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Short communication

Study on the use of boromycin as a chiral selector in capillary electrophoresis

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ABSTRACT

The first application of boromycin as a chiral selector in capillary electrophoresis is described. Given boromycin's insolubility in water, a non-aqueous background electrolyte based on methanol was used for enantiomeric discrimination of selected chiral primary amines (α-methylbenzylamine, *R*,*S*-tryptophanol, *R*,*S*-norepinephrine, *R*,*S*-octopamine, *R*,*S*-p-hydroxynorephedrine and *R*,*S*-2-amino-1-phenylethanol). A basic study of experimental conditions including the influence of boromycin concentration, the composition and concentration of background electrolyte and also the influence of different organic solvents was performed. The best separation condition was 75 mM Tris/50 mM boric acid in methanol, $^{s}_{w}$pH 9.0, with a positive separation voltage. The enantiomeric separation of the primary amines was achieved within 14 min with resolution values greater than 1.5 for the majority of the studied analytes.

1. Introduction

The development and study of new chiral selectors for enantiomeric separations represents an interesting and important area of research. Separation of various types of enantiomers e.g. pharmaceutical preparations, agrochemicals, food components, etc. is required in many areas of science and technology. Capillary electrophoresis (CE) is a highly efficient analytical technique that is particularly suitable for enantiomeric separations because of its very simple method development protocol. Most commonly, CE uses only the addition of a chiral selector to the background electrolyte. Several review articles on the separation of various enantiomers by CE have been published recently [1–4]. Many types of chiral selectors for use in CE [5–8] have been described in last twenty years (namely cyclodextrins and their derivatives, crown ethers, proteins, macrocyclic antibiotics, chiral surfactants, etc.). One important group of chiral selectors is based on macrocyclic antibiotics because they have high number of different stereogenic centers and they can interact with enantiomers by various types of interactions (hydrogen bonds, π–π interactions, coulombic interactions, van der Waals interactions). A variety of different types of these antibiotics were studied as chiral selectors in CE by Armstrong et al. [9–12]. Typical macrocylic antibiotics used in CE are the glycopeptides – vancomycin, teicoplanin and ristocetin A [13–15]. These chiral selectors have good solubility in water based background electrolytes. Some other antibiotics may be suitable for enantioseparations but their solubility in water is very limited or they show better enantiodiscrimination ability in non-aqueous environments. A novel trend is studying water insoluble chiral selectors in non-aqueous capillary electrophoresis (NACE). Chen et al. reported the first used erythromycin lactobionate in methanol based running electrolyte for enantioseparations of two basic drugs duloxetine and propranolol [16,17]. These were the first applications of a macrolide antibiotic as a chiral selector in NACE. Another macrolide antibiotic–azithromycin was used as chiral selector for enantioseparations of chiral drugs in non-aqueous background electrolyte [18].

Boromycin is a macrodiolide antibiotic that contains a stereogenic borate moiety [19,20]. The structure of boromycin is shown in Fig. 1. Boromycin is practically insoluble in water but its solubility is higher in polar organic solvents (methanol, propane-2-ol, acetonitrile) and has even greater solubility in solvents such as *N*,*N*′-dimethylformamide and dimethylsulfoxide.

Boromycin was introduced as a chiral selector by Armstrong et al. [21]. Boromycin covalently bonded to a silica gel support was used as a chiral stationary phase (CSP) in liquid chromatography (LC) and supercritical fluid chromatography (SFC). It was shown to have substantial enantiomeric selectivity for primary amines and aminoalcohols. Boromycin based CSP was used for separations with polar organic mobile phases. Excellent enantioresolution was

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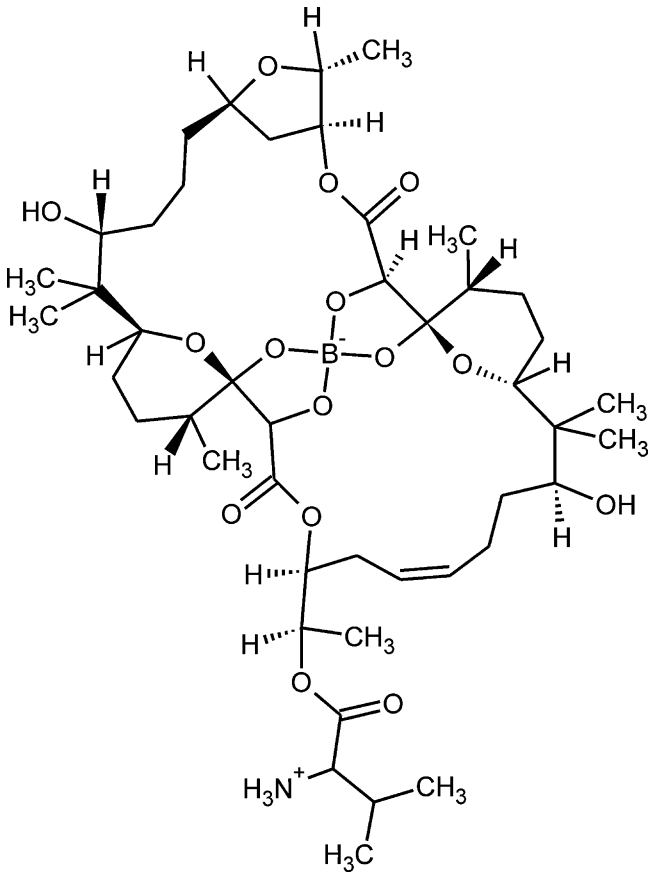

Fig. 1. Chemical structure of boromycin.

achieved for most of the separated primary amines. Boromycin presented an important alternative to chiral crown ethers that have good enantiorecognition ability for primary amines in aqueous environments. Later, another analytical application of boromycin was described. Boromycin was used as a molecular adaptor for a rapid and sensitive stochastic nanopore sensing method for the detection of liquid explosive components [22].

NACE has several notable characteristics for applications in enantiomeric separations. Non-aqueous background electrolytes generate lower Joule heating and so it is possible to use higher ionic strengths buffers. Consequently, higher separation voltages and short capillaries can be used. The mutual solubility of different non-aqueous solvents is commonplace in comparison to the solubility of water in organic solvents. A greater choice of available solvents leads to broader influences on selectivity. Because of the higher solubility of some chiral selectors in organic solvents higher enantioresolutions often can be obtained. Details regarding the use of NACE for enantiomeric separations have been published in several recent reviews [23–25].

The aim of this work is to introduce and evaluate boromycin as a chiral selector in NACE. The influences of boromycin concentration, background electrolyte components and concentrations, and the nature of the organic solvents on enantioselectivity are studied.

2. Materials and methods

2.1. Chemicals and reagents

Boromycin was obtained by fermentation from *Streptomyces antibioticus* (purity > 99%). The chiral primary amines *R*,*S*-α-methylbenzylamine, *R*,*S*-tryptophanol, *R*,*S*-norepinephrine, *R*,*S*-octopamine, *R*,*S*-p-hydroxynorephedrine and *R*,*S*-2-amino -1-phenylethanol were purchased from Sigma (St. Louis, MO, USA). The structures of studied enantiomers are shown in Fig. 2. Boric acid and Tris also were from Sigma (St. Louis, Mo, USA). Sodium hydroxide, sodium acetate anhydrous, sodium formate anhydrous, methanol, acetonitrile, propane-1-ol and propane-2-ol were obtained from Merck (Darmstadt, Germany). All chemicals were analytical grade purity.

The chiral primary amines were dissolved in methanol at a concentration 1 mg mL^{-1} and diluted by methanol to desired lower concentrations.

Running electrolytes were prepared by dissolving appropriate amounts of boric acid, Tris, sodium formate or sodium acetate in methanol and their pH values were measured. The calibration was performed using aqueous standard buffer solutions (phosphate buffer KH_2PO_4/Na_2HPO_4 pH_w 7.00 and borate buffer H_3BO_3/NaCl/NaOH pH_w 10.0). So, $^{s}_{w}$pH values were obtained. Boromycin (concentration range from 2 mM to 20 mM) was dissolved in the running electrolytes and the resulting buffer solutions were sonicated for 5 min.

The solubility of boromycin in the appropriate solvent was determined by visual observation of boromycin solubility. The series of boromycin solution in concentration range from 2 to 30 mM of boromycin (in 2 mM increments) in studied solvents were prepared and sonicated 15 min at laboratory temperature. After sonication the solubility was visually compared and maximum concentration of boromycin that can be dissolved in appropriate solvent was estimated. Such solution could be used for many separations without facing any difficulty with clogged capillary or repeatability of measurements.

2.2. Apparatus

The capillary electrophoresis unit used was a HP 3D Agilent (Waldbronn, Germany) equipped with on-column diode array detector. The separations were performed in fused silica capillary of 48.5 cm total length, effective length 40 cm and 50 μm I.D. (MicroSolv Technology Eatontown, NJ, USA). The capillary cassette was thermostated at 20 °C. Initial activation of the capillary was done by rinsing of the capillary with 1 M NaOH for 30 min, then 30 min with deionized water and at the end 30 min with methanol. The capillary was rinsed 15 min by 0.1 M NaOH, 15 min with deionized water and then with the running electrolyte solution at the beginning of each working day. Between individual analyses the capillary was washed 2 min with the running electrolyte containing the chiral selector. The detection wavelength was 200 nm except for *R*,*S*-α-methylbenzylamine that was detected at the wavelength of 214 nm. Injection was performed by pressure of 50 mbar/5 s. The applied voltage was +20 kV for all experiments. All the measurements were performed five times if not stated otherwise.

3. Results and discussion

3.1. Capillary electrophoresis

Boromycin is practically insoluble in water while its solubility in other organic solvents that are usually used in NACE with UV detection, such as methanol, acetonitrile, propane-2-ol and propane-1-ol, is slightly better [20,21]. First, the solubility of boromycin was tested in the mentioned conventional organic solvents. The solubility of boromycin decreased in the following order: methanol > propane-2-ol > propane-1-ol > acetonitrile. Therefore, methanol was the solvent of the first choice in this study. Methanol can be classified as a polar amphiprotic solvent that can act as both a proton donor and proton acceptor. Therefore,

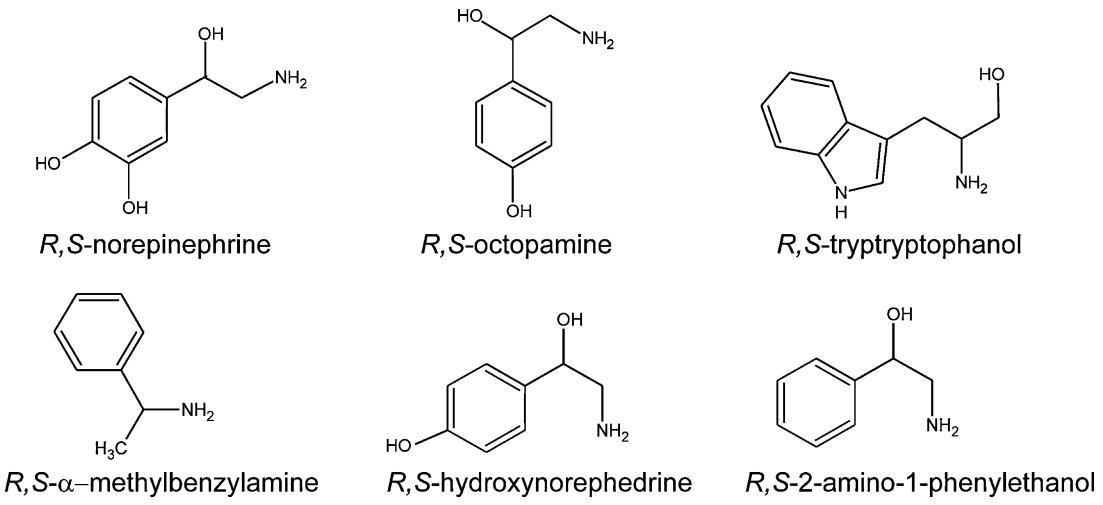

Fig. 2. The structures of studied primary amines.

methanol dissolves well the background electrolyte components that are usually used in non-aqueous capillary electrophoresis. Moreover, methanol has excellent compatibility with UV detection.

Stability of the methanolic solutions of boromycin depends on the pH of the background electrolytes. Acidic ($^{s}_{w}$pH < 3) and very basic electrolytes ($^{s}_{w}$pH > 11) can lead to hydrolysis of boromycin; thus the application of strong acid and strong alkaline pH values of the BGE with boromycin is limited.

Two BGEs, 20 mM ammonium acetate in methanol $^{s}_{w}$pH 7.5 and 20 mM ammonium formate in methanol $^{s}_{w}$pH 7.0, were chosen for the first measurements. At least partial enantioseparation was achieved for all the studied primary amines in these electrolytes. However, the highest values of resolution were lower than 1.5 even though the maximum dissolvable amount of the chiral selector, 20 mM of boromycin, in methanol was used. The exchange of methanol for other solvents, propane-1-ol, propane-2-ol and acetonitrile, did not provide any improvement of enantioresolution for any of the studied primary amines.

A methanolic Tris–boric acid based BGE allowed better resolution of the studied compounds, thus it was chosen for the next experiments. In this electrolyte, all the studied primary amines migrated as cations in the normal polarity mode with their own electrophoretic mobility and in the same direction as the moderate electroosmotic flow.

A study of the influence of boromycin concentration on the resolution of the enantiomers of primary amines was carried out in 75 mM Tris/50 mM boric acid in methanol, $^{s}_{w}$pH 9.0. The amount of boromycin in the running electrolyte varied from 2 mM to 20 mM. Higher amounts of boromycin in the methanol based electrolyte could not be studied because of the limited solubility of boromycin in methanol (20 mM). With increasing boromycin concentration an increase of the resolution values was observed for all the tested chiral analytes, with the exception of *R,S*-α-methylbenzylamine and *R,S*-tryptophanol, see Fig. 3. In the case of *R,S*-α-methylbenzylamine, the only analyte without a hydroxyl group, the highest resolution was observed at 15 mM concentration of boromycin. *R,S*-Tryptophanol was only partially separated at any boromycin concentration.

Then the study was focused on the evaluation of the concentration of $Tris^{+}$ co-ion in the running buffer composed of 50 mM boric acid in methanol with the addition of 20 mM boromycin on enantioselective resolution of the primary amines. The results are depicted in Fig. 4. It should be noted that with the change of Tris concentration in the BGE the $^{s}_{w}$pH value changes, too.

To make sure that pure methanol is the best solvent for the boromycin mediated enantioselective separation, various organic

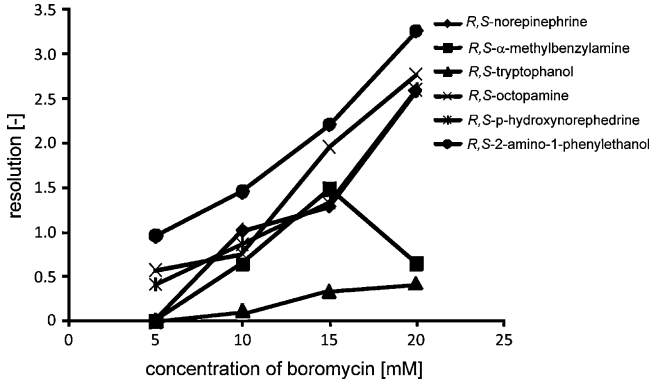

Fig. 3. The influence of boromycin concentration on chiral resolution of primary amines in 75 mM Tris/50 mM borate in methanol, $^{s}_{w}$pH 9.0, *U* = +20 kV.

solvent mixtures with 75 mM Tris/50 mM boric acid were tested. Mixtures of acetonitrile and methanol in *v*/*v* ratios 5:95, 10:90, 20:80, 50:50, 80:20 were studied most in detail. The addition of acetonitrile to methanol based electrolyte (the total concentration of 75 mM Tris/50 mM boric acid was kept constant in the BGE) resulted in decreased enantioresolution of all the studied primary amines. It seemed that the replacement of methanol (amphiprotic solvent) with acetonitrile (aprotic solvent) led to suppression of the interactions between boromycin chiral selector and the studied primary amines. Moreover, the solubility of boromycin decreased with the increase of the amount of acetonitrile in the running electrolyte. Similar results were obtained when propane-1-ol and/or

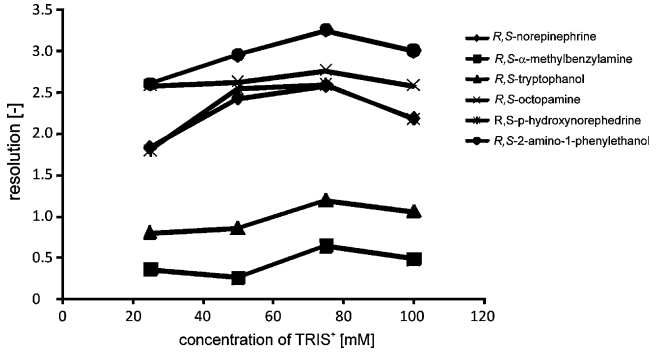

Fig. 4. The influence of $Tris^{+}$ concentration in 50 mM borate buffer in methanol, *U* = +20 kV.

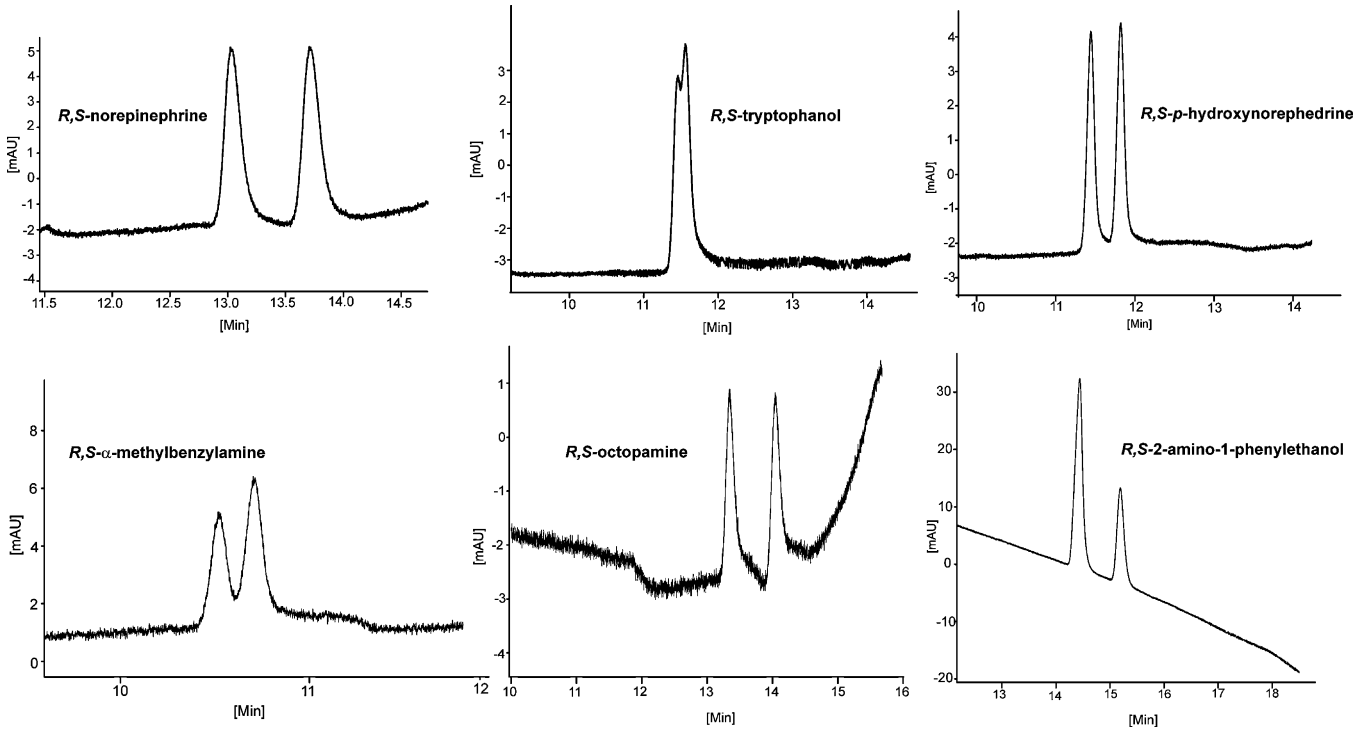

Fig. 5. Representative electrophoregrams of the chiral separation of the studied chiral primary amines. Conditions: 75 mM Tris/50 mM borate in methanol, ${}^{s}_{w}$pH 9.0, U = +20 kV, injection 50 mbar/5 s, concentration of analytes 0.05 mg mL^{-1} in methanol.

propane-2-ol were used in the mixture with methanol or when pure propane-1-ol or propane-2-ol was used. A substantial increase in migration times occurred in the latter BGEs owing to their higher viscosity if compared to methanol and acetonitrile. It can be stated that the best solvent for enantiomeric separation in NACE with boromycin chiral selector is methanol. The corresponding electropherograms of the studied primary amines under the optimized separation conditions are shown in Fig. 5. Baseline separations were achieved for almost all the tested analytes. Only the tryptophanol enantiomers could not be separated because stereogenic center in this molecule is too far away from any suitable interaction group or from the rigid aromatic moiety. Incomplete resolution also was found for methylbenzylamine. These results indicate that interaction with OH groups attached directly to the stereogenic center in the primary amines plays a more important role in the enantiodiscrimination mechanism than NH_2 group itself. The repeatabilities of the enantioresolution values were lower than 3.1% for all studied analytes. The repeatabilities of migration times of separated enantiomers were less than 2.9% intraday and less than 3.5% interday. Repeatability of areas was lower than 3.6% and 4.7% interday and intraday, respectively.

The electrophoretic properties of boromycin have not been published, yet. Therefore, the effective mobility of boromycin was measured in 75 mM Tris/50 mM boric acid in methanol with ${}^{s}_{w}$pH of 9.0. Boromycin migrated as a weak anion at these conditions and the value of its effective mobility was $-12.5 \pm 0.2 \times 10^{-9}$ m^2 V^{-1} s^{-1}. This result evidenced that boromycin chiral selector migrated in the opposite direction to the primary amines and in opposite direction to the electroosmotic flow $\mu_{eo} = 7.40 \pm 0.03 \times 10^{-9}$ m^2 V^{-1} s^{-1} (in the optimized BGE: 75 mM Tris/50 mM boric acid in methanol).

4. Conclusion

It was demonstrated that boromycin, one of the macrolide antibiotic, allows enantiodiscrimination of chiral primary amines in non-aqueous background electrolyte based on methanol as an amphiprotic solvent. This work points out simple applicability of non-aqueous capillary electrophoresis for enantioseparation of primary amines as an alternative to chiral separation of primary amines with crown ethers in aqueous electrolytes. NACE with boromycin as chiral selector can serve also as a complementary method to liquid chromatography with boromycin bonded chiral stationary phase [21].

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