LVSEP Analysis of Cationic Analytes in Non-Aqueous Capillary Electrophoresis

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Abstract
To achieve highly sensitive analyses of cationic analytes with simple experimental procedures in non-aqueous capillary electrophoresis (NACE), large-volume sample stacking with an electroosmotic flow pump (LVSEP) was performed in dynamically polymer coated capillaries. As the dynamic coating polymers, cationic polybrene (PB), and neutral polymer, (hydroxypropyl)methyl cellulose (HPMC), were employed in methanolic media to reverse and suppress the electroosmotic flow. In the analysis of cationic amines, good enrichments were attained with the sensitive enhancement factor (SEF) of 70 and 687 for benzylamine and 1-(1-naphthyl)ethylamine, respectively, with a methanolic running solution containing 1.0% HPMC and 0.5% PB. In the NACE-LVSEP analysis of Ru(bpy)₃²⁺ and Ru(phen)₃²⁺, furthermore, the values of SEF were almost 40 with almost no-loss of the resolution.

Keywords: Non-aqueous capillary electrophoresis; On-line sample preconcentration; LVSEP; Dynamic coating; Cations

1. Introduction
Non-aqueous capillary electrophoresis (NACE) is one of the most important modes in capillary electrophoresis (CE) since specific interaction forces such as hydrogen bonding and hydrophobic interaction enhance the separation ability [1-4]. To improve the sensitivity, several on-line sample preconcentration techniques have been applied to NACE. Among various on-line sample preconcentration techniques in CE [5-12], large-volume sample stacking with an electroosmotic flow pump (LVSEP) has unique characteristics including efficient enrichments by a whole capillary sample-injection, simple experimental procedures based on a voltage application without polarity switching, and high resolutions according to the movement of the stacked analytes zone from the capillary inlet to the outlet [12-20]. In this study, LVSEP was combined with NACE to achieve highly sensitive analyses of cationic compounds with unique selectivities in non-aqueous media. Although there are several reports on LVSEP-NACE of anionic analytes [21-27], the combined technique has not been applied to cationic compounds.

In our previous study [18-20], a fused silica capillary was often permanently coated with poly(vinyl alcohol) (PVA) by a thermal passivation method to obtain a specific electroosmotic flow (EOF). In the PVA coating, however, there are several problems, e.g., need of labor-intensive preparations, capillary clogging, and low success yield of the coating. To simplify the experimental procedures, a dynamic coating technique was applied to LVSEP [13]. In the dynamic coating, only the use of a running solution containing polymers alters the zeta potential of the inner surface of the capillary in the conditioning step and during the CE measurements.

In the LVSEP technique in the thermally PVA-immobilized capillary, on the other hand, only anionic analytes are enriched and separated. To extend the applicability of large-volume sample stacking (LVSS) containing LVSEP to cationic analytes, the capillaries were coated with cationic surfactants and/or polymers [17,28]. Fig. 1 shows the principle of LVSEP for cationic analytes. At first, the capillary is coated with cationic polymers to give weakly and positively-charged surface (Fig. 1a). A low-ionic
strength ($I_1$) sample solution containing cationic analytes is fully injected into the capillary as shown in Fig. 1b. When the normal polarity voltage is applied, the analytes are concentrated by the difference in the $I_2$ between the sample matrix and the background solution (BGS). Since the low-$I_1$ sample solution enhances the EOF velocity ($v_{eo}$) in the capillary, the stacked analytes move to the anode. As the BGS is introduced from the outlet vial into the capillary as depicted in Fig. 1c, $v_{eo}$ is gradually decreased and finally suppressed due to the high-$I_2$ BGS (Fig. 1d). Because the electrophoretic velocity ($v_{ep}$) becomes faster than $v_{eo}$, the stacked analytes zone migrates to the cathodic side (Fig. 1e). Hence, the LVSEP technique requires a faster and slower EOF at the stacking and the separation step, respectively.

In our research group, cationic surfactant, dimethyl-dioctadecylammonium bromide (DODAB), and neutral polymer, polyoxyethylene 40 stearate (POES), were physically immobilized onto the capillary surface to obtain weakly and positively-charged surface for the LVSEP cation analyses in the aqueous system [17]. When the DODAB+POES coating was applied to methanolic media in NACE, unfortunately, the EOF was not reversed. In this study, therefore, a mixture of cationic and neutral polymers was used as the dynamic-coating reagent for LVSEP in NACE. To reverse and suppress the EOF, cationic polymer, polybrene (PB), and neutral polymer, (hydroxypropyl) methyl cellulose (HPMC), were employed, respectively. To evaluate the fundamental performances of NACE-LVSEP, cationic amines and metal complexes were analyzed as standard analytes.

2. Experimental

2.1. Chemicals

A fused silica capillary of 75 μm i.d. was purchased from Polymicro Technologies (Phoenix, AZ, USA), HPMC (Mw 10,000), hexadimethrine bromide (polybrene, PB), tris(2,2'-bipyridine) ruthenium (II) hexafluorophosphate (Ru(bpy)$_3^{2+}$) and tris(1,10-phenanthroline) ruthenium (II) dichloride (Ru(phen)$_3^{2+}$) from Sigma-Aldrich (Tokyo, Japan), and benzylamine (BA), 1-(1-naphthyl)ethylamine (NEA), methanol, thiourea, LiCl and other reagents from Wako (Osaka, Japan). All solutions were prepared with methanol, and filtered through a 0.45 μm pore membrane filter prior to use. For the LVSEP analyses, cationic analytes were dissolved with pure methanol, while in conventional NACE analyses with the methanolic BGS.

2.2. CE measurements

All the CE analyses were performed on a CAPI-3300 system (Otsuka Electronics, Hirakata, Japan) equipped with a diode-array UV detector. Detection wavelength was 210–285 nm. For the CE separation, bare fused-silica capillaries with total/effective lengths of 50/39 cm were employed. They were conditioned with neutral polymer-containing BGSs to suppress and reverse the EOF at 10 kPa for 300 s prior to each run. In the conventional NACE analyses, sample injections were performed electrokinetically at +10 kV for 1.0 s. In the LVSEP analyses, samples were injected at 10 kPa for 300 s (whole capillary injection). In both the normal NACE and NACE-LVSEP analysis, the applied voltage was set at +20 kV. The value of sensitive enhancement factor (SEF) was calculated by comparing the peak height obtained under the LVSEP condition with that in the conventional NACE experiment taking into account the dilution factor. In the determination of the electroosmotic mobility ($\mu_{eo}$) in the dynamically polymer-coated capillary, the outlet vial was filled with the BGS, while the inlet vial was filled with 100 ppm thiourea (EOF marker) dissolved in the BGS. The applied voltage and the UV wavelength were set at +20 kV and 200 nm, respectively. The EOF mobility was calculated from the time to move the EOF marker from the inlet to the detector.

3. Results and discussion

To analyze cationic analytes by NACE-LVSEP, a methanolic mixture of PB and HPMC were used as the dynamic coating reagent. Prior to the LVSEP analysis, BA and NEA were analyzed by the normal NACE with a BGS containing LiCl, HPMC and PB in methanol. As shown in Fig. 2a, two peaks of BA and NEA were detected at longer migration times compared to the LVSEP analysis. The electrophoretic data for cationic amines are summarized in Table 1.

<table>
<thead>
<tr>
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<th>migration time / min</th>
<th>SEF</th>
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<tbody>
<tr>
<td></td>
<td>BA</td>
<td>NEA</td>
</tr>
<tr>
<td>NACE</td>
<td>13.8 (0.6)</td>
<td>28.0 (0.9)</td>
</tr>
<tr>
<td></td>
<td>69.8 (12.4)</td>
<td>687 (11.9)</td>
</tr>
<tr>
<td>LVSEP</td>
<td>15.4 (0.3)</td>
<td>29.1 (1.9)</td>
</tr>
</tbody>
</table>

Values in parentheses are %RSDs ($n = 3$).
Migration times at the concentration of 1.0% HPMC and 0.5% PB, indicating that the EOF was suppressed by the dynamic coating reagent. In the LVSEP analysis, higher and sharper peaks of these amines appeared in spite of using 33~100-fold dilution sample as shown in Fig. 2b. As summarized in Table 1, the values of SEF were 70 and 687 for BA and NEA, respectively. Due to the preconcentration effect by LVSEP, the peak of slowly migrated NEA was especially sharpened relative to the conventional NACE, giving higher SEF. These results demonstrated that PB and HPMC were useful dynamic coating reagents in NACE-LVSEP.

In the PB and HPMC dynamically coated capillary, the EOF mobilities were measured. As summarized in Table 2, the EOF was reversed in the dynamic coating capillary, and the electroosmotic mobility in the BGS-filled capillary ($\mu_{\text{eo,BGS}}$), which should agree with that in the separation step (Fig. 1e), was estimated to be $-2.2 \times 10^{-4}$ cm$^2$/V·s by physically immobilized PB. On the other hand, the EOF mobility in the pure methanol-filled capillary ($\mu_{\text{eo,S}}$), which was almost identical with that in the LVSEP step (Fig. 1c), was significantly enhanced to $-7.1 \times 10^{-4}$ cm$^2$/V·s by the low ionic strength of the medium. Faster $\mu_{\text{eo,S}}$ allowed efficient removal of the vacant sample matrix in the LVSEP step without band broadening of the enriched analytes zone. Since all the electrophoretic mobilities ($\mu_{\text{ep}}$) of the four standard analytes used in this study exceeded $\mu_{\text{eo,BGS}}$, furthermore, they could migrate toward the outlet of the capillary in the separation step. It was confirmed that, therefore, the prepared coating capillary fulfilled the required EOF condition for the LVSEP analysis of cations.

To evaluate the separation performance, Ru(bpy)$_3^{2+}$ and Ru(phen)$_3^{2+}$, whose migration times were closer each other than those of the two amines, were employed in NACE-LVSEP. In the LVSEP analysis, the peak heights of the separated cationic metal complexes were comparable with those in the normal NACE in spite of using 50-fold dilution sample as shown in Fig. 3, and as a result that SEFs for Ru(bpy)$_3^{2+}$ and Ru(phen)$_3^{2+}$ were almost 40. Electrophoretic resolution ($R_S$) in LVSEP was estimated to be 1.13, which was almost comparable with that obtained in the conventional NACE as summarized in Table 3. Since the whole capillary length could be utilized for the separation in almost all the capillary length as shown in Fig. 1d, the loss of $R_S$ was not observed in LVSEP.

To determine the linearity for the quantitation in NACE-LVSEP, a calibration curve with five standards of concentrations, i.e., 1.0, 5.0, 10, 20, and 50 ppm of the two metal complexes, were prepared. Peak areas obtained with NACE-LVSEP were plotted against the concentration. The regression slope, intercept and correlation coefficient were calculated to be 0.0677 mAU/ppm, −0.106 mAU, and 0.991, respectively, for Ru(bpy)$_3^{2+}$, while they were 0.0825 mAU/ppm, 0.980 mAU, and 0.996, respectively, for Ru(phen)$_3^{2+}$. Although good linearity was obtained, RSD values for the SEF were apparently poor, ranging from 61 to 63%, as shown in Table 3. For the quantitative analyses, the introduction of internal standards is essential to improve the

### Table 2. Mobility data in the present system.

<table>
<thead>
<tr>
<th></th>
<th>$\mu_{\text{eo,BGS}}$</th>
<th>$\mu_{\text{eo,S}}$</th>
<th>$\mu_{\text{ep,BA}}$</th>
<th>$\mu_{\text{ep,NEA}}$</th>
<th>$\mu_{\text{ep,Ru(bpy)}}$</th>
<th>$\mu_{\text{ep,Ru(phen)}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$-2.2 \times 10^{-4}$ cm$^2$/V·s</td>
<td>$-7.1 \times 10^{-4}$ cm$^2$/V·s</td>
<td>$+3.4 \times 10^{-4}$ cm$^2$/V·s</td>
<td>$+2.8 \times 10^{-4}$ cm$^2$/V·s</td>
<td>$+3.3 \times 10^{-4}$ cm$^2$/V·s</td>
<td>$+3.2 \times 10^{-4}$ cm$^2$/V·s</td>
</tr>
</tbody>
</table>

Conditions are as in Figs. 2 and 3. $\mu_{\text{ep}}$ values were determined in the BGS.

### Table 3. Electrophoretic data for ruthenium complexes.

<table>
<thead>
<tr>
<th></th>
<th>Migration time / min</th>
<th>$R_S$</th>
<th>SEF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ru(bpy)$_3^{2+}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NACE</td>
<td>15.3</td>
<td>1.25</td>
<td>39.0</td>
</tr>
<tr>
<td>LVSEP</td>
<td>16.5</td>
<td>1.13</td>
<td>40.1</td>
</tr>
<tr>
<td></td>
<td>Ru(phen)$_3^{2+}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(9.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(11)</td>
<td>(16)</td>
<td>(63)</td>
</tr>
<tr>
<td></td>
<td>(6.2)</td>
<td>(17)</td>
<td>(61)</td>
</tr>
</tbody>
</table>

Values in parentheses are %RSDs ($n = 3$).

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**Fig. 2.** (a) Normal NACE and (b) LVSEP analyses of cationic amines. Sample concentrations, (a) 30 ppm BA and 10 ppm NEA, (b) 0.3 ppm BA and NEA; sample injection, (a) +10 kV, 1.0 s, (b) 10 kPa for 300 s (whole capillary injection); BGS, 0.1% LiCl, 1.0% HPMC and 0.5% PB in methanol; UV wavelength, 210 nm.

**Fig. 3.** (a) Normal NACE and (b) LVSEP analyses of cationic metal complexes, Ru(bpy)$_3^{2+}$ and Ru(phen)$_3^{2+}$. Sample concentrations, (a) 50 ppm, (b) 1.0 ppm; UV wavelength, 285 nm. Other conditions are as in Fig. 2.

**Table 3.** Electrophoretic data for ruthenium complexes.
RSDs.

4. Conclusions

In this study, the LVSEP technique was combined with the dynamic coating technique in NACE. As the dynamic coating reagents, a methanolic mixture of HPMC and PB was useful to fulfill the required EOF condition for the NACE-LVSEP analysis of cationic analytes. In the NACE-LVSEP analyses of amines and metal complexes, the values of SEF were ranging from 39 to 687 with almost no-loss of the resolution. Since the developed method eliminated the complicated and labor-intensive procedures for the permanent polymer coating of the capillary, effective preconcentrations by LVSEP can be carried out with simple experimental procedures in NACE, which is also expected to enhance the selectivity by utilizing specific interactions in organic solvent media.

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References