# Efficiency Enhancement in the Capillary Electrophoretic Separation of Aromatic Acids with Organo-Silica Nanoparticles as Pseudo-Stationary Phase

**Jing Wang[1], Yugao Guo[2], Ruijuan Yuan[2], Danning Liu[2],** and **James J. Bao[2,*]**

[1]College of Pharmacy, Nankai University, 94 Weijin Road, Tianjin 30071, P. R. China and [2]Tianjin Key Laboratory for Modern Drug Delivery & High-Efficiency , School of Pharmaceutical Science & Technology, Tianjin University, 92 Weijin Road, Tianjin 300072, P. R. China

## Abstract

**Nanoparticles modified with aminopropyl group were prepared by a single-step electrospray process and used as a pseudo-stationary phase for the separation of anions, (e.g., organic acids in capillary electrophoresis). The nanoparticles form a uniform suspension which is stable in a buffer without adding other stabilizer. The amino containing nanoparticles have significant impact on the electro-osmotic flow (EOF) of the system, which, in turn, affects the overall separation of other analytes in this system. For example, five organic acids, which were not all separable in zone electrophoresis, were fully resolved in the presence of the nanoparticles in a wide range of pH. Various experimental parameters that are related to the electrophoresis including EOF, pH, organic modifier, voltage, concentration of the running buffer and concentration of the nanoparticles have been investigated. The repeatability of the migration time and peak area in this system are better than 5% for both inter- and intra-day evaluation.**

## Introduction

Since the first introduction of micelles into capillary electrophoresis (CE) as a new separation mode for separating neutral analytes by Terabe et al. in 1984 (1), many different categories of other compounds and materials have been added into the electrolyte in CE during the following two decades, such as cyclodextrin (2), nanoparticles (3–5), proteins (6), ionenes (7), linear siloxanes (8), polymers (9–10), liposomes (11), dendrimers (12), and micro-emulsions (13). Most of these materials were used as pseudo-stationary phases (PSPs) for either improving the separation selectivity or enhancing the resolution in CE separation. In CE separation, when these additives were introduced, the relative parameters were changed accordingly, for example, the degree of dissociation, electroosmotic flow, viscosity, and the coefficient of distribution. The migratory behaviour of each analyte changed as well. These changes contributed to the efficiency improvement directly. There is an interaction between PSPs and the analytes when they move in the buffer. By using a PSP, it guarantees that an entirely fresh dynamic stationary phase is used for every new analyte because the phase is continuously replaced. Compared with general capillary electrochromatography (CEC), applying PSPs into the running buffer avoids the complicated and unmanageable steps such as column packing, frit making process or monolithic column preparation (14–15). Among all PSPs, nanoparticles offer more definite advantages. Nano-sized materials have stronger interaction with analytes due to their larger specific surface area (16) and the suspensions containing nano-sized PSPs are much more stable and have better repeatability, high efficiency, and more convenience in operation. Also, nanoparticles are compatible with mass spectrometer (MS) detection which does not hamper electrospay ionization like micelles (9,17).

Nanoparticles must meet certain specific requirements before being used as PSPs (18). Currently, the rapid development of nanotechnology has resulted in many new methods for manufacturing these nanoparticles. Among them, electrospray, a technique widely used in MS for ionization, has been applied in making nano-materials, such as nano-encapsulation (19–20), nano-fiber (21), protein nano-particles (22), nano-silica, titanium, and zirconium oxide (23). Compared with traditional methods, the electrospray method benefits from several characteristics (24–27). First, the size of the particles is controllable from hundreds of micrometers to tens of nanometers. It strongly depends on the liquid viscosity and the ratio of the liquid flow rates. The diameter of the capillary tube used to make these nanoparticles has nothing to do with the sizes of the finally formed actual particles. Second, the particles are non-agglomerated and the size is within a narrowly distributed range. The more uniform, the better is its use in separation. In addition, silica-based nanoparticles containing various organic groups can be prepared by a single-step method involving multiple reagents without any further derivatization.

In our work, tetraethoxysilane (TEOS) and aminopropyltriethoxylsilcane (APTES) were selected as liquid precursors for the synthesis of silica nanoparticles bonded with aminopropyl by using electro-spray technique combining with sol-gel method. The nanoparticles have been reported for their contribution to separation efficiency enhancement in wide-bore electrophoresis

*Author to whom correspondence should be addressed: email jbao007@gmail.com.

system such as PSP, where the ion exchange characteristic played the main role (28). In our study, these nanoparticles were also added into the running buffer as PSP which had obvious influence on electro-osmotic flow (EOF) resulting in a suppressed or reversed EOF, which is more helpful to the separation of anions, (e.g., the acids). Five aromatic acidic compounds were separated completely as they migrated in the same direction of the EOF (*co*-EOF) from the cathode to the anode under low pH. At the same time, other parameters, such as pH, voltage, and buffer concentration have been optimized. The separation results indicated that adding these uniformly made nanoparticles can successfully improve the resolution of this separation.

## Experimental

### Reagents and chemicals

APTES (99%) from Guotai-Huarong New Chemical Materials (Zhangjiagang, China) was used without further purification. TEOS, absolute ethanol, ammonium hydroxide, acetone, phosphoric acid, sodium hydroxide, thiourea, *o*-hydroxybenzoic acid, *p*-aminobenzene sulfonic acid, benzoic aicd, p-toluenesulfonic acid, and *p*-aminobenzoic acid were all of analytical grade, and purchased from Tianjin Kewei Chemical Co., Ltd. (Tianjin, China). Double distilled water DDW purified by a Nanopure II system (Barnstead, San Jose, CA) was utilized throughout the whole experiment. All chemicals were of analytical grade.

The nanoparticles bonded with aminopropyl were prepared in our laboratory had been reported previously (28). The nanoparticles used in this study were in a homogeneous size range of ~200 nm, and the approximate concentration of amino group was about 2.2 mmol/g.

### Apparatus

A HPE 100 CE System (Bio-Rad, Hercules, CA) was used for all of the CE experiments. Data were collected and analyzed using a Chrom Perfect workstation (Justice Innovations, Mountain View, CA). A DL-180A ultrasonic cleaner (Zhixin Instrumental, China) and a model XW-80A Vortex mixer (Kylin Medical Instrumental Plant, China) were used to mix the suspension solution thoroughly. A DZG-403 electric heat vacuum oven (Tianjin Tianyu Laboratory Apparatus Co., Ltd., Tianjin, China) was employed to dry the nanoparticles. Fused silica capillaries of 50 µm i.d. (375 µm o.d.) were purchased from the Yongnian Optic Fiber Plant (Hebei, China).

### Preparation of the suspensions

The suspension containing nanoparticles was prepared by ultrasonication for 15 min without any other additives in the buffer. This suspension remained stable for more than 1 h. Subsequently, sedimentation of the nanoparticles can occur, leading to a decrease in particle concentration and a reduction in the reproducibility of the sample migration times. To achieve the maximum reliability, the suspension was treated in the ultrasonic tank for several minutes before each injection. The nanoparticles were collected by centrifugalization and washing from the suspension for reuse.

### Separation conditions

The stock solution of phosphate based background electrolyte (25 mM) was prepared by dissolving a certain amount of $H_3PO_4$ in distilled water. NaOH (0.1 M) was used to adjust the pH to an appropriate value. Before use, all solutions were filtered through a 0.45-µm membrane (Beiyang Membrane Technologies, Tianjin, China) and degassed by a vacuum followed with ultrasonication. The running buffer was then prepared by mixing adding the nanoparticles into the phosphate based background electrolyte at a certain ratio. Sample solutions were dissolved in the running buffer at proper concentrations. The EOF was determined by using thiourea as the neutral marker which can be detected at 214 nm.

Prior to the actual experiments, the capillary was conditioned with buffer for ~20 min, and then electro-kinetically conditioned at low electric field in order to establish equilibrium of the nanoparticles between the buffer and the inner surface of the capillary. It was only after finishing these steps than a high electric field was applied to the system.

## Results and Discussion

### Effect of the aminopropyl nanoparticles on EOF

EOF is a very important phenomenon in CE, and velocity of EOF is relative to migration time of every analyte. Therefore, it has direct effect on separation efficiency. Attempts have been made to control the EOF for improving specific separations (29–31). The addition of aminopropyl nanoparticles in the running buffers can significantly alter the value of EOF at a broad range of pH as shown in Figure 1. Both the magnitude and direction of the EOF can be changed with the addition of amino containing particles at a different pH. At a lower pH, these nanoparticles contribute to making the inner surface of the separation channel bear a positive charge by dynamic modification. Therefore, the EOF is modified to be helpful for separation of analytes in a single run. Then, under low pH condition, these

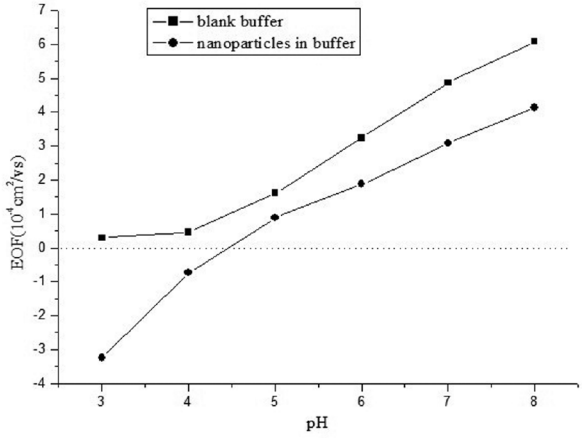


**Figure 1.** Plot of electroosmotic mobility as the function of the mobile phase pH. Conditions: capillary, 40 cm × 50 µm i.d., mobile phase, 25 mmol/L sodium phosphate; nanoparticles, 1.0 mg/mL; voltage, 10.0 kV; injection, EK 8000V, 5 s. EOF marker, thiourea. Detection: UV at 214 nm.

particles carry opposite charge to analytes. Thus, there should be ionic interactions between analytes and nanoparticles. On this point, these particles were also beneficial to optimization of separation.

**Separation of organic acids**

*Separation results*

The separation of five aromatic acids was tested on CE system. As shown in Figure 2A, thiourea, *o*-hydroxybenzoic acid, *p*-aminobenzene sulfonic acid, *p*-toluenesulfonic acid, *p*-aminobenzoic acid, and benzoic aicd were baseline separated within 8 min when the aminopropyl nanoparticles were added into the buffer under the optimized experiment condition, while these acids were not separated in free zone electrophoresis shown in Figure 2B.

The separation result demonstrated that the silica nanoparticles bonded with aminopropyl can suppress and even reverse the direction of the EOF effectively. The analytes went through the capillary from the cathode to the anode and were separated during the electrophoretic separation process. A careful review of the results in Figure 2 proved that the analytes were well separated with the presence of nanoparticles in the buffer, while only the first three acids appeared within 60 min without the nanoparticles. This indicated that the nanoparticles functioned as a direction modifier of EOF to speed up the negatively charged acids to come out of the capillary sooner. The disappearance of the last three peaks indicated that, in the absence of the nanoparticles, if the EOF is in the direction of anode to cathode, the magnitude of the EOF is relatively low and it can only carry the weak acids to move along in the same direction. The net electrophoretic mobility of the more negatively charged acids could overcome the EOF and move in the opposite direction to be detected. Another important factor was the ion-exchange characteristics between the analytes and the nanoparticles. The aromatic acids interacted with the nanoparticles via an ion-exchange mechanism since the particles are positively charged and the acids carry a negative charge. The interaction was stronger for the PSP and analytes migrated in opposite directions. This reversible association between the PSP and analytes is similar to the phenomena of ion-exchange chromatography used for separations of charged analytes. An interesting result was the migration time of *p*-aminobenzoic acid, which has a pKa value of 4.68. At pH 3, the slight positive charge on the amino group made it move slower than the neutral maker. As a result, the migration time of *p*-aminobenzoic acid was longer than that of thiourea (EOF marker).

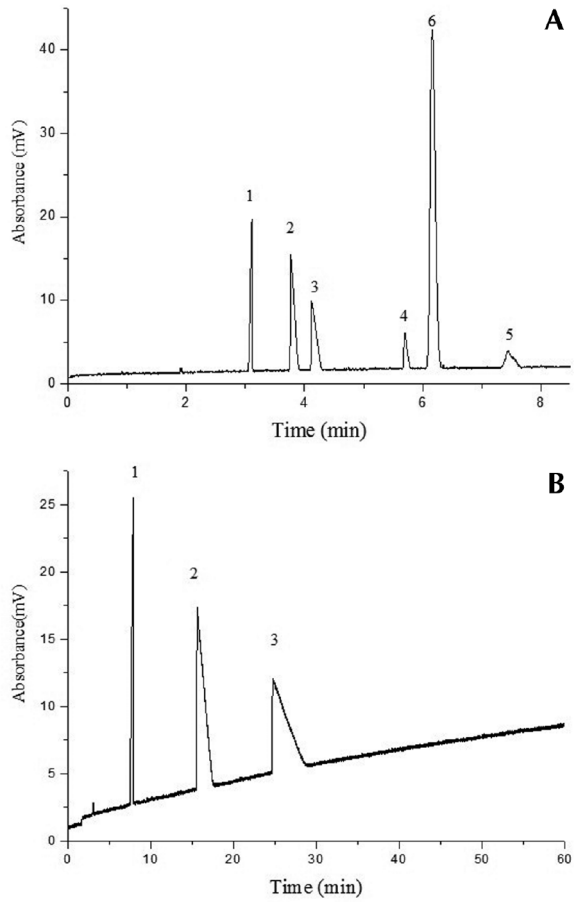


**Figure 2.** Capillary electrophoretic separation of five acids with (A) and without (B) the addition of the nanoparticles in the buffer. Conditions: capillary, 40 cm × 50 µm I.D.; buffer, 25 mmol/L sodium phosphate, pH 3; nanoparticles, 1.0 mg/mL; voltage, 10.0 kV. Injection: EK 8000 V, 5 s. Detection: UV at 214 nm. Analytes: p-toluenesulfonic acid (1); o-hydroxybenzoic acid (2); p-aminobenzene sulfonic acid (3); benzoic acid (4); p-aminobenzoic acid (5); and thiourea (6).

*Influence of pH on the separation*

The buffer pH played an important role in achieving high quality separation because it had complicated effects on EOF, the degree of ionization of electrolytic analytes, and the net charges on nanoparticles. A serial of buffers at pH 3.0, 4.0, 5.0, and 6.0 were evaluated for the separation of the model acids. The results (Figure 3) indicated that pH values higher than 5.0 were not beneficial for the separation of these aromatic acids in this system. In the experiment, analytes were injected from cathode, which means that the migration direction of analytes was opposite to

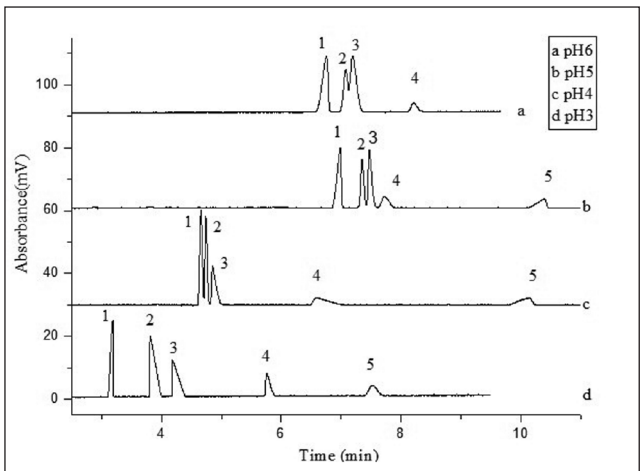


**Figure 3.** Electropherogram shows the separation of model acids in various pHs. Conditions: capillary, 40 cm × 50 µm i.d.; buffer, 25 mmol/L sodium phosphate; nanoparticles, 1.0 mg/mL; voltage, 10.0 kV. Injection: EK 8000 V, 5 s. Detection: UV at 214 nm. p-toluenesulfonic acid (1); o-hydroxybenzoic acid (2); p-aminobenzene sulfonic acid (3); benzoic acid (4); and p-aminobenzoic acid (5).

that of normal EOF. Higher pH would increase the EOF and reduced the charges on the nanoparticles, making it unfavorable for this separation. Under high pH, the normal EOF played the main role in the electrophoresis process. In addition, as nanoparticles were employed as PSPs, a homogeneous and stable dispersion of particles in buffer solution was the key to achieve better results. Two factors (i.e., the size and the charge) were concerned with the particles dispersion. Smaller size is beneficial for keeping stable dispersion in the buffer as it has less gravity effect. The charge status of the particles is also a key factor. Electrostatic force between these particles can also contribute to the steadiness of the dispersion, whose intensity was determined by the degree of charge on these particles. The functional group bonded on these particles is aminopropyl and is more positively charged at a relatively low pH. Based on the above discussion, in combining with the results shown in Figure 3, pH 3.0 was selected as the optimized pH for all further studies.

*Influence of organic modifier on the separation*

The effect of organic modifiers on mobility and selectivity was reported in CZE (32). As an organic modifier, acetonitrile had an important impact on both the EOF and the degree of ionization in the aromatic acids, and its influence on separation in the condition of nanoparticles presented in buffer was investigated. It was found experimentally that the EOF decreased greatly with the increase of acetonitrile in the buffer as shown in Figure 4. A likely reason is acetonitrile suppressed the ionization of the silanol and the amino groups leading to a decrease of the zeta potential (ζ) and thus a decrease of the EOF and retention time accordingly. In this condition, the effect of nanoparticles on the EOF was not reflected obviously. Actually, the separation efficiency decreases in the presence of acetonitrile. On the other hand, there was an obvious change of migration for *p*-aminobenzoic, whose pKa was altered according to the present of acetonitrile. The experiment phenomenon was corresponding to the reference (33).

*Influence of the applied voltage*

With the same experimental conditions as shown in Figure 2B except variable voltage ranging from 8.0 to 15.0 kV, the effect of potential on this separation was evaluated (Figure 5). In general, a higher strength of electric field resulted in faster separation while analytes remained in the same migration order. The EOF increased with the growth of the applied electric field strength. The five acids were separated completely within 5 min when the applied voltage was 15.0 kV. It would take 10 min at 8 kV. Without considering other issues, increasing the voltage could speed up separation and ameliorate selectivity. However, higher voltage would result in higher current and more Joule heat, causing band broadening and the reduction of resolution and selectivity. A plot of separation efficiency, expressed as the average theoretical plate number of all peaks in the electropherogram, versus applied voltage, is shown in Figure 5B. It can be concluded that a relatively high resolution was achieved when the applied voltage was 10.0 kV, which was selected for most of our investigation.

*Effect of buffer concentration*

The EOF and resolution were also influenced greatly by the ionic strength of the buffer. There was an interesting change of charge on *p*-aminobenzoic acid at different concentrations of the buffer shown in Figure 6. It was negatively charged and its migration time was shorter than thiourea when the buffer was 10 mM. As the buffer concentration increased, it gradually changed to positively charged, with the turning point at 15 mM when *p*-aminobenzoic acid and thiourea co-migrated together. For many weak acids, pKa data as function of the temperature are available in the literature (33–34). An investigation reported that the migration time of *p*-aminobenzoic was changed significantly for the temperature altered (35). Owing to the accumulation of Joule heat, the temperature of the system may raise gradually as the concentration increase of the running buffer. As a result, the migration behavior of *p*-aminobenzoic was changed.

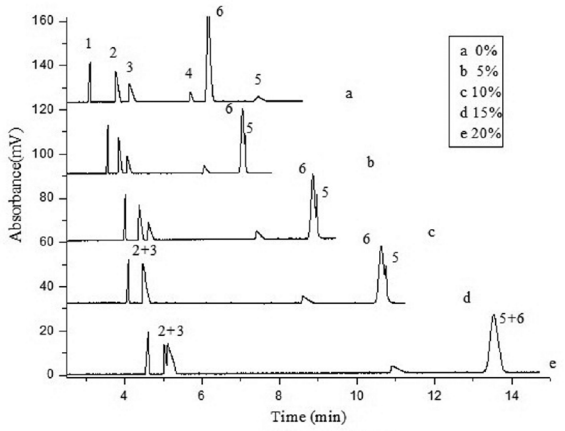


**Figure 4.** Influence of acetonitrile on the separation of the aromatic acids. Conditions: capillary, 40 cm × 50 µm i.d.; buffer, 25 mmol/L sodium phosphate, pH 3; nanoparticles, 1.0 mg/mL; voltage: 10.0 kV. Injection: EK 8000 V, 5 s. Detection: UV at 214 nm. Analytes: p-toluenesulfonic acid (1); o-hydroxybenzoic acid (2); p-aminobenzene sulfonic acid (3); benzoic acid (4); p-aminobenzoic acid (5); and thiourea (6).

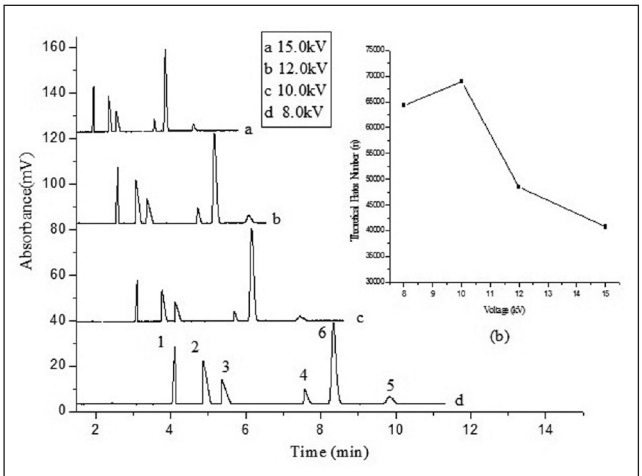


**Figure 5.** Influence of voltage on the separation (A) and efficiency (B) of the aromatic acids. Conditions: capillary, 40 cm × 50 µm i.d.; buffer, 25 mmol/L sodium phosphate, pH 3; nanoparticles, 1.0 mg/mL. Injection: EK 8000 V, 5 s. Detection: UV at 214 nm. Analytes: p-toluenesulfonic acid (1); o-hydroxybenzoic acid (2); p-aminobenzene sulfonic acid (3); benzoic acid (4); p-aminobenzoic acid (5); and thiourea (6).

*Influence of the concentration of the nanoparticles*

As shown in Figure 7, the reversed EOF increased with the concentration of the nanoparticles. As a result, the migration time of the analytes was shorter at higher concentrations. However, when the additive concentration reached 3.0 mg/mL, sedimentation of the nanoparticles appeared and caused many difficulties for experimental operation. It was concluded that the magnitude of the reversed EOF did not increase significantly when too much particles were added in. To have the best performance, there must be equilibrium between the nanoparticles and the inner wall of capillary. Therefore, a concentration of 1.0 mg/mL was the selected as the optimized concentration for the nanoparticles.

*Repeatability*

Under the optimal separation conditions, the migration times and peak areas were employed to evaluate the repeatability of CE with nanoparticles presence in the buffer. For this purpose, six consecutive replica runs were conducted under the same conditions. The results demonstrated that the RSD of migration times were 2% and 3% for intro-day and inter-day, respectively. Also, the peak areas were found to have RSD values between 2–5%. The experimental results further proved the good stability of the suspension with nanoparticles in the buffer to complete with other quantitative methods.

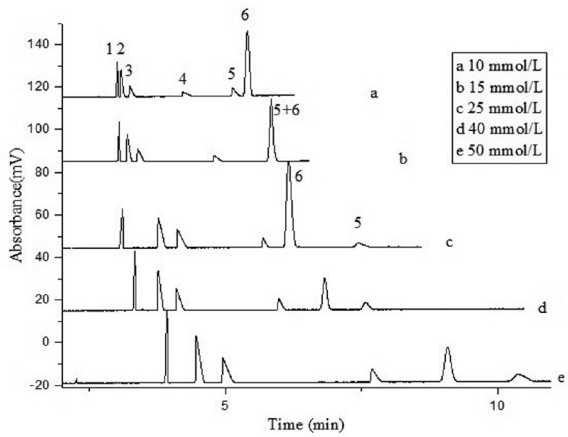


**Figure 6.** Effect of the buffer concentration on separation. Conditions: capillary, 40 cm × 50 µm i.d., pH 3; nanoparticles, 1.0 mg/mL; voltage, 10.0 kV. Injection: EK 8000 V, 5 s. Detection: UV at 214 nm. p-toluenesulfonic acid (1); o-hydroxybenzoic acid (2); p-aminobenzene sulfonic acid (3); benzoic acid (4); p-aminobenzoic acid (5); and thiourea (6).

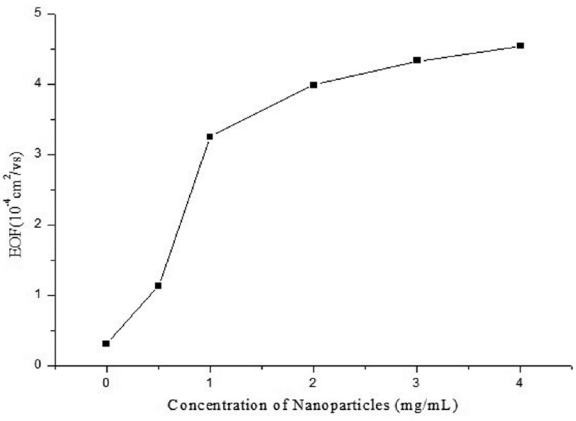


**Figure 7.** Influence of the concentration of nanoparticles on EOF. Conditions: capillary, 40 cm × 50 µm i.d.; buffer, 25 mmol/L sodium phosphate, pH 3; voltage, 10.0 kV. Injection: EK 8000 V, 5 s. Detection: UV at 214 nm.

## Conclusions

An electrophoretic system was used in conjuction with silica-based nanoparticles bonded with aminopropyl group as pseudo-stationary phase for high efficiency separation of a model mixture of five aromatic acids and EOF marker. Five aromatic acids were completely separated in the pH range of 3.0 to 5.0 in the presence of the nanoparticles. The results indicated that the presence of the nanoparticles inside the capillary increased the resolution of the electrophoretic separation significantly. As the nanoparticles were easy to be removed by centrifuge or filtration, they would not bring troubles to next step and also can be reused for other experiments.

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