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Silica nanoparticles for separation of biologically active amines by capillary electrophoresis with laser-induced native fluorescence detection

This paper describes the analysis of biologically active amines by capillary electrophoresis (CE) in conjunction with laser-induced native fluorescence detection. In order to simultaneously analyze amines and acids as well as to achieve high sensitivity, 10 mM formic acid solutions (pH $<$ 4.0) containing silica nanoparticles (SiNPs) were chosen as the background electrolytes. With increasing SiNP concentration, the migration times for seven analytes decrease as a result of increase in electroosmotic flow (EOF) and decrease in their electrophoretic mobilities against EOF. A small EOF generated at pH 3.0 reveals adsorption of SiNPs on the deactivated capillary wall. The decreases in electrophoretic mobilities with increasing SiNP concentration up to 0.3 $\times$ indicate the interactions between the analytes and the SiNPs. Having a great sensitivity (the limits of detection at a signal-to-noise ratio (S/N) = 3 of 0.09 nM for tryptamine (TA)), high efficiency, and excellent reproducibility (less than 2.4% of the migration times), this developed method has been applied to the analysis of urinal samples with the concentrations of 0.50 $\pm$ 0.02 μM, 0.49 $\pm$ 0.04 μM, and 74 $\pm$ 2 μM for TA, 5-hydroxytryptamine, and tryptophan, respectively. The successful examples demonstrated in this study open up a possibility of using functional nanoparticles for the separation of different analytes by CE.

Keywords: Capillary electrophoresis laser-induced native fluorescence / Catecholamines / Silica nanoparticles
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1 Introduction

The determination of catecholamines, such as epinephrine (E), tryptamine (TA), and 5-hydroxytryptamine (serotonin; 5-HT), in biological samples, such as urine and cerebral spinal fluids, is of great interest because they are important neurotransmitters in central and peripheral nervous systems. In addition, their improper regulation has been found to be associated with several neurological disorders, including Parkinson's disease, schizophrenia, anxiety disorders, and memory impairment [1–4]. The side effects of many anticancer drugs are also considered to be mediated by changing the catecholamine levels, particularly in central neuronal cells [5, 6]. However, determination of low concentrations (μM-nM) of catecholamines in complicated biological samples is not an easy task. Thus, there is a great demand for developing techniques that are sensitive and highly efficient.

CE is a powerful separation tool for the analysis of biological samples, which offers a number of advantages, including rapidity, efficiency, and requirement of minute sample volumes [7–11]. CE has been applied to the determination of catecholamines in biological samples for more than one decade [12–21]. Owing to the unique electrochemical characteristics of catecholamines, CE with amperometric detection is usually chosen [12–14]. However, special attention has to be paid to minimize interference from the separation electric field [22–24] and to avoid passivation of the electrodes by the analytes and other materials such as proteins [14].

In addition to electrochemical detection, LIF detection is popular in CE for the determination of catecholamines [15, 16, 18–21]. When using a relatively low-cost visible laser, such as argon-ion laser at 488, derivatization of catecholamines with reagents to form strong fluorescent complexes is required prior to, during, or post CE

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Abbreviations: 5-A-1-N, 5-amino-1-naphthol; **CE-LINF**, capillary electrophoresis in conjunction with laser-induced native fluorescence; **Dopa**, DL-3,4-dihydroxyphenylalanine; **E**, epinephrine; **GNP**, gold nanoparticle; **5-HT**, 5-hydroxytryptamine (serotonin); **Trp**, L-tryptophan; **1-NAA**, 1-naphthylacetic acid; **PEO**, poly(-ethylene oxide); **SiNP**, silica nanoparticle; **TA**, tryptamine; **TEOS**, tetraethyl orthosilicate

separation. However, derivatization may suffer from incomplete reactions and interferences from the side products. In addition, a special configuration between the separation channel and detection area may be needed when conducting on-column derivatization. Furthermore, derivatization becomes problematic when sample volumes are small ($< 1.0\ \mu L$) and/or the analyte concentrations ($<10^{-7}$ M) are low. A poor linearity and irreproducibility are expected, mainly because of inefficient labeling when the analyte concentration is low. With strong intrinsic fluorescence characteristics, catecholamines can be determined by CE in conjunction with laser-induced native fluorescence (CE-LINF) detection using a UV laser. UV lasers, such as Ar^+ ion lasers, XeF excimer laser, metal vapor lasers, and quadruple Nd:YAG laser, have been used in CE-LINF for the determination of catecholamines [18, 19, 21]. With its excellent sensitivity, rapidness, and high resolving power as well as the capability of dealing with extremely small-volume samples, CE-LINF has been applied successfully to the analyses of catecholamines in single cells [18, 19].

From the view point of metabolomics, aiming to obtain a complete set of metabolites, techniques allowing determination of numerous analytes with wide concentration ranges are important. It has been addressed that the accuracy (sensitivity) of diagnosis of several neuron dysfunction related diseases is greater when multiple markers such as catecholamines and their metabolites are determined [25]. However, it is sometimes not an easy issue by a single CE-LINF run. The sensitivity and resolution of amines and acids can be optimized separately at low pH, but they cannot be simultaneously separated in the absence of EOF. Although this problem might be solved by adding surfactants, such as CTAB, to background electrolytes to reverse EOF, it suffers from higher fluorescence background and irreproducibility as a result of Joule heating and/or unstable capillary wall (EOF variation). Similar problems are also found when conducting MEKC [26, 27]. Very recently, we have proposed a CE-LINF approach for the analysis of catecholamines and their metabolites under discontinuous conditions [28]. This stacking and separation approach provides the concentration LODs at S/N = 3 of 0.27 and 0.31 nM for 5-HT and 5-hydroxyindole-3-acetic acid, respectively, when the sample was injected at 15 kV for 360 s. The drawbacks of this approach include unstable baseline, loss of sensitivity as pH increases during separation, and the need for regenerating a high bulk EOF after each run.

To continue our effort in developing techniques for simultaneous determination of biologically active amines and their metabolites, CE-LINF techniques using nanoparticles are interesting. Capillaries and microfabricated channels coated with gold nanoparticles (GNPs) have been employed in CE and microchip CE to enhance the separation efficiency of small isomers [29–34]. It has been suggested that GNPs serve as large surface area platforms for adsorption of organic molecules that alter the interactions of nanoparticles with the capillary wall, the analytes, or both. Recently, we have separately used polymer solutions containing GNPs and polymers-modified GNPs for DNA separation, with the advantages of rapidity, reproducibility, and high efficiency [31–34]. The viscosities of the two separation matrices are extremely low, and thus filling narrow separation channels with these matrices are quite simple. Polymeric nanoparticles as pseudostationary phases have been demonstrated in the separation of three amines in CEC using continuous filling and partial filling techniques [35]. The continuous filling technique does not require time-consuming particle packing or retaining frits. Covalent coating of capillaries with nanoparticles such as polystyrene particles and silica nanoparticles (SiNPs) provides high separation efficiencies for various analytes such as proteins [36–40]. Although the coating is quite stable, tedious coating processes are generally needed.

The aim of this work is demonstration of the separation of amines and acids by CE-LINF using SiNPs. Ten millimolar formic acid (pH < 4.0) containing SiNPs was used as background electrolyte. In this study, we evaluated the impact of SiNPs on EOF and the electrophoretic mobilities of seven analytes (six cations and one anion). Owing to the interactions of SiNPs with the analytes and with the capillary wall, the resolution, speed, sensitivity, and reproducibility of the developed CE approach are optimized.

2 Materials and methods

2.1 Apparatus

The basic design of the separation system has been previously described [41]. Briefly, a high-voltage power supply (Gamma High Voltage Research, Ormond Beach, FL, USA) was used to drive electrophoresis. For safety, the high-voltage end of the separation system was housed in a plexiglass box. The entire CE system was enclosed in a black box with a high-voltage interlock. After passing a UG1 filter (Barrington, Edmund, NJ, USA) to minimize the plasma interference, the light from a diode pumped solid state nanolaser (JDS Uniphase, Manteca, CA, USA) at 266 nm with a 5 mW output was focused with a UV lens. The emitted light from the analytes that were excited by the focused light was collected with a $10\times$ objective (numerical aperture = 0.25) before reaching the photomultiplier tube (PMT) (R928 from Hamamatsu, Shizuoka-Ken, Japan). The amplified current was transferred directly through a 10 kΩ resistor to a 24-bit A/D interface

at 5 Hz (Borwin, JMBS Developments, Le Fontanil, France), and the data were stored in a PC. Fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) with 75 μm ID and 365 μm OD, were used. The zeta potentials of SiNPs in the presence and absence of the analytes were taken using a Malvern Zetasizer 3000 HS (Malvern Instruments, UK).

2.2 Chemicals

Tetraethyl orthosilicate (TEOS) and ammonia (NH_4OH, 25%) were purchased from Fluka (Buchs, Switzerland). Absolute ethanol from Acros (Geel, Belgium) was used as received. Poly(ethylene oxide) (PEO) (M_w 8 000 000), poly(vinylpyrrolidone) (PVP) (M_w 1 300 000), formic acid, TA (pK_a = 10.2), 5-HT (pK_a = 9.8 and 11.1), E (pK_a = 8.66 and 9.95), and DL-3,4-dihydroxyphenylalanine (Dopa, pK_a = 2.32, 8.72, 9.96, and 11.79) were obtained from Aldrich (Milwaukee, WI, USA). 5-Amino-1-naphthol (5-A-1-N, pK_a = 3.97), naphthalene, 1-naphthylacetic acid (1-NAA, pK_a = 4.3), and L-tryptophan (L-Trp, pK_a = 2.46; 9.41) were purchased from Sigma (St. Louis, MO, USA). Sodium chloride was purchased from J. T. Baker (Pittsburg, PA, USA). The pH values of 10 mM formic acid solutions were 2.1 (without adding NaOH), as well as 3.0 and 4.0 that were adjusted with NaOH.

2.3 Preparation and characterization of SiNPs

Base-catalyzed sol-gel reactions of TEOS were conducted to prepare SiNPs [42]. Briefly, 1.5 mL TEOS, 2.0 mL ammonia (25 wt%), 1.0 mL deionized water, and 50 mL ethanol were introduced into a 100-mL conical flask. After the mixture was stirred at room temperature for 3 h, an additional 1.0 mL TEOS was added to the mixture and the reaction was allowed to continue for another 3 h. The product solution was finally subjected to at least six cycles of centrifugation (8000 rpm for 10 min) and washing to remove unreacted precursors. Deionized water and 10 mM formic acid solutions (both 50 mL) were used to wash the precipitates for the first and last three cycles, respectively. The average size of the as-prepared SiNPs was 60 ± 8 nm in diameter, which was obtained from the transmission electron microscopy (TEM) measurements using an H7100 TEM (Hitachi High-Technologies, Tokyo, Japan) operating at 75 kV. The concentration of the original 60-nm SiNPs (density = 2 g/cm^3) was ~89.7 nM, which is denoted by 1.0× in this study. A fluorometer (Aminco-Bowman Series 2, ThermoSpectronic, Pitsford, NY, USA) was used to measure the fluorescence of the analytes in the presence and absence of SiNPs.

2.4 Electrophoretic separation

The deactivated capillaries were dynamically coated with 5.0% PVP for 2 h and then with 0.5% PEO for 8 h. The two-layer dynamic coatings has been found more effective to suppress EOF and minimize the interaction with analytes such as proteins and DNAs when compared to that coated with one layer of either PEO or PVP [32, 43]. We note that if time is an important matter, the dynamic coating process can be shortened to 1 h with each cycle for 30 min. When reproducibility was in question (generally after 10 runs), the capillary was simply treated with PEO for 10 min. Ten millimolar formic acid solutions (pH 2.1–4.0) containing SiNPs (0–2.0×) were used to fill the deactivated capillaries and bare-fused silica capillaries. Prior to separation, the capillaries were equilibrated with the background electrolytes at 10 kV for 10 min. The standard samples prepared in deionized water were injected into capillaries filled with the background electrolytes from the anode end at 1 kV for 10 s. The separations were conducted at 375 V/m (*e.g.*, 15 kV for a 40-cm capillary). In order to determine the EOF under different conditions, 1.0 μM naphthalene solution (without the amines) was injected and analyzed.

2.5 Urine analysis

Ten urine samples were collected from a normal female in a period of 1 month. The samples were collected prior to the analyses and used without any pretreatment process. The samples were injected into capillaries filled with 10 mM formic acid solution (pH 3.0) with or without containing 1.0× SiNPs at 10 kV for 10 s. The separations were conducted at 375 V/m. In order to determine the concentrations of the analytes, 5 μL TA (10–100 μM), 5-HT (10–100 μM), and Trp (0.2–2 μM) were spiked into 495 μL urine samples. Linear plots of the peak heights against the concentrations of the standards were depicted, and the plots were used to determine the concentrations of the analytes in one of the urinal samples.

3 Results and discussion

3.1 Choice of electrolytes

Biologically active amines and acids with benzene or indole residues depicted in Fig. 1 possess highly intrinsic fluorescence characteristics when excited under UV-laser light (the excitation wavelength is 266 nm in this study) and their quantum yields are high at low pH [19]. At pH < 4.0, the amines are cations and can be detected in the cathodic end without EOF. With respect to sensitivity and resolving power, we only tested the separations at

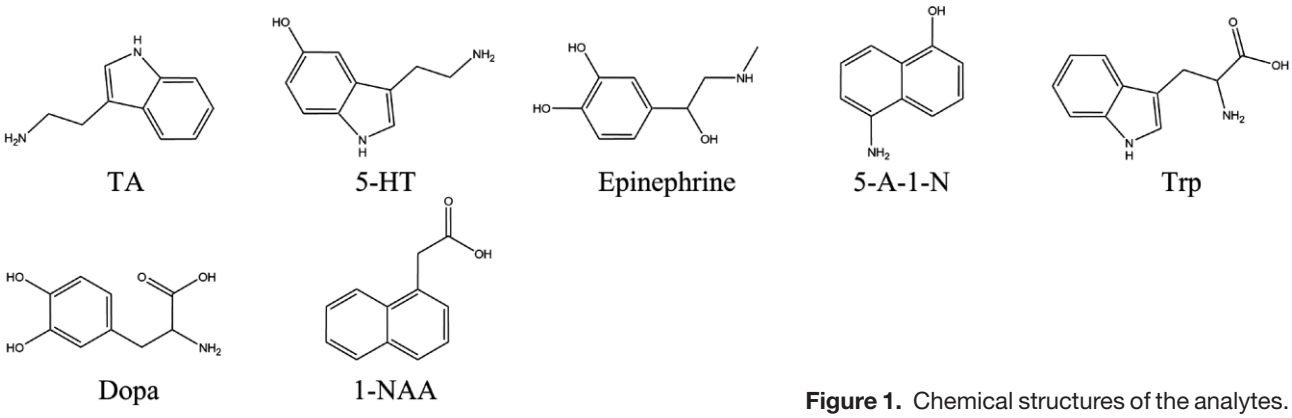

Figure 1. Chemical structures of the analytes.

pH $<$ 4.0. In order to minimize fluorescence quenching as a result of the formation of ion pairs between the analytes and anions such as chloride, acidic background electrolytes prepared from formic acid and NaOH were selected. The advantage of using formic acid over HCl and H_3PO_4 also includes small amounts of Joule heat generated, which minimizes fluorescence quenching caused by collision between the analyte molecules and surrounding molecules such as water.

In this study, 10 mM formic acid solutions (adjusted with NaOH when needed) at pH 2.1, 3.0, and 4.0 with/without containing SiNPs were used as the background electrolytes. Using these solutions, the currents generated at 375 V/cm are all less than 20 μA. Thus, the effect of Joule heating on speed and resolution is negligible. In order to achieve great reproducibility, the capillaries treated with PVP and PEO were used. In our previous study, we found that the coated capillaries were stable at low pH ($<$3.8) for more 100 runs when the capillary was treated with PEO for 5 min after each run [43]. We note that the dynamic coating of PVP on the capillary wall is mainly through hydrogen bonding and the interactions between PVP and PEO are mainly through hydrogen bonding and hydrophobic patches. The electropherogram depicted in Fig. 2A shows that the five amines were nicely separated in 10 min using 10 mM formic acid at pH 2.1. At pH 2.1, the amines are cations and migrate toward the cathode end. The migration times are longer at pH 3.0 (Fig. 2B) than those at pH 2.1; their electrophoretic mobilities are smaller than those at pH 2.1 as a result of deprotonation. Trp and Dopa that are amphiprotic ions and possess very low charged densities at pH 4.0 were not detected in 60 min as shown in Fig. 2C, indicating that EOF is very small ($<10^{-6}$ cm^2/V·s) when using a capillary dynamically coated with PVP and PEO. We note that the degree of the protonation of the ammonium ion residues decreases while the degree of the dissociation of the carboxylic acid

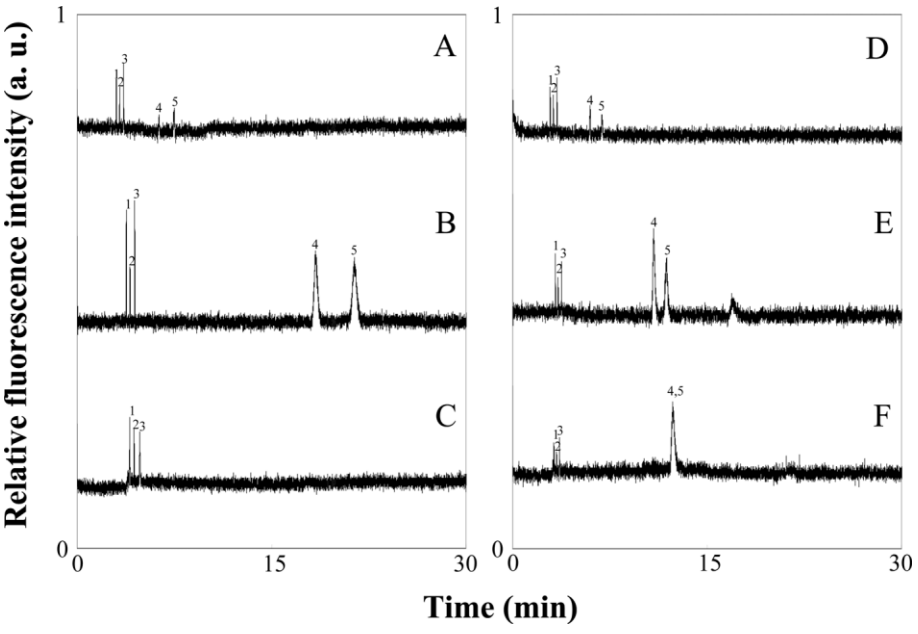

Figure 2. Electropherograms of the amines separated in 10 mM formic acid solutions (A-C) in the absence and (D-F) presence of 1.0× SiNPs at different pH values. (A) and (D): pH 2.1; (B) and (E): pH 3.0; (C) and (F): pH 4.0. Capillary: 40 cm total length and 30 cm effective length. Conditions: electrophoretic injection at 1 kV for 10 s; separation at +15 kV. Peak identities (concentration): 1, TA (1 nM); 2, 5-HT (1 nM); 3, E (0.1 μM); 4, Trp (50 nM); 5, Dopa (1 μM).

residue increases with increasing pH. Sharp peak profiles and excellent reproducibility (RSD values of the migration times for the five analytes are less than 1.7%, $n = 3$) indicate that analyte adsorption on the capillary wall at pH 3.0 is negligible when using the two-layer coated capillary.

Next, we separately tested the separations using 10 mM formic acid solutions containing 1.0× SiNPs at pH 2.1, 3.0, and 4.0. In the presence of SiNPs, the five analytes were nicely separated within 10 and 15 min at pH 2.1 and 3.0, respectively, while the separation of Trp and Dopa was not successful at pH 4.0 (Figs. 2D-F). At pH 2.1, the migration times for the five analytes in the absence and presence of SiNPs are close. The impacts of SiNPs on TA, 5-HT, and E are also insignificant at pH 3.0 and 4.0, while they are apparent for Trp and Dopa at pH 3.0 and 4.0. We point out that in the presence of 1.0× SiNPs, the EOF mobilities at pH 3.0 and 4.0 are 7.6×10^{-5} and 7.7×10^{-5} cm^2/V·s, respectively, which are both greater than that ($<10^{-6}$ cm^2/V·s) at pH 2.1. The EOF mobilities in the presence of SiNPs are also greater than those ($<10^{-6}$ cm^2/V·s) in the absence of SiNPs at the corresponding pH values. Thus, the increase in EOF mobility is the main contributor for slightly shorter migration times for the first three analytes in the presence of SiNPs at pH 3.0 and 4.0. Because the zeta potentials of SiNPs are −0.7, −15.4, and −15.5 mV at pH 2.1, 3.0, and 4.0, respectively, we believe that the increase in the EOF mobility is due to adsorption of SiNPs (increases in the zeta potential) on the capillary wall. It is important to point out that SiNPs have a strong interaction with PEO through hydrogen bonding and hydrophobic patches.

However, the changes in EOF alone cannot explain why the migration times of Trp and Dopa are both less than 12 min at pH 3.0 and 4.0 in the presence of SiNPs, while in the absence of SiNPs they are 18.40 and 21.40 min, respectively, at pH 3.0, and are both longer than 60 min at pH 4.0. We thus suggest that the decrease in the electrophoretic mobility of the analytes (toward anode) as a result of the interactions with SiNPs in the bulk solution is the other reason for the result besides the increases in EOF. We note that the much heavier and less negatively charged SiNPs migrate more slowly against EOF than the analytes do. Thus, the apparent electrophoretic mobilities (the difference between the EOF mobilities and the electrophoretic mobilities of the analytes) in the presence of SiNPs are greater than those without SiNPs at the same pH. The role of SiNPs is similar to those of polymeric NPs and ionic polymers in capillary EKC, in which polymers act as pseudostationary phases to affect the electrophoretic mobilities of analytes and thus the selectivity [35, 44–47]. The interaction between the analytes and SiNPs is also supported with the changes in the zeta potential of the SiNPs from −15.5 to −16.5 mV in the presence of 1.0 μM Trp at pH 4.0. This change is greater than those (less than ± 0.1 mV) in the presence of 1.0 μM TA, 5-HT, and E, which suggests that Trp has a stronger interaction with SiNPs when compared to the other three amines. Because the charge densities of the two analytes and SiNPs are both low, their interactions are mainly through hydrogen bonding and hydrophobic patches. The interaction is further supported by the fact that the native fluorescence intensities (λ_{ex} = 266 nm; λ_{em} = 350 nm) of Trp and Dopa are slightly quenched by SiNPs (not shown). We note that the fluorescence quenching caused by SiNPs is mainly due to collision and energy transfer.

3.2 Impacts of SiNP concentration on separation

Because Dopa and Trp were not separated at pH 4.0 in the presence of SiNPs, we investigated the concentration dependence of SiNPs on the separation of six amines and one acid (1-NAA) at pH 3.0. The electropherogram depicted in Fig. 3A presents that 1-NAA (pK_a = 4.3) that possesses a small negatively charged density at pH 3.0 was not detected in the absence of SiNPs, because of a very small EOF ($<10^{-6}$ cm^2/V·s) at pH 3.0. In the presence of 0.3× SiNPs, the seven analytes were nicely separated as shown in Fig. 3B. The electropherograms depicted in Figs. 3B-D demonstrate that the migration times become shorter with increasing SiNP concentration up to 1.0×. It is interesting to point out that there is a small and broad peak in each electropherogram depicted in Fig. 3B-E. The small peak is probably due to the existence of an unknown neutral impurity from the sample or the change in refractory index (sample prepared in water). We note that when we conducted CE analyses of naphthalene (a neutral marker) without other analytes under conditions used in Figs. 3B-E, there is only a large peak in each electropherogram. When using the migration times for naphthalene to calculate the EOF mobilities, the values are almost the same (less than 1.0%) to those calculated from the times for the small peaks (Table 1). The time for the small peak in each electropherogram was thus used to estimate the EOF mobilities of the system when naphthalene was not added to the samples. The EOF values listed in Table 1 exhibit that EOF increases gradually from 0 to 0.6× SiNPs and slightly increases over the concentration range of 0.6–2.0×. As suggested above, adsorption of SiNPs is the main contributor for the increase in EOF. The results also indicate that adsorption of SiNPs at pH 3.0 reaches saturation around 0.6×.

To further show the interactions between the analytes and SiNPs, the migration times and the electrophoretic mobilities of the seven analytes under different conditions are

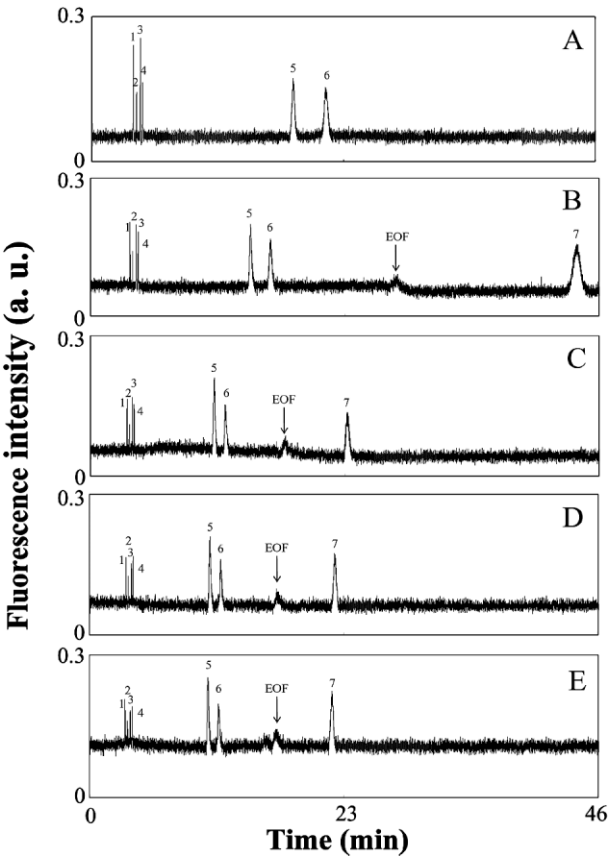

Figure 3. Effect of SiNPs on the separation of seven analytes. The concentrations of SiNPs prepared in 10 mM formic acid (pH 3.0) are (A) 0×, (B) 0.3×, (C) 0.6×, (D) 1.0×, (E) 2.0×. Peak identities (concentration): 1, TA (1 nM); 2, 5-HT (1 nM); 3, E (0.1 μM); 4, 5-A-1-N (0.5 μM); 5, Trp (50 nM); 6, Dopa (1 μM); 7, 1-NAA (1 μM). Other conditions are the same as in Fig. 2.

also listed in Table 1. With increasing SiNP concentration up to 2.0×, the migration times for the analytes decrease, while the EOF mobility increases. Unlikely, the electrophoretic mobilities for the analytes only decrease with increasing SiNP concentration up to 0.3×. We note that the concentration of SiNPs is about 27 nM, which is in the range of the analyte concentrations (1 nM-1 μM). The decreases in the electrophoretic mobilities for various analytes are different, with the smallest change of 0.17 for TA and the largest change of 0.27 for Trp and Dopa. The magnitude of their change agrees with the results revealed in Fig. 2; Trp and Dopa have stronger interactions with SiNPs than the other four amines do. Because of sharp peak profiles and great sensitivity in the presence of SiNPs, we suggest that the analytes were not adsorbed strongly on the surface of SiNPs through hydrogen bonding and hydrophobic patches. This is reasonable since the analytes are water soluble and possess cationic characteristics, while there are only few anionic charges on the SiNP surfaces (a small zeta potential) at pH 3.0. The different impacts of SiNPs on the EOF and the electrophoretic mobility reveal that SiNPs affect the selectivity through alterations of the EOF and interactions with the analytes.

Owing to adsorption of SiNPs on the capillary wall, the interactions of the analytes with the capillary wall decrease, leading to sharper peak profiles. For instance, the peak widths at the half maximum ($w_{1/2}$) are 0.05 and 0.02 min for TA as well as 0.43 and 0.21 min for 1-NAA at pH 3.0 in the absence and presence of 1.0× SiNPs, respectively. Taken together with their greater quantum yields at low pH, the LODs at S/N = 3 for TA and 5-HT are 0.09 and 0.15 nM, respectively, as listed in Table 2. It is

Table 1. Effects of SiNPs on EOF and the mobility shift of seven model analytes

Analyte	μ (10^{-4} cm^2 V^{-1} s^{-1})[a]													
	No SiNPs $\mu_{EOF} < 0.01$		0.3× SiNPs $\mu_{EOF} = 0.47$			0.6× SiNPs $\mu_{EOF} = 0.74$			1.0× SiNPs $\mu_{EOF} = 0.76$			2.0× SiNPs $\mu_{EOF} = 0.79$		
	t_m (min) (RSD%)[b]	μ_{ep}	t_m (min) (RSD%)	μ_{ap}	μ_{ep}	t_m (min) (RSD%)	μ_{ap}	μ_{ep}	t_m (min) (RSD%)	μ_{ap}	μ_{ep}	t_m (min) (RSD%)	μ_{ap}	μ_{ep}
TA	3.86 (1.0)	3.45	3.56 (1.1)	3.75	3.28	3.31 (0.9)	4.03	3.29	3.30 (0.9)	4.04	3.28	3.21 (1.1)	4.15	3.36
5-HT	4.08 (1.3)	3.27	3.81 (1.2)	3.50	3.03	3.52 (0.9)	3.79	3.05	3.51 (0.9)	3.80	3.04	3.42 (1.1)	3.90	3.11
E	4.43 (1.3)	3.01	4.12 (1.3)	3.24	2.77	3.79 (1.0)	3.52	2.78	3.77 (1.0)	3.54	2.78	3.68 (1.2)	3.62	2.83
5-A-1-N	4.65 (1.2)	2.87	4.31 (1.2)	3.09	2.62	3.96 (1.0)	3.37	2.63	3.94 (0.9)	3.38	2.62	3.86 (1.1)	3.45	2.66
Trp	18.40 (1.5)	0.72	14.47 (1.4)	0.92	0.45	11.20 (1.5)	1.19	0.45	10.88 (1.8)	1.23	0.47	10.72 (1.4)	1.24	0.45
Dopa	21.40 (1.7)	0.62	16.26 (1.5)	0.82	0.35	12.19 (1.6)	1.09	0.35	11.88 (1.9)	1.12	0.36	11.63 (2.1)	1.15	0.36
1-NAA	ND[b]	ND	44.04 (2.1)	0.30	−0.17	23.30 (2.1)	0.57	−0.17	22.16 (2.4)	0.60	−0.16	21.90 (2.6)	0.61	−0.18

a) The background electrolytes are 10 mm formic acid (pH 3.0) containing 0–2.0× SiNPs. Other conditions are the same as those in Fig. 3.

b) n = 3; ND, not detected

Table 2. Impact of SiNPs on the LODs of the analytes in CE-LINF at pH 3.0

Analyte	LOD (nM)[a)]				
	SINPs				
	0	0.3 ×	0.6 ×	1.0 ×	2.0 ×
TA	0.07	0.09	0.11	0.12	0.18
5-HT	0.15	0.15	0.19	0.19	0.30
E	6.37	8.82	11.05	14.81	23.08
5-A-1-N	57.69	50.00	62.38	61.22	97.40
Trp	5.63	4.55	4.20	3.90	5.24
Dopa	132.35	115.38	114.75	114.29	172.41
1-NAA	ND[b)]	121.21	126.00	125.36	125.00

a) The background electrolytes are 10 mM formic acid (pH 3.0) containing 0–2.0 × SiNPs. Other conditions are the same as in Fig. 3.
b) Not detected

interesting to note that the sensitivities for TA, 5-HT, and E decrease, while those for 5-A-1-N, Trp, and Dopa slightly increase in the presence of 0.3 × SiNPs. The sensitivity increases for the analytes are mainly due to sharp peak profiles as a result of reduced analyte adsorption in the presence of SiNPs. However, fluorescence quenching is more significant at high SiNP concentrations. Irreproducibility and an unstable baseline (aggregation of SiNPs and greater scattering) also occurred at high SiNP concentrations (*e.g.*, 2.0 ×).

3.3 Comparison of coated and bare silica capillaries

In the absence of SiNPs, analyte adsorption is serious at pH > 4.0 when using a bare fused-silica capillary. Although the seven analytes listed in Table 1 were also nicely separated at pH 3.0 (μ_{EOF} = 5.5 × 10^{-5} $cm^2/V \cdot s$) when using a bare fused-silica capillary, the migration times for Trp, Dopa, and 1-NAA are 13.79, 15.3, and 33.69 min, respectively. In addition to a long separation time, the RSD for the migration time of 1-NAA is 4.5% and the sensitivity is also slightly lower (about threefold) than that in the presence of SiNPs. The problems are mainly due to analyte adsorption on the capillary wall. Although analyte adsorption can be further minimized by decreasing the pH, the loss of resolution between TA and 5-HT as well as 5-HT and E was found. In addition, 1-NAA was not detected within 45 min when conducting the separation at pH 2.1.

The migration times for the three analytes were shortened to 11.05, 12.06, and 22.34, respectively, when using the same capillary filled with the background electrolyte (pH 3.0) containing 1.0 × SiNPs. In addition, the peak widths for the analytes are sharper in the presence of SiNPs. For instance, the $w_{1/2}$ values are 0.05 and 0.02 for TA as well as 0.43 and 0.31 min for 1-NAA, in the absence and presence of SiNPs, respectively. Under the CE condition, μ_{EOF} was 7.6 × 10^{-5} $cm^2/V \cdot s$, which is close to that when using a two-layer coated capillary (Table 1). The sharp peak profile and greater EOF mobility indicate adsorption of SiNPs on the capillary wall. However, the method is irreproducible; the RSD values for the migration times of the seven analytes are all greater than 9.0%, which are much worse than those using the two-layer coated capillary (less than 2.4% as exhibited in Table 1). Irreproducibility is mainly due to aggregation of SiNPs on the capillary wall and analyte adsorption when using a bare fused-silica capillary. By conducting light-scattering microscope measurements, we found that aggregation of SiNPs and GNPs on glass is more serious than those in PVP and PEO coated glass (manuscript in preparation).

3.4 Urine analysis

The results demonstrated in Fig. 3 show that the separation of amines and acids by CE-LINF using SiNPs offers the advantages of speed, efficiency, and repeatability. To test the diagnostic potential of the proposed method, urine samples that were collected from a healthy female in a period of 1 month were analyzed by the proposed method. Although the sensitivity decreases with increasing the concentration of SiNPs (above 0.3 ×), the condition of using a two-layer coated capillary filled with 10 mM formic acid (pH 3.0) containing 1.0 × SiNPs was chosen for the sake of speed. One representative electropherogram is depicted in Fig. 4A. In addition to the three identified analytes (TA, 5-HT, and Trp), the other peaks correspond to benzoic compounds, heterocyclic compounds, peptides, and proteins containing Trp, tyrosine and phenylalanine residues, nucleotides, oligonucleotides, *etc*. Because the compositions in various urine samples are different, depending on several factors such as what she ate and her feeling, we did not intend to quantitatively determine the concentrations of the three identified analytes in all urine samples. Qualitative results from separating the ten samples suggest that the presentmethod is reproducible. The RSD values of the migration times for the three analytes are all less than 3.5% in ten different samples (each with at least three runs). In order to quantitatively determine the concentrations of the three identified analytes, we spiked the standards to one of the urine sample. Using the regression lines listed in Table 3, the concentrations of TA, 5-HT, and Trp were calculated, with the results of 0.50 ± 0.02, 0.49 ± 0.04, and 74 ± 2, respectively, which are in good agreement with the reported data [48, 49].

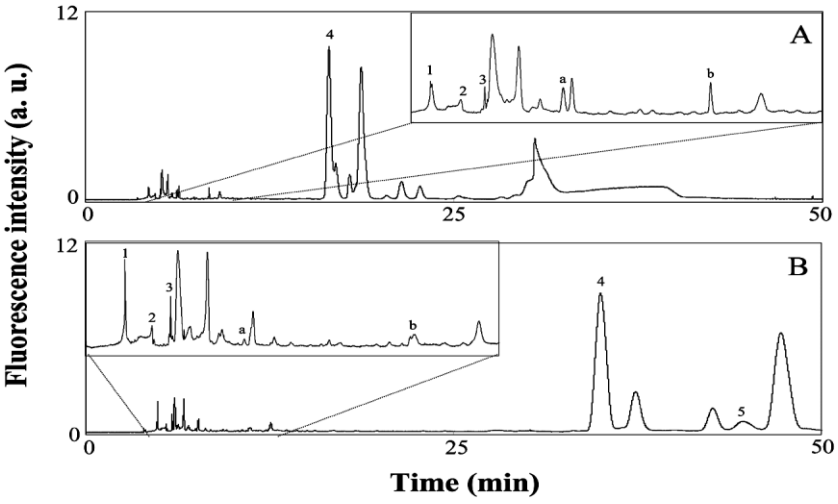

Figure 4. Electropherograms of a urine sample analyzed in 10 mM formic acid (pH 3.0) (A) with and (B) without 1.0 × SiNPs. Peak identities: 1, TA; 2, 5-HT; 3, E; 4, Trp; 5, Dopa. Unidentified peaks a, b, 4, and 5 were marked to show improved separation efficiency and speed in the presence of 1.0 × SiNPs. Other conditions are the same as in Fig. 3D.

For comparison, the urine sample used in Fig. 4A was analyzed in the absence of SiNPs. The electropherogram depicted in Fig. 4B shows that the separation is slower. For example, the migration time for Trp (peak 4) was 35.02 min, which is 16.53 min in the presence of SiNPs. At pH 3.0, only solutes possessing positive charges and intrinsic fluorescence characteristics were detected in the absence of EOF. We point out that peak 5 (Dopa) was detected, which was not resolved from a big peak (18.74 min) in Fig. 4A. Using the regression lines listed in Table 3, the concentrations of TA, 5-HT, and Trp were calculated, with the results of 0.45 ± 0.04, 0.47 ± 0.05, and 85 ± 3 μM, respectively. The differential concentration for Trp between the two approaches is slightly higher when compared to those for the other two analytes, mainly because its corresponding peak partially overlaps with another small peak in the case of using SiNPs. In comparison of the two electropherograms, we found that some of the peak heights (*e.g.*, peaks 4, a, and b) in Fig. 4A are greater, mainly due to reduced adsorption of the solutes on the capillary wall. The peaks migrating after 20 min were not detected in the absence of SiNPs, indicating those peaks corresponding to negatively charged analytes. We also emphasize that the CE approach using SiNPs is rugged for the determination of catecholamines in urine samples.

4 Concluding remarks

To the best of our knowledge, CE-LINF using SiNPs has for the first time been applied to the analysis of biologically active amines at low pH, with LODs of 0.09 and 0.15 nM for TA and 5-HT, respectively. Due to adsorption of SiNPs, a small EOF was generated at pH 3.0, which allows simultaneous analysis of the cationic and anionic analytes. The adsorption of SiNPs on the capillary wall reduces analyte adsorption, leading to highly efficient and reproducible separation results. Alteration of the mobilities of the ana-

Table 3. Quantification of three amines in a urine sample

Analyte	1.0 × SiNPs[a)]			No SiNPs[a)]			
	t_m (min)	Linear regression[b)]	Concentration (μM)	t_m (min)	Linear regression[b)]	Concentration (μM)	Normal level
TA	4.31	$y = 4114\,x + 2058$ $r^2 = 0.986$ (0.1 – 14 μM) $y = 607.0\,x + 299.3$	0.50 ± 0.02	4.87	$y = 3613\,x + 1615$ $r^2 = 0.972$ (0.1 – 14 μM) $y = 495.3\,x + 234.6$	0.45 ± 0.04	6.44 μg/day (0.1 – 0.44 μM)
5-HT	4.74	$r^2 = 0.990$ (0.1 – 14 μM) $y = 102.1\,x + 7554$	0.49 ± 0.04	5.46	$r^2 = 0.985$ (0.1 – 14 μM) $y = 83.70\,x + 7127$	0.47 ± 0.05	1464 μg/day (< 0.94 μM)
Trp	16.56	$r^2 = 0.989$ (2 – 204 μM)	74 ± 2	35.07	$r^2 = 0.985$ (2 – 204 μM)	85 ± 3	25.5 mg/day (~ 604 μM)

a) The conditions are the same as those in Fig. 4.
b) y is the peak height (mV), x is the concentration of standards added, and the linear ranges are shown in parentheses.

lytes in the presence of SiNPs also suggests their interactions with SiNPs. In the presence of SiNPs, the analysis of urine is fast, highly efficient, and reproducible, which clearly indicates the potential of using nanoparticles for the analysis of biological samples by CE. Although we only demonstrated the analysis of urine samples in this study, this method should be suitable for other biological fluids, such as cerebral spinal fluids and cells. Because SiNPs are easily modified with chemicals or biomolecules, the possibility of using different (bio)functional SiNPs opens up a new field of interaction phases (*e.g.*, ionic, hydrophobic, and recognition) for different analytes in CE. One of our particular interests is using biofunctional SiNPs for stacking and separation of proteins in CE.

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