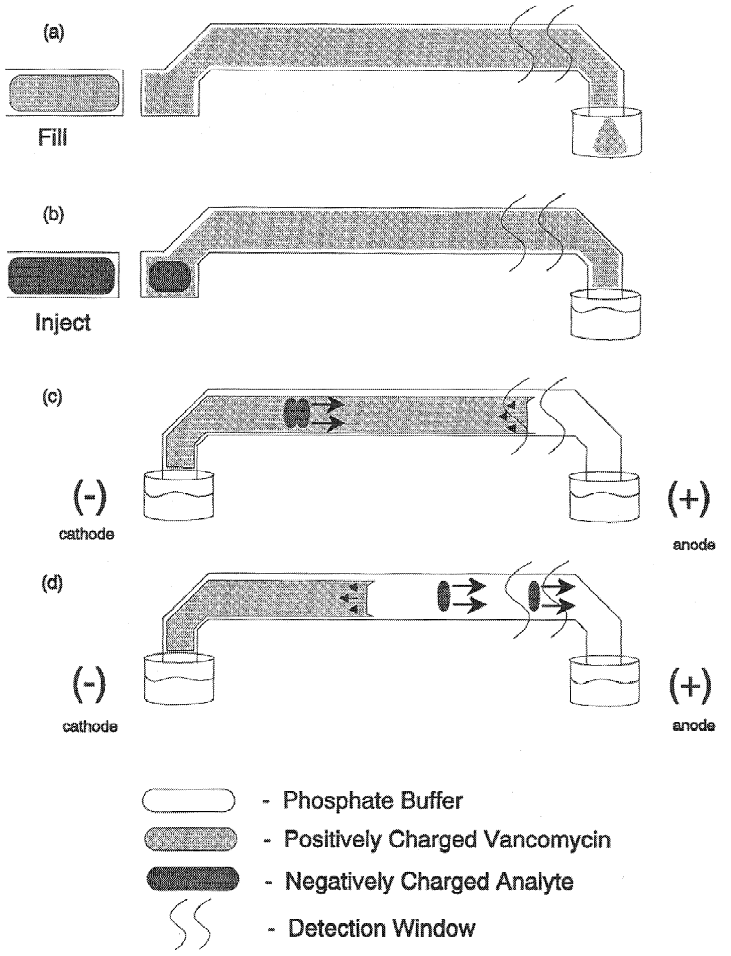

Fig. 4. Schematic shows the partial filling method. (a) The column is filled with running buffer containing chiral selector. (b) The sample is loaded into the capillary. (c) An electrical potential is applied across the capillary column creating a counter current process in which the chiral selector and analyte migrate in opposite directions. (d) Each isomer reaches the detection cell after the chiral selector has already passed [31].

tion mass spectrometry (CE–ESI-MS) [35]. Using vancomycin as the chiral selector they reported the separation of several arylpropionic acids demonstrating that CE–ESI-MS coupling is a viable alternative to more cumbersome HPLC techniques [35].

Strege et al. evaluated A82846B, an analogue of vancomycin shown in Fig. 5, as a chiral selector for anionic compounds using both coated and uncoated fused-silica capillaries [36]. A82846B produced better separations than vancomycin for flurbiprofen, dansylvaline and dansyltryptophan. Vancomycin and A82846B differ significantly in several aspects: its disaccharide amino sugar is epimeric and it contains an additional epi-vancosamine (Fig. 5). The net effect is that A82846B has more basic character with a p*I* of 9 and therefore has a greater positive fractional charge at the same pH used in vancomycin-based separations. This same group introduced recently another vancomycin analog (LY30599) which is a derivative of A82846B [37]. LY30599 differs from A82846B as shown in Fig. 5 in that an additional biphenyl group was added on the primary amine located on the pendant disaccharide. It was used to produce a successful enantioresolution for flurbiprofen [37].

Nair et al. evaluated a crystalline copper complex

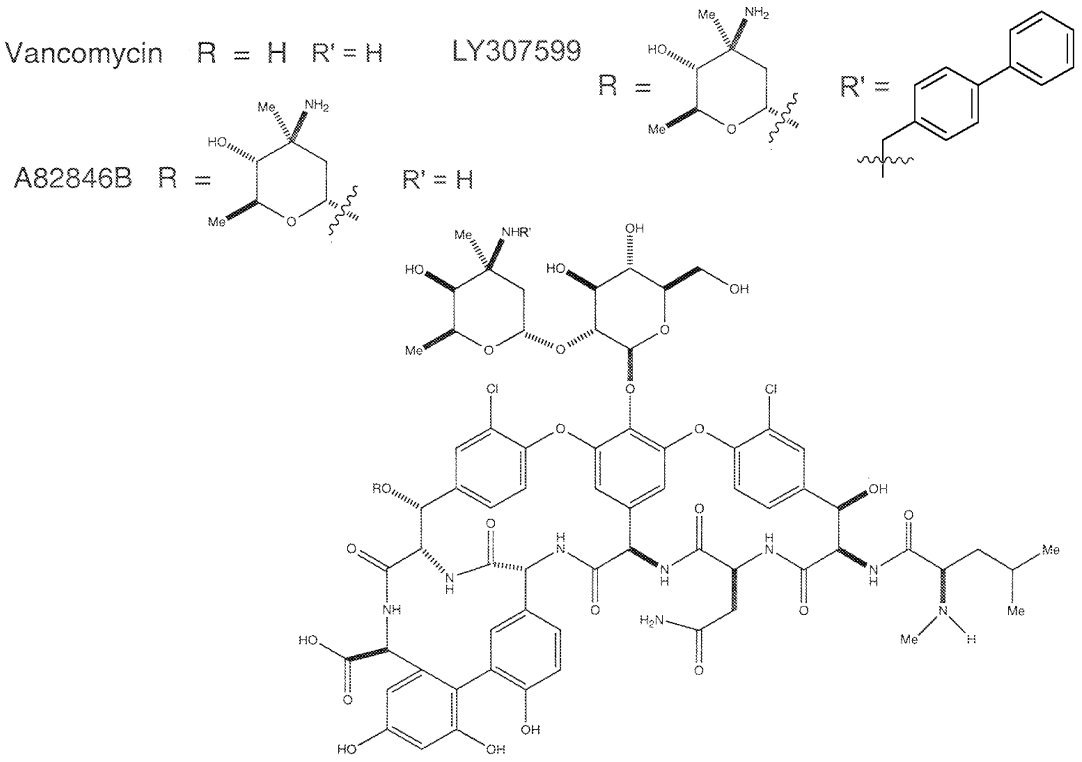

Fig. 5. The structures of vancomycin and the vancomycin analogues A82846B [36] and LY307599 [37].

of vancomycin as a chiral selector to investigate the mechanism of separation [38]. It was shown that when vancomycin is complexed with Cu^{+2}, enantioselectivity is essentially lost. This is believed to be due to the fact that copper binds selectively with the secondary amine group present on the *N*-methylleucine side chain in the aglycon basket of vancomycin, indicating that the secondary amine group located in the aglycon basket appears to be a primary interaction site [38].

3.2.2. Ristocetin A

Ristocetin A appears to be the most useful chiral selector in CE followed by vancomycin and teicoplanin, respectively. Due to its bulkier pendant sugar groups, it binds less strongly to the capillary wall than vancomycin, resulting in shorter analysis times, has greater stability in solution than vancomycin, and is easier to use as a chiral selector [11]. The greatest drawback to using ristocetin A is that it is relatively expensive compared to vancomycin. Over 120 racemates have been successfully resolved using ristocetin A, including the nonsteroidal anti-inflammatory drugs, numerous *N*-blocked amino acids, herbicides, and other biologically important compounds [21]. While ristocetin A is similar to vancomycin, a number of compounds containing a carboxylate group could be resolved using dilute solutions of ristocetin A, that could not be resolved with vancomycin. This included compounds such as mandelic acid and several of its derivatives, tropic acid, β-phenyllactic acid, and 2-bromo-3-methylbutyric acid [11]. The chiral selectivity of ristocetin A was also examined in a countercurrent process using a coated column to suppress electroosmotic flow by Oswald and Ward [22]. Excellent enantioseparations of several nonsteroidal antiinflammatories, dansyl-amino acids, dinitrophenyl derivatives, and other optically active compounds were achieved. The chiral selec-

tivity of ristocetin A also was examined as a function of antibiotic concentration and pH [22].

3.2.3. Teicoplanin

Teicoplanin is unique among the glycopeptide chiral selectors in that it has a long hydrophobic tail that gives it surfactant-like properties. Teicoplanin forms aggregates and micelle formation is favored at low pH [26,39]. Like vancomycin and ristocetin A, concentrations in the range of 1–5 m*M* provided excellent enantioselectivity with resolutions of four or greater not being uncommon. Unlike vancomycin, teicoplanin does not adsorb appreciably to the capillary wall. Teicoplanin has been used to successfully resolve over 100 negatively charged racemic analytes such as nonsteroidal anti-inflammatory drugs, *N*-blocked amino acids, mandelic and lactic acid derivatives, etc. [26]. A series of di- and tripeptides have also been enantioresolved using teicoplanin [40]. The same group also compared the enantioresolving power of vancomycin, teicoplanin and cyclodextrin chiral selectors [41].

It was found that resolution of many enantiomers improved when organic modifiers were added to the run buffer. This was especially true when acetonitrile was added to the run buffer at a concentration of 10–20% (v/v) or greater [26,40]. Small amounts of short chain alcohols tend to precipitate teicoplanin from solution and thus are not feasible as organic modifiers. Organic modifiers are believed to alter and/or inhibit aggregation of the teicoplanin monomers making more teicoplanin molecules available to interact with solutes which results in enhanced enantioselectivity [26].

3.2.4. Avoparcin

Currently there is no commercial source for ''pure'' avoparcin. Avoparcin has been used extensively in the agricultural industry in Europe as a livestock feed additive and was isolated from ''chicken-feed-additive'' to be evaluated as a chiral selector [42]. Avoparcin has three amine groups and tends to adsorb to the capillary wall much stronger than the other glycopeptides. This makes avoparcin more difficult to use since its wall-binding behavior necessitates extensive rinsing of the capillary between analysis. Nevertheless, avoparcin has been shown to enantioresolve several nonsteroidal anti-inflammatory drugs and *N*-blocked amino acids [43]. The authors also examined the effects of changing experimental parameters like chiral selector concentration, pH, and organic modifier as well as compared enantioseparations of various *N*-3,5-dinitrobenoyl-derivatized amino acids using either avoparcin, ristocetin A, teicoplanin or vancomycin [43].

3.2.5. Principle of complementary separations

By changing the glycopeptide antibiotic used the enantioselectivity of the separations can be significantly altered. While the glycopeptide antibiotics have similar structures, they often exhibit different but complementary enantioselectivities. This suggests that the mechanism of separation is similar though not identical. Consequently, if only a partial separation is obtained using one of the glycopeptides, there is an excellent probability that a baseline or better separation may be obtained with one of the other glycopeptides [7,11].

3.2.6. Micelle-mediated separations

The addition of sodium dodecyl sulfate (SDS) micelles in run buffers containing the macrocyclic antibiotics extends the scope of these enantioseparations to hydrophobic and neutral compounds. Using SDS micelles with vancomycin in the run buffer, Rundlett and Armstrong found that efficiencies were enhanced, migration times decreased, and the elution order of some solutes were reversed [44]. The addition of SDS to the run buffer allows for dynamic partitioning of hydrophobic neutral analytes between the vancomycin and micellar components of the run buffer which has the effect of increasing the available elution window for neutral solutes. Since the relative elution order changes from one based electrophoretic mobility in the vancomycin system to one based on the partitioning between the three phases as shown in Fig. 6, unique selectivities can be obtained [44]. Thus, as the concentration of SDS is increased, the migration times of the solutes also increased, and the effective mobilities of the solutes become more negative since the solutes partition into the micelle. It was noted that enantiomeric resolutions were less in vancomycin–micelle systems than resolutions obtained with vancomycin alone. Nair et al. determined the binding constants of solutes in

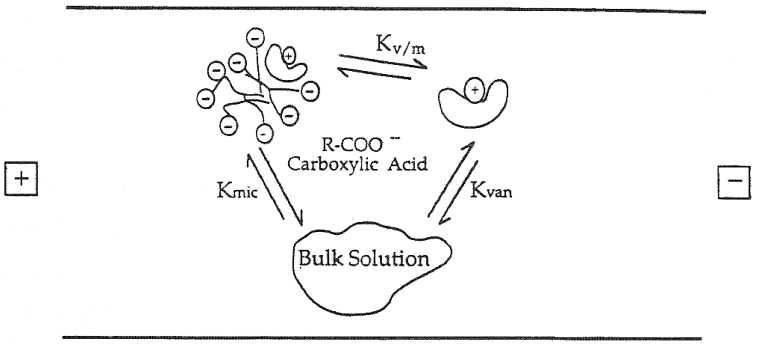

Fig. 6. Representation of the electrophoretic mobility of the analytes, chiral selector and mixed micelles, showing the equilibria of analytes between three phases (bulk aqueous solution, free vancomycin and mixed micelles) [44].

hydroxypropyl-β-cyclodextrin–SDS and vancomycin–SDS systems [45].

The addition of SDS to teicoplanin and ristocetin A-based separations has also been investigated. Ristocetin A, like vancomycin, comicellizes with SDS to form mixed micelles, with the exception that ristocetin A partitions to a greater extent than vancomycin to the SDS micelles [11]. In addition, the reversal of elution order for solutes in the ristocetin A–SDS system was not as consistent or predictable as they were for the vancomycin–SDS system. In general, teicoplanin–SDS systems were similar to vancomycin–SDS systems where analysis times decreased, elution orders reversed, overall enantioselectivity decreased, and efficiency increased in the micelle-containing teicoplanin systems [11].

3.3. The aminoglycosides

The aminoglycosides shown in Fig. 7 are characterized by their low molecular mass and low UV absorbance profiles. Fradiomycin sulfate, kanamycin sulfate and streptomycin sulfate were utilized by Nishi et al. as CE chiral resolving agents [25]. While not true macrocyclic antibiotics, Nishi et al. successfully resolved several chiral compounds such as 1,1′-binaphthyl-2,2′-diyl hydrogenphosphate, 1,1′-binaphthyl-2,2′-dicarboxylic acid, and a synthetic intermediate of clentiazem using one or more of these antibiotic chiral selectors. One potential drawback is their strong adsorption to the capillary wall, which was minimized in this study by the use of coated capillaries. They found that peak shape and enantioresolution was improved by the addition of methanol to the run buffer [25].

4. Chromatographic enantioseparations using the macrocyclic antibiotics

Since their introduction the macrocyclic antibiotic bonded phases have become popular and have proven quite useful as chiral stationary phases (CSPs) in HPLC [10]. The macrocyclic antibiotics vancomycin, ristocetin A, teicoplanin, avoparcin, rifamycin B and thiostrepton have been used for chiral separations. The glycopeptide macrocyclic antibiotics, vancomycin, teicoplanin, ristocetin A and avoparcin appear to have a broader enantioselectivity than the ansamycins or polypeptide thiostrepton. The macrocyclic antibiotics are covalently bound to silica gel via linkage chains employing a variety of chemistries, which ensure their stability while retaining their chiral recognition properties [12,13,15,20]. The macrocyclic glycopeptide antibiotics and thiostrepton are attached to silica gel by reacting them with the carboxylic acid terminated organosilanes, and the macrocyclic antibiotic rifamycin B can be attached via a reaction with amine-terminated organosilanes. The macrocyclic glycopeptide antibiotics can also be attached by reactions with epoxy-terminated organosilanes in a similar method used with cyclodextrins [46], or they can be immobilized by reacting the macrocyclic antibiotics with isocyanate-terminated organosilanes in anhydrous dimethylformate (DMF). The glycopeptide-bonded

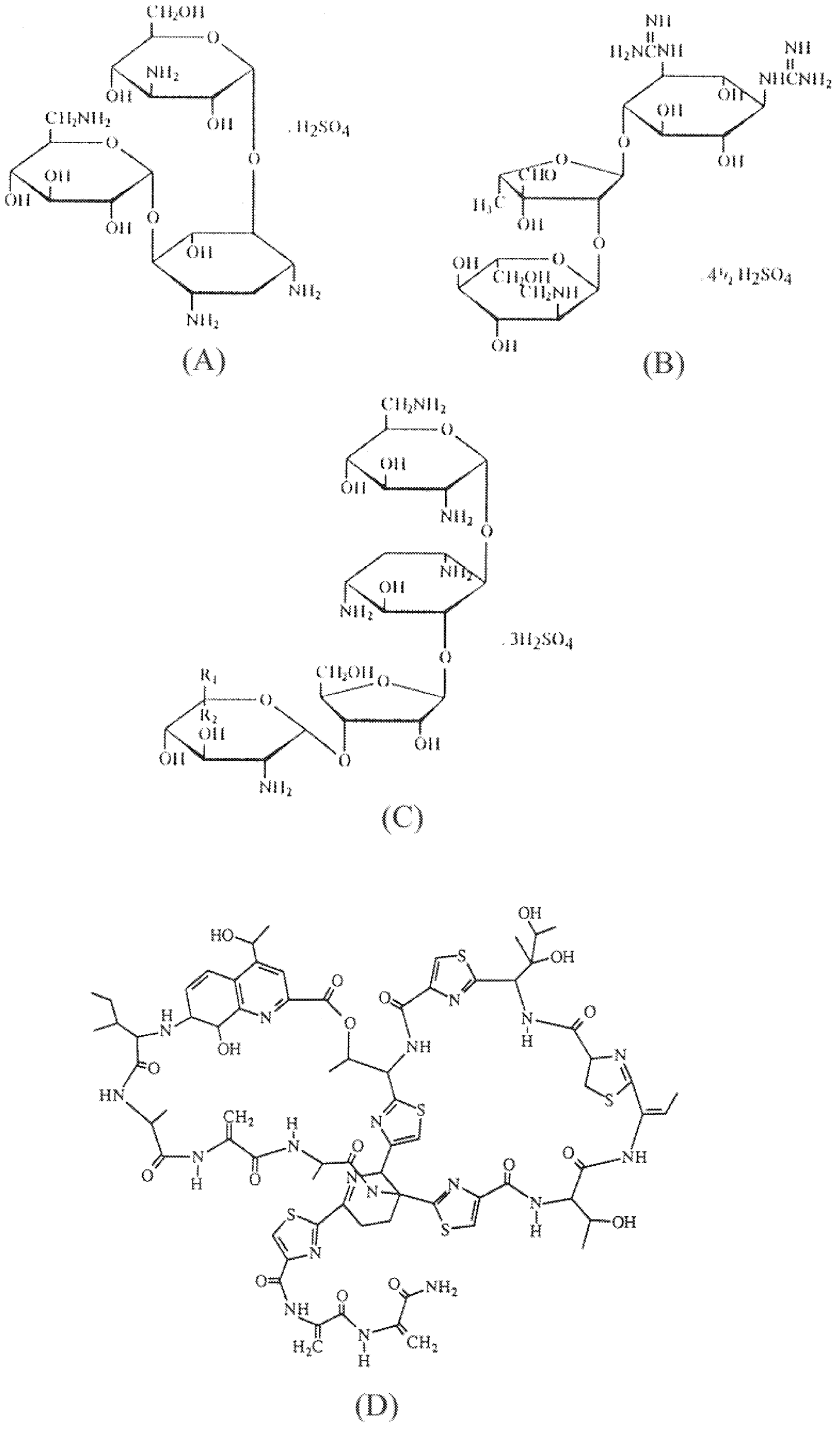

Fig. 7. Chemical structures of aminoglycosidic antibiotics [(A) kanamycin, (B) streptomycin, (C) fradiomycin B (R_1=H and R_2=CH_2NH_2), and fradiomycin C (R_1=CH_2NH_2 and R_2=H)], used as chiral selectors in capillary electrophoresis, and (D) the macrocycle thiostrepton used as bonded stationary phase chiral selectors in HPLC.

phases can be used in normal-phase mode, reversed-phase mode, and polar organic mode to achieve different enantioselectivities or they can be derivatized to alter their enantioselectivity [12,13,15].

The macrocyclic antibiotic bonded phases meet the general criteria that most useful bonded phases have in common, namely, they are sufficiently effective as a separation media, exhibit good mechanical strength to withstand packing and immobilization, and are available in adequate quantities at a reasonable cost. Macrocyclic antibiotic bonded phases are similar to protein-based CSPs; however, they have higher capacities and are more stable. Enantioseparations occur by several different interactions including $\pi-\pi$ complexation, hydrogen bonding, hydrophobic inclusion, dipole stacking, steric repulsions, as well as a combination of interactions. While other chiral stationary phases may produce some of the same interactions, they are usually not available in a single bonded phase with close proximity. Macrocyclic antibiotics can be used in the normal-phase mode without any irreversible change in enantioselectivity or denaturation unlike protein-based CSPs. They also can be used in preparative-scale separations. Commercial versions of these HPLC CSPs have been developed for the glycopeptides. The Chirobiotic V, Chirobiotic T, and Chirobiotic R use vancomycin, teicoplanin and ristocetin A, respectively as chiral bonded stationary phases. A commercial phase of avoparcin should soon be available.

Vancomycin, rifamycin B and thiostrepton were the first macrocyclic antibiotics to be introduced as bonded CSPs [12]. Of these three, only vancomycin demonstrated a broad application potential and was later commercialized as a CSP. Of the 70 plus chiral compounds resolved in that original work, approximately 95% of the 45 compounds separated in the reversed-phase mode were resolved using the vancomycin bonded phase, and approximately 84% of the 31 compounds separated in the normal-phase mode were resolved with vancomycin. Vancomycin was also derivatized with 3,5-dimethylphenylisocyanate (DMP) and evaluated as a CSP [12]. The DMP-vancomycin bonded phase was able to resolve several compounds the vancomycin CSP could not, specifically, hydroxyzine and althiazide. Since this original work, the vancomycin CSP has been used in a number of applications to separate various optically active compounds. The vancomycin CSP has been used to resolve a number of substituted racemic pyridones [14]; aryldihydropyrimidine carboxylates (DHPMs) which are analogs of nifedipine-type dihydropyridine calcium channel modulators [47]; enantiomers of citalopram and its desmethylated metabolites in human plasma [48]; alpha and dansyl-amino acids [49]; cyclic imides, barbiturates, piperdine-2,6-diones, and mephenytoin [50]; semisynthetic ergot alkaloids [51]; and was used recently in enhanced fluidity liquid chromatography (EFLC) [52].

Teicoplanin was introduced shortly after vancomycin as a CSP in HPLC [13]. In the initial study over 90 optically active compounds were resolved and various parameters which affect separation were evaluated. Teicoplanin has been used successfully in a number of applications such as the resolution of native amino acids and dipeptides [53]; salbutamol and its metabolites in biological matrixes [54]; unnatural amino acids and miscellaneous racemates [55]; determination of the enantiomers of albuterol in plasma [56]; resolution of *N-tert*.-butyloxycarbonyl amino acids [57]; and separation of unusual secondary aromatic amino acids [58]. While teicoplanin has several features in common with the vancomycin CSP it differs from the vancomycin CSP in that it has a hydrophobic ‘‘tail’’ and different enantioselective properties. Teicoplanin has often been used in conjunction with vancomycin to separate a wider range of solutes [14,47,49,51].

Ristocetin A is the latest of the glycopeptide CSPs to be introduced commercially as a bonded phase. Using ristocetin A bonded to a silica gel support, over 230 racemates have been separated in the normal-phase mode, reversed-phase mode, or polar organic mode [15]. The retention behavior and selectivity was evaluated in each mode, column stability was found to be excellent and the column appeared to be complementary to the other two glycopeptides CSPs, vancomycin and teicoplanin. The effect of chiral selector coverage and mobile phase composition on enantioselectivity using the ristocetin A bonded phase has been evaluated [59]. Though not commercially available, avoparcin has been isolated and purified from chicken feed additive, and subsequently bonded to silica gel for use as a CSP in HPLC [43].

To date, avoparcin is the fourth glycopeptide

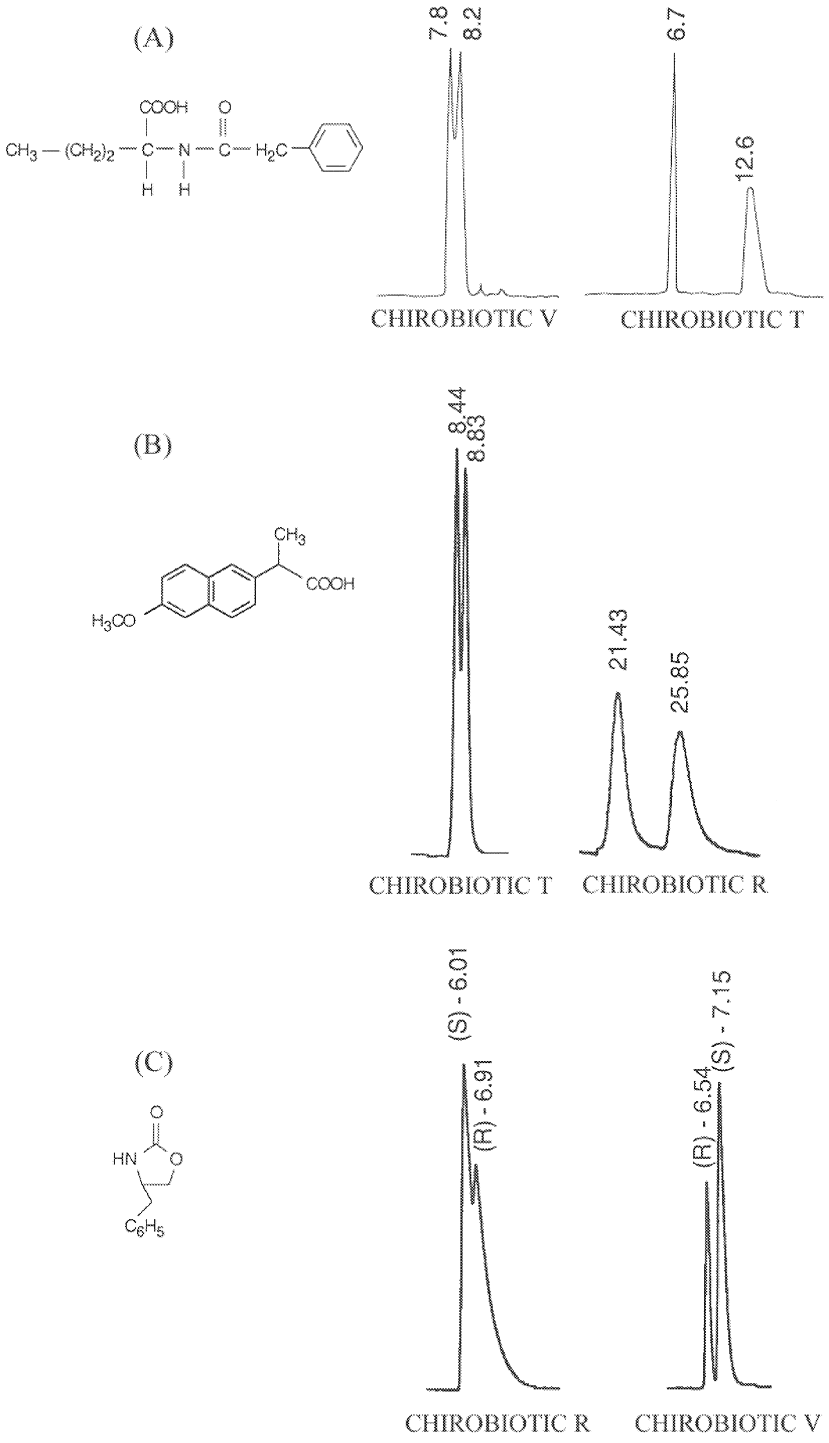

Fig. 8. HPLC chromatograms showing the enantioresolution of: (A) *N*-CBZ-norvaline (MeOH–1% triethylamineacetate (TEAA), 20:80, pH 4.1), (B) naproxen (MeOH–0.1% TEAA, 30:70, pH 4.1 at 1.0 ml/min), and (C) 4-benzyl-2-oxazolidinone (Hex–EtOH, 50:50 at 1.0 ml/min) [60].

macrocyclic antibiotic to be evaluated as a CSP in HPLC. It is structurally related to vancomycin, teicoplanin and ristocetin A, and behaves in a very similar manner. Avoparcin is complementary to the other glycopeptide phases in that it could resolve a number of compounds that the other glycopeptide CSPs could not fully resolve such as verapamil, thyroxine, mephenytoin, and 2-imidazolidine-4-carboxylic acid [43]. In general, the glycopeptide-bonded phases complement one another in their enantioselectivity as shown in Fig. 8 [60]. Three separations are shown in Fig. 8 where one of the bonded-glycopeptide CSPs produces a superior separation for an analyte only partially resolved on one of the other glycopeptide phases. This demonstrates their complementary nature, which is a unique feature characteristic of these CSPs.

Vancomycin has also been used as a chiral mobile phase additive for the TLC resolution of AQC-derivatized amino acids, racemic drugs, and dansyl-amino acids [61]. The authors found that the nature of the stationary phase and the composition of the mobile phase strongly affected resolution. Diphenyl stationary phases with the organic modifier produced the most efficient separations with the shortest analysis times [61].

5. Conclusions

While the number of chiral selectors available has continuously increased, few chiral selectors have had as immediate and significant an impact as the macrocyclic antibiotics. The majority of enantio-separations, especially in CE, can be performed successfully with either the macrocyclic antibiotics and/or cyclodextrins and their derivatives. Their tremendous enantioselectivity, separation efficiency, and short analysis times characterize the macrocyclic antibiotic-based separations. In time, the macrocyclic antibiotics should become as established as the cyclodextrins and find use in routine analytical practice.

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