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Influence of the nature of the electrolyte on the chiral separation of basic compounds in nonaqueous capillary electrophoresis using heptakis(2,3-di-*O*-methyl-6-*O*-sulfo)-β-cyclodextrin

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Abstract

The influence on the enantiomeric resolution of the nature of the cationic BGE component (sodium, ammonium or potassium) and that of the anionic component (chloride, formate, methanesulfonate or camphorsulfonate) as well as the concentration of heptakis(2,3-di-*O*-methyl-6-*O*-sulfo)-β-cyclodextrin (HDMS-β-CD), the selected chiral selector, was studied in nonaqueous capillary electrophoresis (NACE). For this purpose, two D-optimal designs with 33 and 26 experimental points were applied. Three β-blockers (atenolol, celiprolol and propranolol) and three local anesthetics (bupivacaine, mepivacaine and prilocaine) were selected as basic model compounds. Both cationic and anionic BGE components were found to have a deep impact on the enantiomeric resolution of the investigated analytes but it is the cationic component that has shown the strongest influence. Indeed, in some cases, the change of the latter led to a complete loss of enantioresolution. Based on the observed results, two NACE systems were recommended, namely ammonium formate and potassium camphorsulfonate in a methanolic solution containing HDMS-β-CD and acidified with formic acid, in order to separate efficiently the enantiomers of basic drugs.

Keywords: Nonaqueous capillary electrophoresis; Cyclodextrins; Enantiomer separation; Basic drugs; Background electrolyte compostion

1. Introduction

Drug determination has made great progress over the past decades in order to fulfill the requirements of research for the development of new pharmaceuticals and their therapeutic monitoring. When a pharmaceutical molecule is chiral, it is essential to separate and determine its optical isomers since one of the enantiomers can present a different pharmacological activity, be inactive or responsible for toxic effects. With this aim in view, capillary electrophoresis (CE), characterized by its high separation efficiency, constitutes nowadays one of the most commonly used analytical techniques.

The introduction of nonaqueous electrolyte solutions in CE offered new possibilities for changes in separation selectivity, due to the extension of the range of solvent parameters such as the dielectric constant, viscosity, polarity and auto protolysis [1–8]. The use of nonaqueous media in CE has also proved to be a very powerful tool for the enantiomeric resolution of chiral drugs [9].

Various chiral selectors have been tested and, among them, cyclodextrins (CDs) are the most widely used in nonaqueous capillary electrophoresis (NACE) [10]. Besides neutral CDs, charged CD derivatives have been used for enantioseparations in NACE and especially, the new generation of single-isomer sulfated, dimethylated or diacetylated derivatives, which are soluble in methanol [11–16]. In particular, the combination of the anionic β-CD derivative, heptakis(2,3-di-*O*-methyl-6-*O*-sulfo)-β-cyclodextrin (HDMS-β-CD), and potassium camphorsulfonate (camphorSO_3^-) has been successfully applied by our group to separate the optical isomers of a series of basic pharmaceuticals in NACE [17]. The presence of potassium

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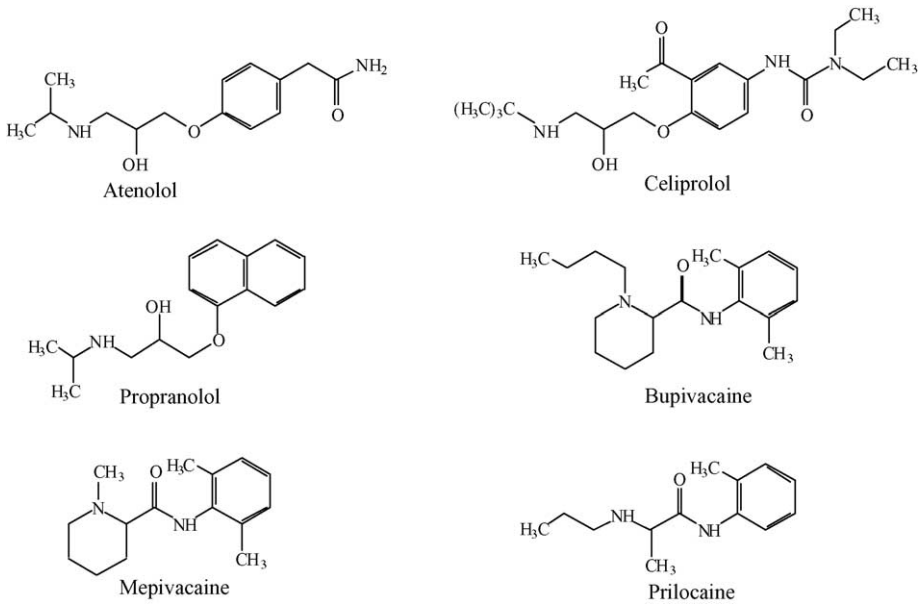

Fig. 1. Structures of the tested basic pharmaceuticals.

camphorSO_3^- was found to be particularly useful for the enantioseparation of compounds with relatively high affinity for the anionic CD. It seems that camphorSO_3^- is acting as a competitor, reducing the affinity for the CD, probably by ion-pair formation with these analytes. Therefore, camphorSO_3^- was only favorable for the enantioresolution of compounds having a relatively high affinity for HDMS-β-CD and for substances presenting a lower affinity, provided the optimal CD concentration could be reached [18].

The aim of this study is to investigate, by means of the methodology of experimental designs, the influence of the cationic and anionic components of the nonaqueous background electrolyte (BGE) on the enantiomeric resolution of β-blockers and local anesthetics in NACE using HDMS-β-CD. Indeed, since it was found that camphorSO_3^- is able to interfere in the enantioseparation process with this CD, it seems to be interesting to evaluate the ability of the BGE components to modify this process in NACE, especially in solvents with lower dielectric constants, which favor the interactions between the entities dissolved herein.

2. Experimental

2.1. Instrumentation

All experiments were carried out on a HP^{3D}CE system (Hewlett-Packard, Waldbronn, Germany) equipped with an autosampler, an on-column diode-array detector and a temperature control system (15–60 ± 0.1 °C). A CE Chemstation (Hewlett-Packard) was used for instrument control, data acquisition and data handling. The elaboration of the experimental designs and all statistical calculations were performed by means of Modde Software version 4.0 (Umetri A, Umea, Sweden). Fused silica capillaries were provided by ThermoSeparation Products (San Jose, CA, USA).

2.2. Chemicals and reagents

Propranolol hydrochloride was supplied by Sigma-Aldrich (St. Louis, MO, USA). Atenolol, bupivacaine, celiprolol, mepivacaine and prilocaine were kindly supplied by different pharmaceutical companies. All these drugs were provided as racemates and their chemical structures are presented in Fig. 1.

HDMS-β-CD was obtained from Antek Instruments (Houston, TX, USA). (1*S*)-(+)-10-Camphorsulfonic and methanesulfonic acids were from Sigma-Aldrich. Buffers were prepared with formic acid 98–100% (Merck, Darmstadt, Germany) at 0.75 M concentration and an electrolyte salt (10 or 40 mM). The considered range of HDMS-β-CD concentration was from 10 to 30 mM. All reagents were of analytical grade. In order to convert (1*S*)-(+)-10-camphorsulfonic and methanesulfonic acids into their sodium, ammonium or potassium salts, a quantity of sodium, ammonium and potassium formate (Sigma-Aldrich) corresponding to the concentration of the acid was added. Methanol from Merck was of LC grade. Buffers were filtered through a Durapore membrane filter (Millex-GV filters, 0.22 μm) from Millipore (Bedford, MA, USA). Samples solutions were filtered through a Polypure polypropylene membrane filter (0.2 μm) from Alltech (Laarne, Belgium) before use.

2.3. Electrophoretic conditions

Electrophoretic separations were carried out with uncoated fused silica capillaries having 50 μm internal diameter and 48.5 cm length (40 cm to the detector). At the beginning

of each working day, the capillary was washed with methanol for 10 min and with the BGE for 10 min. Before each injection, the capillary was washed successively with methanol for 2 min and then equilibrated with the BGE for 2 min. Capillary wash cycles were performed at a pressure of approximately 1 bar. The applied voltage was 25 kV and UV detection was performed at 230 nm. Injections were made by applying a pressure of 50 mbar for a period of 3 s (corresponding to 8.8 nl) and the capillary was thermostated at 15 °C. The sample solutions were prepared by dissolving each analyte at a concentration of approximately 50 μg/ml in methanol. The resolution (R_s) was calculated according to the standard expression based on the peak width at half height [19].

3. Results and discussion

3.1. Experimental designs

In our previous work dealing with the combination of an anionic ion-pairing reagent and an anionic β-CD derivative, the enantiomers of a series of basic pharmaceuticals were resolved by means of HDMS-β-CD and potassium camphorSO_3^- in methanol acidified with formic acid [17,18]. In the present paper, electrophoretic experiments were performed in different BGEs made up of HDMS-β-CD in methanol acidified with 0.75 M formic acid and containing an electrolyte salt. The effects of three factors on the enantiomeric resolution, the selected response, were investigated. Two factors were qualitative—the type of the cationic BGE component (Na^+, NH_4^+ or K^+) and the type of the anionic component [chloride, formate, methanesulfonate (methaneSO_3^-) or camphorSO_3^-]. The third one was quantitative—the concentration of HDMS-β-CD (10–30 mM). The effects of the cationic and anionic BGE components were evaluated at two concentrations, namely 10 and 40 mM. However, it is worth noting that at 40 mM concentration, all chloride salts could not be completely dissolved. Therefore, at the highest concentration, only nine electrolyte salts were studied. Consequently, two experimental designs were carried out: a first design was used to test the effect of twelve salts at 10 mM concentration as well as that of the HDMS-β-CD concentration (10–30 mM) and a second one investigated the influence of nine salts at 40 mM concentration together with that of the HDMS-β-CD concentration (10–30 mM). Three levels were selected for the quantitative factor in order to estimate the quadratic effects. Three β-blockers (atenolol, celiprolol and propranolol) and three local anesthetics (bupivacaine, mepivacaine and prilocaine) were selected as basic model compounds.

The quadratic regression model selected to define, for each electrolyte salt, the relationship between the response and the factors included eight coefficients (the intercept, β_0, the constant terms due to the cation, the anion and the interaction between both components, β_{0c}, β_{0a} and β_{0ca}, the main effects due to the CD concentration, the cation and the anion, β_1, β_c and β_a and one quadratic term, β_{11}), as indicated in the following equation:

$$Y = \beta_0 + \beta_{0c} + \beta_{0a} + \beta_{0ca} + (\beta_1 + \beta_c + \beta_a)X + \beta_{11}X^2 + \varepsilon \quad (1)$$

where Y is the enantiomeric resolution, X is the concentration of HDMS-β-CD and ε is the error term.

Two D-optimal designs were applied. For the first design (10 mM electrolyte concentration), 30 experiments were performed in a random order together with three replicates at the center point. Concerning the second D-optimal design (40 mM electrolyte concentration), 23 experiments were also carried out in a random order together with three replicates at the center point. The Modde software was used to elaborate these designs and to perform all statistical calculations. After having modeled each response, the different coefficients obtained were used to determine, for each investigated analyte and for each studied electrolyte salt, the relationship between the enantiomeric resolution and the HDMS-β-CD concentration. The results obtained for the six basic compounds were then examined one by one.

3.2. Examination of the effects

3.2.1. Propranolol

The influence of the HDMS-β-CD concentration on the resolution of propranolol enantiomers for each potassium and ammonium salt (at 10 mM concentration) is presented in Fig. 2. The results for sodium salts, producing an intermediate effect, are not presented for more clarity. As can be seen in the figure, the curves corresponding to the presence of the ammonium salt have a relatively strong slope, indicating that this cation has the more favorable effect in the sense that it does not hamper the increase of enantioresolution with increasing HDMS-β-CD concentration. In other words, the ion ammonium, being smaller than potassium, seems to be less susceptible to act as a competitor towards the cationic analytes for the formation of a complex with the anionic CD. As for the influence of the anion, the best results were obtained with chloride, producing, like the ammonium ion, the weakest competition effects, compared to formate, methaneSO_3^- and camphorSO_3^-. Indeed, the anionic component of the BGE may also compete with HDMS-β-CD by ion-pair formation with the cationic analytes.

R_s values obtained for each sodium, ammonium and potassium salt (at 40 mM concentration) are presented in Table 1. As can be seen in this Table, when an ammonium or potassium salt was used, resolution was found to change very little (or not at all) with increasing HDMS-β-CD concentration. By contrast, sodium salts, and in particular sodium methaneSO_3^-, had a very favorable effect and in this case, an increase in the CD concentration led to an important increase in resolution.

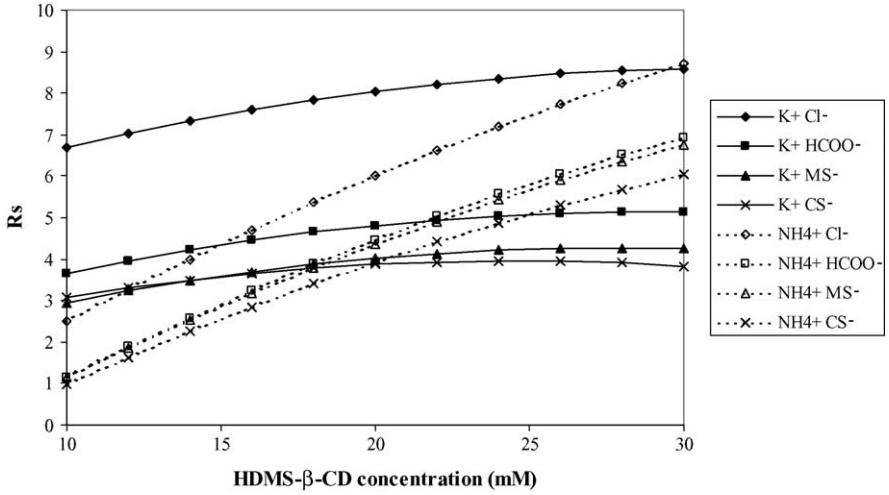

Fig. 2. Influence of the type of electrolyte (at 10 mM concentration) and the HDMS-β-CD concentration on chiral resolution of propranolol. The solid lines represent the potassium salts and the dotted lines, the ammonium salts. Symbols: diamond, chloride; square, formate; triangle, methaneSO_3^- and cross, camphorSO_3^-.

Table 1
Influence of the type of electrolyte and the HDMS-β-CD concentration on chiral resolution of propranolol

40 mM electrolyte		HDMS-β-CD concentration (mM)		
		10	20	30
Na^+	Formate	1.6	2.7	4.8
	MethaneSO_3^-	4.6	5.9	8.2
	CamphorSO_3^-	2.3	3.1	4.9
NH_4^+	Formate	0	0	0.7
	MethaneSO_3^-	0	0	1.5
	CamphorSO_3^-	0.7	0	0.7
K^+	Formate	1.6	1.4	2.2
	MethaneSO_3^-	1.6	1.5	2.5
	CamphorSO_3^-	3.2	2.6	3.1

Calculated R_s values which were negative or lower than 0.5 were arbitrarily settled to 0.

3.2.2. Mepivacaine

Fig. 3 presents R_s values obtained at 30 mM HDMS-β-CD and 10 mM electrolyte. As can be seen in this figure, sodium salts led to a complete loss of enantioresolution, whatever the nature of the anion. No explanation could be found for this amazing phenomenon, especially since HDMS-β-CD is provided as a sodium salt, and therefore, sodium is always present in the BGE. Nevertheless, it is important to remember that the CD is dissolved in methanol, characterized by a low dielectric constant. Consequently, the dissociation of the sodium salt of the CD is certainly very low in this medium, especially since the CD has seven sulfonate groups, which should strongly retain sodium through electrostatic interactions. A slightly better enantiomeric resolution was observed with the potassium salts, compared with the ammonium ones. As for the nature of the anion, it seems to have no significant impact.

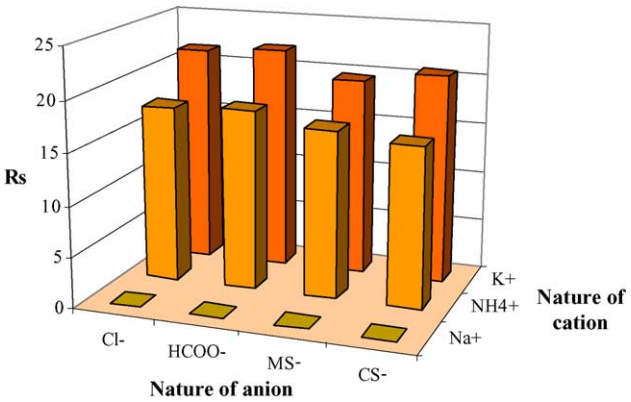

Fig. 3. Influence of the type of electrolyte (at 10 mM concentration) on chiral resolution of mepivacaine in the presence of 30 mM HDMS-β-CD.

The resolution of mepivacaine enantiomers obtained at 40 mM electrolyte concentration is presented in Fig. 4. At this concentration, the effect of sodium salts on the enantioresolution did not change: they led to the lowest R_s values. The presence of potassium or ammonium salts did not hamper the increase of R_s with increasing the HDMS-β-CD concentration.

3.2.3. Bupivacaine and prilocaine

From the results obtained for bupivacaine and prilocaine enantiomers, it appeared that the behavior of these two analytes was similar. Therefore, only the data for bupivacaine are presented. It was also noted that the type of anion had no influence on the enantioresolution. Consequently, the resolutions presented for bupivacaine are the mean values obtained for the different potassium, sodium and ammonium salts.

At 10 mM electrolyte concentration, the presence of potassium salts was clearly unfavorable (Fig. 5A). Indeed, the relationship between enantioresolution and the CD concentration presents a negative slope. By contrast, the slope was relatively strong for the ammonium salts, which have a favorable effect at this concentration.

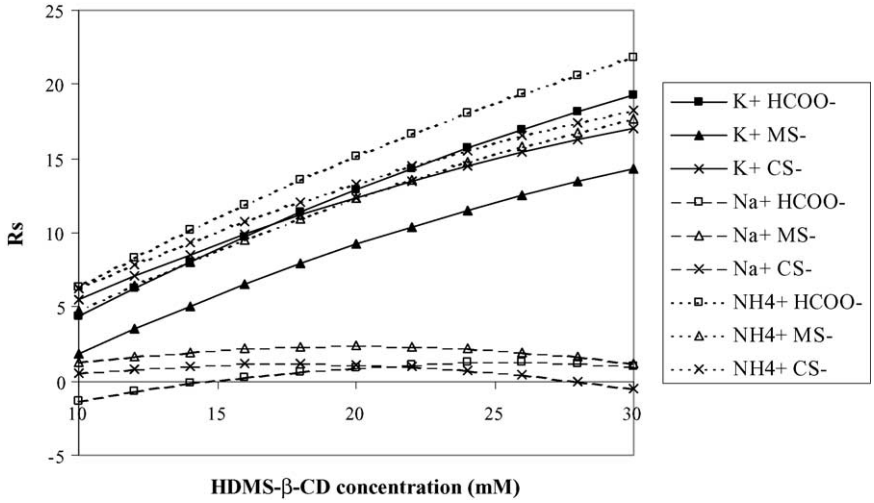

Fig. 4. Influence of the type of electrolyte (at 40 mM concentration) and the HDMS-β-CD concentration on chiral resolution of mepivacaine. The solid lines represent the potassium salts, the dotted lines, the ammonium salts and the dashed lines, the sodium salts. Symbols: square, formate; triangle, methaneSO_3^- and cross, camphorSO_3^-.

The influence of the HDMS-β-CD concentration on the resolution of bupivacaine enantiomers for each kind of salt at 40 mM concentration is presented in Fig. 5B. At this higher concentration, the potassium salts have a favorable effect and shift the optimal CD concentration to a higher value, compared to the ammonium salts for which the optimal concentration of HDMS-β-CD is reached within the experimental domain. Indeed, the curve corresponding to the ammonium salts passes through a maximum, located between 25 and 30 mM CD.

Finally, the enantiomers of bupivacaine could not be completely separated when sodium salts were used, as it was observed for mepivacaine.

3.2.4. Atenolol

R_s values obtained for each sodium and potassium salt (at 40 mM concentration) are presented in Table 2. It should be noted that the results obtained in the presence of ammonium salts are not included in the Table since R_s values obtained with ammonium formate, methaneSO_3^- and camphorSO_3^- were almost always lower than 0.5. As can be seen in this Table, the highest resolution values were observed with potassium salts at 10 mM CD concentration and among potassium

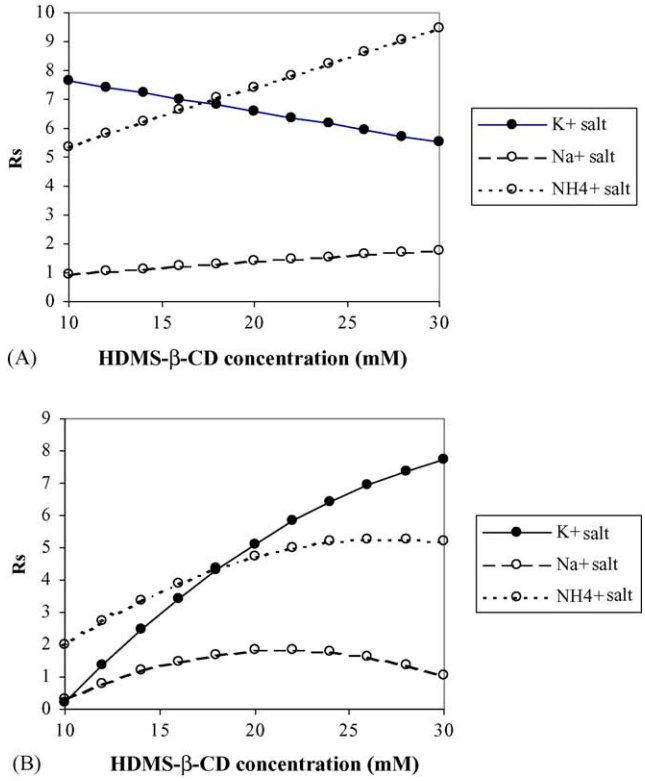

Fig. 5. Influence of the type of electrolyte and the HDMS-β-CD concentration on chiral resolution of bupivacaine at (A) 10 mM and (B) 40 mM electrolyte concentration. The solid lines represent the potassium salts, the dotted lines, the ammonium salts and the dashed lines, the sodium salts.

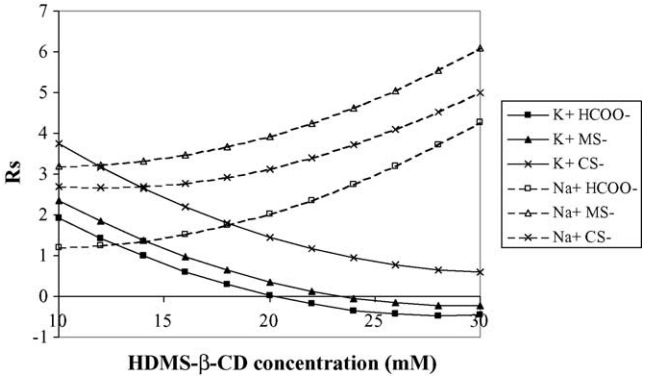

Fig. 6. Influence of the type of electrolyte (at 40 mM concentration) and the HDMS-β-CD concentration on chiral resolution of celiprolol. The solid lines represent the potassium salts and the dashed lines, the sodium salts. Symbols: square, formate; triangle, methaneSO_3^- and cross, camphorSO_3^-.

salts, it is camphorSO_3^- that leads to the best result, i.e. the most competing anion. As for the sodium salts, methaneSO_3^- gives rise to a relatively high chiral separation, as it was previously observed for propranolol.

3.2.5. Celiprolol

The influence of the HDMS-β-CD concentration on resolution of celiprolol enantiomers for each potassium and sodium salt (at 40 mM concentration) is presented in Fig. 6.

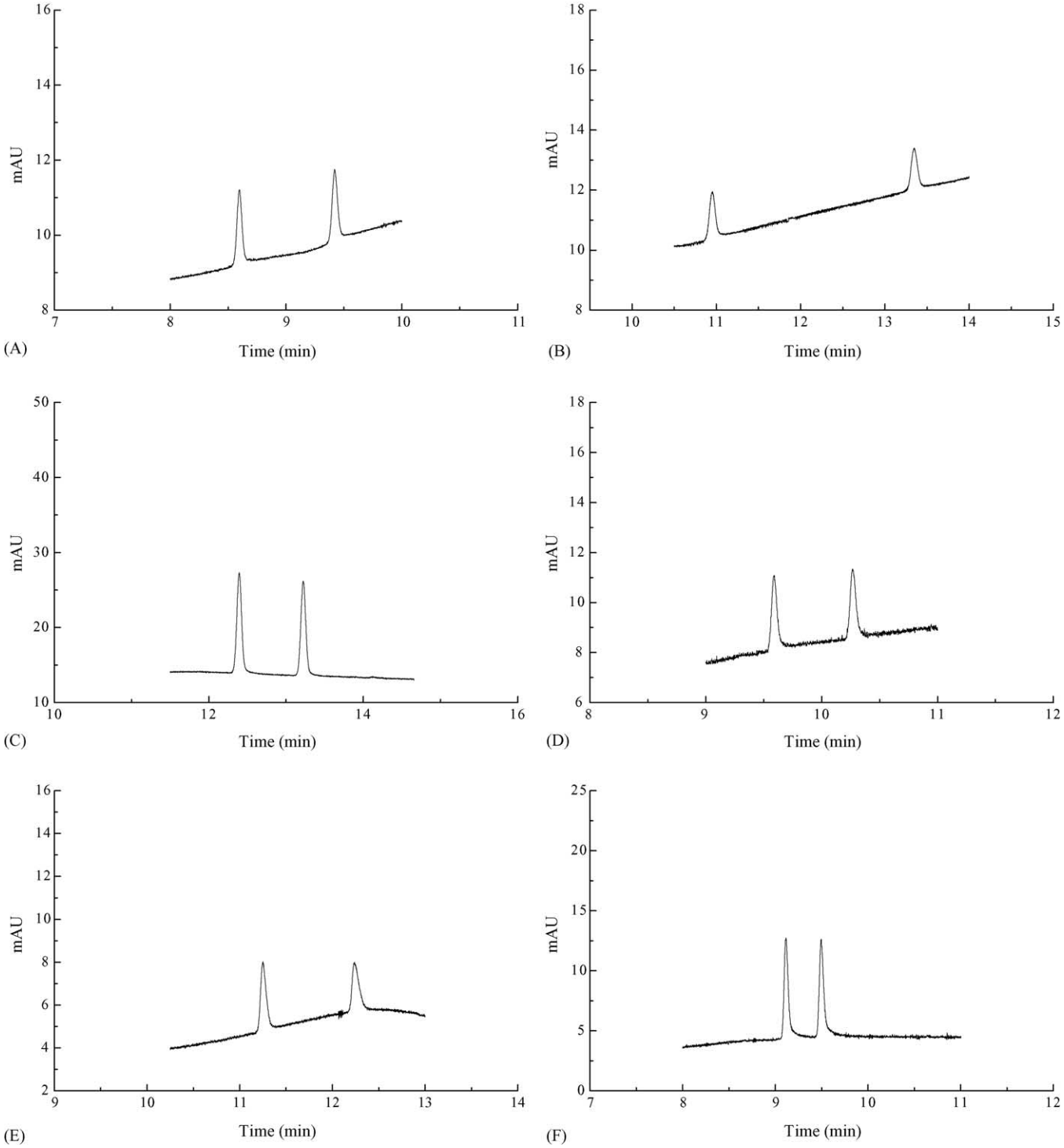

Fig. 7. Electropherograms obtained under the two recommended systems. Buffers: (A) bupivacaine, (B) mepivacaine and (C) propranolol: 30 mM HDMS-β-CD, 10 mM ammonium formate and 0.75 M formic acid in MeOH. (D) Prilocaine: 30 mM HDMS-β-CD, 40 mM potassium camphorSO_3^- and 0.75 M formic acid in MeOH. (E) Atenolol and (F) celiprolol: 10 mM HDMS-β-CD, 40 mM potassium camphorSO_3^- and 0.75 M formic acid in MeOH. Other conditions as described in Section 2.3.

Table 2
Influence of the type of electrolyte and the HDMS-β-CD concentration on chiral resolution of atenolol

40 mM electrolyte		HDMS-β-CD concentration (mM)		
		10	20	30
Na^+	Formate	0	0	0.9
	MethaneSO_3^-	2.3	2.1	4.7
	CamphorSO_3^-	1.3	0.7	2.2
K^+	Formate	6.8	0	0
	MethaneSO_3^-	3.5	0	0
	CamphorSO_3^-	8.6	0.7	0

Calculated R_s values which were negative or lower than 0.5 were arbitrarily settled to 0.

The results for ammonium salts are not presented for the same reason as mentioned for atenolol. As can be seen in this figure, the tendency is the same as for atenolol: resolution decreases with increasing CD concentration in the case of the potassium salts. Nevertheless, by contrast to what was observed for atenolol, sodium salts produced higher R_s values than potassium salts. In this case, the highest resolution was observed when a BGE made up of 30 mM HDMS-β-CD and 40 mM sodium methaneSO_3^- was employed.

3.3. Recommended NACE systems

The influence of the type of electrolyte ions on the enantiomeric resolution of the investigated analytes was clearly demonstrated. Not only the choice of the chiral selector but also the ionic composition of the BGE in which the selector has to be dissolved was found to play a crucial role in these NACE systems. It is, however, useful for the analysts to have generic methods at their disposal in order to separate efficiently and rapidly the enantiomers of a series of basic compounds. With this aim in view, two electrolyte salts seem to be particularly interesting, namely ammonium formate and potassium camphorSO_3^-. Indeed, with a methanolic solution containing 10 mM ammonium formate and 30 mM HDMS-β-CD acidified with 0.75 M formic acid, the enantiomers of bupicavaine, mepivacaine and propranolol could be resolved in a very satisfactory way (R_s values: 9.6, 17.8 and 6.9, respectively). Fig. 7A–C show electropherograms of the three analytes obtained under these experimental conditions. Not only this electrolyte can generate high enantiomeric resolution values but it should also represent an excellent choice in the case of on-line coupling of CE to MS, due to its good volatility. Finally, a second electrolyte that seems to be very interesting is potassium camphorSO_3^-. As can be seen in Fig. 7D–F, a methanolic solution containing 40 mM potassium camphorSO_3^- and acidified with 0.75 M formic acid could lead to an efficient separation of the enantiomers of prilocaine in the presence of 30 mM HDMS-β-CD (R_s value: 8.0) and those of atenolol and celiprolol, this time in the presence of 10 mM HDMS-β-CD (R_s value: 8.5 and 3.7, respectively). Moreover, potassium camphorSO_3^- has also proved to be an interesting electrolyte salt to resolve the enantiomers of a series of other basic drugs substances [17,18].

4. Conclusion

The type of the BGE has shown to have a strong influence on the enantioresolution of basic compounds in NACE using HDMS-β-CD. Even if the type of the anionic BGE component can influence the enantioseparation, it is mainly the cationic component that has a strong impact. Indeed, sodium salts lead to a complete loss of enantioresolution for mepivacaine and to the lowest resolution for bupivacaine and prilocaine enantiomers. Competition seems to be the main reason of the effects of the BGE components, competition either for complexation with HDMS-β-CD (in the case of the cationic BGE component) or for ion-pairing with the basic analytes (in the case of the anionic BGE component). But further experiments should be carried out to confirm this hypothesis.

In order to resolve efficiently and rapidly the enantiomers of basic drugs, two NACE systems are then recommended, namely ammonium formate and potassium camphorSO_3^- in a methanolic solution containing HDMS-β-CD and acidified with formic acid.

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