

## Research Article

# Evaluation of Poly(Glycidyl Methacrylate) Nanocoating for Chiral Separation with Glu- $\beta$ -CD as Chiral Selector in Capillary Electrophoresis

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In this paper, a capillary coated with poly(glycidyl methacrylate) nanoparticles (PGMA NPs) was prepared and applied to construct a capillary electrophoresis (CE) enantioseparation system with glucosyl- $\beta$ -cyclodextrin (Glu- $\beta$ -CD) as a chiral selector. The PGMA NP coating can be easily introduced through a simple ring-opening reaction. Two basic drugs were used as models to evaluate the separation performance of the PGMA coating. Under the optimal conditions selected, the separation of the two enantiomers was obtained.

## 1. Introduction

The human body and the two enantiomers show different pharmacodynamics and pharmacokinetic properties, which means controlling the quality of the drug enantiomers is the key factor in the modern pharmaceutical industry. The development of effective and fast chiral drug analysis methods has attracted more and more attention [1–3]. Among various separation techniques, capillary electrophoresis (CE) has proven to be a useful method for chiral drugs due to its high efficiency, simplicity, low consumption, and flexibility of separation methods (e.g., micellar electrokinetic chromatography (MEKC), nonaqueous capillary electrophoresis (NACE), and capillary electrochromatography (CEC)) [4–6]. With further research, the traditional separated CE model has many limitations. Therefore, the induction of new type of materials to modify the inner wall of the capillary and enhance the selectivity and efficiency of CE has become a commonly used method [7, 8].

After being modified by materials such as ionic liquids, nanoparticles, and even phospholipid vesicles, capillaries may have many characteristics, including inhibition of electroosmotic flow (EOF) and local enrichment of chiral selector [9, 10]. In the past few decades, nanoparticles (NPs) have been used in many fields, including application as column coating for preparing capillary columns [11, 12]. NPs can provide unique advantages, such as favorable surface-to-volume ratio, significant mechanical strength, good chemical stability, and easy modification characteristics [13, 14]. Due to specific physical and chemical properties, they can be easily connected to the capillary to improve the column capacity and efficiency of chiral CE [15–20]. Mayer and Schurig reported the first chiral open tubular CEC system based on Chirasil-Dex- (permethyl- $\beta$ -cyclodextrin chemically linked to dimethylpolysiloxane-) modified column for enantioseparation. This method was developed in 1992 [21]. Our group explored an HKUST-1-functionalized CE column for the enantiomeric separation of several racemic

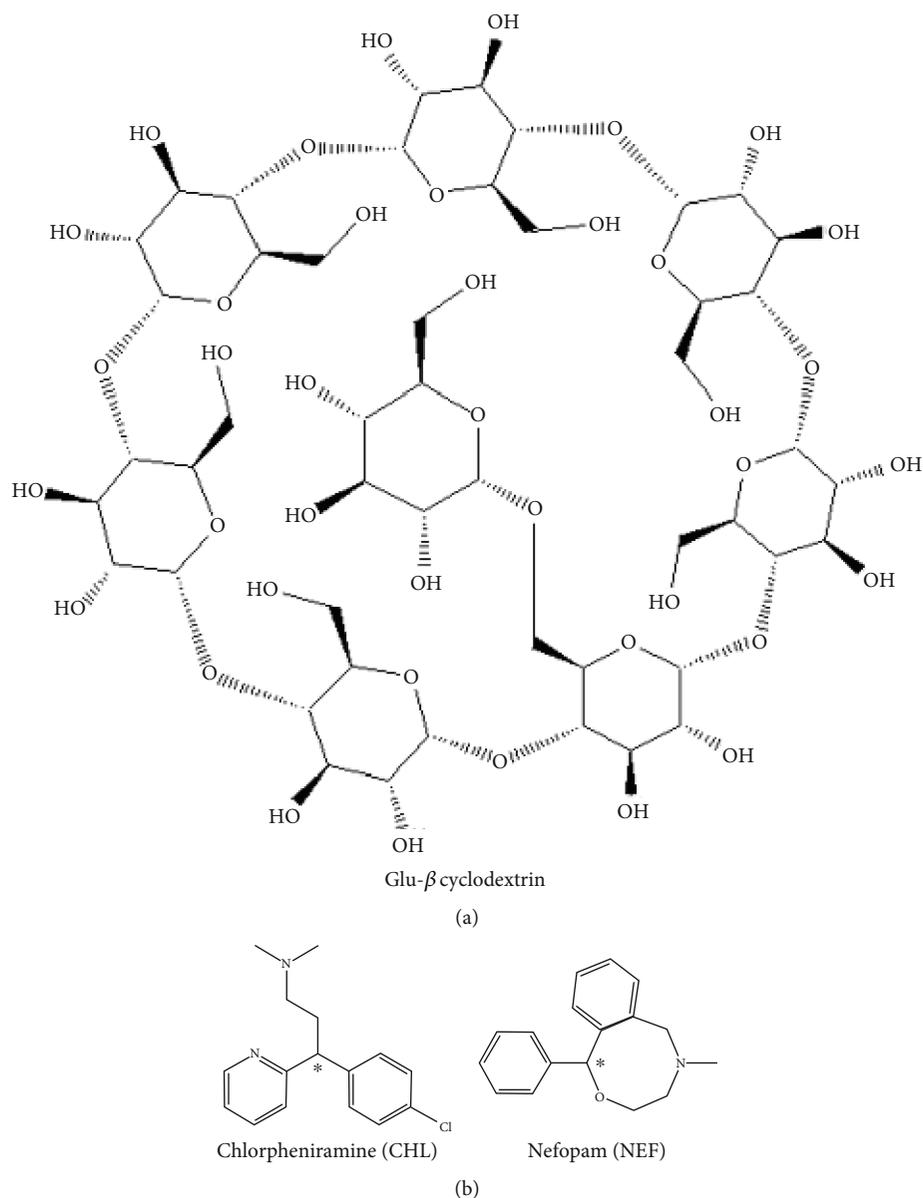


FIGURE 1: Structure of Glu- $\beta$ -CD and racemic drugs.

drugs with CM- $\beta$ -CD as the chiral selector in 2019. Compared with the uncoated bare column system, the HKUST-1 coating system has significantly improved separation for enantiomers [22].

In this study, poly(glycidyl methacrylate) nanoparticles (PGMA NPs) were immobilized on the inner wall of the capillary through a simple ring-opening reaction [23–26]. Then, Glu- $\beta$ -CD was used as chiral selectors in the PGMA-coated column to construct a Glu- $\beta$ -CD/PGMA column separation system. Two essential drugs were selected as models for evaluating enantioselectivity of PGMA nanocoating. Compared with bare column, the new system shows a satisfactory separation performance improvement. The main influencing factors such as chiral selector concentration and background electrolyte (BGE) pH are systematically studied.

## 2. Experimental

**2.1. Materials and Reagents.** HPLC grade methanol was purchased from Jiangsu Hanbang Science and Technology Co., Ltd. (Nanjing, China). 3-Aminopropyltriethoxysilane (APTES) and glycidyl methacrylate (GMA) were purchased from Aladdin Chemical Reagent Co., Ltd. (Shanghai, China). Phosphoric acid, sodium hydroxide, hydrochloric acid, and sodium tetraborate decahydrate (all analytical grades) are purchased from Nanjing Chemical Reagent (Nanjing, China). Glu- $\beta$ -CD (purity > 99) and racemic drugs (nefopam (NEF) and chlorpheniramine (CHL)) are provided by Jiangsu Food and Drug Administration (Nanjing, China). The structure is shown in Figure 1. All experimental water is ultrapure water.

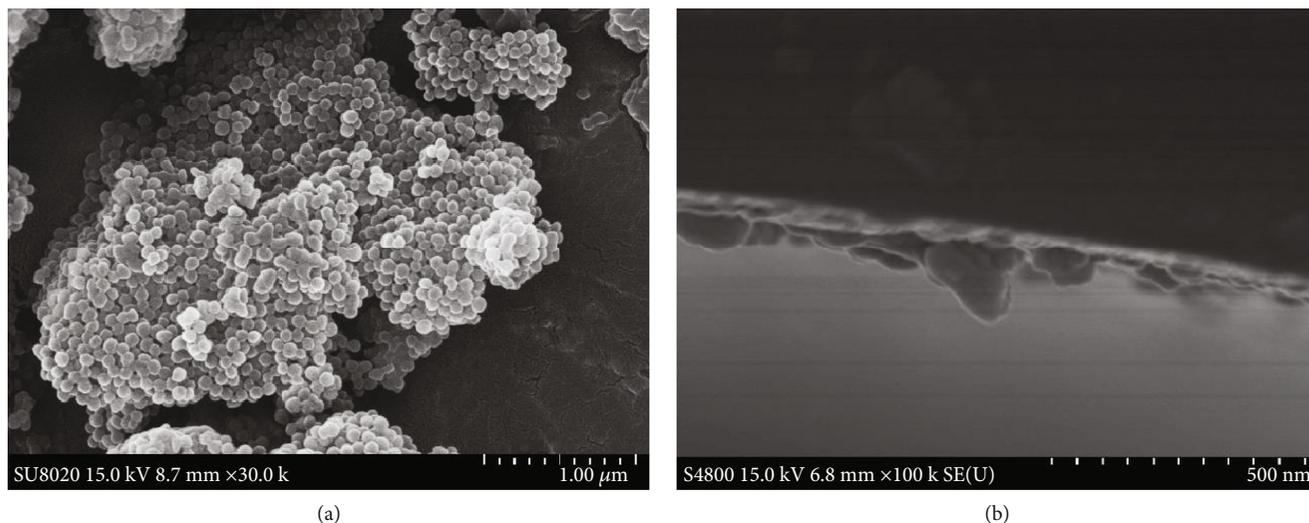


FIGURE 2: SEM of (a) PGMA nanoparticles and (b) PGMA-coated capillary column.

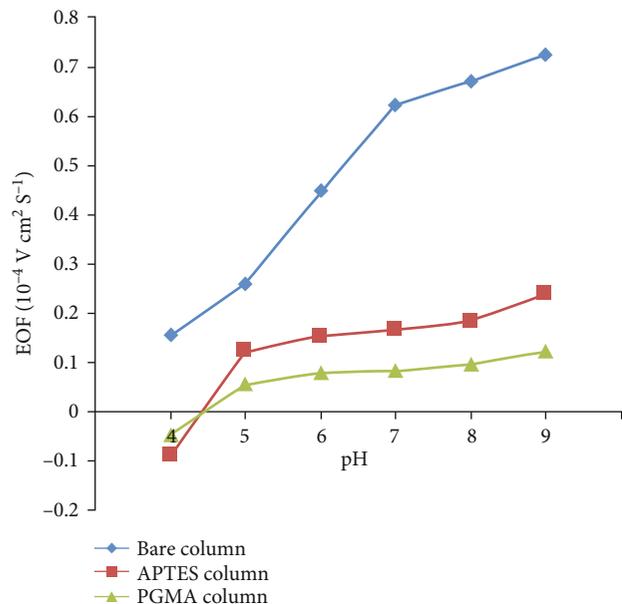


FIGURE 3: Influence of buffer pH value on the EOF mobility for different columns. Conditions: BGE (pH 4.0–9.0); EOF marker, 0.5 mg/mL thiourea; applied voltage, 15 kV.

**2.2. Apparatus.** A CL1030 CE system (Beijing Huayang Liming Instrumental Co., Beijing, China), equipped with a UV detector (190–700 nm) and a 30 kV high-voltage power supply, was used to perform all electrophoretic experiments. This article used a capillary column with an inner diameter of 33 cm (effective length 24.5 cm) × 75 I.D. The uncoated fused silica capillary tube was provided by Hebei Yongnian Ruifeng Co., Ltd. The morphology of PGMA nanoparticles was characterized by scanning electron microscopy (SEM) (Japan Hitachi SU8010). The PGMA nanocoating in the capillary was characterized by a field emission scanning electron microscope (FESEM, Zeiss Supra, Germany). Between successive injections, flush the capillary column with 0.01 M

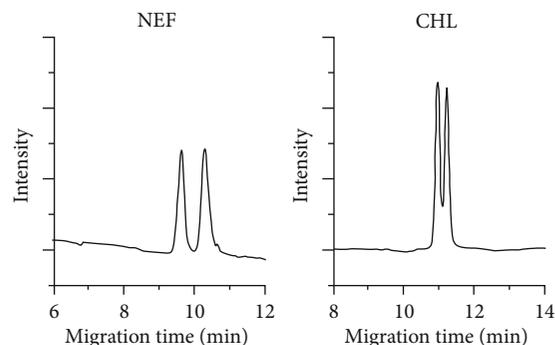


FIGURE 4: Enantioseparation electropherograms of NEF and CHL (0.5 mg·mL<sup>-1</sup>) in PGMA nanocoating with Glu-β-CD as chiral selector. Conditions: 20 mM phosphate buffer; Glu-β-CD concentration, 60 mM; pH, 6.20; applied voltage, 15 kV.

NaOH for 60 s, flush with distilled water for 100 s, and then flush with BGE until the baseline is stable. The racemic drug (0.5 mg·mL<sup>-1</sup>) was dissolved in a dual solvent mixed with methanol and distilled water (50 : 50, v/v). Phosphate buffers (20 mM) with various pH values were prepared for electroosmotic flow (EOF) measurement. Thiourea was chosen as the neutral marker. BGE is freshly prepared by dissolving GLU-β-CD/AL in a buffer solution with a specific pH value and then adjusted to the desired pH value by adding a small amount of sodium hydroxide solution using a microsyringe. Before use, all solutions are filtered through a 0.45 μm pore membrane filter and degassed by ultrasonic treatment.

### 2.3. Preparation of PGMA-Coated Capillary Column

**2.3.1. Synthesis of PGMA Nanoparticles.** We followed the methods of Sun et al. [27]. 20 mM NaHCO<sub>3</sub>, 20 mM Na<sub>2</sub>CO<sub>3</sub>, 10% (w/v) GMA, and 0.3% (w/v) sodium lauryl sulfate in 100 mL water were introduced into 250 mL three-necked circle. After adding 0.2% (w/w) potassium persulfate, the mixture was stirred at 70°C for 12 h. The residual monomer was removed through a syringe (pressure 0.1 MPa) and

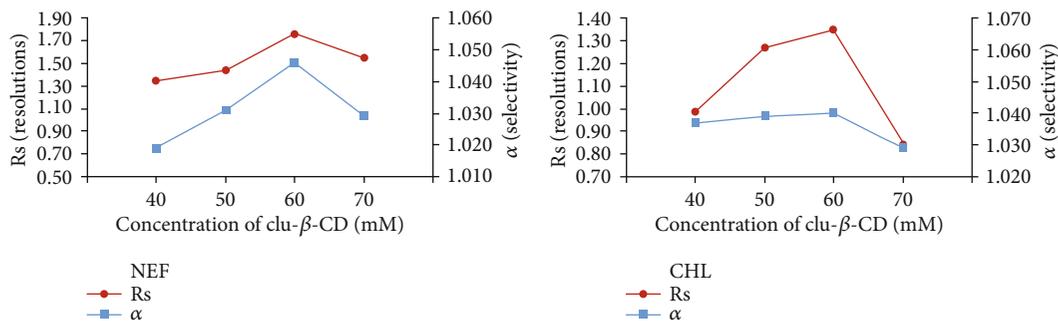


FIGURE 5: Effect of Glu- $\beta$ -CD concentration for enantioseparation in PGMA nanocoating system with NEF and CHL ( $0.5 \text{ mg}\cdot\text{mL}^{-1}$ ) as model drugs. Conditions: 20 mM phosphate buffer with selected Glu- $\beta$ -CD concentration (40-70 mM); buffer pH, 6.20; applied voltage, 15 kV.

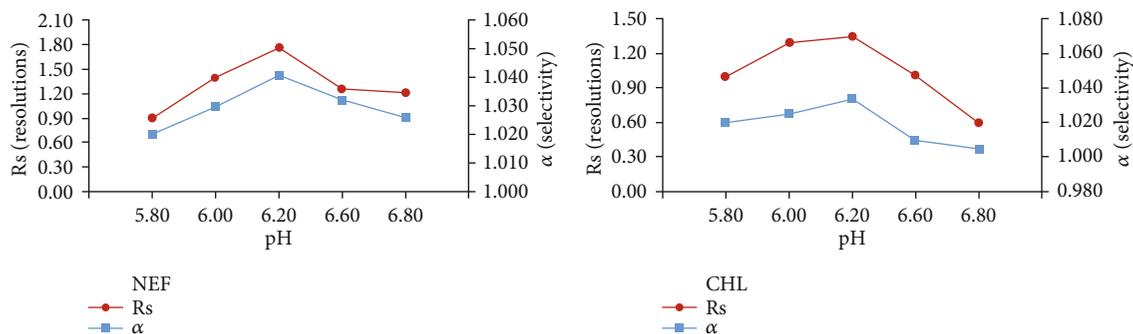


FIGURE 6: Effect of buffer pH for enantioseparation in PGMA nanocoating system with NEF and CHL ( $0.5 \text{ mg}\cdot\text{mL}^{-1}$ ) as model drugs. Conditions: 20 mM phosphate buffer with selected pH range (5.80-6.80); Glu- $\beta$ -CD concentration, 60 mM; applied voltage, 15 kV.

washed several times. Finally, a white powder was obtained under vacuum at room temperature.

**3.2.2. Preparation of PGMA Nanocoating.** In our experiment, a 33 cm (24.5 cm effective length)  $\times$  75  $\mu\text{m}$  I.D. fused silica capillary was used. First, wash the capillary with 1.0 M NaOH (1 h),  $\text{H}_2\text{O}$  (30 min), and 1.0 M HCl (30 min) in sequence, and then, purge it with methanol for 30 min. After removing the methanol with nitrogen, the capillary was heated in a vacuum oven at  $100^\circ\text{C}$  for about 1 hour. After cooling to room temperature, the APTES solution (50%,  $v/v$ ) prepared in methanol was pumped through the capillary for 30 min. Then, the capillary column was sealed with rubber and reacted in a water bath at  $55^\circ\text{C}$  for 12 hours. In order to obtain the PGMA coating, the obtained capillary was rinsed with 0.5 mg/mL PGMA nanoparticles (pH 8.5, 50 mM phosphate buffer) for about 30 min at room temperature. The filled capillary was sealed and allowed to react at  $55^\circ\text{C}$  for 4 h. Wash the capillary with deionized water to remove excess PGMA nanoparticles. Repeat the above process 3 times.

### 3. Results and Discussion

**3.1. Characterization of PGMA Nanoparticles and PGMA Nanocoating.** The morphology of PGMA nanoparticles has been clearly characterized by SEM, as shown in Figure 2(a). All PGMA nanoparticles are spherical, with a uniform average diameter and an about 100 nm narrow particle size distribution. Figure 2(b) shows the SEM image of the inner surface

of the PGMA-coated column. Very obvious spherical protrusions can be observed, which indicates that the PGMA nanoparticle coating has been successfully immobilized to the inner wall of the capillary.

**3.2. Measurement of EOF.** EOF is a key influence due to the fact that it can reflect the ionic situation of capillary inner surface. Here, a range of pH 4.0–pH 9.0 with thiourea as electroosmotic flow marker in the uncoated capillary, APTS-modified capillary, and PGMA-coated column was investigated. As Figure 3 shows, EOF of the APTS-modified column and PGMA-coated column was negative in pH 4, which is due to the positive charge of the amino group (residual amino for the PGMA-coated column). After immobilization of PGMA NPs, the EOF was lower than that in the APTS column, which was due to the reaction of PGMA with the amino group. It is worth noting that mild EOF change with pH is favorable for obtaining high stability in migration time.

**3.3. Performance of PGMA Nanocoating with Glu- $\beta$ -CD as Chiral Selector.** The capillary coating can suppress EOF and reduce the adsorption of analytes, thereby improving separation efficiency. NEF and CHL have been tested to evaluate the modification of PGMA coating. Using 20 mM phosphate buffer and a limited amount of Glu- $\beta$ -CD, the drug is analyzed under optimized separation conditions in the PGMA-coated capillary. From Figure 4, the enantiomeric separation performance of NEF and CHL in PGMA coating system has been obtained.

### 3.4. Optimal Separation Conditions

**3.4.1. Effect of Glu- $\beta$ -CD Concentration for Enantioseparation in PGMA Nanocoating System.** The concentration of the chiral selector is a key factor affecting the separation of enantiomers. In order to determine the optimal selection agent concentration for the system, a series of concentrations were studied using a 20 mM phosphate buffer solution. As shown in Figure 5, the migration time of racemic drugs all increase with the increase of Glu- $\beta$ -CD concentration. The reason for this phenomenon may be the enhanced interaction between the chiral selector and the enantiomer led by local enrichment of Glu- $\beta$ -CD in the PGMA nanocoating. And the increase in the viscosity of BGE may be the other reason. As the concentration of the chiral selector increases,  $R_s$  and  $\alpha$  of the tested drug first increase and then decrease. The maximum value is obtained when the concentration of Glu- $\beta$ -CD reaches 60 mM. Therefore, 60 mM Glu- $\beta$ -CD was chosen for the next experiment.

**3.4.2. Effect of Buffer pH for Enantioseparation in PGMA Nanocoating System.** The pH of the buffer is an important parameter of the separation system [28]. It determines the degree of ionization of the analyte and the chiral selector and the ion state of the capillary wall. Therefore, optimizing BGE pH is usually a key strategy for optimizing separation. A pH range of BGE was studied using a 20 mM phosphate buffer solution containing an optimized concentration of Glu- $\beta$ -CD to isolate the selected drugs. As shown in Figure 6,  $R_s$  and  $\alpha$  in the two PGMA-coated column systems have the same trend, increasing as the pH increases from 5.80 to 6.00. The change trend of resolution gradually decreased from pH 6.20 to 6.80, and the best separation effect was obtained at 6.20.

## 4. Conclusion

In this study, a PGMA nanocoating-based enantiomeric separation system with Glu- $\beta$ -CD as the chiral selector was established to separate enantiomers. In this PGMA-coated column system, the enantiomeric separation results of the NEF and CHL have been obtained. PGMA nanocoating can achieve satisfactory stability of EOF. In addition, by evaluating influencing factors such as Glu- $\beta$ -CD concentration and BGE pH, the best separation resolution can be obtained with pH 6.20 and 60 mM Glu- $\beta$ -CD. This PGMA nanocoating can provide a support to the development of more new types of modification nanomaterials for enantioseparation.

## Data Availability

Due to the confidentiality before public, this manuscript has no associate data.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

## Authors' Contributions

Jin Xu and Xuecheng Sun contributed equally to this work.

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