

# Silica nanoparticle-mediated, Electric field-triggered sensitivity enhancement strategy for Capillary electrophoretic detection of proteins

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## Article Info

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## Abstract

We report in this paper the improved capillary electrophoresis (CE)-UV detection sensitivity of proteins based on silica nanoparticle-mediated, electric field-triggered (SNM-EFT) strategy. During the process, the capillary was first filled with acidic separation buffer, followed by injection of a segment of alkaline treating solution containing silica nanoparticles (SNPs). After the inlet end of the capillary was dipped into the protein solution containing SNPs, a high voltage was applied across the capillary. The SNPs in the protein solution became aggregate, promoting the SNP-protein conjugation, by which transformation of the protein structures took place. During CE, the unfolded protein desorbed from SNPs, generating stronger signal than the native one due to the more effective exposure of tryptophanyl residues to the polar buffer. The parameters of the treatment protocols, e.g., pH of the treating solution, concentration of the SNPs presented, the voltage and duration of the electric field applied, had notable effects on the detection sensitivity. Significant improvement in CE-UV response was obtained by the EFT at  $-8 \text{ kV} \times 60 \text{ s}$  on the protein standards dissolved in 4 mM sodium tetraborate, 1 mM boric acid and 0.015% SNPs.

**Keywords:** Capillary electrophoresis; Proteins; Silica nanoparticles; Electric field; sensitivity

**Abbreviations:** SNPs, silica nanoparticles; LZ, lysozyme; BSA, bovine serum albumin; HB, hemoglobin; PEO, polyethylene oxide; CE, capillary electrophoresis

## Introduction

Nanoparticles (NPs) can conjugate with proteins via hydrophobic patches, hydrogen bonding and columbic interactions owing to the large surface area of NPs [1,2] and to the simultaneous presence of hydrophobic, hydrophilic, cationic, and anionic groups at the surface of protein molecules [3]. The NP-protein conjugation is size-dependent, higher degree conjugation takes place with aggregated NPs [4]. Moreover, such interaction often results in tertiary conformational transformation (unfolding) of the protein [5-8].

Capillary electrophoresis (CE) combines well-known advantages of speedy separation, high efficiency, small amount of sample and solvent consumption, and high automation [9-12]. Due to these unique advantages, it has been extensively employed in proteome research. Not surprisingly, NPs have been hyphenated to CE [13-17] and chip-based electrophoresis system [18-20] for protein separation. For example, a buffer containing surfactant-capped gold nanoparticles (AuNPs) allowed simultaneous separation of acidic and basic proteins in a single run [13,14]. By employing lipid-based

liquid crystalline NPs as pseudostationary phase (PSP), green fluorescent protein (GFP) and its mutants were baseline separated at neutral pH [17]. Moreover, silica nanoparticles (SNPs) acting as PSPs could also render enhanced resolution for proteins [16]. Liu and co-workers reported TiO<sub>2</sub> NP-coated open-tubular capillary electrochromatographic separation of proteins [15], in which conalbumin (ConA) and apo-transferrin (apoTf) of similar molecular weight could be baseline separated.

However, in contrast to the large number of reports enhancing the resolution, few reports were dedicated to investigate their applicability in sensitive detection of proteins by CE. The main reason might be that both the fluorescence emission and UV absorbance decrease upon adsorption of protein to nanoparticles [7,21]. Nonetheless, such NP-protein conjugation might be adopted under some circumstances for sensitive protein detection. Studies revealed that the refolding rates of some proteins, such as lysozyme [22], are very slow after deconjugation. The surrounding environments of the amino acid residues in the detached, unfolding proteins might be different than those in the normal proteins. In this context, the UV-active amino acid residues, i.e., tyrosine, tryptophan and phenylalanine, might have different molar absorptivities which would lead to different detection sensitivities. This strategy can be readily realized in CE techniques due to its quick analysis and ease in tuning the chemical properties of the running buffer to facilitate the deconjugation under the electric field.

The aim of this work is to demonstrate the proof-of-principle application of the silica nanoparticle-mediated, electric field-triggered (SNM-EFT) sensitivity enhancement strategy for CE-UV detection of unfolded proteins following the decomposition of the SNP-protein complexes. SNPs were employed as model nanoparticles for investigation. To initialize the SNP-protein complexation, a capillary was first filled with separation buffer followed by injection of a segment of alkaline treating solution containing SNPs. After the inlet end of the capillary was dipped into the protein solution containing SNPs, a high voltage was applied across the capillary. A low conductivity zone formed at the interface between the acidic CE buffer and the alkaline treating solution due to neutralization, whereby the SNPs aggregated, moving to the injection vial and promoting the SNP-protein conjugation. The SNM-EFT parameters, e.g., pH and the SNPs concentration of the treating solution, the electric voltage and duration of EFT, were investigated. Notable improvement in sensitivity was observed for UV detection of the model proteins, especially for lysozyme, suggesting the potential of the strategy in CE analysis of proteins.

## Materials and methods

### Reagents and solutions

#### Reagents

Hemoglobin (HB) and lysozyme (LZ) were from Sigma (St. Louis, MO, USA); bovine serum albumin (BSA, fraction V) was purchased from Amresco (Solon, OH, USA). PEO (polyethylene

oxide, Mr 1 000 000) was supplied by Alfa Aesar (Ward Hill, MA, USA). The SNPs with average diameter of 20 nm and purity of 99.9% were bought from Nanjing Nano High-Tech (Jiangsu, China). All the other chemicals were of analytical grade. Double-distilled water was used to prepare buffers and solutions throughout the experiment.

### Solutions

SNP suspension at 1% (w/v) was prepared by gradually adding precisely weighed SNPs to double-distilled water under vigorous stirring. Stock solutions of 100 mM phosphoric acid, 100 mM sodium tetraborate and 100 mM boric acid were employed for preparing buffer solutions of desired concentrations and pH values. Working solutions of protein standards (mixture of 50 mg•L<sup>-1</sup> each) were prepared by mixing the individual stock solutions (10000 mg•L<sup>-1</sup> in double-distilled water) with appropriate volumes of borate buffer, SNPs suspension and diluting with double-distilled water.

### Capillary electrophoresis

The CE-UV system consisted of a DW-P303-1AC capillary electrophoresis high-voltage power supply (Sanchuan High Tech, China) and a CE-10UV detector (Johnsson Separation Science, Liaoning, China) operated at 210 nm. Signal from the detector was acquired and processed with HW2000 chromatography station (Qianpu, Jiangsu, China). A 50-cm long polyimide-coated fused-silica capillary (40 cm in effective length) of 75- $\mu$ m i.d. and 375- $\mu$ m o.d. (Yongnian Photoconduction Fibre, Hebei, China) was used for CE. The fresh capillary was consecutively rinsed for 30 min with 1 M NaOH, 10 min with double-distilled water and 5 min with separation buffer. Each electrolyte solution was filtered through a 0.22- $\mu$ m membrane filter (Jiuding High Tech, Beijing, China). Samples were hydrodynamically injected into the capillary by raising the inlet reservoir 20.0 cm for 30 s. Electrophoretic separations were carried out at a voltage of 11 kV under ambient temperature.

### SNM-EFT procedure

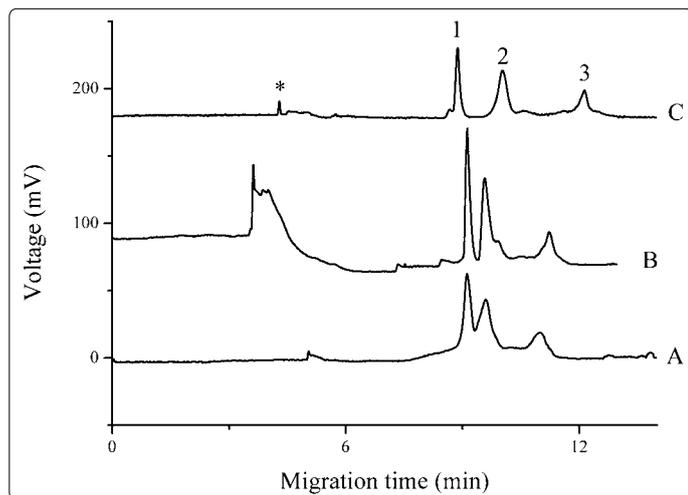
The capillary was first rinsed for 2 min with the separation buffer, followed by hydrodynamic injection of a segment of treating solution by siphon (the optimum parameters were 20 cm  $\times$  40 s). After that, the inlet was immersed into the sample vial containing the protein standard solution and the outlet was immersed into the separation buffer. A negative voltage was then applied between the sample and the outlet vials for a desired duration. The capillary was flushed with running buffer for 2 min after the procedure, and the treated protein solution was vigorously shaken before injection.

## Results and discussion

### Optimization of CE conditions

Background electrolyte (BGE) of 20 mM phosphoric acid (pH 1.89) was employed to suppress the deprotonation of silanol groups of the capillary inner wall and, therefore, to suppress the wall-adsorption of the positively charged proteins. In order to further improve the separation

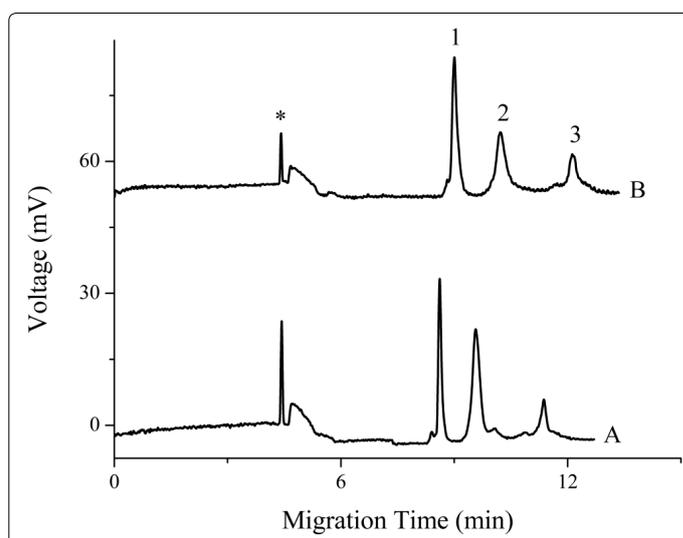
performances, PEO was added to the buffer, and its concentration was optimized. Improved peak intensities were observed in the presence of 0.05% SNPs (Figure 1B vs. 1A). The buffer containing 0.2% PEO offered the highest resolutions for the proteins (Figure 1C) although the overall sensitivity of the three proteins was slightly lower than that of Figure 1B. Addition of PEO to the buffer increases the buffer viscosity, resulting in reduced injection volume and consequently the decreased peak heights of the analytes. Taking into account the parameters of resolution and sensitivity, we chose BGE consisting 20 mM phosphoric acid and 0.2% PEO for the further experiments.



**Figure 1.** Influence of buffer additives. Separations were carried out with BGE of 20 mM phosphoric acid (A), which was added to 0.05% SNPs (B) or 0.2% PEO (C). Proteins were dissolved to concentrations of  $50 \text{ mg} \cdot \text{L}^{-1}$  each in treating solution. Electrophoresis was conducted at 11 kV and UV detection was performed at 210 nm. Peak identities: 1, LZ; 2, BSA; 3, HB; \*, systemic peak. Electropherograms were offset for clarity.

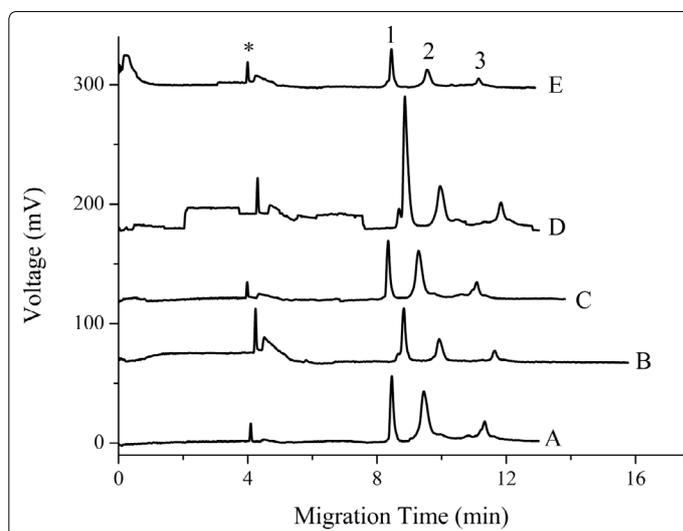
### Preliminary experiments on SNM-EFT

Experiments were carried out to explore the influence of SNPs and electric field on the detection sensitivity. Two kinds of treating solutions were employed, i.e., 4 mM sodium tetraborate and 1 mM boric acid at pH= 9.11 with and without 0.015% SNPs, they are denoted treating solutions A and B, respectively. Our preliminary experiments suggested that application of positive high voltage did not change the detection sensitivity of the proteins (Figure 1S of the Supporting Information); so, a negative voltage was employed. We initially employed same treating solutions for injecting into the capillary and for dissolving the protein standards. Compared to treating solution A (Figure 2A, without EFT), using treating solution B did not result in higher resolution; on the contrary, lower peak heights (Fig. 2B, without EFT) were observed, probably due to the higher solution viscosity in the presence of 0.015% SNPs. With EFT, utilizing treating solution A (Figure 2C) did not bring about noticeable changes in detection sensitivity as compared with Figure 2A. However, when EFT was applied to treating solution B, remarkable improvement of the peak heights was obtained, especially for lysozyme. The results suggest the potential of SNM-EFT strategy in sensitive detection of proteins.



**Figure 1S.** Effect of positive high voltage on detection sensitivity. Sample treatment techniques: Proteins dissolved in 4 mM sodium tetraborate, 1 mM boric acid and 0.015% SNPs; the solution was treated for 60s at a voltage of (A) 0 kV; (B) +8 kV.

For better understanding the mechanisms, the SNM-EFT experiments were conducted using different treating solutions for pre-injection and for dissolving protein standards. With pre-injection of treating solution A, the detection sensitivities of the proteins (Figure 2E) were similar to those in Figure 2B. Likewise, peak heights of the proteins close to Figure 2A were observed with pre-injection of treating solution B.



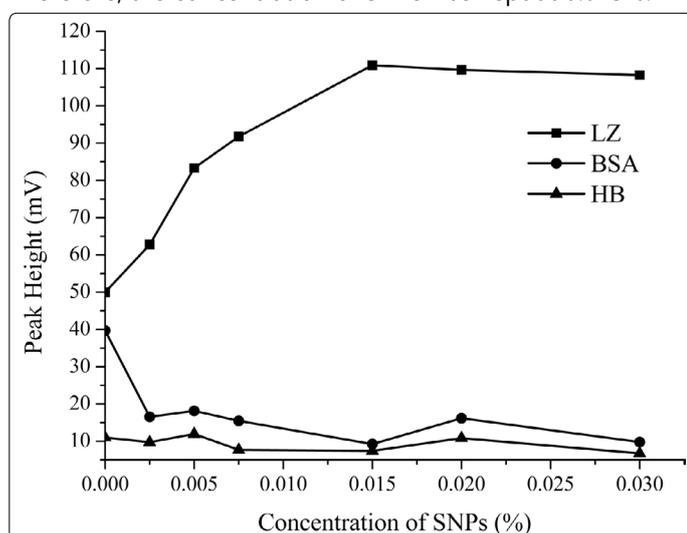
**Figure 2.** Effect of sample treatment techniques on detection sensitivity. Separation buffer: 20 mM phosphoric acid and 0.2% PEO. Peak identities: 1, LZ; 2, BSA; 3, HB; \*, systemic peak. Preparation and treatment of the standards: (A) Proteins dissolved in treating solution A, without EFT; (B) proteins dissolved in treating solution B, without EFT; (C) proteins dissolved in treating solution A; EFT:  $-8 \text{ kV} \times 60\text{s}$ ; (D) proteins dissolved in treating solution B, EFT:  $-8 \text{ kV} \times 60\text{s}$ . In (A)-(D), the plug of treating solution in each trace, introduced by  $20 \text{ cm} \cdot 40\text{s}$ , was the same with that used in preparing the corresponding protein standards. (E) proteins were dissolved in treating solution B; treating solution A was injected into the capillary ( $20 \text{ cm} \cdot 40\text{s}$ ); EFT:  $8 \text{ kV} \times 60\text{s}$ . Other conditions were the same as those in Figure 1.

During EFT process, a pH junction was formed at the boundary between the alkaline treating solution and the acidic separation buffer. The hydrogen ion ( $\text{H}^+$ ) from the BGE

intruded into the treating solution plug under electric field and reacted with OH<sup>-</sup> and B(OH)<sub>4</sub><sup>-</sup>, forming a low-conductivity zone where SNPs began to aggregate [23,24]. These aggregates were carried into the cathodic sample vial by EOF, broadening the primary particle size distribution (PPSD) and consequently accelerating the aggregating rate of the SNPs [25], promoting the SNP-protein conjugation.

### Effect of SNP concentration and pH

Figure 3 depicts that under an EFT treatment of -8 kV × 60 s, the peak height of LZ increases gradually with the increasing concentration of SNPs until 0.015%, from which the peak height kept at a relatively stable level. However, the peak height of HB did not show significant improvement in the presence of SNPs; moreover, addition of SNPs even caused slightly decreased response of BSA. We suggest that presence of SNPs improves the viscosity of the sample solution, resulting in low injection volume and, hence, low detector response. Therefore, the concentration of SNPs was kept at 0.015%.



**Figure 3.** Dependence of peak heights on the concentration of SNPs presented in the treating solution. The protein concentrations were 50 mg·L<sup>-1</sup> each. The treating solution in the experiment contained 4 mM sodium tetraborate, 1 mM boric acid and varying concentrations of SNPs from 0 to 0.03%. The other conditions were the same as those in Figure 2

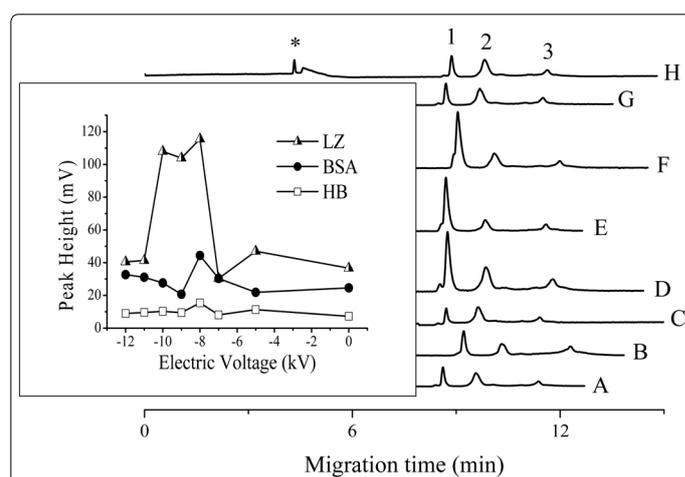
To study the influence of treating solution pH, proteins were dissolved in different treating solutions of 4 mM H<sub>3</sub>PO<sub>4</sub> (pH= 2.11), 10 mM NaH<sub>2</sub>PO<sub>4</sub> (pH= 4.27) and 4 mM sodium tetraborate (pH= 9.11). All solutions were added with SNPs to 0.015%. Application of electric field did not lead to enhanced detection sensitivity with proteins dissolved in acidic treating solution; but it did in alkaline solutions.

Adsorption of LZ onto SNPs was influenced by the nanoparticle size and solution pH. Multilayer adsorption and greater conformational change occurred with proteins attached on SNPs of larger size [4]. High solution pH promotes these progresses [4].

### Effect of electric voltage and treating duration

Significant enhancement in the response of LZ was observed at negative voltages ranged from -8 to -10 kV (Figure 4). LZ is positively charged in buffer of pH 9.11 because its

isoelectric point is ca 11. The results reveal that the complementary electrostatic interaction facilitates the adsorption of LZ to SNPs [4,22]. It is interesting to find that the negatively charged BSA and HB, which should be electrostatically repulsive to the SNPs, also indicate considerable enhancement in detection sensitivity. The hydrophobic interaction might be responsible for the adsorption of proteins to SNPs under this circumstance [7]. Moreover, it was reported [26] that BSA and HB possess low internal stability; they are "soft" and are prone to adsorb on all surfaces irrespective of electrostatic interactions.



**Figure 4.** Effect of electric voltage on detection sensitivity. The sample solutions underwent EFT treatments for 60s at the following voltages: (A) 0 kV; (B) -5 kV; (C) -7 kV; (D) -8 kV; (E) -9 kV; (F) -10 kV; (G) -11 kV; (H) -12 kV. Figures in the inset: dependence of peak heights of proteins on the electric voltages. The other conditions were the same as those in Figure 2.

The treating time is another important factor influencing the sensitivity; the peak height of LZ increased with the duration first to a maximum at 60 s, then decreased with further extended duration.

In the SNM-EFT strategy, high voltage generates high EOF intensity and large amount of SNP aggregates, favoring high sensitivity. Nevertheless, high migration velocities of the ions in the capillary disturb the pH junction and, even worse, under some circumstances, for example, the long EFT time, the acidic buffer may enter the sample vial. The former does not favor formation of aggregated SNPs, whereas the latter will weaken the protein-SNP interaction [4,27].

## Conclusions

We report the proof-of-principle application of SNM-EFT strategy for sensitive CE-UV detection of proteins. Influences of SNP concentration and pH of the treating solution, the treating electric voltage and duration were investigated. The SNP aggregates generated during the EFT promoted the aggregation of the SNPs in the standard solution and, as a result, favored the SNP-protein interaction. The unfolded protein desorbed from the SNPs during CE could produce enhanced UV-absorbance signal. Due to the great variety of nanoparticles and the wide pH range of the CE buffers employed in proteome research, we expect the method opens up new opportunities for sensitive detection of more proteins.

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**Conflicts of Interest:** The authors have declared no conflict of interest.

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