# Another Approach Toward over 100 000-Fold Sensitivity Increase in Capillary Electrophoresis: Electrokinetic Supercharging with Optimized Sample Injection

**ARTICLE** *in* ANALYTICAL CHEMISTRY · JANUARY 2011

Impact Factor: 5.64 · DOI: 10.1021/ac102661b · Source: PubMed



**4 AUTHORS**, INCLUDING:

Zhongqi Xu
Donghua University
**27** PUBLICATIONS **394** CITATIONS
SEE PROFILE

Takeshi Hirokawa
Hiroshima University, retired
**153** PUBLICATIONS **2,744** CITATIONS
SEE PROFILE

*Anal. Chem.* **2011,** *83,* 398–401

# Another Approach Toward over 100 000-Fold Sensitivity Increase in Capillary Electrophoresis: Electrokinetic Supercharging with Optimized Sample Injection

**Zhongqi Xu,[†,‡] Kentaro Nakamura,[†] Andrei R. Timerbaev,[§] and Takeshi Hirokawa*[,†]**

*Department of Applied Chemistry, Graduate School of Engineering, Hiroshima University, Higashi-hiroshima 739-8527, Japan, College of Chemistry, Chemical Engineering and Biotechnology, Donghua University, Shanghai 201620, China, and Vernadsky Institute of Geochemistry and Analytical Chemistry, Russian Academy of Sciences, Moscow 119991, Russia*

**Electrokinetic supercharging (EKS) is a powerful and practical method for multifold in-line concentration of various analytes prior to capillary electrophoresis (CE) analysis. However, a problem of insufficient sensitivity has always existed when trace analyte quantification by EKS-CE is a target, especially when coupled with conventional detectors. Normally this requires a greatly increased amount of analyte injected without separation degradation. In this contribution, we have shown that it is possible to substantially improve analyte loading and hence CE method detectability by modifying sample introduction configuration. The volume of sample vial was increased (from typical 500 μL to 17 mL), the common wire electrode was replaced by a ring electrode, and the sample solution was stirred. With these alterations, more analyte ions are accumulated within the effective electric field during electrokinetic injection and then maintained as focused zones due to transient isotachophoresis. The versatility of the customized EKS-CE approach for sample concentration was demonstrated for a mixture of seven rare-earth metal ions with an enrichment factor of 500 000 giving detection limits at or below 1 ng/L. These detection limits are over 100 000 times better than can be achieved by normal hydrodynamic injection, 1000 times better than the sensitivity thresholds of inductively coupled plasma atomic emission spectrometry (ICP-AES), and even close to those of inductively coupled plasma mass spectrometry (ICPMS).**

Considerable interest in enhancing concentration sensitivity of capillary electrophoresis (CE) assays has led to the development of an array of different preconcentration approaches capable of providing impressive improvements in the concentration detection limits (DL). From the viewpoint of robustness and enrichment power, the preference should be given to those systems that can operate in in-line fashion, i.e., within the same sole capillary where the following separation takes place.[1−5] Most of the in-line concentration techniques are based on the principle of stacking that is commonly referred to a (strong) decrease in analyte electrophoretic velocity achieved via changes in electric field strength or by a chemical way.[6,7] These add the advantage of simplicity as no specific material to concentrate analytes is required to be in-built into the separation capillary. Although the enrichment factors over 1000 are feasible through the stacking of fairly diluted samples following electrokinetic injection, there is a range of analytical situations where lower DLs are necessary. To address this issue, a number of tailor-made in-line methods that typically engage several preconcentration mechanisms were developed. In general, these systems rely on longer electrokinetic injections and focusing the sample zone using a complementary stacking principle or maintaining it sharp by means of counterbalancing the electrophoretic movement of a stacking boundary. Specifically, Quirino and Terabe[8] combined two in-line techniques, sample stacking with electrokinetic injection and sweeping, and this arrangement afforded improvements in sensitivity for cationic analytes by nearly 1 000 000-fold. Davis et al.[9] and Breadmore[10] presented counterflow isotachophoretic (ITP) stacking to greatly improve the detectability of CE by taking advantage of continuous electrokinetic sample injection against a virtually stationary ITP boundary generated by a counterflow. Their systems mainly differ in the origin of the counterflow: the sum of hydrodynamic and electroosmotic flow (EOF)[9] and the EOF itself,[10] respectively.

* To whom correspondence should be addressed. Phone: +81-824-247610. Fax: +81-824-247608. E-mail: hiro77@hiroshima-u.ac.jp.
[†] Hiroshima University.
[‡] Donghua University.
[§] Vernadsky Institute of Geochemistry and Analytical Chemistry.

(1) Breadmore, M. C. *Electrophoresis* **2007**, *28*, 254–281.
(2) Mala, Z.; Křivánková, L.; Gebauer, P.; Boček, P. *Electrophoresis* **2007**, *28*, 243–253.
(3) Simpson, S. L., Jr.; Quirino, J. P.; Terabe, S. *J. Chromatogr., A* **2008**, *1184*, 504–541.
(4) Puig, P.; Borrull, F.; Calull, M.; Aguilar, C. *Anal. Chim. Acta* **2008**, *616*, 1–18.
(5) Aranas, A. T.; Guidote, A. M., Jr.; Quirino, J. P. *Anal. Bioanal. Chem.* **2009**, *394*, 175–185.
(6) Mala, Z.; Slampová, A.; Gebauer, P.; Boček, P. *Electrophoresis* **2009**, *30*, 215–229.
(7) Breadmore, M. C.; Thabano, J. R. E.; Dawod, M.; Kazarian, A. A.; Quirino, J. P.; Guijt, R. M. *Electrophoresis* **2009**, *30*, 230–248.
(8) Quirino, J. P.; Terabe, S. *Anal. Chem.* **2000**, *72*, 1023–1030.
(9) Davis, N. I.; Mamunooru, M.; Vyas, C. A.; Shackman, J. G. *Anal. Chem.* **2009**, *81*, 5452–5459.
(10) Breadmore, M. C. *Electrophoresis* **2008**, *29*, 1082–1091.



10.1021/ac102661b 
Published on Web 12/13/2010

More recent work by Breadmore and Quirino[11] has further improved this approach by creating an EOF-counterbalanced stationary ITP stacking boundary under field-amplified conditions and thus attaining 100 000-fold lowered DL for inorganic and organic anions. Several other approaches to several 100 000-fold improvements in detection sensitivity (compared with typical hydrodynamic injection) can be found in recent literature, and readers are directed to excellent reviews on this topic by Breadmore et al.[7] and Aranas et al.[5]

As an alternative combined stacking technique, electrokinetic supercharging (EKS) was introduced by Hirokawa et al.[12] The concept of EKS implies a long electrokinetic injection to introduce the greatest amount of analyte followed by a transient ITP (tITP) stacking step to refocus the injected analytes into a sharp zone. Subsequent work on EKS has been recently reviewed,[13] with a major focus on theoretical and computer simulation analysis of critical factors that affect the performance of electrokinetic injection and tITP. As was also highlighted in that review, research efforts of these authors and groups of Gareil, Haddad, Peltre, and others have proven potentially universal applicability of EKS, including metal ions, pharmaceuticals, peptides, DNA fragments, and proteins, importantly both in capillary and microchip formats. Even though reductions in the DL of 3−4 orders of magnitude were shown to be feasible, further improvements in sensitivity are anticipated, provided that the extent of injection is additionally enhanced. For instance, the sample volume found in vials which are used with current CE instrumentation (typically 0.5 mL vials) may not be sufficient to hold enough analytes within the effective electric field for the period of injection. Supposedly, this inadequacy can be resolved by optimization of the sample introduction design.

We present here a simple modification to the standard EKS-CE procedure that offers possibly the lowest DL of inorganic cations ever reported by CE. This new protocol involves electrokinetic injections from a bigger, 17 mL sample vial by applying voltage via a ring platinum electrode, positioned around the capillary inlet, and continuous stirring of sample solution. The motivation behind this modification was to ultimately allow the injection of larger sample volumes for tITP stacking of cations while keeping the efficiency and resolution intact. This approach provides concentration factors approaching 500 000-fold that makes EKS-CE adequate to perform ultratrace multicomponent analysis.

## EXPERIMENTAL SECTION

**Apparatus.** All experiments were conducted on an CAPI-3200 instrument (Otsuka Electronics, Osaka, Japan) and with polyimide-coated fused-silica capillaries (Otsuka Electronics) of 75 $\mu$m i.d. with a length of 50 cm, 37.7 cm to the detector, thermostatted at 25 °C. Single-wavelength indirect UV detection was performed using a photodiode array detector at 214 nm. A constant voltage was applied as a positive potential to the inlet vial for sample injection and separation.

**Chemicals.** All chemicals were analytical grade or better and used without purification. 4-Methylbenzylamine (4-MB), 2-hydroxyisobutyric acid, malonic acid, hydroxypropyl cellulose, and 2-ethylbutyric acid (2-EB) were obtained from Sigma-Aldrich (Japan). Stock solutions of each rare-earth metal were prepared from their chlorides (Mitsuwa Kagaku, Osaka, Japan) at a concentration of 2.5 $\mu$M and diluted in Milli-Q water as required. All sample and electrolyte solutions were prepared in Milli-Q water.

**Electrophoresis.** New capillaries were conditioned with 1.0 M NaOH, Milli-Q water, and BGE for 10 min each prior to use. Between separations, the capillary was conditioned with Milli-Q water and electrolyte for 2 and 3 min, respectively.

Injections were performed by placing the sample either in the standard vial (0.5 mL) or in a large sample vial (17 mL) and applying voltage for a designated time. In the latter case, the electrode immersed into the vial was of a wire or a ring type. During sampling from the large vial using the ring electrode, stirring of the sample solution was performed by means of a magnetic stirrer.

The separation electrolyte was a solution of 10 mM 4-MB, 4 mM 2-hydroxyisobutyric acid, 0.4 mM malonic acid, and 0.1% hydroxypropyl cellulose with pH 4.8 adjusted by adding 2-EB. The co-ion of the electrolyte (the protonated form of 4-MB) possesses a higher electrophoretic mobility than that of all of analytes and hence plays the role of leading ion. During electrokinetic injection when the cationic analytes are locally depleted in the sample zone, their functions are taken over by the hydrogen ion ($H^+$), including transit of the migration current. $H^+$ is formed in the sample vacancy zone due to dissociation of 2-EB, accumulates after the analyte ions, and thus acts as the terminating ion to create the tITP state.

All DLs given hereafter were calculated according to 3 times the signal-to-noise ratio (S/N), which was defined as maximum peak height divided by the background noise.

## RESULTS AND DISCUSSION

To improve the experimental design of EKS preconcentration method, it is essential to understand the behavior of various parameters influencing the performance of electrokinetic injection. In previous work,[14] it was shown that analytes occurring in an effective electric field can only be introduced into the capillary, whereas a remaining part of the sample could be lost because of slow transport of the analyte ions. Therefore, along with injection time and applied voltage, other factors, e.g., the position of the electrode to the capillary inlet,[14,15] may affect the amount of the injected analytes. For instance, with a prolonged distance between the end of capillary inlet and electrode, it was possible to inject an increased amount of rare-earth metal ions, resulting in a 10-fold enhancement in sensitivity.[14] This lowered the DL down to 0.02 $\mu$g/L. Although this is the lowest DL reported for these analytes in CE, the potential of electrokinetic injection was not fully exploited with regard to other influential parameters such as the sample volume available to injection, electrode configuration, etc. (see Figure 1 for the stepwise sampling optimization pursued here).

(11) Breadmore, M. C.; Quirino, J. P. *Anal. Chem.* **2008**, *80*, 6373–6381.
(12) Hirokawa, T.; Okamoto, H.; Gaš, B. *Electrophoresis* **2003**, *24*, 498–504.
(13) Xu, Z. Q.; Timerbaev, A. R.; Hirokawa, T. *J. Chromatogr., A* **2009**, *1216*, 660–670.
(14) Hirokawa, T.; Koshimidzu, T.; Xu, Z. Q. *Electrophoresis* **2008**, *29*, 3786–3793.
(15) Boer, T. D.; Ensing, K. *J. Chromatogr., A* **1997**, *788*, 212–217.

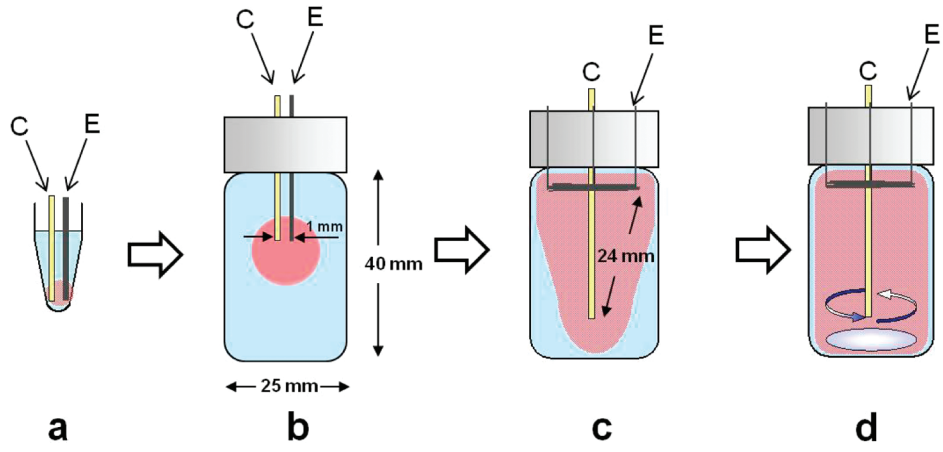


**Figure 1.** Schematic of sequential alterations of the injection setup implemented to improve the sensitivity of EKS-CE: a wire electrode in (a) a standard and (b) a large-volume sample vial; a ring electrode in a large-volume sample vial (c) without and (d) with stirring. The red zone depicts the actual part of the sample solution subjected to injection (not to scale). C and E stand for capillary and electrode, respectively.

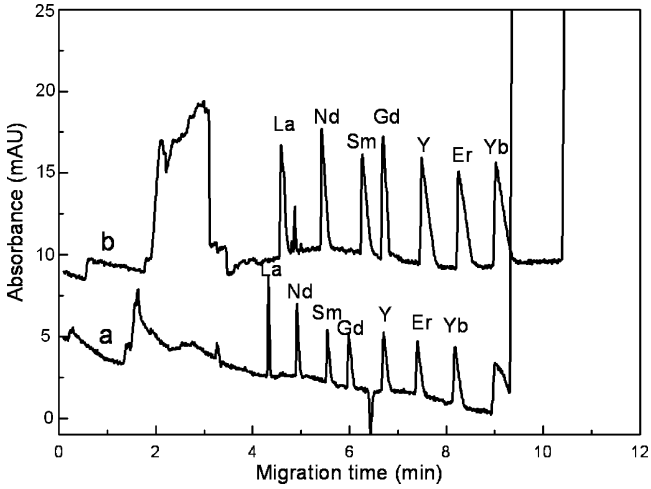


**Figure 2.** Electropherograms showing the influence of varying the sample volume. Injections from (a) 0.5 and (b) 17 mL inlet vials at 10 kV for 250 s. Sample: 100-fold diluted at 25 nM. Separation voltage: 20 kV.

The fact that the former factor produces a certain influence on sensitivity can be seen in Figure 2. A simple replacement of standard 0.5 mL injection vial by one with a volume of 17 mL led to increased loadings, and hence the DLs of test analytes improved by a factor of about 3. This is due to a greater amount of analytes that experience the effect of electric field despite the same voltage and capillary versus electrode configuration, i.e., identical effective field strength. However, the observed increase is lower than more than 30-fold difference in sample volumes, suggesting that the maximized injection has been not yet reached. We explain this by the impact of slow transport rather than insufficient strength of the electric field. (Note the depletion of the analytes in the vicinity of the capillary end, which actually allocates the sample region effective for injection.) Moreover, a system peak, resulting from the absence of UV-absorbing probe (i.e., 4-MB) in the sample solution (seen in Figure 2), overlaps most of the rare-earth peaks at longer injection times (not shown).

It appeared that the electric field strength would grow significantly when a common wire electrode is substituted by a ring electrode assembled around the separation capillary inlet (see Figure 1c). When electrokinetic injection is initiated, field enhancement will force the analytes to move rapidly in the capillary where they stack by the tITP principle. Indeed, the ring electrode afforded more sample to inject, the separation being not deteriorated and the stacked sample zone having time to be destacked and resolved (but still remains under ITP conditions with the wire electrode; Figure S-1 in the Supporting Information). The expected improvement in injected analyte resulted in peak heights 3−5 times higher than when the wire electrode is in use. Evidently, the more amplified the field, the larger is the region where analytes are subjected to injection. Nonetheless, experimental data suggest that this approach has the potential to further improve sensitivity, as even with the ring-electrode configuration only a part of sample ions migrates into the capillary during injection (and then undergoes stacking).

In attempt to rectify this shortcoming, stirring of the sample was concomitantly applied to accelerate the supplement of analytes when they were depleted in the effective field. This resulted in an impressive increase in sensitivity (see below) so that the peaks of rare-earths injected from a 25 pM (ca. 5.0 ng/L) solution are still measurable using customary indirect UV detection, as shown in Figure 3. One can also witness that in spite of a prolonged, 300 s injection, the peaks remain fairly well-focused, with plate numbers reaching up to values of $(2.8-9.7) \times 10^4$ (see Table 1) due to the high stacking power of EKS. However, it is important to note that stirring has an impact on peak area repeatability, elevating it to 10−15% RSD, as the agitating rate controlled by a magnetic stirrer could barely be kept constant. DLs for the analytes obtained under EKS-CE conditions shown in Figure 3 are exceptionally low. They range from 0.4 to 1.3 ng/L (3−7 pM) and are presumably the lowest ever attained by CE for these ions. Thus, injections made from a large sample volume at high electric field strength, offered by the ring-shape electrode, and with analyte transport enhanced by stirring, represent a practical way to sensitivity enhancement of around 500 000 (the calculation is presented in the Supporting Information). DLs of rare-earth elements attained here are over 100 000 times lower than can be achieved with a hydrodynamic injection,[16,17] at average 64 000

(16) Macka, M.; Nesterenko, P.; Andersson, P.; Haddad, P. R. *J. Chromatogr., A* **1998**, *803*, 279–290.

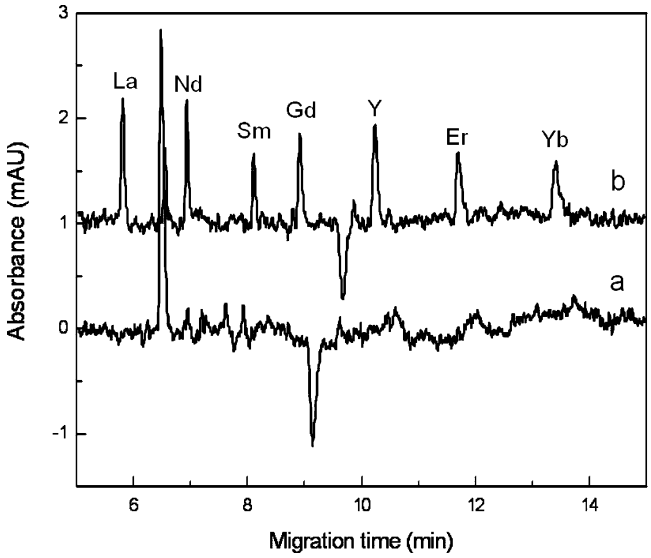


**Figure 3.** Electropherograms of (a) blank and (b) 25 pM sample analyzed at the optimized EKS conditions (as shown in Figure 1d). Sample: 100 000-fold diluted at 25 pM. Injection: 20 kV for 300 s. Separation voltage: 20 kV.

times lower compared to that reported using field-amplified sample stacking,[18] and from 20- to 300-fold over the previously published EKS systems.[12,14] Furthermore, the DLs given in Table 1 are at least 1000 times lower than those typically achieved by inductively coupled plasma atomic emission spectrometry (ICP-AES) (0.1−2 $\mu$g/L) and even comparable to those of inductively coupled plasma mass spectrometry (ICPMS) (0.1−0.9 ng/L).

(17) Verma, S. P.; García, R.; Santoyo, E.; Aparicio, A. *J. Chromatogr., A* **2000**, *884*, 317–328.
(18) Hirokawa, T.; Okamoto, H.; Ikuta, N. *Electrophoresis* **2001**, *22*, 3483–3489.

**Table 1. Detection Limits and Efficiencies for Rare-Earth Cations Concentrated Using Optimized EKS[a]**

| | detection limit | | |
|---|---|---|---|
| cation | ng/L | pM | plate number ($\times 10^4$) |
| $La^{3+}$ | 0.4 | 3.0 | 5.4 |
| $Nd^{3+}$ | 0.5 | 3.0 | 7.6 |
| $Sm^{3+}$ | 0.8 | 6.0 | 9.7 |
| $Gd^{3+}$ | 0.7 | 4.0 | 7.4 |
| $Y^{3+}$ | 0.4 | 4.0 | 6.4 |
| $Er^{3+}$ | 1.0 | 6.0 | 5.1 |
| $Yb^{3+}$ | 1.3 | 7.0 | 2.8 |

[a] Conditions: are the same as those in Figure 3.

Summarizing, a simple and inexpensive alteration of electrokinetic injection procedure, easily adopted with commercial instrumentation, allows for improved DL for a range of cationic analytes translating to several 100 000-fold improvements in detection sensitivity compared with typical injection in CE. On the top of that, the time has arrived when ultratrace analysis by EKS-CE would be practical or routine. Advantageously, the modified injection layout presented here will be useful for any other in-line preconcentration protocol based on electrokinetic sample injection given a complementary enrichment technique that has the potential to effectively stack the injected analytes.

## SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

Received for review October 8, 2010. Accepted November 28, 2010.

AC102661B