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Review

Chiral analysis by capillary electrophoresis using antibiotics as chiral selector

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Abstract

The separation of chiral compounds by capillary electrophoresis (CE) is a very interesting field of research in different areas such as pharmaceutical, environmental, agricultural analysis etc. The separation of two enantiomers can be achieved in CE using a chiral environment interacting with the two analytes on forming diastereoisomers with different stability constants and thus different mobilities. A wide number of chiral selectors have been employed in CE and among them glycopeptide antibiotics exhibited excellent enantioselective properties towards a wide number of racemic compounds. Vancomycin, ristocetin A, rifamycins, teicoplanin, kanamycin, streptomycin, fradiomycin, and two vancomycin analogues, added to the background electrolyte (BGE), are the antibiotics studied by CE running the separation in untreated and/or coated fused-silica capillary. Due to adsorption and absorption phenomena, some drawbacks can be expected when using bare fused-silica capillary, e.g., changes of electroosmotic flow (EOF), broaden peaks, reduced efficiency and low sensitivity. Coated capillary and counter current mode can be the solution to overcome the above mentioned problems. This review surveys the separation of enantiomers by CE when macrocyclic antibiotics are used as chiral selector. The enantioselectivity can be easily controlled modifying several parameters such as antibiotic type and concentration, pH, ionic strength and concentration of the background electrolyte, organic modifier etc. The paper also presents a list of the latest chiral separations achieved by CE where antibiotics were used as chiral selector. © 1998 Published by Elsevier Science B.V.

Keywords: Reviews; Enantiomer separation; Chiral selectors; Enantioselectivity; Buffer composition; Antibiotics

Contents

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1. Introduction

In the field of enantiomeric separation the search for new chiral selectors together with the optimisation of sensitive, efficient and fast analytical methods are always of growing interest. In fact the separation of optical isomers represents an important topic especially in pharmaceutical and environmental fields where many drugs and agrochemicals are racemic compounds administered in several cases as pure enantiomer. New analytical methods with the ability to carry out enantiomeric separation in short time, at low cost and with high efficiency and resolution, are very often required for e.g., monitoring the stereoselective synthesis of enantiomers, chiral purity control of drugs, pharmacokinetic studies, biological activity of two enantiomers etc.

Capillary electrophoresis (CE) has proven to be a challenging separation tool for the stereoselective analysis of several classes of compounds using different separation mechanisms. Compared with other analytical techniques commonly used for chiral separations, such as high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC) and gas chromatography (GC), CE exhibits several advantages including high separation efficiency and resolution power, feasibility and fast analysis together with low costs for the minimum consumption of reagent and samples and automation.

The majority of CE applications dealing with resolution of chiral compounds has been performed using the direct separation method. Compared with the indirect method where chemical reaction with derivatizing compounds are needed, the direct separation method analyses unmodified enantiomers in the presence of a chiral environment. The chiral selector either dissolved in the background electrolyte (BGE) or bound to the capillary wall or immobilised into a gel, is forming labile diastereoisomers during the electrophoretic run moving towards the detector with different velocities if possessing diverse stability constants [1–3].

Several resolution mechanisms can be selected for chiral separations by CE on the basis of physicochemical properties of the studied analytes, namely ligand exchange, inclusion complexation, optical micelles solubilization, affinity and ion-pairing interactions [2,4].

The usefulness of macrocyclic antibiotics, as chiral selectors, for enantioseparations in several analytical techniques such as TLC [5], HPLC [6] and CE [7] has been shown by Armstrong and coworkers.

As documented in published papers [2,8–10], macrocyclic antibiotics have been successfully used as chiral agents for the stereoselective analysis of several classes of compounds by CE. In fact in the chiral recognition process the macrocyclic antibiotics exhibit both very high selectivity, which is typical of the chiral selectors of biological origin and high molecular weight such as proteins, and high efficiency, characteristic of the host–guest complexing agents, which exceeds that of cyclodextrins [9]. They possess several functional groups which are responsible for multiple stereoselective interactions. The ionizable groups of their structure determine their positive or negative charge, depending on buffer pH and composition, and make them, in most cases, soluble compounds in aqueous buffers as the BGE commonly used in CE. Although macrocyclic antibiotics exhibit very similar physico-chemical properties, they show a different stereoselective power. Rifamycins B and SV have been successfully used for the chiral resolution of cationic and anionic compounds, respectively [11,12]. Vancomycin [7,9,13,14], teicoplanin [15], ristocetin A [16], fradiomycin, kanamycin and streptomycin sulphates [17] showed stereoselectivity towards anionic substances. Recently two different vancomycin analogues, namely A82846B [18] and LY307599 [19], have been used to analyze negatively charged compounds.

Besides the high resolution power of these chiral agents, their use in CE shows some drawbacks mainly due to the lack of detection sensitivity from their strong UV absorption and also due to their adsorption on the capillary wall.

Several papers report studies trying to elucidate the mechanism of chiral recognition of the macrocyclic antibiotics considering the interactions with cavities and ravines, the role of the separation environment (buffer composition), etc. [9,10,20–23]. In fact, as more recently stated, the theory of the three points of interaction is moving from a static towards a dynamic understanding of this process taking into account both the spatial arrangements of

the three interaction points and the contribution of the achiral components of the microenvironment [24,25].

The aim of this paper is to illustrate the state of the art concerning the use of this new class of chiral selectors in CE mainly emphasising glycopeptide antibiotics which have found most popular use. The physico-chemical properties, the advantages and disadvantages as well as the main applications of macrocyclic antibiotics in CE are also considered, drawing strategies to overcome the limitations in their use.

2. Physico-chemical properties of macrocyclic antibiotics and enantiomeric resolution mechanism.

Several macrocyclic antibiotics have been used in CE as chiral selectors, namely rifamycins B and SV, ristocetin A, vancomycin, teicoplanin, kanamycin, streptomycin, fradiomycin B and C, A82846B and LY307599 vancomycin analogues, and among them vancomycin became the most employed compound. Fig. 1 shows the molecular structures of the main antibiotics used in CE as chiral selectors. The second molecular structure of vancomycin has a characteristic basket shape with three fused macrocyclic rings and two side chains (a carbohydrate dimer and *N*-methylleucine). It contains 18 asymmetric centers and several functional groups such as carboxylic, hydroxyl, amino and amido groups and aromatic rings. The three chargeable groups (two amines and one carboxylic) are mainly responsible for vancomycin's ionization state depending on buffer pH and composition (six different values of pK have been measured) [7].

Vancomycin is very soluble in water and polar aprotic solvents, less soluble or insoluble in higher alcohols and less polar organic solvents [7]. In aqueous solution vancomycin can dimerize depending on solution type and vancomycin concentration.

Vancomycin solutions have been shown to be stable if stored at low temperature and in buffers of pH value in the range 3–6 [7,8].

It has been shown that this antibiotic is a very effective chiral selector for the enantiorecognition of anionic compounds, particularly for those containing carboxylic groups in their structure. The selectivity of vancomycin towards carboxylic acids is related to the presence of the amine groups and, in fact, better enantiomeric separations are obtained at acidic buffer pH values below or close to the isoelectric point (p*I*) of the antibiotic.

Ristocetin A and teicoplanin have molecular structures very similar to that of vancomycin. Ristocetin A is characterized by four fused macrocyclic rings, one tetrasaccharide moiety and two monosaccharides together with 38 stereogenic centers. As with vancomycin, it has been used for the resolution of anionic compounds but exhibits different stereoselectivity resolving enantiomers which were not separated by the vancomycin [8].

With respect to vancomycin and ristocetin A, teicoplanin is unique in possessing an hydrophobic acyl side chain attached to a 2-amino-2-deoxy-β-D-glucopyranosyl moiety which activates its surface and enables the formation of micellar aggregates (critical micellar concentration, CMC, about 0.18 m*M* in unbuffered aqueous solutions [10]). As a consequence of its molecular structure teicoplanin shows a slighter solubility in water than the other macrocyclic antibiotics [10,15].

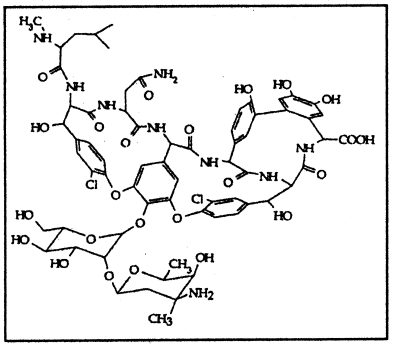
Fig. 2 shows the effect of pH on electrophoretic mobility of vancomycin, teicoplanin and ristocetin A in 0.1 *M* phosphate solution. All these compounds can be both positively or negatively charged depending on the pH of BGE. The plots of vancomycin and ristocetin A have almost the same trend showing a zero mobility (isoelectric point, p*I*) at pH 7.2 and 7.5, respectively.

Teicoplanin shows a different behavior exhibiting a very slight anionic character even at acidic pH (p*I*~3.8) [10].

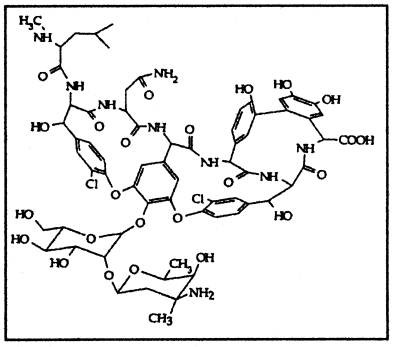
Rifamycins belong to the class of ansamycin antibiotics with a characteristic ansa structure. They can be used in either positive or negative charged mode or neutral. They are very soluble in water and in light alcohols and acetone. Rifamycin B possesses nine stereogenic centers. It has been used whilst negatively charged for the resolution of single ring cationic compounds whereas rifamycin SV has been employed as a neutral chiral selector for the resolution of anionic analytes with two aromatic rings [11,12].

All macrocyclic antibiotics strongly absorb in the

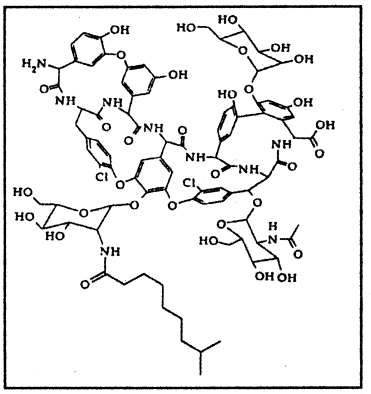
GLYCOPEPTIDES



RISTOCETIN A



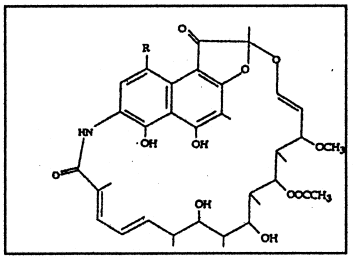
VANCOMYCIN



TEICOPLANIN

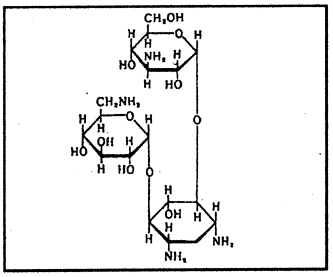
ANSAMYCINS

rifamycin B R=OCH_2COOH
rifamycin SV R=OH

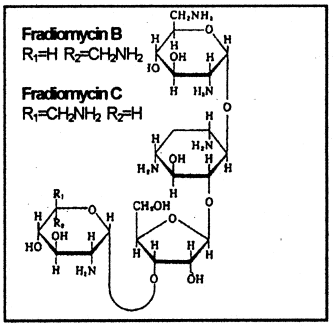


RIFAMYCIN

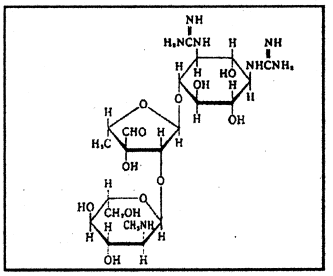
AMINOGLYCOSIDES



KANAMYCIN



FRADIOMYCIN



STREPTOMYCIN

Fig. 1. Chemical structure of the antibiotics mainly used in CE for chiral separations.

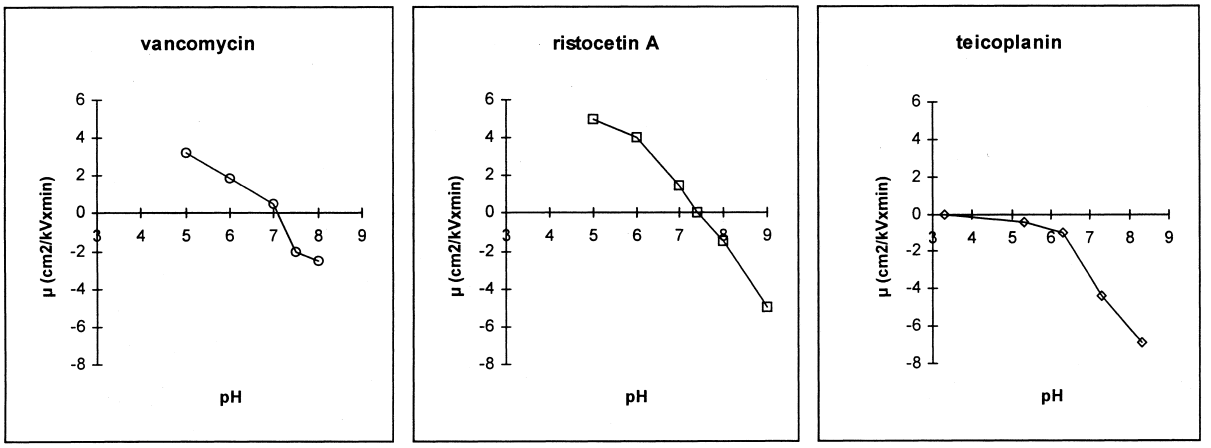


Fig. 2. Effect of buffer pH on electrophoretic mobility of vancomycin, ristocetin A and teicoplanin. (modified from Ref. [10]).

UV region commonly used in CE for detection of analyzed compounds. However in most cases the use of very low concentrations (0.5–5 m*M*) of these chiral selectors, due to their high stereoselectivity, allows the analyst to perform direct UV detection at wavelengths higher than 254 nm. As an alternative method indirect UV detection can be used, e.g., the enantiomeric separation of amino alcohols with rifamycin B at 254 nm [11].

As reported by Armstrong's group, the chiral selector shape, the tridimensional structure of the molecule and the different space arrangements of the functional groups are responsible for the different stereoselective capability of these macrocyclic antibiotics [10]. The aglycon portion of these antibiotics, which is of three fused macrocyclic rings for vancomycin and four for ristocetin A and teicoplanin respectively, can exhibit different morphological characteristics such as the openness of the aglycon basket and the degree of helical twist. The twist degree does not seem to depend on the molecular size, in fact vancomycin, which possesses the smallest macrocyclic ring, has the highest twist degree [10].

The primary interaction between the glycopeptide chiral selectors and the analytes seems to involve charge–charge or electrostatic interactions. Hydrogen bonding, steric repulsion, hydrophobic, dipole–dipole and π–π interactions are considered as secondary interactions [10].

Macrocyclic antibiotics, and particularly vancomycin, exhibit a very high separation efficiency which exceeds that of cyclodextrins. This property can be ascribed to the low association constant and the fast kinetics of the separation process. Furthermore the high molecular mass of vancomycin (eventually increased by the dimerization) limits the diffusion process of the sample bands increasing the efficiency of the separation [9].

In a recent paper Nair and Izzo [26] elucidated vancomycin's enantioselective binding site by studying both the X-ray cristal structure and the stereoselective capability of a copper–vancomycin complex. They concluded that the secondary amine plays a key role in the enantioresolution capability of vancomycin in aqueous solutions. In fact the copper atoms, bound to the secondary amine of vancomycin, strongly reduced the enantiorecognition of the complex exhibiting a lower resolution than the native vancomycin towards the studied acidic enantiomers.

3. Chiral analysis by CE using macrocyclic antibiotics

As previously mentioned, in CE, the direct separation method is the simplest way in order to achieve chiral resolution. The macrocyclic antibiotic is simply added to the selected BGE at the appropriate concentration and the electrophoretic run performed.

Armstrong et al. [7] introduced the use of macrocyclic antibiotics as chiral selectors in CE resolving a wide number of racemic compounds in bare fused-silica capillaries filled with a BGE containing vancomycin; detection was done directly at 254 nm. Further studies have been carried out using rifamycin B as the chiral selector and revealing analytes by indirect UV detection [11,12].

When untreated fused-silica capillaries are used for the analysis and antibiotics are the chiral selectors, some drawbacks have to be expected, e.g., adsorption on the capillary wall and lack of detection sensitivity in the low wavelengths used in CE (<254 nm).

Macrocyclic antibiotics can differently interact with the capillary wall of fused-silica columns due to adsorption depending on their properties and chemical structure. Among the glycopeptide antibiotics vancomycin most strongly exhibits this phenomena which is very much reduced in teicoplanin, probably due to the more hydrophobic character due to the presence of only one amino group and to the formation of micellar aggregates which minimize the process. Ristocetin A besides, possessing physicochemical properties very similar to vancomycin, has a larger molecular structure which contributes to reduce adsorption on the capillary wall [10].

As a consequence of chiral selector adsorption the following effects have been observed: (1) reduced sensitivity, (2) dramatic drop of electroosmotic flow (EOF) causing longer analyte migration times, (3) peak broadening and reduced efficiency.

Several strategies can be adopted to avoid/minimize the adsorption phenomena, e.g., optimising the capillary washing procedure, working at a pH close to the p*I* of the antibiotics, adding organic solvents to the BGE, using coated capillaries or performing the separation in counter current process.

In order to remove the adsorbed antibiotics from the untreated capillary wall is generally advised to wash for long time with water or with the BGE antibiotic free. However 0.01 *M* sodium hydroxide can be flushed for about 15 min to obtain complete removal of all the adsorbed compounds [9]. Another way to minimize the interactions between glycopeptide antibiotics and the capillary wall is to increase the BGE concentration and the applied voltage [10].

As an alternative method Wan and Blomberg [27] proposed to work at buffer pH values close to the p*I* of the vancomycin or to add 2-propanol to the buffer. However when changing the buffer pH the stability of antibiotics solutions have to be taken into account in order to avoid chiral selector degradation.

The use of coated capillary is an interesting approach to control the adsorption phenomena, the electroosmotic flow, the separation efficiency and the detection sensitivity. However it has been reported [9] that the decomposition products of vancomycin can interact with the wall of coated capillary and thus efficient capillary washings between runs are necessary in order to restore the performance of the capillary and the substance detectability, e.g., long duration washings with the running buffer-antibiotic free is recommended [9,13,28].

Finally the counter current process can be advantageously used in order to reduce the above discussed drawbacks. This method is realised by using a buffer pH at which the chiral selector and the analyte possess opposite charges and thus are migrating in the inverse direction (counter current). The counter current process can be performed in the partial filling mode where only part of the capillary is filled with the BGE-chiral selector leaving the detector path free of antibiotic. This technique has been introduced by Valtcheva et al. [29] using an enzyme (cellobiohydrolase I) as the chiral selector for the enantiomeric resolution of several drugs. When the voltage is applied, the antibiotic moves towards the cathode (if positively charged) never passing through the detector cell allowing the detection of the analytes (anionic substances) with the highest sensitivity. This method has been successfully applied for the resolution of compounds of pharmaceutical and environmental interest using vancomycin as the chiral selector [13,28,30] also allowing the performance of quantitative analysis with good repeatability, linearity and reproducibility data [13,28].

A similar method has been shown by Ward et al. [30] filling the whole capillary with the BGE-chiral selector and suppressing the EOF. In this case the detector was antibiotic free only after a certain time and enantiomers moving with relatively high mobility cannot be analyzed at the optimum detection conditions.

Another interesting advantage to be remarked on

using the counter-current mode is represented by the combination of CE with mass spectrometry (CE–MS). This has been shown analyzing several non steroidal anti-inflammatory drugs enantiomers in urine samples with vancomycin [31]. The method allowed the analysis without any antibiotic interference. The presence of vancomycin ions in the MS source enormously reduces the sensitivity of this detector due to the competition of analytes-chiral selector ions.

Fig. 3 shows a scheme of electrophoretic separation of anions with a positively charged antibiotic (counter current - partial filling mode).

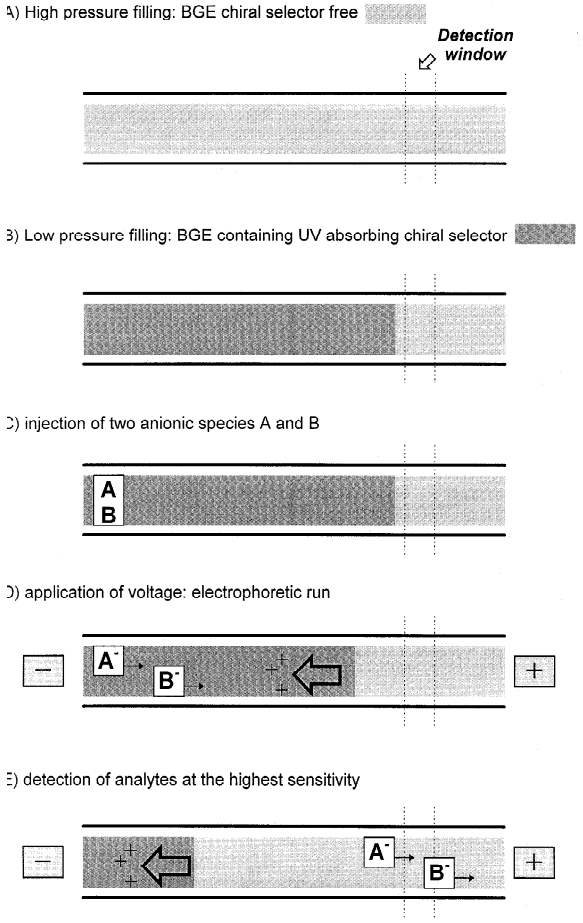


Fig. 3. Scheme of the electrophoretic separation of two anionic enantiomers by CE using the counter-current partial-filling method and suppressing the electroosmotic flow.

4. Experimental parameters influencing the enantiorecognition

The improvement of the stereoselectivity in CE when using antibiotics as chiral selector can be obtained by modifying the BGE and acting on experimental parameters such as antibiotic type and concentration, buffer pH, BGE composition (buffer type, ionic strength), BGE additives (micellar phases, organic modifiers), column temperature, etc. In this section several examples concerning the effect of the above mentioned parameters on chiral resolution will be discussed.

4.1. Effect of antibiotic concentration

The effect of antibiotic concentration has been studied by several groups performing enantiomeric separations in either uncoated or coated capillaries [7–9,11–13,15,16,27,28,30,32,33]. The increase of chiral selector concentration caused a general increase of both resolution and migration time of the studied analytes (decrease of electrophoretic mobility). When working with untreated capillary and in presence of EOF, a reduction of electrophoretic mobility should cause a decrease of migration time in contrast with the longer analysis time obtained by Armstrong et al. [7] using vancomycin for the separation of naproxen, dansyl-valine and iopanoic acid. This behavior has been explained by the authors considering the decrease of EOF produced by the adsorption of vancomycin on the capillary wall. It is interesting to remark that, very often, an increase in the antibiotic concentration can mainly decrease the mobility of one of the two enantiomers [9,30,34]. This behavior is very similar to that of other affinity chiral selectors such as proteins and is the consequence of the very high enantiospecifity of the recognition process. As an example, Fig. 4 shows this effect in the enantiomeric separation of 9-fluorenylmethyl chloroformate (FMOC) derivatized dipeptides with teicoplanin.

Usually the range of antibiotic concentration studied is very limited for the high stereoselectivity exhibited by this group of chiral selector even at very low levels (0.5–5 m*M* for vancomycin, teicoplanin and ristocetin A). Higher concentrations of rifamycins (25 m*M*) had to be used for the chiral

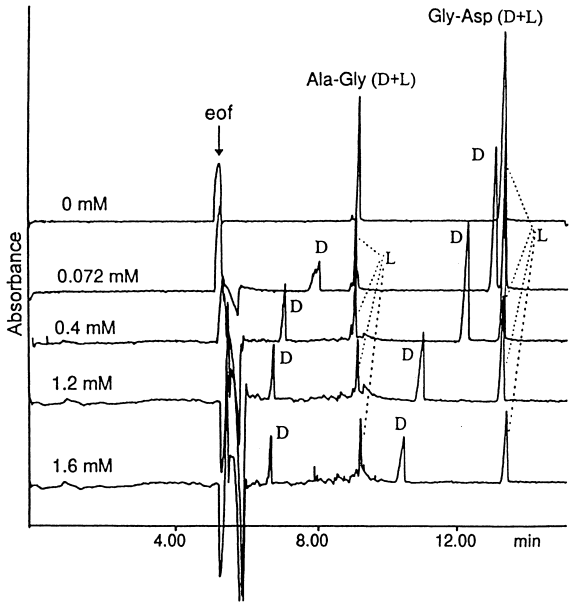


Fig. 4. Electropherogram of the enantiomeric separation of FMOC derivatized dipeptides at different concentrations of teicoplanin. Capillary: 62.5 cm (41.5 cm effective length)×50 um I.D. BGE: 25 m*M* phosphate–Tris buffer, pH 6.25, 40% (*v*/*v*) acetonitrile and 0–1.6 m*M* of teicoplanin. Applied voltage: 25 kV, 17 μA. Capillary temperature: 25°C. (From Ref. [34], with permission).

resolution of amino alcohols [11] and other drugs [12].

4.2. Effect of buffer composition and pH

The type of buffer and/or its ionic strength can influence the mobility of the antibiotics modifying their charge, their p*I* and the BGE viscosity. It has been reported that the composition of the BGE is important for the formation of homodimers in solution by the glycopeptide antibiotics, and particularly for vancomycin dimerization and aggregation state (micelles) of teicoplanin [9,15,32,34].

The chemical composition and the concentration of the buffer can affect the baseline stability, the peak shape and the separation selectivity [33]. Armstrong et al. [11] observed, in the enantiomeric separation of terbutaline, norphenylephrine and metoprolol with rifamycin B, a decrease of resolution and an increase of migration time after the addition to the BGE of 0.05 and 0.10 *M* of NaCl. While, when studying the effect of buffer concentration, the best resolution, have been obtained at 0.1 *M* of phosphate buffer (the studied concentration range was 0.05–0.15 *M*). The use of diluted buffers is recommended to obtain high separation selectivity [9].

The pH of the BGE is another very important experimental parameter to be controlled when antibiotics are used as chiral selectors in CE. In fact the charge of both the antibiotic and the analytes can be modulated, changing this parameter can result in different mobilities and electrostatic interactions being obtained. Furthermore the change of the pH can influence the stability of the antibiotic solution. Usually it is convenient to work in the pH range 4–7 to avoid hydrolysis of amide bonds, macrocyclic ring opening or cleaveage of sugar moieties [8].

Different behaviors can be recorded by modifying the pH of the BGE depending on the type of antibiotic used. For instance the best enantiomeric resolutions of the compounds separated by using rifamycins were achieved at the highest pH investigated (pH 7) while in the case of vancomycin, the increase in pH usually caused a loss of resolution [7,8,13,28]. However several separations, with relatively high resolution, have been obtained using vancomycin at pH 7 (see Table 1 at the end of this paper).

4.3. Effect of organic modifier

Organic modifiers such as methanol, acetonitrile and 2-propanol have been added to the BGE containing antibiotics for chiral CE separations influencing both enantioselectivity and migration time. Armstrong et al. [7,16] showed that among the organic modifiers used (methanol, acetonitrile and 2-propanol), 2-propanol resulted to be the most effective additive for the improvement of the chiral resolution of several analytes using either vancomycin or ristocetin A, e.g., naproxen, dansyl-valine, 2-(3-chlorophenoxy)-propionic acid, ketoprofen, 3-methoxymandelic acid and 1-benzocyclobutenecarboxylic acid.

Table 1
Enantiomeric separation of racemic compounds by CE using antibiotics as chiral selector

Compounds	Chiral Selector	BGE	Detection	Ref.
1,1′-Binaphthyl-2,2′-dicarboxylic acid (BNC)	Kanamycin (3%)	20 m*M* phosphate buffer pH 8.2 and 30% methanol	220 nm	[17]
1,1′-Binaphthyl-2,2′-dicarboxylic acid (BNC)	Fradiomycin (3%)	20 m*M* phosphate buffer pH 7.5 and 30% methanol	220 nm	[17]
1,1′-Binaphthyl-2,2′-dihyl hydrogen phosphate (BNP)	Streptomycin (3%)	20 m*M* phosphate buffer pH 8.1 and 30% methanol	220 nm	[17]
1,1′-Binaphthyl-2,2′-dihyl hydrogen phosphate (BNP)	Fradiomycin (3%)	20 m*M* phosphate buffer pH 7.5 and 30% methanol	220 nm	[17]
5-(4-Hydroxyphenyl)-5-Phenylhydantoin	Vancomycin (2 m*M*)	50 m*M* phosphate buffer pH 7.0 and 50 m*M* SDS	254 nm	[37]
Alprenolol	Rifamycin B (25 m*M*)	0.1 m*M* phosphate buffer pH 7–2-propanol (30:70, *v*/*v*)	Indirect UV 350 nm	[12]
Alprenolol	Rifamycin B (25 m*M*)	0.1 *M* phosphate buffer pH 7–2-propanol (60:40, *v*/*v*) (*R*=0.7)	Indirect UV 254 nm	[11]
Amino acids, *N*-acetyl (2m*M*) (flurophenylalanine, phenylalanine)	Vancomycin	0.1 *M* phosphate buffer pH 6.0	254 nm	[7]
Amino acids, *N*-acetyl (4-fluorophenylalanine, phenylalanine)	Ristocetin A (2 m*M*)	0.1 *M* phosphate buffer pH 6.0 or 7.0	254 nm	[16]
Amino acids, *N*-acetyl (phenylalanine, *m*-fluorophenylalanine)	Teicoplanin (2 m*M*)	0.1 *M* phosphate buffer pH 6 (0–10% acetonitrile)	254 nm	[15]
Amino acids, 2-(9-anthryl)ethyl chloroformate (AEOC) (Ala, Leu, Gln, Phe, Ser, Thr)	Vancomycin (2 m*M*)	50 m*M* phosphate buffer pH 7.5, 0 or 10% 2-propanol	254 nm	[27]
Amino acids Aminoquinolyl carbamate (AQC) (methionine, selenomethionine, ethionine, cystine,	Vancomicin (0.1–5 m*M*)	20 m*M* 3-morpholinopropenesulfonic (MOPS) –Tris pH 7	254 nm	[32]
selenocystine, tryptophan, *N*-2,4-dinitrophenyl-methionine)	(0.1–1.5 m*M*)	20 mM MOPS–Tris pH 7 (polyacrylamide coated capillary)	254 nm	[33]
Amino acids, AQC (alanine, valine, leucine, isoleucine, isoserine, homoserine, norvaline, α-amino-*n*-butyric acid, proline, 3,4-dihydroxyproline, α-aminopimelic acid, α-aminoadipic acid, asparagine, threonine, ornithine, phenylalanine, tryptophan, *m*-tyrosine, serine, norleucine,	Ristocetin A (2 m*M*)	0.1 *M* phosphate buffer pH 6.0 or 7 and 0–30% 2-propanol	254 nm	[16]

(*Continued overleaf*)

Table 1. Continued

Compounds	Chiral Selector	BGE	Detection	Ref.
methionine, indolelactic acid, nipecotic acid, citrulline, 3-fluorophenylglycine, 3-fluorophenylalanine, dihydroxyphenylalanine, amino-3-phenylpropionic acid, 4-chlorophenylalanine)				
Amino acids, AQC (alanine,valine, leucine,isoleucine, serine, ornitine, threonine, phenylalanine, tyrosine, *m*-tyrosine, tryptophan, methionine, norleucine, norvaline, α-amino-*n*-butyric acid, proline, 3,4-dehydroproline, homoserine, β-aminoisobutyric acid, citrulline)	Vancomycin (5 m*M*)	0.1 *M* phosphate buffer pH 4.9 or 7	254 nm	[7]
Amino acids, AQC (3-fluorophenylglycine, 3-fluorophenylalanine, 2-fluorophenylalanine, dihydroxyphenylalanine, 3-amino-3-phenylpropionic acid)	Vancomycin (2 m*M*)	0.1 *M* phosphate buffer pH 6	254 nm	[7]
Amino acids, AQC (alanine, valine, leucine, isoleucine, isoserine, homoserine, ornitine, phenylalanine, tryptophan, tyrosine, *m*-tyrosine, methionine, serine, norleucine, indolelactic acid, cycloserine, norvaline, α-amino-*n*-butyric acid, proline, α-aminopimelic acid, α-aminoadipic acid, asparagine, threonine, citrulline,3-fluorophenylglycine,3-fluorophenylalanine, dihydroxyphenylalanine, 4-chlorophenylalanine,	Teicoplanin (2 m*M*)	0.1 *M* phosphate buffer pH 6 (acetonitrile 0–30%)	254 nm	[15]

(*Continued overleaf*)

Table 1. Continued

Compounds	Chiral Selector	BGE	Detection	Ref.
glutamic acid)				
Amino acids, *N*-benzoyl (alanine, valine, methionine, leucine)	Ristocetin A (2 m*M*)	0.1 *M* phosphate buffer pH 6.0	254 nm	[16]
Amino acids, *N*-benzoyl (alanine, valine, methionine, leucine, tryptophan, methionine, norleucine, norvaline)	Vancomycin (2 m*M*)	0.1 *M* phosphate buffer pH 6.0 or 7.0	254 nm	[7]
Amino acids, *N*-benzoyl (valine, leucine)	Teicoplanin (2 m*M*)	0.1 *M* phosphate buffer pH 6 (0–20% acetonitrile)	254 nm	[15]
Amino acids, carbobenzyloxy (alanine, valine, leucine, serine, phenylalanine, tyrosine, tryptophan, methionine, norleucine, norvaline)	Vancomycin (1 m*M*)	0.1 *M* phosphate buffer pH 6.0	254 nm	[7]
Amino acids, carbobenzyloxy (valine, leucine, serine, phenylalanine, tyrosine, aspartic acid)	Teicoplanin (2 m*M*)	0.1 *M* phosphate buffer pH 6(0–10% acetonitrile)	254 nm	[15]
Amino acids, Carbobenzyloxy (alanine, valine, leucine, serine, glutamic acid, methionine)	Ristocetin A (2 m*M*)	0.1 *M* phosphate buffer pH 6.0	254 nm	[16]
Amino acids, Dansyl (valine, leucine, threonine, phenylalanine, tryptophan, methionine, norleucine, norvaline, α-amino-*n*-caproic acid, α-amino-*n*-caprylic acid, serine)	Vancomycin (5 m*M*)	0.1 *M* phosphate buffer pH 4.9 or 6	254 nm	[7]
Amino acids, Dansyl (glutamic, phenylalanine, valine)	Vancomycin 1 or 2 m*M*)	0.1 *M* phosphate buffer pH 6 (polyacrylamide coated capillary)	254 nm	[30]
Amino acids, Dansyl (Ala, Leu, Gln, Phe, Ser, Thr)	Vancomycin (2 m*M*)	50 m*M* phosphate buffer pH 7.5, 0 or 10% 2-propanol	254 nm	[27]
Amino acids, Dansyl (aspartic)	Rifamycin SV (25 m*M*)	0.1 m*M* phosphate buffer pH 7–2-propanol (30:70, *v*/*v*)	Indirect UV 350 nm	[12]
Amino acids, Dansyl (valine, leucine, apsartic acid, phenylalanine, tryptophan, methionine,	Ristocetin A (2 m*M*)	0.1 *M* phosphate buffer pH 6.0	254 nm	[16]

(*Continued overleaf*)

Table 1. Continued

Compounds	Chiral Selector	BGE	Detection	Ref.
norleucine, norvaline, α-amino-*n*-butyric acid, α-amino-*n*-caprylic acid, serine)				
Amino acids, Dansyl (norleucine, norvaline)	Teicoplanin (2 m*M*)	0.1 *M* phosphate buffer pH 6 (acetonitrile 0–30%)	254 nm	[15]
Amino acids, Dansyl (valine, tryptophan, norleucine, norvaline, leucine, α-amino-*n*-butyric acid, phenylalanine)	Vancomycin (2 m*M*)	50 m*M* phosphate buffer pH 7.0 and 21–103 m*M* SDS	254 nm	[36]
Amino acids, *N*-3,5-dinitrobenzoyl (phenylglycine, leucine)	Teicoplanin (2 m*M*)	0.1 *M* phosphate buffer pH 6 (10–30% acetonitrile)	254 nm	[15]
Amino acids, *N*-3,5-dinitrobenzoyl (leucine, phenylglycine, phenylalanine)	Vancomycin (5 m*M*)	0.1 *M* phosphate buffer pH 7.0 or 6.0	254 nm	[7]
Amino acids, 2,4-dinitrophenyl (methionine, norleucine, norvaline, α-amino-*n*-butyric acid, amino-*n*-caprylic acid, glutamic acid, ethionine)	Ristocetin A (2 m*M*)	0.1 *M* phosphate buffer pH 7.0 and 0–30% 2-propanol	254 nm	[16]
Amino acids, 2,4-dinitrophenyl (methionine norleucine, norvaline, α-amino-*n*-butyric acid, citrulline)	Vancomycin (5 m*M*)	0.1 *M* phosphate buffer pH 7.0	254 nm	[7]
Amino acids, 2,4-dinitrophenyl (norleucine, norvaline, α-amino-*n*-butyric acid)	Teicoplanin (2 m*M*)	0.1 *M* phosphate buffer pH 6 (acetonitrile 0–30%)	254 nm	[15]
Amino acids, 2,4-dinitrophenyl (ethionine, citrulline, glutamic acid)	Teicoplanin (5 m*M*)	0.1 *M* phosphate buffer pH 6	254 nm	[15]
Amino acids, 2,4-dinitrophenyl (norleucine, norvaline, α-amino-*n*-butyric acid)	Teicoplanin (2 m*M*)	0.1 *M* phosphate buffer pH 6 (acetonitrile 0–30%)	254 nm	[15]
Amino acids, *N*-3,5-dinitropyridyl (alanine, leucine, serine, phenylalanine)	Vancomycin (5 m*M*)	0.1 *M* phosphate buffer pH 7.0	254 nm	[7]
Amino acids, *N*-3,5-dinitropyridyl	Teicoplanin (2 m*M*)	0.1 *M* phosphate buffer pH 6 (0–10% acetonitrile)	254 nm	[15]

(*Continued overleaf*)

Table 1. Continued

Compounds	Chiral Selector	BGE	Detection	Ref.
(leucine, serine, phenylalanine, norvaline)				
Amino acids, *N*-3,5-dinitropyridyl (alanine, tryptophan, methionine)	Teicoplanin (5 m*M*)	0.1 *M* phosphate buffer pH 6	254 nm	[15]
Amino acids, FMOC (ala, Asn, Asp, Cys, Gln, Glu, His, Ile, Leu, Lys, Met, Nleu, Nval, Phe, Pro, Ser, Trp, Tyr, Val)	Vancomycin (1 or 2 m*M*)	50 m*M* phosphate buffer pH 7.5, 0 or 10% 2-propanol	254 nm	[27]
Amino acids, FMOC (valine, alanine, 3,4-dihydroxyphenylserine, citrulline, norleucine, serine, proline, homoserine, isoserine, α-aminoadipic acid, α-aminopimelic acid, threonine, β-amino-isobutyric acid, 3,4-dehydroproline)	Ristocetin A (2 m*M*)	0.1 *M* phosphate buffer pH 7.0 and 0–10% 2-propanol	254 nm	[16]
Amino acids, FMOC (valine, citrulline, norleucine, serine, proline, tyrosine, *o*-tyrosine, leucine homoserine, α-, aminoadipic acid, α-amino-*n*-butyric acid, norvaline)	Teicoplanin (2 m*M*)	0.1 *M* phosphate buffer pH 6 (acetonitrile 0–30%)	254 nm	[15]
Amino acids, *N*-formyl (tryptophan, phenylalanine)	Ristocetin A (2 m*M*)	0.1 *M* phosphate buffer pH 6.0	254 nm	[16]
Amino acids, *N*-formyl (tryptophan, phenylalanine)	Vancomycin (2 m*M*)	0.1 *M* phosphate buffer pH 6.0	254 nm	[7]
Amino acids, *N*-formyl (phenylalanine)	Teicoplanin (2 m*M*)	0.1 *M* phosphate buffer pH 6	254 nm	[15]
Amino acids, PHTH (α-amino-*n*-butyric acid, methionine, valine)	Ristocetin A (2 m*M*)	0.1 *M* phosphate buffer pH 6.0	254 nm	[16]
Amino acids, Phthaldialdehyde (PHTH) (α-amino-*n*-butyric acid, methionine, glycyl-norleucine, glycyl-α-*n*-butyric acid, glycyl-tyrosine)	Vancomycin (2 m*M*)	0.1 *M* phosphate buffer pH 6.0	254 nm	[7]

(*Continued overleaf*)

Table 1. Continued

Compounds	Chiral Selector	BGE	Detection	Ref.
Amino acids, PHTH (methionine)	Teicoplanin (2 m*M*)	0.1 *M* phosphate buffer pH 6.0 % acetonitrile)	254 nm	[15]
Amphetamine	Rifamycin B (25 m*M*)	0.1 m*M* phosphate buffer pH 7–2-propanol (30:70, *v*/*v*)	Indirect UV 350 nm	[12]
Atenolol	Rifamycin B (25 m*M*)	0.1 *M* phosphate buffer pH 7–2-propanol (60:40, *v*/*v*) (*R*=0.7)	Indirect UV 254 nm	[11]
Bemethan	Rifamycin B (25 m*M*)	0.1 *M* phosphate buffer p H 7–2-propanol (60:40, *v*/*v*)	Indirect UV 254 nm	[11]
Bromacil	Vancomycin (2 m*M*)	95% 50 m*M* phosphate buffer pH 7.0–5% methanol and 25 m*M* SDS	254 nm	[37]
Carboxylic acids [2-phenoxypropionic, 2-chlorophenoxy)propionic, 2-(3-chlorophenoxy)propionic, 2-(4-chlorophenoxy)propionic, dibromohydrocinnamic, indolelacetic, *trans*-4-cotinine, 3-(4 hydroxyphenyl)lactic, iopanoic, iophenoxie, 2-(4-hydroxyphenoxy)propionic, 2-(4-nitrophenyl)propipnic, proglumide, β-phenyllactic, 3-phenylbutyric, tropic, 2-bromo-3-methylbutyric, *p*-chloromandelic, 3-hydroxy-4-methoxymandelic, hexahdroxymandelic, 2-methoxymandelic, 3-methoxymandelic, *O*-acetylmandelic, mandelic, benzocyclobutene]	Ristocetin A (2 m*M*)	0.1 *M* phosphate buffer pH 6.0 or 7.0 and 0–10% 2-propanol	254 nm	[16]
Carboxylic acids [3-oxo-2-indan, 3-[4-carbonyl]-PROXYL, 3-methyl-2-phenylbutyric, 2-phenylbutyric, α-aminothiophenacetic, hydroxymandelic, 1, 4-dihydro-2-methylbenzoic, 2-(4-dihydronitrophenyl)propionic amephoterin, atrolactic)	Ristocetin A (2 m*M*)	0.1 *M* phosphate buffer pH 6.0 or 7.0 and 0–10% 2-propanol	254 nm	[16]
Carboxylic acids (2-phenoxypropionic, 2-(2-chlorophenoxy)propionic, 2-(3-chlorophenoxy)propionic, 2-(4-chlorophenoxy)propionic, dibromohydrocinnamic, indolelacetic, *trans*-4-cotinine, 3-(4-	Vancomycin (2 m*M*)	0.1 *M* phosphate buffer pH 6.0	254 nm	[7]

(*Continued overleaf*)

Table 1. Continued

Compounds	Chiral Selector	BGE	Detection	Ref.
hydroxyphenyl)lactic, iopanoic, iophenoxic, 2-(4-hydroxyphenoxy)propionic 2-(4-nitrophenyl)propionic, methotrexate, proglumide, atrolactic)				
Carboxylic acids (2-phenoxypropionic, 2-(2-chlorophenoxy)propionic, 2-(3-chlorophenoxy)propionic, 2-(4-chlorophenoxy)propionic, proglumide, indolelactic, *trans*-4-cotinine, iopanoic, β-phenyllactic, tropic, 2-bromo-3-methylbutyric, *p*-chloromandelic, 3-hydroxymandelic, hexahydromandelic, 3-methoxymandelic, *O*-acetylmandelic, mandelic, benzocyclobutene, 5-methoxy-2-indanone, 5-methoxy-2-indanone acetic, 3-oxo-2-indan, 3-[4-carbonyl]proxyl, 2-phenylbutyric, α-aminothiophenacetic, amethoptorin, atrolactic, di-*O*,*O*′-toluoyl tartaric)	Teicoplanin (2 m*M*)	0.1 *M* phosphate buffer pH 6 (0–30% acetonitrile)	254 nm	[15]
Coumachlor	Vancomycin (2 m*M*)	90% 50 m*M* phosphate buffer pH 7.0–10% acetonitrile and 50 m*M* SDS	254 nm	[37]
Ephedrine	Rifamycin B (25 m*M*)	0.1 *M* phosphate buffer pH 7–2-propanol (60:40, *v*/*v*)	Indirect UV 254 nm	[11]
Epinephrine	Rifamycin B (25 m*M*)	0.1 m*M* phosphate buffer pH 7–2-propanol (30:70, *v*/*v*)	Indirect UV 350 nm	[12]
Epinephrine	Rifamycin B (25 m*M*)	0.1 *M* phosphate buffer pH 7–2-propanol (60:40, *v*/*v*)	Indirect UV 254 nm	[11]
Glutethimide	Rifamycin SV (25 m*M*)	0.1 m*M* phosphate buffer pH 7–2-propanol (30:70, *v*/*v*)	Indirect UV 350 nm	[12]
Herbicides (dichlorprop, diclofop, fenoprop, fenoxaprop, flamprop, fluazifop, fenoxaprop, mecoprop)	Vancomycin (6 m*M*)	75 m*M* Britton–Robinson buffer pH 5 (polyacrylamide coated capillary)	205 nm	[28]
Hexobarbital	Rifamycin SV (25 m*M*)	0.1 m*M* phosphate buffer pH 7–2-propanol (30:70, *v*/*v*)	Indirect UV 350 nm	[12]
Isoproterenol	Rifamycin B (25 m*M*)	0.1 *M* phosphate buffer pH 7–2-propanol (60:40, *v*/*v*)	Indirect UV 254 nm	[11]
Loxiglumide	Vancomycin (3 m*M*)	50 m*M* phosphate buffer pH 6 (polyacrylamide coated capillary)	205 nm	[13]
Metanephrine	Rifamycin B (25 m*M*)	0.1 *M* phosphate buffer pH 7–2-propanol (60:40, *v*/*v*)	Indirect UV 254 nm	[11]

(*Continued overleaf*)

Table 1. Continued

Compounds	Chiral Selector	BGE	Detection	Ref.
Metaprolol	Rifamycin B (25 m*M*)	0.1 m*M* phosphate buffer pH 7–2-propanol (30:70, *v*/*v*) *R*=0.7	Indirect UV 350 nm	[12] [12]
Metaproterenol	Rifamycin B (25 m*M*)	0.1 *M* phosphate buffer pH 7–2-propanol (60:40, *v*/*v*)	Indirect UV 254 nm	[11]
Metoprolol	Rifamycin B (25 m*M*)	0.1 *M* phosphate buffer pH 7–2-propanol (60:40, *v*/*v*) (*R*=0.8)	Indirect UV 254 nm	[11]
Normetanephrine	Rifamycin B (25 m*M*)	0.1 *M* phosphate buffer pH 7–2-propanol (60:40, *v*/*v*)	Indirect UV 254 nm	[11]
Non-steroidal antiinflammatory drugs (flurbiprofen)	LY307599 M_r, 1731 (4.0 m*M*)	100 m*M* borate buffer pH 9.2 and 15% methanol	254 nm	[19]
Non-steroidal antiinflammatory drugs (carprofen, cicloprofen, flurbiprofen, ibuprofen, indoprofen, ketoprofen, naproxen, suprofen)	Vancomycin (2,5 or 5 m*M*)	75 m*M* Britton Robinson buffer pH 5 (polyacrylamide coated capillary)	206 nm	[39]
Non-steroidal antiinflammatory drugs (flurbiprofen, fenoprofen, ibuprofen, indoprofen, ketoprofen, suprofen)	Vancomycin (1 or 2 m*M*)	0.1 *M* phosphate buffer pH 6 (polyacrylamide coated capillary)	254 nm	[30]
Non-steroidal antiinflammatory drugs (flurbiprofen, indoprofen, carprofen, ketoprofen, suprofen, fenoprofen, naproxen)	Ristocetin A (2 m*M*)	0.1 *M* phosphate buffer pH 6.0	254 nm	[16]
Non-steroidal antiinflammatory drugs (flurbiprofen, indoprofen, carprofen, ketoprofen, suprofen, fenoprofen, ibuprofen, naproxen)	Vancomycin (5 m*M*)	0.1 *M* phosphate buffer pH 7.0	254 nm	[7]
Non-steroidal antiiflammatory drugs (flurbiprofen, indoprofen, carprofen, ketoprofen, suprofen)	Vancomycin (2 m*M*)	50 m*M* phosphate buffer pH 7.0 and 21–103 m*M* SDS	254 nm	[36]
Non-steroidal antiinflammatory drugs (indoprofen, carprofen, ketoprofen, suprofen, fenoprofen)	Teicoplanin (2 m*M*)	0.1 *M* phosphate buffer pH 6(0–10% acetonitrile)	254 nm	[15]
Norepinephrine	Rifamycin B (25 m*M*)	0.1 m*M* phosphate buffer pH 7–2-propanol (30:70, *v*/*v*) *R*=0.9	Indirect UV 350 nm	[12]
Norepinephrine	Rifamycin B (25 m*M*)	0.1 *M* phosphate buffer pH 7–2-propanol (60:40, *v*/*v*)	Indirect UV 254 nm	[11]
Normethanephrine	Rifamycin B (25 m*M*)	0.1 m*M* phosphate buffer pH 7–2-propanol (30:70, *v*/*v*)	Indirect UV 350 nm	[12]
Norphenylephrine	Rifamycin B (25 m*M*)	0.1 *M* phosphate buffer pH 7–2-propanol (60:40, *v*/*v*)	Indirect UV 254 nm	[11]
Octopamine	Rifamycin B (25 m*M*)	0.1 m*M* phosphate buffer pH 7–2-propanol (30:70, *v*/*v*)	Indirect UV 350 nm	[12]

(*Continued overleaf*)

Table 1. Continued

Compounds	Chiral Selector	BGE	Detection	Ref.
Octopamine	Rifamycin B (25 m*M*)	0.1 *M* phosphate buffer pH 7–2-propanol 60:40, *v*/*v*)	Indirect UV 254 nm	[11]
Oxprenolol	Rifamycin B (25 m*M*)	0.1 m*M* phosphate buffer pH 7–2-propanol (30:70, *v*/*v*)	Indirect UV 350 nm	[12]
Oxprenolol	Rifamycin B (25 m*M*)	0.1 *M* phosphate buffer pH 7–2-propanol 60:40 (*v*/*v*) (*R*=0.4)	Indirect UV 254 nm	[11]
Peptides (Ala–Gly, Ala–Gly–Gly, Ala–Ala, Ala–Leu, FMOC–Leu–Gly, AEOC–Leu–Gly, AQC–Leu–Gly, FMOC–Leu–Gly–Gly, AEOC–Leu–Gly–Gly, AQC–Leu–Gly Gly, Dansyl–Leu–Gly–Gly, Leu–Leu, Gly–Ala, Gly–Asn, Gly–Asp, Gly–Leu, Gly–Met, Gly–Phe, Gly–Val)	Teicoplanin (1.2 m*M*)	25 m*M* phosphate–*Tris* pH 6.25, 40% acetonitrile	254 nm	[34]
Peptides, AEOC (Gly–Leu), Dansyl (Gly:Leu)	Vancomycin (0.5 m*M*)	50 m*M* phosphate buffer pH 7.5, 0–10% 2-propanol	254 nm	[27]
Peptides, FMOC (Gly–Ala, Gly–Leu, Gly–Phe, Leu–Gly, Ala–Leu, Leu–Ala)	Vancomycin (0.5 m*M*)	50 m*M* phosphate buffer pH 7.5, 0–10% 2-propanol	254 nm	[27]
Peptides, FMOC (Ala–Gly, Gly–Ala, Gly–Leu, Gly–Phe, Leu–Gly)	Vancomycin (1 m*M*)	25 m*M* HEPES buffer pH 7.6, 0 or 25 or 50 m*M* SDS	254 nm	[38]
Peptides, FMOC (Ala–Ala, Ala–Gly, Ala–Gly–Gly, Ala–Leu, Gly–Ala, Gly–Asn, Gly–Asp, Gly–Leu, Gly–Met, Gly–Phe, Gly–Val, Leu–Ala, Leu–Gly, Leu–Gly–Gly, Leu–Leu)	Vancomycin (1 m*M*)	50 m*M* phosphate buffer pH 7.6	254 nm	[38]
Pindolol	Rifamycin B (25 m*M*)	0.1 m*M* phosphate buffer pH 7–2-propanol (30:70, *v*/*v*) (*R*=0.3)	Indirect UV 350 nm	[12]
Propanolol	Rifamycin B (25 m*M*)	0.1 m*M* phosphate buffer pH 7–2-propanol (30:70, *v*/*v*)	Indirect UV 350 nm	[12]
Pseudo-ephedrine	Rifamycin B (25 m*M*)	0.1 *M* phosphate buffer pH 7–2-propanol (60:40, *v*/*v*)	Indirect UV 254 nm	[11]
Quinolonecarboxylic acids (ofloxacin and its related substances, DU-6859)	Vancomycin (5 m*M*)	0.1 *M* acetate buffer pH 4.0	300 nm	[14]
Salbutamol	Rifamycin B (25 m*M*)	0.1 *M* phosphate buffer pH 7–2-propanol (60:40, *v*/*v*)	Indirect UV 254 nm	[11]
Synephrine	Rifamycin B (25 m*M*)	0.1 *M* phosphate buffer pH 7–2-propanol (60:40, *v*/*v*)	Indirect UV 254 nm	[11]
Terbutaline	Rifamycin B (25 m*M*)	0.1 *M* phosphate buffer pH 7–2-propanol (60:40, *v*/*v*)	Indirect UV 254 nm	[11]
Warfarin	Vancomycin (2 m*M*)	50 m*M* phosphate buffer pH 7.0 and 25 m*M* SDS	254 nm	[37]

In another work [11] the effect of acetonitrile, methanol, ethanol, 1-propanol, 2-propanol and 2-propanol–0.5% methyl *tert*.-butyl ether mixture, separately added to the BGE containing rifamycin B, on the enantiomeric resolution and mobility of terbutaline, norphenylephrine and metoprolol was studied. The 2-propanol enhanced the resolution of the evaluated enantiomers. Increased resolution, decreased mobility and EOF were observed after increasing the concentration of the organic modifier.

The addition of 10% 2-propanol to the run buffer at pH 7.5, containing vancomycin as the chiral selector, gave rise to a decrease in enantiomeric resolution for almost all FMOC-amino acids studied. However the presence of an organic modifier improved the repeatability of migration times [35]. Wan and Blomberg [34] investigated the effect of teicoplanin and acetonitrile concentration on resolution and efficiency using a factorial design experiment where the separation of FMOC-Gly–Leu was observed. The results showed that the optimum experimental conditions were found using 25 m*M* phosphate–Tris buffer with 40% of acetonitrile at pH 6.25 and 1.2 m*M* teicoplanin.

From the examples reported above it can be observed that the effect of organic modifiers can be different depending on the type of the antibiotic used and analyte.

4.4. Effect of micellar phases

The composition of the running BGE, containing the antibiotic as chiral agent, can be modified by the addition of micellar phases to the buffer, e.g., sodium dodecyl sulfate (SDS). Rundlett and Armstrong [36] showed that the addition of SDS to the buffer containing vancomycin can be a good approach for improving efficiency, decreasing analysis time and controlling the order of enantiomers elution of chiral anionic analytes. Several dansyl-amino acid enantiomers have been separated with 50 m*M* phosphate buffer pH 7 containing 2 m*M* of vancomycin and 25 m*M* SDS; by increasing the SDS concentration up to 50 m*M*, better resolution was achieved [36]. It has been further demonstrated that the addition of a micellar phase to the buffer/antibiotic BGE also allowed the use of the high resolving power of antibiotics towards chiral neutral compounds performing their separation in a CE system. The separation of bromacil, bendroflumethiazide and 5-(4-hydroxyphenyl)-5-phenylhydantoin enantiomers with a buffer containing SDS-vancomycin are some examples [37].

The use of SDS as a buffer modifier enormously improved the efficiency of the separation of several FMOC derivatized peptide enantiomers with vancomycin. The 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) buffer at pH 7.60 and 1 m*M* vancomycin was modified with either 25 or 50 m*M* SDS. The addition of the surfactant to the chiral BGE caused an increase in EOF and a decrease in both the selectivity and migration times. Furthermore a reversal of migration order was observed [38].

4.5. Effect of capillary temperature

Finally it is worthy to remark that the capillary temperature has to be taken into account for method optimization in CE chiral analysis. In fact an increase of temperature causes a decrease of buffer viscosity and thus a change in the mobility of both analytes and antibiotics. Furthermore the stability of the chiral selector and the kinetic of the resolution mechanism can be influenced by this parameter. We studied the enantiomeric resolution of several acidic herbicides using vancomycin as the chiral selector changing the capillary temperature (20–30°C) and the best resolution and selectivity were achieved at the lowest value [28].

5. Main applications in chiral CE analysis using antibiotics

Chiral CE using antibiotics as chiral selector additives to the BGE has been successfully applied for the enantiomers separation of a wide number of compounds belonging to such different classes as amino acid derivatives, peptides, drugs, amino alcohols, organic anions etc. The electrophoretic analy-

ses have been carried out using both untreated or coated capillaries; good repeatability for migration time and/or peak areas has also been shown. Table 1 shows several examples of chiral separations by CE using different antibiotics as chiral selectors.

6. Conclusions

Enantiomeric separations can be easily achieved by CE by adding antibiotics to the BGE as chiral selectors and using either untreated or coated capillaries. The separated zones can be monitored by both direct or indirect UV detection.

Macrocyclic antibiotics have been demonstrated to be excellent chiral selectors exhibiting high enantioselectivity and resolution power at very low concentrations towards a wide variety of chiral compounds.

When using antibiotics as chiral selectors some drawbacks can be expected due to the adsorption and absorption phenomena influencing both the efficiency and sensitivity of the CE method. Several approaches have been proposed in order to overcome the above mentioned problems, e.g., modifying the BGE (pH, ionic strength) or using coated capillaries. Furthermore a counter-current mode can be advantageously employed in order to obtain the optimum conditions. An appropriate pH is selected so that the analytes and antibiotic are moving in opposite direction leaving the detector path free of chiral selector working with the highest sensitivity. This method has been shown useful for very good quantitative analysis [13,28].

According with the properties of analytes and antibiotics several parameters (pH, temperature) and operating conditions (type of capillary, BGE type, concentration and ionic strength, BGE additives, voltage, etc.) can be modified to improve the enantiomeric resolution and the separation efficiency. Furthermore the addition of a micellar phase to the buffer allows to use these chiral selectors also for the resolution of uncharged racemic compounds.

A wide number of enantiomeric separations have been summarised in Table 1 showing the analytical potential of this new class of chiral selectors.

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