Journal of Chromatography A, 1306 (2013) 97–103

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography A

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

Evaluation of the enantioselectivity of glycogen-based synergistic system with amino acid chiral ionic liquids as additives in capillary electrophoresis

Qi Zhang [a], Yingxiang Du [a,b,c,*]

[a] Department of Analytical Chemistry, China Pharmaceutical University, Nanjing 210009, PR China
[b] Key Laboratory of Drug Quality Control and Pharmacovigilance (Ministry of Education), China Pharmaceutical University, Nanjing 210009, PR China
[c] State Key Laboratory of Natural Medicines, China Pharmaceutical University, Nanjing 210009, PR China

ARTICLE INFO

Article history:
Received 22 February 2013
Received in revised form 14 July 2013
Accepted 15 July 2013
Available online 19 July 2013

Keywords:
Capillary electrophoresis
Enantiomeric separation
Chiral ionic liquids
Amino acid ionic liquids
Glycogen
Synergistic system

ABSTRACT

In this paper, two novel amino acid chiral ILs, tetramethylammonium-L-arginine (TMA-L-Arg) and tetramethylammonium-L-aspartic acid (TMA-L-Asp), were applied for the first time in CE enantioseparation to evaluate their potential synergistic effect with glycogen as chiral selector. As observed, significantly improved separation of tested enantiomers were obtained in the chiral ILs/glycogen synergistic systems compared to the single glycogen separation system. Several primary parameters affecting the enantioseparation, such as amino acid ILs (AAILs) concentration, glycogen concentration and buffer pH, were systematically investigated. An achiral tetramethylammonium hydroxide ionic liquid (TMA-OH) modified separation system was also evaluated to validate the superiority of the novel chiral ILs/glycogen synergistic systems. To further optimize the overall synergistic systems, the effect of three other parameters, including buffer concentration, applied voltage and capillary temperature were simultaneously analyzed by a central composite design (CCD), and excellent enantioseparations were achieved with the optimized parameters. The results indicate that the application of chiral ILs/glycogen synergistic systems is a promising way in chiral separation science.

1. Introduction

Enantiomeric separation is an important subject in pharmaceutical analysis. The popularity stems from the fact that very often enantiomers of racemic drugs may exhibit different physiological activities as well as different even opposite pharmacokinetic and pharmacodynamic effects [1,2]. Various analytical techniques have been developed for enantioseparation. Among them capillary electrophoresis (CE) has been found to be a powerful alternative to chromatography for chiral separations over the last few decades due to its several known advantages such as high separation efficiency, short analysis time, convenient change in separation condition and extremely small volume requirements for sample and separation media [3–7].

The most common approach for enantiomeric separation in CE involves the addition of chiral selectors into the running buffer. Thus various kinds of chiral selectors have been developed, including cyclodextrins [8,9] and their derivatives [9,10], macrocyclic antibiotics [11,12], proteins [13], polysaccharides [14], etc. However, in many cases, satisfactory enantioseparation could not be achieved in the conventional separation system using one single chiral selector. Consequently, during the past years, research has been performed to enhance enantioselectivity in CE by introducing different types of additives into the electrolyte, and the combined use of more than one chiral recognition reagent to improve chiral separation has drawn increasing attention [15,16].

Room-temperature ionic liquids (ILs), which are a group of organic salts with melting point close to or below room temperature, have been studied extensively in recent years. They have many unique physicochemical characters, such as negligible vapor pressure, good thermal stability, relatively high ionic conductivity, designable properties and good redox-robustness (wide electrochemical potential windows) [17–20]. ILs has successfully been applied to various areas, such as electrochemical reactions, replacing conventional organic solvent in organic or inorganic synthesis, stationary phases in GC and mobile phases additive in HPLC [21–26]. In CE, ILs have already been used as electrolyte [27], running buffer modifiers [28], and supported coatings on the capillary wall [29]. Chiral ILs, which have a either chiral cation, chiral anion, or both, are particularly attractive for their potential applications

* Corresponding author at: Department of Analytical Chemistry, China Pharmaceutical University, No. 24 Tongjiaxiang, Nanjing, Jiangsu 210009, PR China. Tel.: +86 25 83221790; fax: +86 25 83221790.
E-mail address: du_yingxiang@126.com (Y. Du).

0021-9673/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.chroma.2013.07.053

to chiral discrimination. However, only a few papers have reported the application of chiral ILs in CE for enantiomeric separation, and the chiral ILs synergistic systems reported in these few studies were all based on cyclodextrins (CDs) and their derivatives [30–34]. To the best of our knowledge, there has been no prior attempt to investigate the enantioselectivity of chiral ILs synergistic system based on polysaccharides selector.

Recently, we have reported the use of glycogen as a novel polysaccharides selector for enantioseparation of some chiral compounds [35]. Glycogen is an electrically neutral and branched polysaccharide of high molecular weight playing a role in many tissues of the human and animal body as a storage carbohydrate. It has an average molecular weight of several million of Dalton, with-(1,4)-linked glucose subunits and-(1,6)-linked branching [36]. Glycogen possesses not only high solubility but also low viscosity in water. With the lack of conjugated double bond, aromatic rings and C=O bond, etc. in the structure, it also exhibits very weak UV absorption. Amino acid ionic liquids (AAILs) are synthesized from natural amino acid, thus most AAILs have stereogenic centers. Compared with other chiral ILs, AAILs have many advantages such as stable chirality, high biocompatibility and good biodegradability, etc. [37–39]. In this paper, tetramethylammonium-L-arginine (TMA-L-Arg) and tetramethylammonium-L-aspartic acid (TMA-L-Asp), two novel amino acid chiral ILs which have never been reported before, were first applied in CE enantioseparation to evaluate their potential synergistic effect with glycogen as the chiral selector. We presented details of the glycogen-based synergistic system with chiral ILs as buffer additives for enantioseparation by CE.

2. Experimental

2.1. Chemicals and reagents

Glycogen (>90%) was purchased from Sigma (St. Louis, MO, USA). tetramethylammonium-L-arginine (TMA-L-Arg), tetramethylammonium-L-aspartic acid (TMA-L-Asp) and tetramethylammonium hydroxide (TMA-OH) ionic liquids (purity > 98%) were purchased from Shanghai Cheng Jie Chemical Co., Ltd. (Shanghai, China), the structures of glycogen and three ILs are shown in Fig. 1. Nefopam hydrochloride (NEF, pKa 8.98), citalopram hydrobromide (CIT, pKa 9.78), duloxetine hydrochloride (DUL, pKa 9.70), were supplied by Jiangsu Institute for Food and Drug Control (Nanjing, China). All these drug samples are racemic mixtures. Tris (hydroxymethyl) aminomethane (Tris) was purchased from Shanghai Huixing Biochemistry Reagent (Shanghai, China). Nylon filters (0.45 mm) were purchased from Jiangsu Hanbon Science and Technology (Nanjing, China). Phosphoric acid and sodium hydrogen were of analytical reagent from Nanjing Chemical Reagent (Nanjing, 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). The samples were introduced hydrodynamically for 5 s (injection pressure 50 mbar). All separations were carried out at 10–20 °C

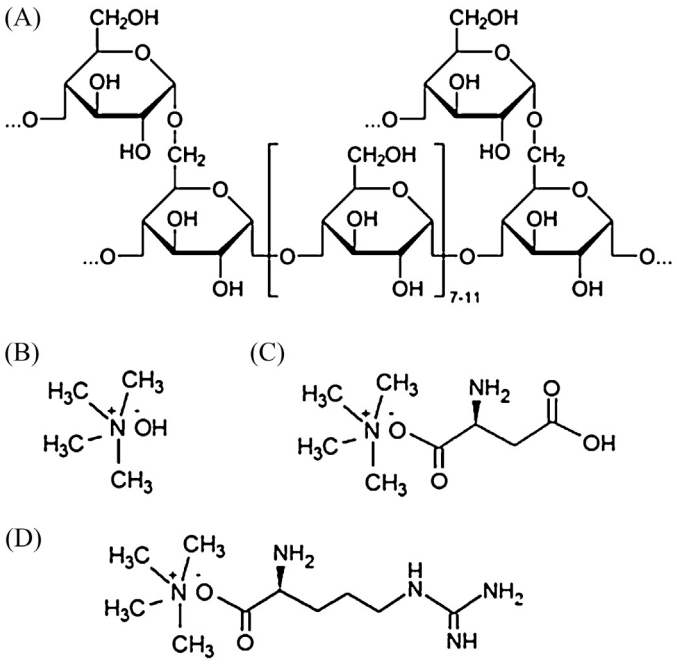

Fig. 1. Chemical structure of, (A) glycogen, (B) TMA-OH, (C) TMA-L-Asp, (D) TMA-L-Arg.

using a voltage in the range of 15–25 kV. The wavelength for detection was 237 nm (CIT), 230 nm (DUL), or 220 nm (NEF). The CE system was operated in the 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 back-ground electrolyte (BGE) consisted of 40 mM Tris solution (if not stated otherwise), adjusted to specified pH value with H_3PO_4 (10% v/v). The running buffer solutions were freshly prepared by dissolving appropriate amounts of glycogen and/or ILs 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 (0.6 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.

Experimental design and data analysis were performed by using the software Stat-Ease Design-Expert trial Version 8.0. For three factors, a central composite design was used consisting of 20 experiments, with 6 replicates of a central point. Experiments were combinations of the independent variables in the ranges: buffer concentration 20–60 mM, applied voltage 15–25 kV, capillary temperature 10–20 °C (As shown in Table 2A). The levels studied were selected considering the results of our previous exploration experiment. All experiments were performed in random order to minimize the effects of uncontrolled factors that may introduce bias in the measurements [40,41].

2.4. Calculations

The resolution (Rs) and selectivity factor (α) of the 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