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Research Article

A novel hybrid mode of sample injection to enhance CZE sensitivity for simultaneous determination of a pyridine-triphenylborane anti-fouling agent and its degradation products

We developed a novel hybrid sample injection mode (HSIM) that presents the combination of electrokinetic injection and vacuum injection to enhance detection sensitivity in CZE. Samples were introduced using both vacuum and electrokinetic injections simultaneously, with a water plug injected into the capillary prior to sample introduction (i.e. similarly to field-amplified sample injection, FASI). Using a sample mixture containing an anti-fouling agent applied to ship hulls, pyridine-triphenylborane and its degradation products (diphenylborinic acid, phenylboronic acid, and phenol) dissolved in ACN, the length of water plug, time, and voltage for sample introduction were optimized. The signal intensity (peak height) was found to be up to a 30-fold increased using HSIM by applying 4 kV for 4 s at the inlet end of the capillary as the cathode with supplementary vacuum in comparison with only vacuum injection for 4 s. The LODs (at a S/N of 3) for pyridine-triphenylborane, diphenylborinic acid, phenylboronic acid, and phenol were 0.88, 1.0, 21, and 23 μg/L, respectively. At the level of 0.04 mg/L, the RSDs ($n = 4$, intra-day) for the above analytes were in the ranges of 1.9–11, 4.3–9.2, and 0.34–0.66% for peak area, peak height, and migration time, respectively. The HSIM is a simple and promising procedure useful for enhancing the sensitivity for both low-and high-mobility ions in CZE.

Keywords:
Electrokinetic injection / Field-amplified sample injection / Hybrid sample injection mode / Pyridine-triphenylborane / Sensitivity enhancement
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1 Introduction

CE is now a mature technique for separation-based analysis that implies several advantages, such as high separation efficiency, minimum requirements for sample and reagents, and (much) more rapid analysis, over other separation techniques [1]. A UV–vis absorbance detector is generally used in CE. However, the concentration sensitivity of CE-UV is fairly poor because a small inner diameter of the capillary limits the optical path length for detection. Samples are conventionally injected into the capillary using hydrodynamic injection (HDI), e.g. under action of vacuum or pressure or electrokinetic injection (EKI). In addition to the conventio modes, different types of sample injection procedures h: been proposed, including in-line sample concentrat techniques, to enhance the CE sensitivity: large volu.... sample stacking [2–5], field-amplified sample injection (FASI) [6–16], ITP stacking [17, 18], ACN-mediated stacking [19–22], electrokinetic supercharging [23–28], etc. These procedures were shown to be effective but some of them were somewhat complicated in design and implementation.

As mentioned above, both the HDI and EKI are the most popular injection mode in CE and have different features [29]. A sample volume injected into the capillary can be easily estimated for the HDI mode. In addition, any analytes including neutral, positive, and negative ones in spite of their actual mobilities can be introduced without changing the sample composition. However, with HDI, severe zone broadening may occur due to a laminar flow generated during the injection [6]. On the other hand, in the EKI mode, it is more difficult to estimate the sample amount injected because the actual amount is dependent on

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Abbreviations: **DPB**, diphenylborinic acid; **EKI**, electrokinetic injection; **FASI**, field-amplified sample injection; **HDI**, hydrodynamic injection; **HSIM**, hybrid sample injection mode; **MPB**, phenylboronic acid; **PTPB**, pyridine triphenylborane

analyte mobilities and transport number of the analytes. In some analytical situations, this feature could be advantageous because analytes will be injected and then determined selectively. Importantly, the concentration stack is rather generated than the zone broadening when using EKI. However, its reproducibility is generally worse than that of HDI for the reasons discussed in [30]. To underscore, each sample injection mode has its advantages and disadvantages. Therefore, it appears that if the sample can be injected using both modes simultaneously, a combined injection mode could produce certain gains by taking advantages of individual injection modes, especially when the sample contains ions, which have higher and lower mobilities simultaneously as in the present case.

An anti-fouling agent is usually applied to ship hulls to prevent worsening of fuel consumption rates resulting from the buildup of marine organisms, such as barnacles and bivalves, which with time become attached to the surfaces of ship hulls. One anti-fouling agent, pyridine-triphenylborane (PTPB), is frequently used in some Asian countries because of its proven effectiveness [31]. To elucidate its degradation products and their toxicities to marine organisms, it is important to develop an analytical method for these compounds. We have previously developed a CZE method with direct UV detection for the simultaneous determination of PTPB and its degradation products such as diphenylborinic acid (DPB), phenylboronic acid (MPB), and phenol [32]. Additional improvement of the LODs is desirable for making the quantification of lower concentrations of these compounds feasible and thus the method more useful. In the present study, we proposed a novel hybrid sample injection mode (HSIM), which was the combination of vacuum injection and EKI to improve sensitivity. In this way, samples were introduced into the capillary using both vacuum and EKI simultaneously, with a short water plug injected into the capillary prior to sample introduction. Using a sample mixture containing PTPB, DPB, MPB, and phenol in ACN, injection conditions (time of water plug injection, time, voltage of sample introduction, etc.) were examined and optimized. The performance of the proposed HSIM was compared with the conventional sample injection modes. Tůma et al. mentioned that the sample could be electrokinetically and hydrodynamically injected into the capillary if the split-flow injector was used with the capillary inlet oriented against the BGE flow [33]. However, this geometric arrangement was not tested further. To the best of our knowledge, the present study might be the first attempt to use different injection modes simultaneously as a simple procedure to enhance the CZE sensitivity.

2 Materials and methods

2.1 Instrumentation

The CE apparatus used throughout this study was equipped with a UV–vis absorbance detector (270A-HT; Perkin-Elmer Foster City, CA, USA). Usually, in this apparatus samples can be introduced into the capillary either electrokinetically or by applying vacuum. However, it was also possible to introduce samples using both vacuum injection and EKI simultaneously. The rise time for the detector was set at 0.5 s. A polyimide-coated fused-silica capillary column was used (75 μm id × 375 μm od; GL Sciences, Tokyo, Japan). The total length of the column was 72 cm; its effective length was 50 cm. The peak area, peak height, and migration time were measured using a Chromato-Integrator (D-2500; Hitachi, Tokyo, Japan). The pH measurements were carried out using a pH meter (F-22; Horiba, Kyoto, Japan).

2.2 Reagents

All reagents were of analytical-reagent grade and used as received. PTPB, DPB, and MPB were obtained from Hokko Chemical (Tokyo, Japan). Phenol was the product of Nacalai Tesque (Kyoto, Japan). The individual stock solutions (1000 mg/L) of PTPB, DPB, MPB, and phenol were prepared in ACN purchased from Nacalai Tesque. To keep the stability of stock solutions for a longer time, 1% v/v pyridine (Nacalai Tesque) was added and the solutions were then covered with an aluminum foil and kept at 4°C to prevent their degradation. Standard solutions used for the examination of analytical conditions and building-up the calibration graphs were prepared by serial dilutions of stock solutions with ACN. The pH of the BGE (a 20-mM solution of sodium tetraborate) was adjusted to 9.8 using 1 M NaOH (Nacalai Tesque). The BGE was filtered through a 0.45-μm membrane filter (Advantec Toyo Kaisha, Tokyo, Japan) before use. Distilled, deionized water, obtained from an automatic still (WG220; Yamato Kagaku, Tokyo, Japan), and a Simpli Lab-UV high-purity water apparatus (Nihon Millipore, Tokyo, Japan) were used throughout.

2.3 Procedure

New capillaries were pretreated by flushing with 1 M NaOH for 40 min and then with water for 10 min. Before the first analysis of each day, the capillary was washed with water for 5 min, 1 M NaOH for 5 min, and water for 10 min. The detection wavelength was set at 200 nm. The capillary was thermostated at 30°C. The following optimum analytical conditions were established. Between runs, the capillary was flushed with 0.1 M NaOH for 3 min, and then filled with the BGE for 3 min. After that, water was injected by applying vacuum (16.9 kPa) for 1 s (corresponding to 21 nL) and the sample solution was injected into the capillary using both vacuum and voltage (4 kV for 4 s, at a negative polarity at the capillary inlet end) simultaneously. A positive voltage of 15 kV was applied for separation. Each step was run automatically. After each analysis, 700 μL of the sample was newly filled in the sample vial. Calibration graphs were prepared using synthetic standards.

Electrophoretic mobilities were calculated as follows [34]:

$$\mu_{ep} = \mu_a - \mu_{eof} \quad (1)$$

$$\mu_a = lL/t_a V \quad (2)$$

$$\mu_{eof} = lL/t_{eof} V \quad (3)$$

where μ_{ep} is electrophoretic mobility of an analyte, μ_a apparent mobility, μ_{eof} is the EOF mobility, l is effective length of the capillary, L total length of the capillary, t_a the migration time of the analyte, V the applied voltage, and t_{eof} is the migration time of EOF marker. The first peak in electropherograms that corresponds to acetonitrile or pyridine was used as the EOF marker to calculate the EOF mobility.

3 Results and discussion

3.1 Sample injection mode

Schematics of sample injection modes generally used in CE is illustrated in Fig. 1. Any analytes including neutral, positive, and negative ones regardless of their actual mobilities can be introduced using HDI (Fig. 1A). On the other hand, either positive or negative analytes can only be injected using EKI (Fig. 1B) if the EOF is negligible or suppressed. To improve the sensitivity and reproducibility of EKI, a short water plug is injected into the capillary prior to sample introduction (FASI, Fig. 1C). Analytes are then stacked around the interface between BGE and the water plug. We proposed here a new sample introduction procedure, HSIM, which was the combined sample introduction mode of HDI and EKI. Using HSIM (Fig. 1D), any analytes can be introduced similarly to HDI, but increased amounts of charged analytes (either cations or anions) are injected compared to those using single HDI. Furthermore, a part of charged analytes may be stacked at the beginning of HSIM because of high electric field. The analytes in the present study were anions, which moved to the detector end because of higher EOF after the sample was introduced using HSIM, as shown in Fig. 1E.

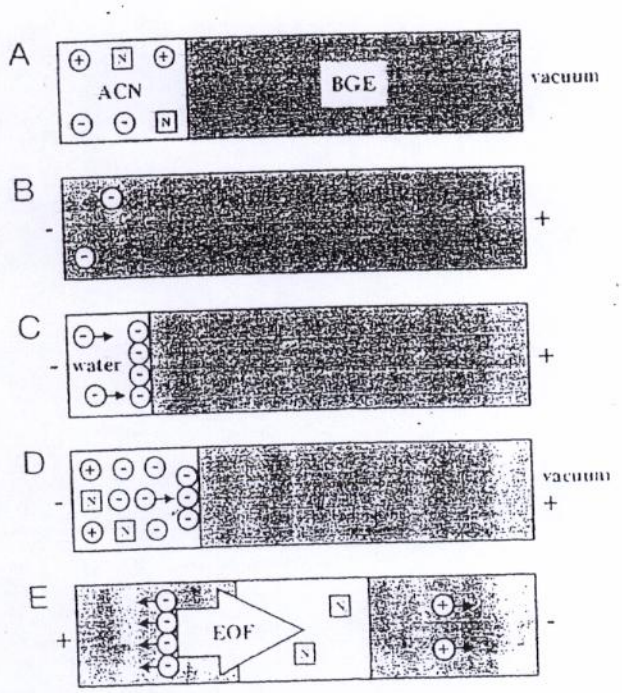

Figure 1. Schematics of sample injection modes. (A) HDI (vacuum), (B) EKI, (C) FASI, (D) HSIM, (E) migration of analytes for HSIM. ACN, sample solvent; +, cation; -, anion; N, neutral species.

3.2 Sensitivity enhancement potential of HSIM

To verify the sensitivity enhancement potential of HSIM regarding PTPB, DPB, MPB, and phenol, the following experiments were carried out. A mixture of 0.4 mg/L PTPB, DPB, MPB, and phenol were analyzed using three modes of injection and the results are compared in Fig. 2. Peak heights for PTPB, DPB, MPB, and phenol notably increased using HSIM with an enhancement factor of 12, 12, 1.5, and 1.3, respectively, compared to vacuum injection (cf. Fig. 2A and C). The sample was also injected using EKI immediately after vacuum injection (Fig. 2B) and peak heights using HSIM increased by a factor of 5.2, 3.3, 2.2, and 1.2, respectively, were observed in comparison with the results obtained by the successive injection. It was presumed that a sufficient amount of analytes could not move across the

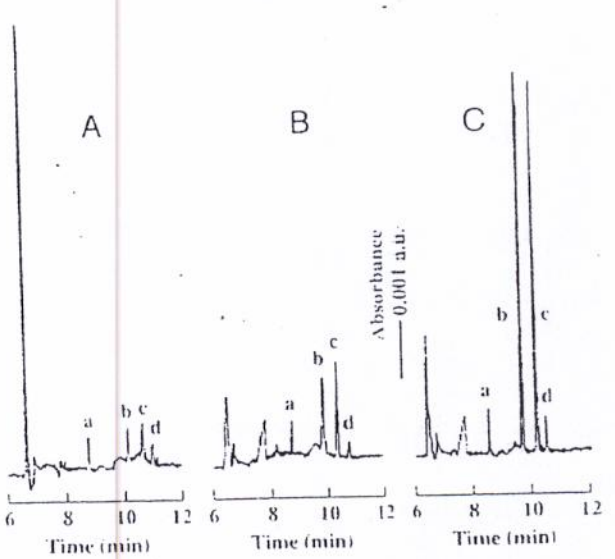

Figure 2. Electropherograms of a standard solution of phenol, PTPB, DPB, and MPB obtained with different injection modes. (A) Vacuum injection for 1 s (21 nL); (B) vacuum injection for 1 s and then electrokinetic injection at 1 kV for 1 s; (C) HSIM at 1 kV for 1 s. CZE conditions: capillary, 50/72 cm · 75 μm id; BGE, 20 mM sodium tetraborate adjusted to pH 9.8 with 1 M NaOH; voltage, 15 kV; wavelength for detection, 200 nm. Sample, 0.4 mg/L of each analyte in ACN. Peak identification: a, phenol; b, PTPB; c, DPB; d, MPB.

boundary between essentially nonaqueous sample zone and aqueous BGE zone because of the lack of current in the case of successive injection. When the sample was injected using only EKI, no peaks were observed. In general, it is difficult to introduce extremely low-mobility ions and neutral species into the capillary using the EKI mode without applying a correct polarity voltage to generate EOF. However, both higher and lower mobility ions and neutral species can be simultaneously injected using HSIM. It was deemed that HSIM would be a promising sample injection mode for samples, which contain both ionic and nonionic substances. Therefore, the following experiments were performed in order to establish optimum HSIM conditions.

3.3 Effect of water plug

It was reported that the sensitivity and reproducibility could be improved by injecting a water plug into the capillary prior to EKI (i.e. using FASI) [22]. Therefore, the injection time for the water plug was varied between 0 and 2 s using HSIM. As can be seen from Fig. 3, the peak height for PTPB decreased with increasing injection time. Also, the highest peak was obtained for DPB when the injection time was 1 s, whereas for phenol the peak height increased with the injection time up to 0.5 s, leveled off around 0.5–1.5 s, and then decreased. The peak height for MPB was almost constant up to 1.5 s and then decreased. When the injection time was 1 s, the RSDs of peak heights for PTPB, DPB, MPB, and phenol were in the range from 3.7 to 9.2%. The RSDs for 1-s injections were almost the same as those for 1.5 s (2.9–8.3 s), smaller than those for 0.5 s (6.2–37%), 2 s (5.1–10%), and without the water plug (3.4–22%). Therefore, optimum injection time of the water plug adopted in the subsequent experiments was 1 s.

3.4 Effect of sample injection time and voltage

The sample injection time was varied between 1 and 5 s using HSIM. The peak height for PTPB increased with the injection time up to 2 s, almost leveled off when going to 4 s, and then declined. The peak height for DPB was almost constant with the injection time up to 5 s. The peak height for MPB increased with the injection time up to 3 s and almost leveled off. The peak height for phenol increased up to an injection time of 5 s. The baseline separation was not observed for PTPB and DPB at 5 s. On the other hand, the RSDs of peak-height for all analytes at the sample injection time in the range of 1–4 s were 7.1–13, 5.2–12, 5.6–14, and 1.6–9.3%, respectively. Therefore, 4 s was chosen as the optimum sample injection time.

The injection voltage was varied between 1 and 5 kV using HSIM with the sample injection time for 4 s. The results are illustrated in Fig. 4. The peak height for PTPB, DPB, and MPB increased with increasing the injection voltage up to 4 kV and then decreased. The peak height for phenol was almost constant up to 4 kV and then decreased. The RSDs of peak height for the sample injection voltage ranging from 1 to 4 kV were 1.6–9.3, 2.2–11, 1.8–13, and 2.9–9.5%, respectively. Therefore, 4 kV was further used as the optimum injection voltage. Peak heights for PTPB, DPB, MPB, and phenol using HSIM at 4 kV for 4 s (Fig. 5B) were 30, 22, 1.8, and 1.0 times higher, respectively, when compared to the results obtained with only vacuum injection for 4 s (Fig. 5A). The peak heights of MPB and phenol obtained using HSIM were much lower than that of PTPB or DPB. To elucidate the reasons, the electrophoretic mobilities of PTPB, DPB, MPB, and phenol in the BGE (pH 9.8) were calculated using the procedure described in Section 2.4. The results were 15.8×10^{-5}, 17.3×10^{-5}, 18.3×10^{-5}, and 12.4×10^{-5} cm^2 V^{-1} s^{-1}, respectively; the EOF was 52.2×10^{-5} cm^2 V^{-1} s^{-1}. There was not much difference among these electrophoretic mobilities. The electrophoretic mobilities of MPB and phenol in the sample

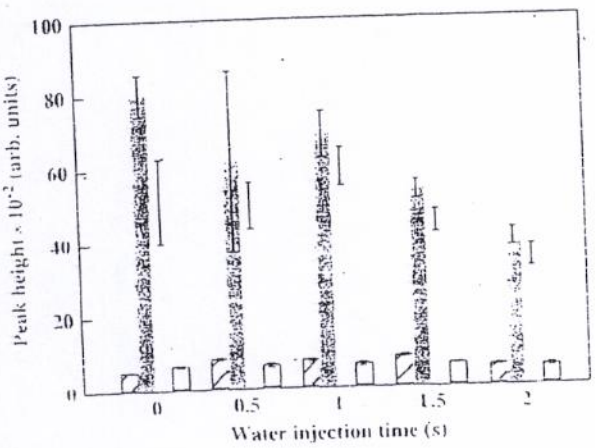

Figure 3. Effect of injection time of water plug on the peak height. Each bar corresponds to phenol, PTPB, DPB, and MPB, respectively, from left to right. Injection: HSIM at 5 kV for 1 s. Other conditions are as in Fig. 2.

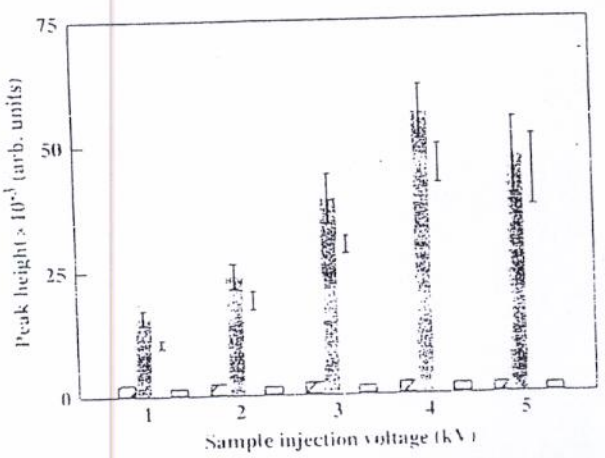

Figure 4. Effect of sample injection voltage on the peak height. Water plug was vacuum injected for 1 s (21 nL) prior to sample injection. CZE conditions and bars assignment are as in Fig. 3.

solution prepared using ACN are probably lower than those in the aqueous BGE (pH 9.8). In addition, molar absorptivities for PTPB, DPB, MPB, and phenol were estimated as 2.3×10^5, 9.2×10^4, 5.2×10^5, and 6.5×10^4 L mol^{-1} cm^{-1}, respectively (for example, concentration of PTPB was 1.56×10^{-6} M, absorbance 2.64×10^{-3}, optical path length 7.5×10^{-1} cm, giving a molar absorptivity of 2.3×10^5). The molar absorptivities for MPB, phenol, and DPB were 77, 72, and 60% lower than that for PTPB, respectively. The differences were another reason for the lower peak heights for MPB and phenol.

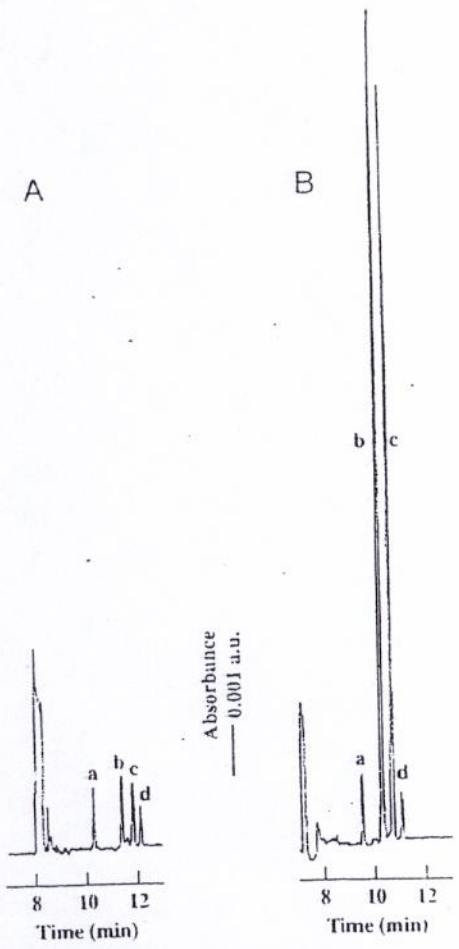

Figure 5. Electropherograms of a standard solution of phenol, PTPB, DPB, and MPB obtained with (A) vacuum injection for 4 s (84 nL) and (B) HSIM at 4 kV for 4 s. Identification of peaks are as in Fig. 2 and other conditions are as in Fig. 4.

Charge states for the four analytes of interest were presumed as follows. As mentioned in our previous paper [32], MPB changed to an anion type under a basic condition because of the coordination of the hydroxide ion to the boron. It can be presumed that the charge state for MPB is −1 in the BGE (pH 9.8) because of its pK_a value (8.8) [35, 36]. The chemical form of PTPB in water was supposed to be mainly triphenylborate after liberating pyridine. Probably, the coordination of the hydroxide ion could be happened in the cases of PTPB and DPB, similarly to MPB. It can be also presumed that the charge state for DPB is −1 in due account of its pK_a value (6.2) [36, 37]. The charge state for PTPB seems to be similar to other analytes although the pK_a of PTPB is unknown. On the other hand, the charge state for phenol could be −1 because of its pKa value (10) [38].

3.5 Calibration graphs

Calibration graphs for PTPB, DPB, MPB, and phenol were linear using both the peak area and peak height as analytical response. Regression equations relating the area or height response (y) to concentration for PTPB, DPB, MPB, and phenol (x, 0–0.1 mg/L) are accommodated in Table 1. Table 1 presents also the RSDs and LODs for the four analytes using the proposed HSIM-CZE method. The RSDs (intra-day) of peak area for PTPB, DPB, MPB, and phenol were obtained as 1.9–11%, for peak heights 4.3–9.2%, and for migration times 0.34–0.66%. The LODs were improved 35, 30, 2.4, and 1.2 times for PTPB, DPB, MPB, and phenol compared to the LODs obtained using vacuum injection.

4 Concluding remarks

We developed a novel combined sample injection mode for CZE, the potential of which was tested for the determination of PTPB and its degradation products. DPB, MPB, and

Table 1. Precision and LODs of PTPB, DPB, MPB, and phenol using HSIM

Analyte	RSD (intraday, %)[a]			LOD (μg/L, S/N = 3)		Regression equation[b] (correlation coefficient)	
	Area	Height	Time	hSIM	Vacuum[c]	Area	Height
PTPB	7.1	5.7	0.49	0.88	25	$y = 1.59 \times 10^3 x - 6.43$ (0.9967)	$y = 3.62 \times 10^2 x - 0.995$ (0.9965)
DPB	1.9	4.3	0.43	1.0	30	$y = 7.65 \times 10^2 x - 0.419$ (0.9799)	$y = 1.68 \times 10^2 x + 0.208$ (0.9872)
MPB	7.8	6.8	0.34	21	50	$y = 62.2x - 0.246$ (0.9855)	$y = 14.3x - 0.0421$ (0.9925)
Phenol	11	9.2	0.66	23	29	$y = 26.2x - 0.305$ (0.9719)	$y = 7.63x - 0.0590$ (0.9889)

a) Sample: 0.04 mg/L of PTPB, DPB, MPB, and phenol in ACN, $n = 4$. CZE conditions as in Fig. 5B.
b) In the regression equation, the x value is the concentration of analytes (0–0.1 mg/L) and the y value is the peak area/peak height.
c) At 4 s.

phenol. It was revealed that HSIM is a simple and promising approach for injection of both low- and high-mobility ions. We intend to examine further the effect of the vertical distance between the tip of electrode and the capillary end in order to improve the sensitivity and reproducibility of HSIM-CZE [24, 39].

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The authors have declared no conflict of interest.

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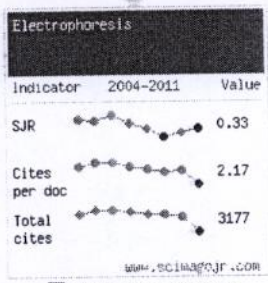

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Citation vs. Self-Citation