



Escherichia coli adhesive coating as a chiral stationary phase for open tubular capillary electrochromatography enantioseparation



Qifeng Fu^{a,*}, Kailian Zhang^a, Die Gao^a, Lujun Wang^a, Fengqing Yang^c, Yao Liu^c, Zhining Xia^{b,c,**}

^a Department of Medicinal Chemistry, Southwest Medical University, Luzhou 646000, China

^b Innovative Drug Research Centre and School of Pharmaceutical Sciences, Chongqing University, Chongqing 400030, China

^c School of Chemistry and Chemical Engineering, Chongqing University, Chongqing 400030, China

HIGHLIGHTS

- Bacteria were firstly introduced in OT-CEC as a chiral stationary phase for chiral separation.
- Enantioseparation of ofloxacin enantiomers was achieved on *E. coli* coated open tubular capillary column.
- Bacterial stationary phases may be used to predict the possible difference in antibacterial activities of chiral compounds.

GRAPHICAL ABSTRACT



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ABSTRACT

Bacteria, the microorganism with intrinsic chirality, have numerous fascinating chiral phenomena such as various chirality-triggered biological processes and behaviors. Herein, bacteria were firstly explored as novel chiral stationary phases in open-tubular capillary electrochromatography (OT-CEC) for enantioseparation of fluoroquinolone enantiomers and simultaneous separation of six fluoroquinolone antibiotics. The model strain, i.e. non-pathogenic *Escherichia coli* (*E. coli*) DH5 α , was adhered onto the inner surface of positively charged polyethyleneimine (PEI) modified capillaries based on the bacterial adhesion characteristics and strong electrostatic interaction. The morphology and thickness of the bacteria adhesive coatings in the capillary were characterized by field emission scanning electron microscopy (FESEM) and atomic force microscopy (AFM). Baseline separation of ofloxacin and partial separation of lomefloxacin enantiomers could be achieved by the *E. coli* coated columns. The preparation parameters including the coating time and concentration of bacteria that affecting the chiral resolution were intensively investigated. The electrophoretic parameters, including pH, buffer concentration and applied voltage, were also optimized. The developed method was validated (linearity, LOD, LOQ, intra-day, inter-day and column-to-column repeatability and recovery) and successfully utilized for the quantitative analysis of ofloxacin enantiomers in the ofloxacin tablets. Moreover, only a slight decrease in the separation efficiency was observed after 90 consecutive runs on the *E. coli*@capillary. These results demonstrated that bacteria are promising stationary phases for chiral separation in CEC.

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* Corresponding author.

** Corresponding author. Innovative Drug Research Centre and School of Pharmaceutical Sciences, Chongqing University, Chongqing 400030, China.

E-mail addresses: fuqifeng1990@163.com (Q. Fu), tcm_anal_cqu@163.com (Z. Xia).

1. Introduction

The enantioseparation of chiral compounds is of great importance in the pharmaceutical analysis [1,2]. Various chromatographic separation techniques such as high performance liquid chromatography (HPLC) and capillary electrophoresis (CE) have been widely utilized for chiral separation by using plenty of chiral selectors as stationary or pseudo-stationary phases [1–4]. To date, a variety of naturally occurring chiral selectors and their derivatives have been utilized for enantioseparation, ranging from small molecules such as cyclodextrins [2] and macrolide antibiotics [5] to biomacromolecules such as polysaccharides [6], proteins [7] and nucleic acids [8].

On the other hand, the elementary building blocks and functional units of living organisms in nature are composed of polysaccharides, proteins, nucleic acids and some other essential biomacromolecules, which may process chirality. Consequently, the living organisms, including bacteria, are typical chiral systems and various chiral phenomena are pervasive [9]. For instance, plenty of microorganisms have been used in biocatalytic asymmetric synthesis of chiral compounds [10,11]. Many bacteria tend to be more susceptible to a single enantiomer of antibiotics [12,13]. Some bacterial colonies can develop chiral morphology during their growth [14]. Besides, chiral flow patterns have also been observed in bacterial swarms [15]. Thus, given that bacteria possess intrinsic chirality at the macroscopic and microscopic scales, they may potentially be used as the chiral selectors for chiral separation. However, there are few researches on the possible use of bacteria in chiral separation. In our previous study, the potential utilization of bacteria as chiral selectors in chiral CE has initially been verified and baseline separation of ofloxacin enantiomers could be achieved by partial filling of *E. coli* or *Pseudomonas aeruginosa* as pseudostationary phases [16]. However, unsatisfactory reproducibility and poor separation efficiency were also observed. Therefore, it is essential to develop new applicable approaches to make better use of the stereoselective properties of bacteria in chiral separation.

Most bacteria prefer a surface-bound lifestyle to a nomadic existence and may eventually form biofilms, which are structured community of bacteria embedded in a self-produced matrix of extracellular polymeric substances [17]. The biofilms caused by the surface adhesion of bacteria are usually considered as troublesome issue, which may result in biofouling and bacterial infection [18]. Consequently, various approaches have been presented to inhibit the adhesion of bacteria [19–21]. In particular, the steric effects of bacterial adhesion on surfaces of chiral biointerface materials have been observed previously [22,23]. In contrast, considering the interfacial adhesion tendency and stereoselective properties of bacteria, it is an intriguing possibility that the adhered bacteria could be used as novel chiral stationary phases for chiral separation. To verify the assumption, we attempt to fabricate a novel chiral stationary phase consisting of bacteria for chiral separation by open-tubular capillary electrochromatography (OT-CEC). As one type of capillary electrochromatography (CEC) columns, OT-CEC is promising for chiral separation because it has some advantages compared with packed and monolithic CEC columns, such as the ease of column preparation, the absence of bubble formation and low back-pressure [24]. Nevertheless, it is difficult to immobilize sufficient amounts of bacteria on the electro-negative unmodified capillary inner surface because of the electrostatic repulsion. Recently, Riekkola's group successfully immobilized bacterial biofilms on the positively charged poly-L-lysine modified capillaries for the characterization of interactions between biofilms and antimicrobial compounds [25]. However, the exploitation on the potential

use of bacteria adhesive coating as chiral stationary phase for chiral separation has not yet received enough attention.

In the present study, inspired by their strong surface adhesion and stereoselective properties, we firstly introduce bacteria as chiral stationary phases in OT-CEC for chiral separation. For a proof-of-concept demonstration, non-pathogenic *E. coli* strain DH5 α was used as the model bacteria to fabricate bacteria coated columns. To immobilize these bacteria on capillary inner surface, the columns were pre-modified with positively charged polyethyleneimine (PEI), which could promote the adhesion of bacteria via electrostatic interaction [26,27]. The preparation parameters including the coating time and concentration of bacteria that affecting the chiral resolution were intensively investigated. The morphology and thickness of the bacteria adhesive coatings in the capillary were characterized by field emission scanning electron microscopy (FESEM) and atomic force microscopy (AFM). The fabricated *E. coli* coated columns are tested by the enantioseparation of fluoroquinolone antibiotics. Consequently, baseline separation of ofloxacin and partial separation of lomefloxacin enantiomers could be achieved. The fabricated bacteria coated columns possess good repeatability and stability. To the best of our knowledge, there is no report on the immobilization of bacteria as novel chiral stationary phases for chiral separation. In addition to possessing the enantioseparation efficiency, bacterial stationary phases may be used to evaluate the interaction between bacteria and chiral antimicrobial compounds under different conditions e.g. external pH and ionic concentration as well as to reveal the possible difference in antibacterial activities of potentially chiral antimicrobial compounds simultaneously.

2. Materials and methods

2.1. Chemicals and materials

E. coli DH5 α was provided by the School of Pharmacy and Bioengineering of Chongqing University of Technology. Nutrient broth and agar powder were brought from Jikong Biotechnology Co., Ltd (Shanghai, China). The racemates of ofloxacin, lomefloxacin and gatifloxacin were obtained from Adamas Reagent Co., Ltd (Shanghai, China). Levofloxacin and PEI solution with an average molecular weight of 750,000 Da (50% w/v in H₂O) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ofloxacin tablets (claimed to contain 0.1 g ofloxacin active ingredient) was purchased from Double-Crane Pharmaceutical Co. Ltd (Beijing, China). Acridine orange and ethidium bromide were purchased from Sangon Biotech Co. Ltd (Shanghai, China). Other reagents such as sodium hydroxide (NaOH), hydrochloric acid (HCl), phosphoric acid, sodium chloride (NaCl), disodium hydrogen phosphate (Na₂HPO₄) and dimethyl sulfoxide (DMSO) were all analytical grade and purchased from KeLong Chemical Reagent Co., Ltd. (Chengdu, China). The ultrapure water used in this study was purified with an AK's laboratory pure water system from the Kangning Lab ultrapure Equipment Co., Ltd. (Chengdu, China). Uncoated fused silica capillaries with a dimension of 50 μ m i.d. $\times$ 375 μ m o.d. (48.5 cm total length and 40.0 cm effective length) were obtained from Ruifeng Chromatographic Devices Co., Ltd. (Yongnian, Hebei, China).

2.2. Instrumentations

All the CE experiments were performed on an Agilent 7100 3D CE system (Agilent Technologies, Waldbronn, Germany) equipped with a diode array detector and an Agilent ChemStation software. The analytes were injected using a pressure of 50 mbar for 5 s. The

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