Enantioseparation of Ofloxacin by Ligand Exchange Capillary Electrophoresis Using L-Histidine Modified Nanoparticles as Chiral Ligand

L-histidin modifiye nanopartiküllerin kiral ligand olarak kullanılması ve ligand değişim kapiler elektroforez yöntemi ile ofloksasinin enantiyoayrılması

Research Article

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ABSTRACT

A novel application of chiral ligand-exchange capillary electrophoresis (LE-CE) was developed with polymeric nanoparticles as a chiral ligand and Cu^{2+} as a central ion. Nanoparticles (NPs) were prepared by polymerization of N-methacryloyl-L-histidine methyl ester (MAH) and ethylene dimethacrylate (EDMA). NPs were characterized by elemental analysis, fourier transform infrared spectroscopy (FTIR), atomic force microscopy (AFM). Average particle size and size distribution of NPs were also performed. Elemental analysis of MAH for nitrogen stoichiometry was found as 0.2 mmol/g polymer. CE systems that contain NPs in running buffer can be thought as pseudocapillary electrochromatography. Using this approach, enantiomer separation of ofloxacin was carried out by using LE-CE. The results demonstrated that NPs with chiral functionalized group interacted differently with structural enantiomers of ofloxacin. Factors affecting chiral resolution were studied. The optimum running conditions for the enantioseparation of ofloxacin were found to be a background electrolyte (BGE) (pH 4.7) containing 70% ACN, 10 mM $CuSO_4$, 40 mM $(NH_4)_2SO_4$ and 30 mg/mL NPs. Under these conditions, the enantioseparation of ofloxacin was successfully achieved. With this system, R-ofloxacin and S-ofloxacin (levofloxacin) were used to analysis capsules in the ofloxacin tablets.

Key Words

Ligand exchange, Capillary electrophoresis, ofloxacin, polymeric nanoparticles

ÖZET

L-histidin modifiye nanopartiküllerin kiral ligand ve Cu^{2+}'nin merkez iyon olarak kullanılması ile yeni bir kiral ligand değişim kapiler elektroforez yöntemi geliştirildi. İlk olarak, MAH ve EDMA'nın polimerizasyonu ile polimerik nanopartiküller hazırlandı. Nanopartiküller atomik kuvvet mikroskobu (AFM), FTIR ve elemental analiz ile karakterize edildi. Nanopartiküllerin elementel analizi sonucu polimerde 0.2 mmol/g azot olduğu bulundu. Elektrolit çözeltisi içerisinde nanopartiküller içeren kapiler elektroforez sistemi yalancı kapiler elektrokromatografi yöntemi olarak bilinir. Bu yaklaşım ile ofloksasinin enantiyoayırımı, ligand değişim kapiler elektroforez ile yapıldı. Kiral grup içeren nanopartiküller farklı şekilde ofloksasinin yapısal enantiomerleri ile etkileşim gösterdi. Kiral ayırmayı etkileyen faktörler incelendi. Ofloksasin için enantiyoayırma koşulları %70 ACN, 10 mM $CuSO_4$, 40 mM $(NH_4)_2SO_4$ (pH 4.7) ve 30 mg/mL nanopartikül olarak optimize edildi. Bu yöntem ile ofloksasinin enantiyoayrılması gerçekleştirildi ve ofloksasin tabletleri ile uygulması yapıldı.

Anahtar Kelimeler

Ligand değişimi, Kapiler elektroforez, ofloksasin, polimerik nanopartiküller

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Abbreviations:
EDMA, Ethylene dimethacrylate
LE, Ligand exchange
MAH, N-methacryloyl-L-histidine methyl ester
NPs, Nanoparticles
CE, Capillary Electrophoresis
EOF, Electroosmotic Flow
ACN, Acetonitrile
CEC, Capillary Electrochromatography

INTRODUCTION

Capillary electromigration techniques such as capillary electrophoresis (CE) and capillary electrochromatography (CEC) continues to grow. Enantioseparations using these techniques have become a rapidly burgeoning field in chiral separation science [1-12]. Chiral separations by electromigration techniques are performed in different methods. Among other types, ligand exchange capillary electrophoresis (LE-CE) has been extensively carried out using different chiral selectors with central ions such as Cu^{2+}, Zn^{2+}, Ni^{2+} [13-15]. Gassmann et al. firstly used Cu^{2+} complex with L-histidine as a chiral selector and successfully achieved in chiral separation of dansyl amino acids by LE-CE [16]. From that time now on, a lot of chiral compounds including pharmaceuticals, amino acids, foods as well as biologically active compounds have enantioseparated using LE-CE active technique [14,17,18].

Ofloxacin is one of the most commonly used fluoroquinolone antimicrobial included two enantiomers, R-ofloxacin and S-ofloxacin (levofloxacin). It is well known that the two enantiomers of chiral drugs exhibit different pharmacological and toxicological properties [19]. Therefore, the two enantiomeric forms of many drugs have highlighted the need for enantioselective separation. In chiral drugs, the desired pharmacologic effect is largely due to one enantiomer, however, its converse may be responsible for significant noxious side-effects. Pharmacological researches have shown that the antibacterial activity of S-ofloxacin is almost 128 times higher than that of R-ofloxacin [20,21]. Several researches using high performance liquid chromatography (HPLC) and CE have been documented in determination and enantioseparation of ofloxacin [22-24].

Chiral ligand exchange chromatography of ofloxacin by HPLC has been performed. However, this technique requires either special chiral columns for effective analysis of ofloxacin or chiral mobile phase additives such as cyclodextrin. It is well known that some major advantages of chiral separations by electromigration techniques in comparision with HPLC are the low consumption of chiral selector and mobile phase as well as more high plate numbers. On the other hand, to date, there have not any report on chiral LE-CE of ofloxacin in the literature.

Since nanoparticles (NPs) have high surface area, their usage in separation science is very important. Recently, applications of NPs as stationary phase of electromigration techniques have drawn attention. There have been some reports in the literature using NPs for electromigration [25-28]. Various kinds of nanoparticles such as polymeric NPs, silica NPs, gold NPs, fullerenes and carbon nanotubes have been utilized to enhance the separation efficiency of electromigration techniques [29].

In this study, L-histidine modified polymeric NPs are demonstrated as stereospecific interaction phase according to LE-CE principle. We present a novel application to enantiseparation of ofloxacin with the NPs. Efficient enantioseparation of ofloxacin has been observed with high selectivity after optimization of chromatographic parameters such as NPs concentration, pH and organic modifier composition of eluent.

EXPERIMENTAL

Chemical

Ethylene dimethacrylate (EDMA), NaOH, R-ofloxacin and S-ofloxacin were purchased from Sigma-Aldrich Chemical (Milwaukee, WI, USA). Ofloxacin tablets from TEVA pharmaceuticals were provided by a local pharmacy. N-methacryloyl-L-histidine methyl ester (MAH) was supplied from Nanoreg (Ankara, Turkey). Fused-silica capillary (i.d. 50 μm and, o.d. 360 μm) was supplied by Polymicro Technologies (Phoenix, AZ, USA). $CuSO_4.5H_2O$ was supplied by Salzburg vitriol. Acetonitrile (ACN), was of HPLC grade and supplied from Merck A.G (Darmstad, Germany). The other chemicals were of analytical grade.

Instrumentation

CE experiments were performed on a Prince CE-C-760 (Prince Technologies B.V. Cornelis Houtmanstraat 267825 VG Emmen, The Netherlands) equipped with a photodiode array dedector, a high voltage power supply (–30 kV and +30 kV). The elemental analysis of NPs were performed using a Leco Elemental Analyzer (Model CHNS-932). The Zeta Sizer (Malvern Instruments, Model 3000 HSA, England) was used for the measurement of the average poly(EDMA-MAH) NPs size and size distribution. FTIR spectrum analysis of NPs was carried out using a FTIR spectrophotometer (Perkin-Elmer, Spectrum One instrument, USA). The size of NPs was also analyzed by atomic force microscopy (AFM). (Digital Instruments, MMafm-2/1700 EXL)

The preparation of poly(EDMA-MAH) NPs

The poly(EGDMA–MAH) NPs were produced by surfactant free emulsion polymerization. The polymerization was performed according to our previous study procedure with minor modifications as reported elsewhere [30]. Firstly the dispersion phase was prepared with 0,5g poly(vinyl alcohol) dissolved in 50 mL deionized water. Then the monomer mixture EGDMA/MAH (0.85 mL/500 mg with respectively) was added to dispersion phase. Before the polymerization, potassium persulphate (KPS) was added to the monomer phase and nitrogen gas was blown through the medium for about 1–2 min to remove dissolved oxygen. KPS (initiator) concentration in monomer phase was 0.44 mg/mL. Polymerization was carried out in a constant temperature shaking bath at 70°C, under nitrogen atmosphere for 24 h. After the polymerization, the NPs were cleaned by washing with methanol and water several times to remove the unreacted monomers. For this purpose, the NPs were precipitated and collected with the help of a centrifuge (Beckman Coulter, Allegra 64R Centrifugen, U.S.A.) which is the rate of 25,000 g for 1 h and resuspended in ethanol and water several times. The washed NPs were resuspended in deionized water and this suspension was liyophilized to obtain dry NPs. The dry NPs were weighed and added in run buffer.

Enantioseparation conditions of LE-CE

All experiments were carried out in a long capillary mode of Prince-CE-C-760 (anode at inlet and cathode at oulet). A capillary with a total length of 36 cm and an effective length 29 cm was used. The inner wall of the capillary column was treated with 0.2 M NaOH for 30 min, followed by rinsing with pure water for 5 min, and then run buffer for 30 min. A solution of 10 mM $CuSO_4.5H_2O$, 40 mM $(NH_4)_2SO_4$ (60/40%, v/v) for run buffer was prepared. CE running buffer was composed of the solution and ACN (30/70%, v/v), respectively. At the begining of each day throughout analysis, the column flushed with water for 5 min, and then run buffer 20 min. Samples were injected hydrodynamically at 0.6 psi for 9 s and separated at +20 kV and 25°C. The analytes were detected at 293 nm. The mixture of R-ofloxacin (1 mg/mL) and levofloxacin (3 mg/mL) were prepared in water. 2 mg sample of drug was mixed with 3 mL mobile phase for 2 h. The solutions were filtered for further analysis. Capacity factors for each enantiomers and separation factors (α) were calculated according to our previous work [12].

RESULT and DISCUSSIONS

The preparation and characterization of poly(EDMA-MAH) NPs

The average size of poly(EDMA-MAH) NPs was performed using zeta sizer. The results were assembled in Figure 1A. The size of NPs quantified as 111.5 nm. As can also be shown in here, the polydispersity index was determined as 0.172. These results confirmed that NPs had the average of size with 110 nm. The elemental analysis for NPs showed that MAH for nitrogen was found as 0.2 mmol/g polymer. Polymerization ingredients don't contain nitrogen except MAH. This result is suitable according to the our previous study [30]. FT-IR spectrum of NPs was shown in Figure 1B. As can be shown here, the peak at 1733,64 cm^{-1} from carbonyl in the structure of EDMA and 1637,44 cm^{-1} due to N-H bending in the MAH structure. Therefore, these results indicated the composing of poly(EDMA-MAH) NPs. On the other hand, the size of NPs was also verified using AFM image in Figure 2, where the average size of NPs was around 110 nm.

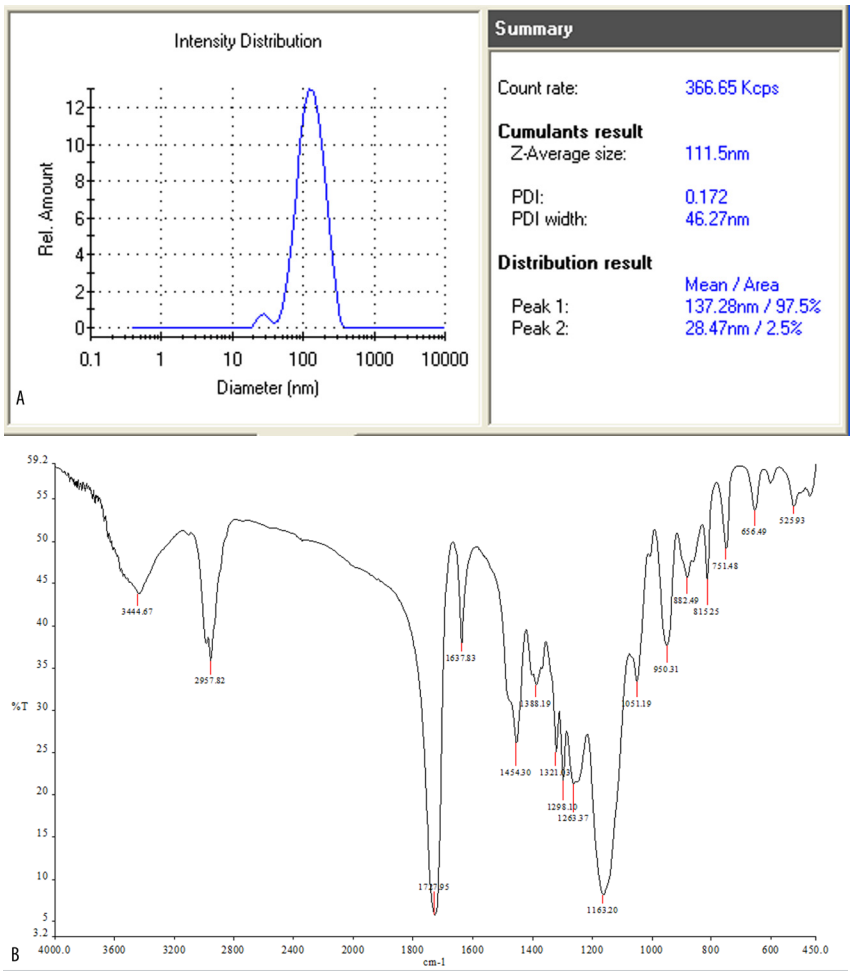

Figure 1. (A) Particles size distribution of poly(EDMA-MAH) NPs, (B) FTIR spectrum of poly(EDMA-MAH) NPs.

Ligand exchange mechanism

The priciple of chiral ligand exchange is based on complexes between ligands and a central ions, typically a metal ion. Cu^{2+} includes divalent metal ions. Enantioseparation by ligand exchange is restricted to analytes with two or three electron donating groups such as amino acids, hydroxy acids, amino alcohols or diols [31]. Ofloxacin as shown in Figure 3 has two relevant ionizable functional groups, a basic piperazinyl group and a carboxylic acid group. Chen and Tobo used L-phenyl alanine amide as a chiral selector and Cu^{2+} as a central ion and prepared monolithic silica column according to LE-CEC principle. They showed that the chiral discrimination based on the principle of ligand exchange attributing to the exchange of one ligand in the Cu^{2+} complex on the stationary phase an analyte ligand, forming ternary mixed copper complexes. Similarly, in our current study, the enantioselectivity depends on the different interactions between NPs and the complex consisting of Cu^{2+} and ofloxacin enantiomers. On the other hand, the same method of ligand exchange enantioseparation of ofloxacin in HPLC was demonstrated in literature [22].

Effects of concentrations of NPs and Cu^{2+} ions

In LE-CE, the ratio of ligand to central ion is of great importance for optimal resolution. In this study, while keeping the Cu^{2+} concentration constant, the NPs concentration was varied. Vice versa Cu^{2+} concentration was varied while keeping NPs concentration constant. The effects of concentration of NPs and Cu^{2+} were examined using different concentrations ranging from 5.0 to 60.0 mM. The results were shown in Figure 4. For NPs, the best results were achieved using

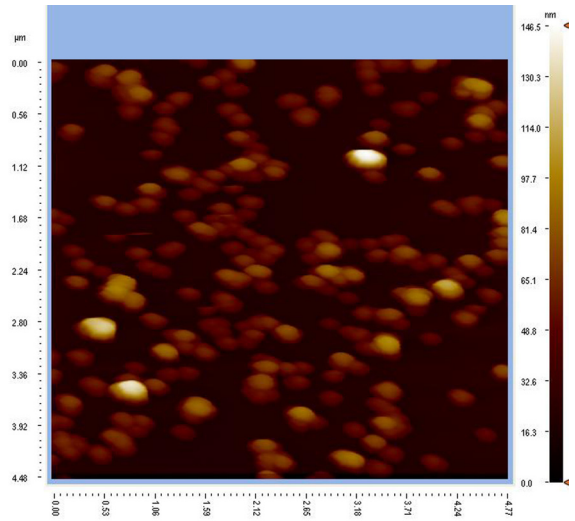

Figure 2. AFM image of poly(EDMA-MAH) NPs.

20 mM. When increasing concentration of NPs in run buffer, the aggregation of NPs was observed. For that reason, the resolution was not improved. On the other hand, the best result for Cu^{2+} was 10 mM. The optimal ratio of NPs and Cu^{2+} is 2:1, respectively. Considering these data, the ratio was selected as 2:1 for further investigations.

Effect of pH

The influence of pH on the optical resolution was investigated from 2.5 to 7.5. The results were shown in Figure 5A. As pH increases from 2.5 to 4.7, the separation factor (α) increase. This should be explained that ofloxacin are positively charged by means of piperazinyl group in the structure. On the other hand, the corresponding Cu^{+2} complexes are enhanced. Thus, the chiral separation factor improved. However, when increasing pH from 4.7 to 7.5, the chiral resolution of ofloxacin decreased. The imidazole moiety in MAH structure, which is responsable for chiral separation, may reduce the extent of complexation of the Cu^{+2} ternary complex because H^+ ions will complete with Cu^{+2} ions for the amin groups in the imidazole structure. Therefore, according to investigations above, pH 4.7 was selected for further studies of ofloxacin enantioseparation.

Effect of ACN content

Since electroosmotic flow (EOF) affect on the separation factor in electromigration techniques such as CE and CEC, it is important to control EOF in electrochromatographic enantioseparations. ACN content in mobile phase affects the magnification of EOF. Therefore, in this study, the effect of mobile phase content on separation factors of ofloxacin enantiomers was also investigated. The results were presented in Figure 5B. While keeping constant NPs concentration and increasing ACN content in the mobile phase, the separation factor was improved. It was also observed the increasing of EOF. As can be shown Figure 5B, ACN content as 70% (v/v) was selected for the further enantioseparation studies.

Enantioseparation studies of ofloxacin by LE-CE

Boer et al. performed the enantiseparation of ofloxacin using an anionic cyclodextrin-derivative with or without combination with a neutral cyclodextrin-derivatives, as chiral selector (s) in an electrokinetic chromatography system. [33] For this study, mobile phase as 0.35

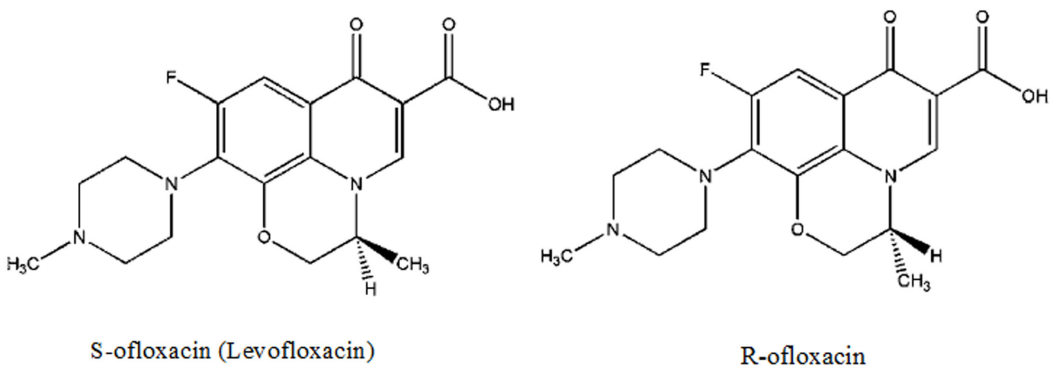

Figure 3. Structures of ofloxacin enantiomers.

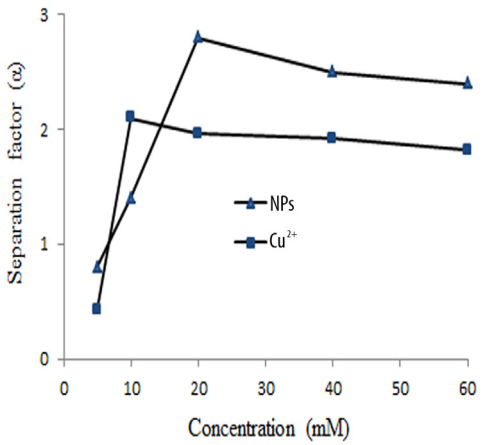

Figure 4. Effects of concentrations of NPs and Cu^{2+} on separation factor (α). Conditions: Buffer (0-60 mM $CuSO_4$, 40 mM $(NH_4)_2SO_4$, pH 4.7) with 5-100 mg/mL NPs, 70/30% (v/v); injection, 0.6 psi, 9 s; applied voltage, 20 kV; detection, 293 nm.

mM sulfated β-cyclodextrin was dissolved in a 50 mM phosphate buffer, pH 2.5, and at 15°C. The separation factor up to 1.045 was achieved [33]. Shi et al. prepared molecularly imprinted polymer microparticles for CEC separation of ofloxacin enantiomers [34]. Using mobile phase containing ACN up to 90% (v/v), the separation factor for ofloxacin was observed as 1.53. In our study, optimized chromatographic conditions as 70/30 % (v/v) ACN, 5 mM $CuSO_4$, 20 mM $(NH_4)_2SO_4$ and 30 mg/mL (20 mM) polymer nanoparticles were chosen. The electrochromatograms of ofloxacin enantioseparations were given in Figure 6. Using optimized chromatographic conditions, the enantioseparation of ofloxacin without NPs was presented in Figure 6A. On the other hand, Figure 6B shows the electrochromatographic enantioseparation of ofloxacin with NPs. These results showed that NPs has important effect on the chiral separation. The separation factor up to 2.9 was succesfully achieved. Finally, this system was applied to ofloxacin tablets. The results were shown in Figure 6C. Under optimized enantioseparation conditions, R-and S-ofloxacin in the ofloxacin tablets were detected succesfully.

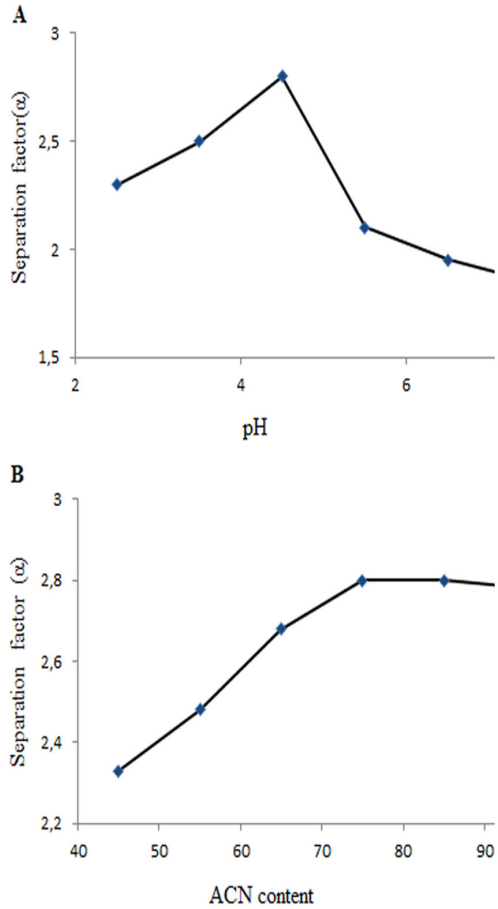

Figure 5. (A) Effect of pH. Conditions: mobile phase: Buffer (10 mM $CuSO_4$, 40 mM $(NH_4)_2SO_4$) with 30 mg/mL NPs, 70/30% (v/v); injection, 0.6 psi, 9 s; applied voltage, 20 kV; detection, 293 nm.,
(B) Effect of ACN content. Conditions: mobile phase, ACN (in the range of 45-95%): buffer (10 mM $CuSO_4$, 40 mM $(NH_4)_2SO_4$) with 30 mg/mL NPs, (v/v); injection, 0.6 psi ,9 s; applied voltage, 20 kV; detection, 293 nm.

CONCLUSIONS

Ofloxacin enantiomers were successfully enantio separated by LE-CE using polymer NPS with chiral functionalized group. Cu^{2+} was used as central ion. Chromatographic conditions such as concentrations of NPs and Cu^{2+}, pH and ACN content were optimized. Separation factor up to 2.9 was achieved. The enantioseparation efficiency of ofloxacin enantiomers in this study using NPs was far better than those obtained methods by both electromigration and liquid techniques. The technique with NPs has shown promising features for enantioseparations.

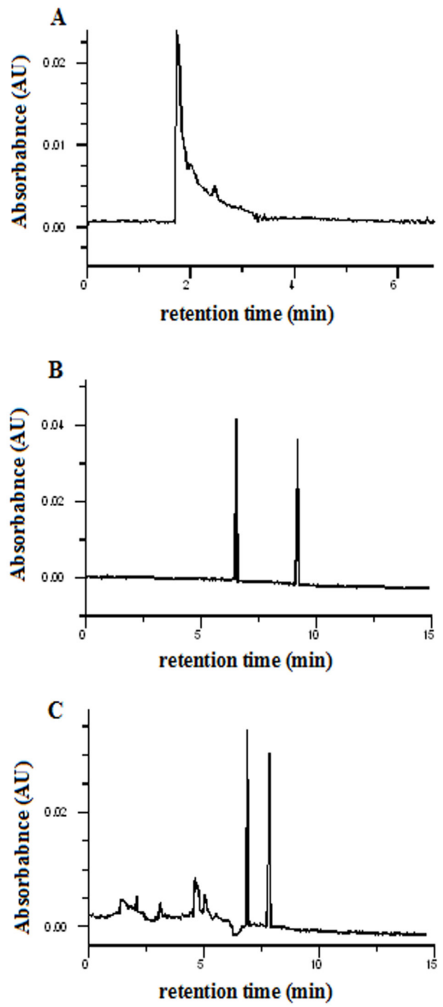

Figure 6. Enantioseparation of ofloxacin by LE-CE. (A) The enantioseparation of ofloxacin without NPs. Conditions: mobile phase, ACN; buffer (10 mM $CuSO_4$, 40 mM $(NH_4)_2SO_4$, pH 4.7) 70/30% (v/v); injection, 0.6 psi ,9 s; applied voltage, 20 kV; detection, 293 nm. (B) The enantioseparation of ofloxacin with NPs. Conditions: mobile phase, ACN: buffer (10 mM $CuSO_4$, 40 mM $(NH_4)_2SO_4$, pH 4.7) with 30 mg/mL NPs, 70/30% (v/v); injection, 0.6 psi ,9 s; applied voltage, 20 kV; detection, 293 nm. (C) The enantioseparation of ofloxacin in ofloxacin tablets using NPs, the conditions as the same (B).

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