Journal of Chromatography A, 1462 (2016) 146–152

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Three chiral ionic liquids as additives for enantioseparation in capillary electrophoresis and their comparison with conventional modifiers

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ARTICLE INFO

Article history:
Received 7 April 2016
Received in revised form 18 July 2016
Accepted 25 July 2016
Available online 26 July 2016

Keywords:
Capillary electrophoresis
Enantioseparation
Chiral ionic liquids
Synergistic system
Conventional modifiers

ABSTRACT

The combined use of chiral ionic liquids (ILs) and conventional chiral selectors in CE to establish synergistic system has proven to be a convenient and effective approach for enantioseparation. In this work, three amino acid chiral ILs, tetramethylammonium-L-arginine (TMA-L-Arg), tetramethylammonium-L-hydroxyproline (TMA-L-Hyp) and tetramethylammonium-L-isoleucine (TMA-L-Ile), were first applied in CE enantioseparation to investigate their potential synergistic effect with hydroxypropyl-β-cyclodextrin (HP-β-CD). Markedly improved separations were obtained in the chiral ILs/HP-β-CD synergistic systems compared with single HP-β-CD system. Parameters, such as the chiral ILs concentration, HP-β-CD concentration, buffer pH, applied voltage and capillary temperature, were optimized. A systematic comparison of chiral ILs with conventional (commonly used) modifiers was also performed, including the use of achiral ILs, conventional salts and molecular organic solvents. In addition, the chiral configuration of ILs was investigated to demonstrate the existence of synergistic effect between chiral ILs and HP-β-CD. All these results indicate that chiral ILs, as additives for CE chiral separation, has significant superiority over conventional modifiers in certain cases.

1. Introduction

Ionic liquids (ILs) are a broad class of salts with melting points close to or below room temperature. They are usually composed of an organic cation (e.g. imidazolium, pyridinium or tetraalkyl ammonium) and numerous organic or inorganic anions (e.g. tetrafluoroborate, hexafluorophosphate or hydroxide). Compared with conventional salts or molecular organic solvents, ILs have been extensively studied in recent years due to their unique physical and chemical properties, such as negligible vapor pressure, good thermal stability, relatively high ionic conductivity, as well as moderate dissolvability. Besides, it is feasible to design and synthesize various task-specific ILs by changing their anion–cation combinations [1–4].

The development of methods for chiral separation is a topic of interest in pharmaceutical science. This interest stems from the fact that, in many cases, enantiomers of a racemic drug may exhibit different pharmacological and toxicological properties [5,6]. A variety of analytical techniques have been developed for enantioseparation over the past decades [7–9]. In addition to conventional chromatographic techniques (e.g. HPLC, GC), CE has been shown to be a high-performance separation tool for chiral separation due to its several advantages such as high separation efficiency, low cost, simplicity, as well as high flexibility [10–12]. The most common approach for CE enantioseparation is the addition of different chiral selectors (e.g. cyclodextrins and their derivatives, saccharides, antibiotics, etc.) to the running buffer [13–15]. In some cases, however, satisfactory enantioseparation could not be achieved using one single chiral selector without any modification. Therefore, the addition of additives/modifiers in combination with chiral selectors has attracted considerable interest [16–19].

The use of ILs in CE has proven to be a promising approach to improve chiral separation [19,20]. It has been reported that achiral ILs are able to modify the conventional chiral separation system by affecting the magnitude or direction of electroosmotic flow (EOF) and the ionic strength of running buffer, etc. Also, the peak tailing of some basic analytes could be suppressed to some extent by the competitive adsorption of IL cations on the capillary inner wall [21,22]. Chiral ILs, which have either a chiral cation or a chiral anion, or both, are particularly attractive for their potential applications in chiral discrimination [17,23–25]. A prominent advantage of chiral

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http://dx.doi.org/10.1016/j.chroma.2016.07.066
0021-9673/

ILs compared with achiral ILs is that they can usually bring extra "enantiorecognition" capability while retaining the "system modification" capability. However, only a limited number of literatures have reported the application of chiral ILs as additives in CE [26–33] and, to the best of our knowledge, no systematic comparison of chiral ILs with conventional additives/modifiers has been made to demonstrate the superiority of these chiral ILs synergistic systems.

In this paper, three amino acid chiral ILs, tetramethylammonium-L-arginine (TMA-L-Arg), tetramethylammonium-L-hydroxyproline (TMA-L-Hyp), and tetramethylammonium-L-isoleucine (TMA-L-Ile), were first applied to evaluate their synergistic effect with a classical chiral selector, hydroxypropyl-β-cyclodextrin (HP-β-CD). The comparison of chiral ILs as additives with conventional additives/modifiers, including achiral ILs, conventional salts and molecular organic solvents, was investigated in details.

2. Experimental

2.1. Chemicals and reagents

Amlodipine besylate (AML, pKa 8.6), nefopam hydrochloride (NEF, pKa 9.0), duloxetine hydrochloride (DUL, pKa 9.7) and propranolol hydrochloride (PRO, pKa 9.5) were supplied by Jiangsu Institute for Food and Drug Control (Jiangsu, China). All these drug samples are racemic mixtures. TMA-L-Arg, TMA-L-Hyp, TMA-L-Ile, TMA-D-Arg (purity > 99%), and tetramethylammonium hydroxide (TMA-OH, 25%) were purchased from Shanghai Chengjie Chemical Co., Ltd. (Shanghai, China). The structures of the enantiomers and ILs are shown in Fig. 1. HP-β-CD (purity > 98%, average substitution degree: 5.2) were purchased from Zibo Qianhui Biotechnology Co., Ltd. (Shandong, China). Nylon filters (0.45 mm), methanol, ethanol and acetonitrile (ACN), all of HPLC grade, were purchased from Jiangsu Hanbon Sci. & Tech. Co., Ltd. (Jiangsu, China). Sodium hydroxide, phosphoric acid, and thiourea were of analytical grade purchased from Nanjing Chemical Reagent Co., Ltd. (Jiangsu, China). Double distilled water was used throughout all the experiments.

2.2. Apparatus

Electrophoretic experiments were performed with an Agilent 3D CE system (Agilent Technologies, Waldbronn, Germany), which consisted of a sampling device, a power supply, a photodiode array UV detector (wavelength range from 190 to 600 nm) and a data processor. The whole system was driven by Agilent ChemStation software (Revision B.02.01) for system control, data collection and analysis. It was equipped with a 50 cm (41.5 cm effective length) × 50 μm id uncoated fused-silica capillary (Hebei Yongnian County Reafine Chromatography Ltd., Hebei, China). Sample injections were performed by pressure (50 mbar, 5 s). All separations were carried out at 10–20 °C using a voltage in the range of 15–25 kV. The wavelength for detection was 237 nm (AML), 230 nm (DUL), 220 nm (NEF) or 225 nm (PRO). The CE system was operated in a conventional mode with the anode at the injector end of the capillary. A new capillary was first rinsed with 1.0 M NaOH (30 min), followed by 0.1 M NaOH (20 min) and water (20 min), respectively. At the beginning of each day, the capillary was flushed with 0.1 M NaOH (10 min) followed by water (10 min). Between consecutive injections, the capillary was rinsed with 0.1 M NaOH, water and running buffer for 3 min each.

2.3. Procedures

The background electrolyte (BGE) consisted of 40 mM Tris solution (if not stated otherwise), was adjusted to a specified pH value with H_3PO_4 (10% v/v). The running buffer solutions were freshly prepared by dissolving appropriate amounts of HP-β-CD and/or other additives in BGE, and then adjusting pH exactly to a desired value by adding a small volume of H_3PO_4 (10% v/v) using a microsyringe. The racemic samples (1.0 mg/mL) were dissolved in distilled water. Running buffers and samples were filtered with a 0.45 μm pore membrane filter and degassed by sonication prior to use.

2.4. Calculations

The resolution (Rs) and selectivity factor (α) of enantiomers were calculated from $Rs = 2\,(t_2 - t_1)/(w_1 + w_2)$ and $\alpha = t_2/t_1$, where t_1 and t_2 are the migration times of the two enantiomers, and w_1 and w_2 are the widths of their peaks at the baseline. The EOF was expressed by the equation, $\mu_{eof} = (L{\cdot}l)/(V{\cdot}t_0)$, where L, l, V and t_0 are total capillary length, effective capillary length, applied voltage and migration time of thiourea (a neutral marker), respectively.

3. Results and discussion

It has been shown theoretically and experimentally that chiral ILs are able to recognize enantiomers [17]. The use of amino acid chiral ILs in MEKC or CZE for direct CE enantioseparation was also reported, which sufficiently proved their enantiorecognition capability [23,34]. However, not all amino acid chiral ILs can be used as a sole chiral selector because, for some of them, their enantiorecognition capability may not be strong enough to achieve observable enantioseparation when used alone. In this work, AML, DUL, NEF and PRO were selected as model drugs. TMA-L-Arg, TMA-L-Hyp and TMA-L-Ile were at first used as chiral selectors for direct enantioseparation, however, no separation was found under test conditions. Hence we focused our effort on investigating the synergistic effect of these chiral ILs with a conventional and classical chiral selector, HP-β-CD.

3.1. Separation performance of different chiral systems

3.1.1. Comparison of chiral ILs with achiral ILs and conventional salt

The enantioseparation performance of four chiral systems, including single HP-β-CD system; HP-β-CD/chiral ILs synergistic system, HP-β-CD/achiral ILs (TMA-OH) system, and HP-β-CD/conventional salt (TMA-Cl) system were investigated, respectively. Under the test conditions, the enantiomers were all partially or baseline resolved in the single HP-β-CD system with Rs values in the range of 0.96–1.59 (see Table 1). With the addition of chiral ILs, the EOF decreased significantly. This may be attributed to the increased ionic strength of the running buffer, as well as the adsorption of IL cations (TMA^+) on the capillary inner surface [20]. The prolonged migration time would provide more chances for enantiorecognition during the separation process. Also, the peak tailing of some basic analytes could be suppressed to some extent by the competitive adsorption of IL cations on the capillary inner wall. As expected, remarkably improved Rs and α were obtained in these chiral ILs synergistic systems compared with the single HP-β-CD system.

Here, one may question whether the decreased EOF is the main or only reason for the improved separation. To investigate this, a comparative study was performed using an achiral IL (TMA-OH) and a conventional salt (TMA-Cl) as additives. As observed, the two additives which have the same cations can also deduce the EOF to a similar extent (Table 1). However, their performance was significantly poorer than chiral ILs, which indicates that the structures and properties of the chiral part of ILs have a direct influence on enantiorecognition process (representative electropherograms are shown in Fig. 2). With regard to the three chiral ILs, TMA-L-Arg showed better enantioselectivities than TMA-L-Hyp and TMA-L-Ile