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RESEARCH ARTICLE

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Study on characteristics of bias caused by FI–CE split flow electrokinetic injection

Abstract The characteristics of bias caused by split-flow electrokinetic injection (SEKI), a new type of sample injection method used in coupled flow injection–capillary electrophoresis system (FI–CE), was investigated using pseudoephedrine hydrochloride, a basic drug, and ibuprofen, an acidic drug, as model analytes. It was found that bias imposed by SEKI under the condition of continuous sample matrix/running buffer was similar to that done by electrokinetic injection (EKI). The linearity of calibration curve provided by SEKI was similar to that offered by non-bias hydrodynamic injection (HDI) but significantly better than that obtained by EKI. These features were exploited to improve analytical performances in simultaneous determination of the minor ingredient of pseudoephedrine hydrochloride and the major ingredient of ibuprofen in a pharmaceutical preparation. Detectability of 0.7 mg/l for pseudoephedrine hydrochloride was achieved at a sample throughput rate of 24 times per hour, which is 30% lower than that obtained by HDI-based conventional CE. Relative standard deviations (RSDs) of 2.8% for the minor ingredient and 1.2% for the major ingredient were produced in 11 runs of a test solution containing 13.1 mg/l pseudoephedrine hydrochloride and 81.4 mg/l ibuprofen. This is an improvement compared to that obtained by HDI-based conventional CE. Analytical results for two batches of compound ibuprofen tablets by the SEKI-based FI–CE approach were in good agreement with that obtained by a conventional high performance liquid chromatographic method.

Keywords capillary electrophoresis, flow injection, electrokinetic injection, hydrodynamic injection, split-flow electrokinetic injection, pseudoephedrine hydrochlororide, ibuprofen

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1 Introduction

Hydrodynamic injection (HDI) and electrokinetic injection (EKI) are the two most frequently used techniques for sample introduction in capillary electrophoresis (CE). In a recent review on EKI, Krivacsy et al. [1] made a comprehensive comparison between HDI and EKI, and pointed out that EKI suffers problems of discrimination for differently charged species (so-called bias), poor precision and narrow dynamic range. Nevertheless, the simple operation and instrumentation required by EKI make it an access for interfacing CE to other hydrodynamic-flow analytical methods such as flow injection analysis.

Recently, a series of reports on the combination of flow injection (FI) to capillary electrophoresis have been published [2–5]. In these works, a vertical flow-through-cell was employed to interface the FI to CE. While a sample zone was delivered by carrier solution (also the CE running buffer) to travel through the flow-through-cell where the inlet of a capillary and a platinum electrode were inserted, a small fraction of the sample zone was electrokinetically introduced into the capillary and most portion of the sample zone was drained out of the cell. Thus the injection approach was termed as split-flow electrokinetic injection (SEKI). The coupled FI–CE system provides the advantage of allowing multiple injections to be consecutively carried out in one electrophoretic run without interruption of the applied voltage, resulting in improved precision and sample-throughput rate. Unfortunately, SEKI still suffers bias for differently charged species. To our best knowledge, however, no one has made a comparison in the features of

bias between EKI and SEKI.

The compound ibuprofen tablet is a pharmaceutical preparation for the treatment of cold. It is composed of a major active ingredient of ibuprofen (IB, 200 mg per tablet) and a minor active ingredient of pseudoephedrine hydrochloride (PE, 30 mg per tablet). HPLC is regulated by The Ministry of Health, China [6] for the determination of the two active ingredients. Recently, we developed a capillary zone electrophoresis approach for assay of the tablet [7]. Using HDI for sample introduction, the obtained peak-area ratio of IB to PE was greater than 10, despite the fact that the wavelength for on-column UV detection was selected so that the absorbance ratio of PE to IB was in favor of PE. Thus, it was hard to prepare a test solution in which both the major and minor components were within the concentration ranges for accurate and precise quantification. Most often when the concentration of major component (IB) was made within the upper-limit of its calibration curve, the corresponding concentration of minor component (PE) was close to the limit of quantification, resulting in poor precision for the minor component (PE).

In the present work, with ibuprofen (an anionic species) and pseudoephedrine hydrochloride (a cationic species) being used as model analytes, the bias caused by dynamic SEKI in FI–CE was compared to that done by static EKI in conventional CE. It was found that SEKI produced not only significant bias in favor of positively charged species but also better dynamic linearity of the calibration curve and precision as compared to EKI. These features of SEKI-based FI–CE were utilized to improve analytical performances in the simultaneous determination of minor cationic pseudoephedrine hydrochloride and major anionic ibuprofen in pharmaceutical preparations.

2 Experimental

2.1 Apparatus

A Beckman P/ACE 2100 capillary electrophoresis system was used to perform CE separation. When the CE instrument was coupled to a flow injection system, a constant voltage was supplied by a separate voltage supplier (Beijing New Technology Institute, Beijing, China) whose anode was grounded (The Beckman instrument-equipped voltage supplier grounds cathode). A 27-cm (effective length 20 cm) uncoated fused-silica capillary with 75 μm i.d., 375 μm o.d. (Yongnian Optical Fiber, Yongnian, Hebei, China) was used for separation. On-column UV detection was performed at 214 nm. Analytical data were acquired using an IBM-PC486 computer processed by Gold System software..

A model 2000 of programmable FI processor (Zhaofa Institute for Lab. Automation, Shenyang, China) equipped with two variable-speed peristaltic pumps and an eight-channel valve was used for automated sample injection. The flow-through-cell used in this work was the same as that described by Chen and Fang [4].

2.2 Reagents and solutions

Ibuprofen and pseudoephedrine hydrochloride standards were donated by the National Institute for the Control of Pharmaceutical and Biological Products, China. A mixed standard solution containing 700 mg/l ibuprofen and 100 mg/l pseudoephedrine hydrochloride were made by diluting individual stock solutions of ibuprofen (1 400 mg/l in 0.1 mol/l sodium hydroxide solution) and pseudoephedrine hydrochloride (1 000 mg/l in water) with water. Working standard series were prepared by appropriate dilution of the mixed standard solution with running buffer. A running buffer of 0.025 mol/l phosphate buffer (pH 8.1) was prepared with disodium hydrogen phosphate and sodium dihydrogen phosphate. Except for ibuprofen and pseudoephedrine hydrochloride, the chemicals used were of analytical grade or better, and deionized water was used throughout the work.

2.3 Sample preparation

Twenty compound ibuprofen tablets were ground. A portion of about 0.15 g of the powder was accurately weighed into a 100-ml volumetric flask, then 30 ml of 0.1 mol/l sodium hydroxide solution was added. After sonication for 15 min, it was diluted to mark with water. The undissolved excipients were filtrated off. A 5-ml filtrate was pipetted into a 50-ml volumetric flask, and diluted to the volume with the running buffer.

2.4 Procedure for FI–CE analysis

At the beginning of each working day, the capillary was rinsed, from the outlet to the inlet of the capillary sequentially, with 0.1 mol/l sodium hydroxide solution for 5 min, water for 2 min, and running buffer for 5 min. Then a constant voltage of 10 kV was applied to the capillary until a steady baseline was observed. Then the FI program was initiated. In step 1, with the valve in the “fulfill” position (Fig. 1a), sample solution was delivered by pump 1 to load the sampling loop, meanwhile carrier solution, which also functioned as running buffer, was driven by pump 2 at a flow of 0.5 ml/min to pass the flow-through cell. Step 1 lasted 30 seconds. At the beginning of step 2, the valve automatically switched to the “injection” position (Fig. 1b), pump 1 stopped and pump 2 maintained its rotation speed. Thus, the sample plug was injected into the carrier stream and transported at 0.5 ml/min to pass the flow-though cell, where a small fraction of the sample was electrokinetically introduced into the separation capillary. The remaining part of the sample plug was withdrawn out of the cell by pump 2 to waste. Step 2 also lasted 30 s. Then, the speed of pump 2

was reduced to maintain a carrier flow of 0.2 ml min^{-1}, until the commencement of the next cycle of operation. This standby step lasted 90s, during which electrophoresis separation was performed in the CE system. Usually, 15 injections were consecutively run by the coupled FI–CE system without interruption of the applied voltage and between-run-flushing of the capillary. After that, the capillary was flushed as described above, and prepared for next use.

3 Results and discussion

3.1 Separation conditions

It has been shown in our previous work [7] that, with a 37 cm×75 μm capillary and a separation voltage of 20 kV, ibuprofen and pseudoephedrine could be easily separated by capillary zone electrophoresis run with a phosphate buffer of pH 8.1. This applied voltage was too high for the present work, because the applied voltage for injection in FI–CE was the same as that for separation. A high injection voltage would cause deterioration in column efficiency and precision. Thus a lower voltage of 10 kV in combination with a shorter capillary 27 cm×75 μm was adopted in the present work, while the running buffer was kept unchanged. It was found that under these modified conditions, the two species could be satisfactorily separated (resolution >8) within 3 min.

The experimental parameters of an FI system, such as sampling volume and flow rate of the carrier steam, were optimized to obtain similar sensitivity as that provided by HDI-based conventional CE.

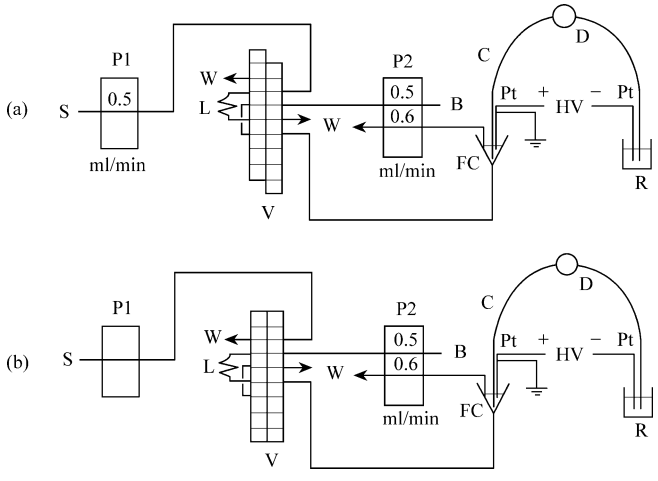

Fig. 1 Experimental set-up of coupled FI–CE system. (**a**) Loading, (**b**) Injection

C：capillary; D：UV-detector; FC：the flow-through-cell for the split-flow injection; B：running buffer solution; L：sampling roop, 40μl；P1,P2：peristaltic pumps; R：buffer reservoir； S：sample solution；V：8-channel valve; W：waste; HV: high-voltage supplier

3.2 Behavior of the split-flow electrokinetic injection

To characterize the bias caused by SEKI, signals of differently charged species obtained with SEKI were compared to those obtained with HDI (the non-bias injection mode) and EKI (the bias injection mode). The linearity of calibration curves produced by the different injection modes was also compared. To make sense of the linearity comparison, injection conditions such as time duration, applied pressure or voltage were carefully selected so that the amounts of the analytes introduced into the capillary by different injection methods were close to each other. Since conductivity of sample matrix may significantly affect the behaviors of SEKI and EKI, both the injection modes were applied not only under continuous sample matrix/running buffer (CMB) condition, i.e., there was no difference in conductivity between the background electrolyte of sample matrix and the running buffer, but also under discontinuous sample matrix/running buffer (DMB) condition which may induce an amplified field [8] for EKI or SEKI. Taking HDI as standard reference, the normalized peak-height ratio and peak-area ratio of pseudoephedrine hydrochloride to ibuprofen was used to depict the bias extent of the injections. As seen in Table 1, under the CMB condition, both EKI and SEKI showed significant discrimination against anionic ibuprofen despite the bias imposed by SEKI being a little weaker than that by EKI. Under the DMB condition, the discrimination produced by EKI became so strong that negatively charged ibuprofen was totally refused entry into the capillary, resulting in infinite ratios for both peak-height and peak-area. Discrimination produced by SEKI under DMB condition was much smaller than that caused by EKI under DMB condition, although it was almost increased by a factor of ten (for peak height) in comparison to that obtained by SEKI under the CMB condition. The significant difference in bias extent between SEKI and EKI under the DMB condition may be ascribed to partial mixing of sample zone with the carrier solution during the sample delivery in the FI system. The mixing lessened the conductivity difference between the sample zone and carrier solution (which also served as running buffer for CE) and, in turn, reduced the extent of field amplification for SEKI under the DMB condition.

Linearity of calibration curves was another investigated characteristic of the injection methods. For pseudoephedrine hydrochloride in the concentration range of 10–300 mg/l, excellent linearity was observed for HDI, SEKI and EKI under the CMB condition (Table 2). Under the DMB condition, the linearity degraded as a result of convex-shaped calibration curves being obtained for HDI, SEKI and EKI. Nevertheless, the linearity obtained by SEKI under this condition was almost the same as that by HDI but significantly better than that by EKI. For ibuprofen in the same concentration range, however, the linearity was much worse: even under the CMB condition the calibration curves became convex-shaped, with the worst being again produced by EKI. The significant difference in the dynamic

linearity between SEKI and EKI was most possibly related to the fact that SEKI took place in a flowing sample zone, while EKI was carried out in a static sample solution. In the latter case, partial ion exhaustion might occur in the region close to capillary inlet during injection.

Table 1 Peak-height ratio and peak-area ratio of psuedoephedrine hydrochloride to ibuprofen observed with various sampling modes[a]

Injection Modes[b]	Peak height ratios		Peak area ratios	
	Injection in continuous sample matrix[c]	Injection in discontinuous sample matrix[d]	Injection in continuous sample matrix[c]	Injection in discontinuous sample matrix[d]
Hydrodynamic injection	1.00	1.00	1.00	1.00
Electrokinetic injection	1.79	-[e]	2.73	-
Split-flow electrokinetic injection	1.48	14.07	2.57	16.00

[a]The concentrations of both pseudoephedrine hydrochloride and ibuprofen are 120 mg/l.

[b]Conditions for hydrodynamic injection: 3.45 kPa×4 s; Conditions for electrokinetic injection: 10 kV×3 s; Conditions for split-flow electrokinetic injection: 40 μL test solution was driven to pass the flow-through cell at the flow rate 0.5 ml/min and under a 10 kV voltage.

[c]Sample solution prepared with running buffer.

[d]Sample solution prepared with 10-fold diluted running buffer.

[e]Not detected because ibuprofen failed to be introduced into the capillary by electrokinetic injection under the condition of discontinous sample matrix/running buffer.

Table 2 Correlation coefficients of linear regression of external standard curves obtained with different injection methodes [a]

Injection modes[b]	Pseudoephedrine hydrochloride		Ibuprofen	
	Injection in continuous sample matrix[c]	Injection in discontinuous sample matrix[d]	Injection in continuous sample matrix[c]	Injection in discontinuous sample matrix[d]
Hydrodynamic injection	0.9996	0.9980	0.9975	0.9947
Electrokinetic injection	0.9999	0.9893	0.9936	-[e]
Split-flow electrokinetic injection	0.9997	0.9972	0.9973	0.9775

[a]The concentrations of both pseudoephedrine hydrochloride and ibuprofen in standard series are 10, 60, 120, 200, 240, 300 mg • L^{-1}.

[b]Injection conditions are the same as described in Table 1.

[c]Sample solution prepared with running buffer.

[d]Sample solution prepared with 10-fold diluted running buffer.

[e]Not detected because ibuprofen failed to be introduced into the capillary by electrokinetic injection under the condition of discontinuous sample matrix/running buffer.

3.3 Application

In literatures, the bias was discussed as one major drawback of EKI, and various methods [9, 10] have been reported to correct it. Krivacsy et al. pointed out in their review [1] that the mobility bias should be considered as a special feature of EKI injection and should not or cannot be corrected. Nevertheless, analysts usually prefer HDI to EKI for conventional CE quantitative analysis whenever HDI is feasible. The reason might be due to the relatively poorer precision and narrower dynamic linearity produced by EKI. As can be seen from the above discussions, SEKI under CMB condition can not only produce bias in favor of the positively charged species but also offer linear calibration curves in a concentration range close to that offered by HDI. One can also find that the precision of SEKI-based FI–CE is no worse than that observed by HDI. Thus, use of the SEKI-based FI–CE system for simultaneous determination of the minor component of positively-charged pseudoephedrine hydrochloride and the major component of negatively-charged ibuprofen in pharmaceutical preparations would produce more accurate results in addition to the merits of high sample throughput rate and good precision.

3.3.1 Analytical performances

Table 3 lists the RSDs, produced by the FI–CE with SEKI, for 11 determinations of a test solution made with tablets. Reproducibility of the present system is significantly better than that reported in the previous work using HDI-based conventional CE [7]. Regression equation of the external calibration for pseudoephedrine hydrochloride in the range of 5～40 mg/L^{-1} was Y=5.0×10^{-3} C-3.7×10^{-3} (Y: peak area, C: analyte concentration in mg/L^{-1}) with a correlation coefficient of 0.9996. The equation for ibuprofen in the range of 30～240 mg/L^{-1} was Y=8.6×10^{-3} C+1.2×10^{-2} with a correlation coefficient of 0.9993. The recoveries of the 10.0 mg/L^{-1} pseudoephedrine hydrochloride and 60.0 mg/L^{-1} ibuprofen spiked into a sample solution containing approximately the same levels of the analytes were 103±0.8% (mean±S.D., n=4) for pseudoephedrine hydrochloride and 100±1.9% for ibuprofen. The detection limits defined as 3 σ were 0.7 mg/L^{-1} for pseodoephedrine

hydrochloride and 0.8 mg/L^{-1} for ibuprofen. Compared to the detection limits of 1.0 mg/L^{-1} for pseodoephedrine hydrochloride and 0.8 mg/l for ibuprofen obtained by HDI-based conventional CE [6], the detectability of PE was improved. Figure 2a shows the electropherogram obtained by SEKI-based FI–CE for a test solution made with the tablets, while Fig. 2b shows electropherogram obtained by HDI-based conventional CE for the same test solution. The benefits obtained by SEKI is clearly seen in the balance of the responses of major and minor components. The analysis speed offered by the FI–CE was 24 times per hour.

Table 3 Precision of eleven runs for a sample solution containing 13.1 mg/L^{-1} pseudoephedrine hydrochloride and 81.4 mg/l ibuprofen

Components	RSD for peak area (%)		RSD for peak height (%)	
	Founded by this method	Reported by reference[7][a]	Founded by this method	Reported by reference[7][a]
Pseudoephedrine hydrochloride	2.8	5.9	1.7	2.9
Ibuprofen	1.2	3.8	0.8	1.9

a: obtained with a sample solution containing 9.5 mg/l pseudoephedrine hydrochloride and 66.7 mg/l ibuprofen.

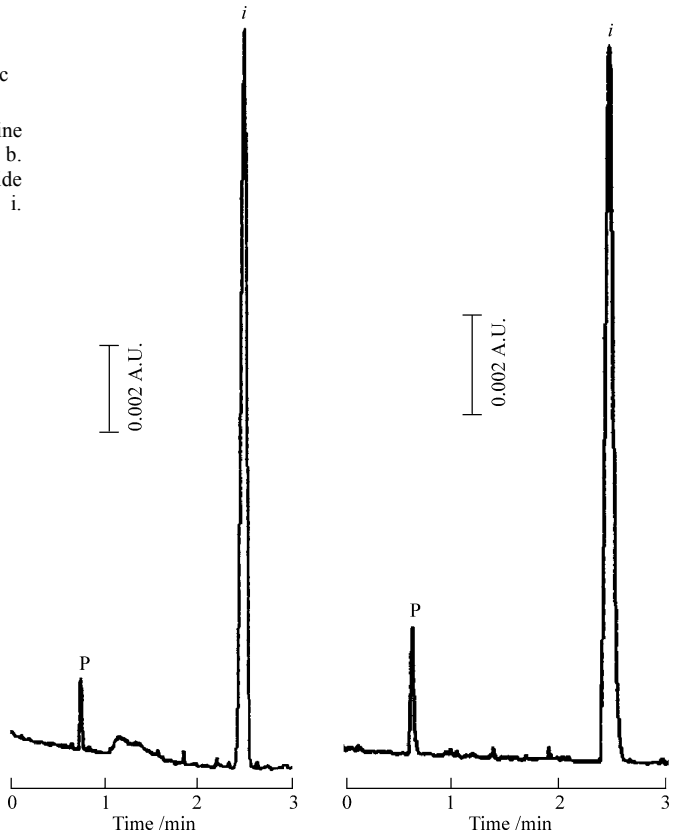

Fig. 2 Electophoregrams of the sample solution with hydrodynamic injection (**a**) and with split-flow electrokinetic injection (**b**)

The sample solution containing 13.1 mg/L^{-1} pseudoephedrine hydrochloride and 81.4 mg/l ibuprofen. a. H_p/H_i=0.092; b. H_p/H_i=0.16. H_p means peak height of pseudoephedrine hydrochloride and H_i that of ibuprofen. p. pseudoephedrine hydrochloride; i. Ibuprofen.

3.3.2 Analytical results of the pharmacuetical formulation

Two batches of the compound ibuprofen tablet were analyzed by the SEKI-based FI–CE. The results are listed in Table 4. The results obtained by the developed FI–CE approach were in good agreement with that obtained by the regulated HPLC method.

Table 4 Analytical results of compound ibuprofen tablet (mg per tablet)

Samples	Labeled		Founded by this method [a] (mean±SD)		Founded by HPLC [a, b] (mean±SD)	
	Pseudoephedrine hydrochloride	Ibuprofen	Pseudoephedrine hydrochloride	Ibuprofen	Pseudoephedrine hydrochloride	Ibuprofen
Tablet 1	30	200	32.1±0.7	201±0.9	32.0±0.5	201±1.4
Tablet 2	30	200	29.4±0.4	208±2.1	30.7±0.3	207±1.1

[a] : n=3

[b] : See Ref. [7].

4 Conclusions

Under the CMB condition the bias property of SEKI used in coupled FI–CE system is similar to that of EKI used in conventional CE. Dynamic linearity of calibration curve obtained by SEKI-based FI–CE is similar to that obtained by HDI-based conventional CE but significantly better than that obtained by EKI-based conventional CE. The bias property enhances the detectability of cations while the better dynamic linearity guarantees the accuracy of quantification by external standard calibration method. These characteristics can be exploited for simultaneously accurate determination of minor cationic components and major anionic components in pharmaceutical formulations.

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References

1. Krivacsy Z., Gelencser A., Hlavay J., Kiss G. and Sarvari Z., Electrokinetic injection in capillary electrophoresis and its application to the analysis of inorganic compounds, J. Chromatogr. A, 1999, 834(1/2): 21–44
2. Fang Z. L., Liu Z S. and Shen Q., Combination of flow injection with capillary electrophoresis. Part I. The basic system, Anal. Chim. Acta, 1997, 346(2): 135–143
3. Liu Z S. and Fang Z. L., Combination of flow injection with capillary electrophoresis. Part 2. Chiral separation of intermediate enantiomers in chloramphenicol synthesis, Anal. Chim. Acta, 1997, 353(2/3): 199–205
4. Chen H. W and Fang Z L., Combination of flow injection with capillary electrophoresis.: Part 5. Automated preconcentration and determination of pseudoephedrine in human plasma, Anal. Chim. Acta, 1999, 394(1): 13–22
5. Luo J W., Chen H W. and He Q. H., Determination of cimetidine in human plasma by use of couple-flow injection, solid-phase extraction, and capillary zone electrophoresis, Chromatographia, 2001, 53(5/6): 295–300
6. The Pharmacopoeia Commission of the Ministry of Health People's Republic of China, Drug Standard of Ministry of Health (NO: WS-308(X-263)-95), 1995, 1–3
7. Chen H. W., Huang D. H., Chen Q. J. and Li H. L., Rapid determination of active constituents in compound ibuprofen tablets by capillary zone electrophoresis, Chinese J. Chromatogr., 1998, 16(4): 289–292
8. Chien R. L. and Burgi D. S., On-column sample concentration using field amplification in CZE, Anal. Chem., 1992, 64(8): A489–A496
9. Van der Moolen J. N., Boelens H. F. M., Poppe H. and Smit H. C., Origin and correction of bias caused by sample injection and detection in capillary zone electrophoresis, J. Chromatogr. A, 1996, 744(1/2): 103–113
10. Qi S Z., Huang A J. and Sun Y. L., Compensation of two kinds of bias in capillary zone electrophoresis and its use in internal normalization quantitation, Anal. Chem., 1996, 68(8): 1342–1346