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Evaluation of the enantioseparation capability of the novel chiral selector clindamycin phosphate towards basic drugs by micellar electrokinetic chromatography

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ABSTRACT

To date, a series of chiral selectors have been utilized successfully in capillary electrophoresis (CE). Among these various chiral selectors, macrocyclic antibiotics have been demonstrated to represent powerful enantioselectivity towards many chiral compounds. Differing from macrocyclic antibiotics, the use of lincosamide antibiotics as chiral selectors has not been reported previously. In our recent work, clindamycin phosphate belonging to the group of lincosamides has been first used as a chiral selector in capillary zone electrophoresis (CZE). In this paper, a micellar electrokinetic chromatography (MEKC) method has been developed for the evaluation of enantioseparation capability of this novel chiral selector towards several racemic basic drugs. As observed during the course of this work, clindamycin phosphate allowed excellent separation of the enantiomers of nefopam, citalopram, tryptophan, chlorphenamine, propranolol and metoprolol, as well as partial enantioresolution of tryptophan methyl ester and cetirizine. In this MEKC chiral separation system, different types of anionic surfactants, organic additives and background electrolytes were tested, and satisfactory enantioseparations of basic drugs above-mentioned were achieved using sodium dodecyl sulfate (SDS) as the surfactant, isopropanol as the organic additive, and phosphate as the background electrolyte. Furthermore, both migration times and enantioseparation of the analytes were influenced by several experimental parameters such as pH of the BGE, clindamycin phosphate and SDS concentrations, phosphate and isopropanol concentrations, and applied voltage. Consequently, the effects of these factors on enantioseparations of the studied basic drugs were systematically investigated in order to evaluate the stereoselectivity of clindamycin phosphate in MEKC.

1. Introduction

Capillary electrophoresis (CE) has been regarded as an attractive separation technique by reason of its high resolution efficiency, short analysis time, and the small amounts of analytes and running solution additives required [1–4]. Over the past decade, CE has also become a powerful technique for the separation of enantiomers [5–10]. Micellar electrokinetic chromatography (MEKC) is an important branch of CE, which has become one of the most popular techniques for the separation of enantiomeric compounds due to its high resolving power and capability of separating both ionic and neutral compounds [11]. Advantages of MEKC include high efficiency, fast analysis, and a powerful flexibility in rapidly tuning or changing the running buffer composition and subsequently the selectivity of the separation [12]. The MEKC technique, first introduced by Terabe et al. [13] for analysis of neutral analytes, uses a surfactant at a concentration above its critical micellar concentration (CMC) in the background electrolyte (BGE). One of the important properties of surfactant is the CMC, defined as a concentration below which the surfactant is in a solution as a monomer and above which practically all additional surfactant forms micelles [14]. The MEKC usually utilizes negatively charged micelles formed from anionic surfactants such as sodium dodecyl sulfate (SDS), which constitutes the pseudostationary phase (PSP). The separation is achieved by differential partitioning of analytes between the PSP and the bulk aqueous phase [15]. Electrostatic, hydrophobic interactions as well as hydrogen bonding with hydrophilic core of the surfactant play important roles in separation of analytes. What's more, chiral separations in MEKC are dependent on the use of different chiral selectors (cyclodextrins and their derivatives, macrocyclic antibiotics, calixarenes, etc. [16–19]) in the BGE in combination with various achiral surfactants. An alternative way

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is to use a chiral surfactant above its CMC in the BGE. The chiral surfactant forms micelles with stereogenic centers at the surface and it acts as the chiral selector. The chiral surfactants used in MEKC include natural chiral surfactants (such as digitonin [20], saponins [21] and bile salts [22]), synthetic chiral surfactants and high-molecular-mass surfactants [23].

Since Armstrong and co-workers [24,25] demonstrated that vancomycin was a useful chiral selector, macrocyclic antibiotics including glycopeptides, ansamycins, aminoglycosides and polypeptides have proved to be a powerful class of chiral selectors for both HPLC and CE. These antibiotics possess several characteristics that allow them to interact with analytes and serve as chiral selectors. They have a number of stereogenic centers and functional groups, allowing them to have multiple interactions with chiral molecules. Electrostatic or charge–charge, dipole–dipole, π–π, hydrophobic interactions, hydrogen bonding and steric repulsion are assumed to be the interactions responsible for chiral recognition [17,18,26]. Additionally, due to the presence of ionogenic groups in their structure they can be either positively or negatively charged as well as uncharged depending on the buffer pH [26]. Nevertheless, these macrocyclic antibiotics exhibited some drawbacks such as strong absorption in the UV wavelengths, low solubility in the water and adsorption on the capillary wall [27]. On account of this fact, we have been searching for other new types of antibiotics that can be used as chiral selectors for CE. Recently, we have reported the use of a new antibiotic, clindamycin phosphate (CP) belonging to the lincosamides family as a novel chiral selector in capillary zone electrophoresis (CZE) [28]. By comparison to macrocyclic antibiotics, the use of lincosamide antibiotics as chiral selector has not been reported before. CP has a molecular mass of 505, and consists of an amide portion, an amino portion (tertiary amine), a phosphate ester portion and an aglycon portion in each molecule (see Fig. 1). Compared to macrocyclic antibiotics, it possesses not only high solubility but also low viscosity in both water and alcohols. Furthermore, with the lack of aromatic rings in the structure, it exhibits very weak UV absorption.

In this paper, we further investigated and evaluated the enantiorecognition capability of this chiral selector towards several basic drugs with anionic surfactant in MEKC. In regard to the use of antibiotics as chiral selectors in MEKC, to date, only the glycopeptides have been used in conjunction with micelles. The composition of the running BGE, containing antibiotic as the chiral selector, can be modified by the addition of micellar phase to the buffer. Let's take glycopeptide antibiotics including vancomycin, teicoplanin and ristocetin A as examples. Rundlett and Armstrong [25] 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

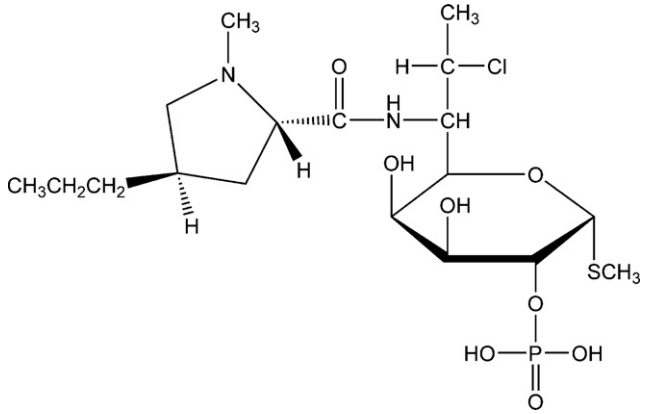

Fig. 1. Structure of clindamycin phosphate (CP) employed in this paper.

analytes. For example, several dansyl-amino acid enantiomers have been separated with 50 mM phosphate buffer (pH 7) containing 2 mM vancomycin and 25 mM SDS; by increasing the SDS concentration up to 50 mM, better resolution was achieved [25]. The addition of SDS to teicoplanin and ristocetin A-based separations has also been investigated. Ristocetin A, like vancomycin, comicellized with SDS to form mixed micelles, with the exception that ristocetin A partitioned to a greater extent than vancomycin to the SDS micelles [29]. In addition, teicoplanin–SDS systems were similar to vancomycin–SDS systems where analysis times decreased, elution orders reversed, and efficiency increased in the micelle-containing teicoplanin systems [29]. On the basis of these valuable results, in this work we presented details of enantioseparations of the studied drugs using CP as a chiral selector with anionic surfactant in MEKC.

2. Experimental

2.1. Chemicals and reagents

CP (purity > 99%, its structure is shown in Fig. 1) was supplied by Jiangsu Institute for Food and Drug Control (Nanjing, China). Propranolol hydrochloride (β-blocker, PRO), tryptophan (a member of α-amino acid, TRY), tryptophan methyl ester (TME), ephedrine hydrochloride (adrenergic agent, EPH), laudanosine (titanic poison, LAU), lomefloxacin hydrochloride (antimicrobial drug, LOM), ketoprofen (analgesic drug, KET), ibuprofen (analgesic drug, IBU), naproxen (analgesic drug, NAP), and pranoprofen (analgesic drug, PRA) were purchased from Sigma (St. Louis, MO, USA). Nefopam hydrochloride (analgesic drug, NEF), citalopram hydrobromide (psychotolytic drug, CIT), chlorphenamine maleate (antihistaminic drug, CHL), metoprolol tartrate (β-blocker, MET), cetirizine hydrochloride (antiallergic agent, CET), oxazepam (psychotolytic drug, OXA), mitiglinide (hypoglycemic agent, MIT), and nateglinide (hypoglycemic agent, NAT) were supplied by Jiangsu Institute for Food and Drug Control. All these drug samples were racemic mixtures.

Methanol, ethanol, isopropanol and acetonitrile, all of HPLC grade, were purchased from Jiangsu Hanbon Sci. & Tech. Co., Ltd. (Nanjing, China). SDS, sodium tetradecyl sulfate (STS), sodium octyl sulfate (SOS), sodium hydroxide, hydrochloric acid, phosphoric acid, dipotassium hydrogen phosphate, sodium tetraborate decahydrate, tris(hydroxymethyl)aminomethane (Tris), all of analytical grade, were purchased from Nanjing Chemical Reagent Co., Ltd. (Nanjing, China). Double distilled water was used throughout all the experiments.

2.2. Apparatus

Electrophoretic experiments were performed with an Agilent 3D capillary electrophoresis system (Agilent Technologies, Waldbronn, Germany), which consisted of a sampling device, a power supply, a photodiode array UV detector (wavelength range from 190 nm 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 43.5 cm (35 cm effective length) × 50 μm i.d. uncoated fused-silica capillary (Hebei Yongnian County Reafine Chromatography Ltd., Hebei, China). A new capillary was flushed for 10 min with 1 M HCl, 10 min with 1 M NaOH and 10 min with water respectively. At the end of each day it was flushed successively with 0.1 M NaOH (5 min) and water (5 min). Between consecutive injections the capillary was rinsed with 0.1 M NaOH, water and running buffer for 2 min each. Sample injections were performed by pressure (50 mbar, 4 s). Enantioseparations were performed at a constant voltage in a range