이학석사 학위논문

Headspace-no drop microextraction for capillary electrophoresis

모세관 전기영동을 위한 상공-비 방울 미세추출법

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서울대학교 대학원
화학부 분석화학전공

이 혜 려

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이 논문을 이학석사 학위논문으로 제출함.

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Abstract

Headspace-no drop microextraction for capillary electrophoresis

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In liquid phase microextraction, high enrichment factors can be obtained using an acceptor phase of small volume. By hanging an acceptor drop at the separation capillary tip, single drop microextraction (SDME) can be in-line coupled with capillary electrophoresis (CE). The small surface-to-volume ratio of the drop enables high enrichment factors to be obtained in a short time. One practical issue in SDME is how to keep the drop attached to the capillary stably. Here, we present novel but extremely simple no drop microextraction (NDME) using the liquid inside the capillary as an acceptor phase, without forming a drop at the capillary tip. As a first example, NDME has been combined with headspace (HS) extraction. Simply by placing a capillary filled with a basic run buffer in the HS above an acidic donor solution, volatile acidic analytes were extracted into the acceptor phase in the capillary. After extraction, electrophoresis of the extracts in the capillary was carried out. Owing to the

robust nature of the acceptor phase, the extraction temperature and time ranges of HS-NDME can be extended significantly, compared to HS-SDME. The enrichment factors for chlorophenols in a standard solution were up to 1100 under an optimal HS-NDME condition of 80℃ for 15 min. For a complex sample such as red wine, an acceptor phase with an increased buffer capacity was used obtaining detection limits of about 20 nM. The whole procedures of HS-NDME-CE were carried out automatically using built-in programs of a commercial CE instrument.

Keywords:

Capillary electrophoresis (CE); Headspace-no drop microextraction (HS-NDME); Sample preparation

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CONTENTS

ABSTRACT i

CONTENTS iii

LIST OF FIGURES AND TABLES iv

1 INTRODUCTION 1

2 EXPERIMENTAL SECTION 4

2.1 Reagents 4

2.2 CE 4

2.3 Headspace-no drop microextraction process 5

3 RESULTS AND DISCUSSION 8

3.1 Optimization of HS-NDME 8

3.1.1 Pre-injection of the HS phase 8

3.1.2 The donor and acceptor phases 8

3.1.3 Extraction temperature and time 12

3.2 Analytical performance 15

3.3 HS-NDME vs. HS-SDME 17

3.4 Chlorophenols in red wine 19

4 CONCLUSIONS 23

REFERENCES 24

ABSTRACT IN KOREAN 27

ACKNOWLEDGEMENTS IN KOREAN 28

LIST OF FIGURES AND TABLES

Figure 1. HS-NDME procedures; (1) HS extraction, (2) Pre-injection, (3) Waiting, and (4) Electrophoresis.

Figure 2. Optimization of HS-NDME conditions; (a) Pre-injection volume, (b) Waiting time, (c) Donor pH, and (d) Donor volume.

Figure 3. EFs of HS-SDME vs. the extraction time and temperature.

Figure 4. Chlorophenols in standard solutions.

Figure 5. Chlorophenols in red wine.

Table 1. HS-NDME performance.

Table 2. HS-NDME of chlorophenols in red wine.

1 INTRODUCTION

High performance separation and many other advantages such as a small volume sample requirement of capillary electrophoresis (CE) come from the fact of using a narrow-bore fused silica capillary as a separation column. However, the small dimensions of the capillary also cause poor detection sensitivity, especially for commonly used absorbance detection. One effective means to overcoming this drawback is through a sample preconcentration approach, which can be divided into schemes before, during, and after sample injection. The schemes before injection are usually based on off-line or in-line solid phase extraction (SPE) and liquid phase extraction (LPE).[1-4] One example of the schemes during injection is field amplified sample injection (FASI).[5] The schemes after injection, usually called on-line sample preconcentration techniques, are the most widely used; examples include field amplified sample stacking (FASS), transient isotachophoresis (tITP), sweeping, and dynamic pH junction.[6-12] In order to obtain even higher detection sensitivity, different techniques can be combined[13] as in electrokinetic supercharging combining FASI and tITP.[14-16]

Single drop microextraction (SDME) is a method of performing LPE before injection by hanging a single drop of an acceptor phase on the capillary tip.[17-22] The drop volume is in the range of several tens to hundred nanoliters matching the sample volume as required by CE well and facilitating in-line coupling of SDME and CE without modification of existing homemade or commercial CE instruments.[23,24] Due to

the very large surface-to-volume ratio and sample volume-to-acceptor phase volume ratio, quite high enrichment factors (EFs) can be obtained in a short extraction time.[24,25] In addition, there is a desalting effect since inorganic ions cannot easily pass through the organic phase.[26,27] These advantages of SDME-CE have been demonstrated in sample cleanup and enrichment processes for acids, bases, zwitterions, and chiral compounds in various sample matrices.[28-34] A combination of SDME-CE and on-line sample preconcentration techniques was also demonstrated.[35-37] Recently, SDME-CE was applied to headspace (HS) extraction[38-43] of chlorophenols in a complex sample such as red wine.[44]

We report an extremely simple but novel improvement over SDME, no drop microextraction (NDME) eliminating the hanging drop from SDME. A liquid plug inside the separation capillary, instead of a hanging drop, is used as an acceptor phase. Analytes from a donor phase are extracted through the opening of the capillary. Although the flux of the extracted analytes is limited by the small opening, all of the extracted analytes are automatically injected into an acceptor of volume far smaller than that of SDME, achieving much higher EF values. Thus NDME can be regarded as sample preconcentration before and during injection. Moreover, given that the acceptor phase is well protected by the capillary, the extraction conditions can be adjusted without much concern over the drop stability, in contrast to SDME.

As a first example, NDME-CE was applied to HS extraction. In HS-NDME-CE, the separation capillary inlet was placed in the HS over the sample donor solution. The

evaporated analytes in the HS were extracted into the acceptor phase inside the capillary. Due to the lack of a hanging drop, the present scheme was extraordinarily simple and fully automatic. Also, owing to the robust nature of the acceptor phase, the extraction temperature and time ranges can be extended significantly. Under the optimal condition of 15 min extraction at 80℃, the EFs obtained for chlorophenols in an aqueous acidic donor phase were up to 1100. The limits of detection (LODs; *S/N* = 3) were in a low nanomolar range with UV absorbance detection. HS-NDME-CE was also applied to red wine samples. Due to the complex matrix of red wine, an acceptor of increased buffer capacity was used. The LODs for the chlorophenols in red wine were about 20 nM.

2 EXPERIMENTAL SECTION

2.1 Reagents

2,6-Dichlorophenol (2,6-DCP), 2,3,6-trichlorophenol (2,3,6-TCP), and 2,4,6-TCP were from Aldrich (Milwaukee, WI, USA). Sodium tetraborate decahydrate and HPLC-grade HCl were from Sigma (St. Louis, MO, USA). Boric acid was from Merck (Darmstadt, Germany). HPLC-grade methanol was from J. T. Baker (Phillipsburg, NJ, USA). Deionized water was obtained with a NANOpure II System (Barnstead, Dubuque, IA, USA). 40 mM stock solutions of 2,6-DCP, 2,3,6-TCP, and 2,4,6-TCP were prepared in methanol and stored in the dark at 4℃ before use. A 240 mM borate buffer was prepared with sodium tetraborate decahydrate, and the pH was adjusted to 9.2 by titrating with a saturated boric acid solution. Standard samples for CE were prepared by diluting the corresponding stock solutions with the borate buffer. Sample donor solutions for HS-NDME were prepared by diluting the corresponding stock solutions with 1 mM HCl. Wine samples were prepared by adding 60 μL of a standard sample to 1140 μL of red wine without any further acidification. Every solution except for the donor phase was filtered through a 0.45-μm syringe filter (Whatman, Clifton, NY, USA) before use.

2.2 CE

CE was performed with a P/ACE MDQ CE system (Beckman, Fullerton, CA, USA) equipped with a UV detector. The dimensions of a fused silica capillary

(Polymicro Technologies, Phoenix, AZ, USA) were 60 cm (50 cm to the detector) × 50 μm id × 363 μm od. The sample tray of the CE instrument was modified to accommodate a jacketed beaker to control the sample vial temperature as previously reported.[24] The run buffer for CE was the borate buffer of pH 9.2 described above. Before each run, the capillary was conditioned with 0.1 M NaOH, water, and run buffer each for 5 min at 40 psi. For electrophoresis, a normal potential of +20 kV was applied across the capillary and the absorbance at 214 nm was monitored. The capillary temperature was set to 20℃.

2.3 Headspace-no drop microextraction process

Fig. 1 shows the HS-NDME-CE procedures. (1) The sample donor vial was capped with a perforated vial cap (#144648, Beckman), which was then covered with a household wrap. The donor temperature was controlled by circulating water through the jacketed beaker using a thermostat (LAUDA, Lauda-Königshofen, Germany). The separation capillary was filled with a run buffer, which could also be used as an acceptor phase. If the acceptor phase was not equal to the run buffer, an acceptor phase was injected at 0.3 psi for a desired duration of time. Without removing the wrap, the inlet tip of the capillary was inserted through the wrap and placed in the HS above the donor solution. The capillary outlet placed in an empty vial was at the same height as the inlet to prevent the movements of liquids inside the capillary. The extraction was then performed and the analytes were enriched into the acceptor phase inside the capillary through the capillary opening. (2) The HS phase was pre-injected into the capillary before separation so as not to lose the enriched analytes. (3) After the inlet

and outlet of the capillary were placed in vials of run buffer, 30 s elapsed so that the pre-injected HS phase could be dissolved away. (4) Electrophoresis was then carried out.

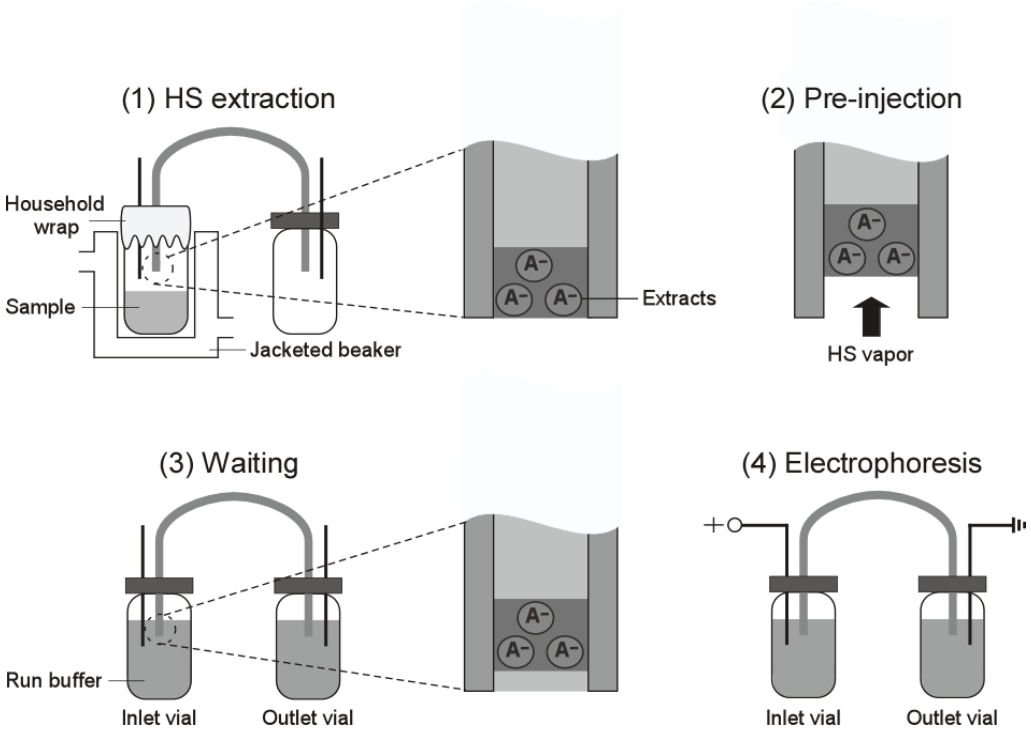

Figure 1. HS-NDME procedures; (1) HS extraction, (2) Pre-injection, (3) Waiting, and (4) Electrophoresis.

3 RESULTS AND DISCUSSION

3.1 Optimization of HS-NDME

3.1.1 Pre-injection of the HS phase

After inserting the capillary inlet into the run buffer vial, it took about 5 s for our commercial CE instrument to apply an electric potential for an electrophoresis step. The analytes enriched near the entrance surface of the acceptor phase in the capillary were then lost during the process time. In order to solve this problem, the acceptor phase plug, enriched with the analytes, was pushed into the capillary by injecting the HS phase vapor after extraction. Then the capillary inlet was inserted into the run buffer vial and a certain amount of time was allowed to elapse without applying an electric potential before an electrophoresis step was started. This was done for the gaseous plug of the HS phase at the capillary inlet entrance to dissolve away, reinstating the electrical connection. The optimal waiting time was 30 s, and a longer waiting time resulted in lower EF values. Fig. 2(a) shows the changes in the EF values as the pre-injection volume (% capillary volume) was increased from 0 to 0.4% with 5 min HS-NDME at 60℃ and a waiting time of 30 s. The EFs for 2,4,6-TCP, 2,3,6-TCP, and 2,6-DCP were respectively 50, 50, and 30 without pre-injection but increased to 290, 300, and 220 with pre-injection of the optimal 0.2% of the capillary volume.

3.1.2 The donor and acceptor phases

For further optimization of the extraction conditions, the results obtained with 5

min HS-NDME at 60℃ and a waiting time of 30 s were compared. To promote the evaporation of the acidic analytes into the HS, the donor phase pH was adjusted for the analytes to be in their neutral forms. Fig. 2(c) shows the changes in the analyte peak areas as the donor pH was varied from 1.5 to 4 by adding HCl. Considering both the EF and relative standard deviation (RSD) values, we set the pH of the donor solution to 3. The optimal donor phase volume in a 2 mL sample vial was set to 1.1 mL after comparing the peak areas obtained with 0.9 to 1.5 mL donor phases.

In our previous HS-SDME-CE using a large drop with a Teflon sleeve support, the sample vial cap was covered with aluminum foil to reduce the loss of volatile analytes in the HS phase at an elevated temperature.[44] The aluminum foil had to be removed manually immediately before the capillary entered the sample vial, not to block the passage of the capillary with a hanging drop. On the other hand, in HS-NDME-CE, the sample vial cap was covered with a household wrap which could be passed through easily by the capillary without a hanging drop. Thus, the wrap did not have to be removed manually and HS-NDME-CE was carried out automatically using a commercial CE instrument. The EFs of 2,4,6-TCP, 2,3,6-TCP, and 2,6-DCP were 15, 13, and 25 without a wrap and 14, 13, and 23 with a wrap at 25℃, respectively. They were 380, 500, and 220 without a wrap and 510, 650, and 340 with a wrap at 80℃, respectively. The increase in the EF values with the wrap became more pronounced as the extraction temperature was increased; at 25℃, the EF values changed little with or without a wrap, while the EF values increased more than 30% with a wrap at 80℃. The RSDs of peak areas were decreased with a wrap at the all temperature ranges; at 25℃ from 35 to 16%, from 35 to 16%, and from 32 to 18%; at 80℃, from 38 to 16%,

from 35 to 21%, and from 41 to 35%, respectively for 2,4,6-TCP, 2,3,6-TCP, and 2,6-DCP. Hence, the sample vial was covered with a wrap even during the capillary pretreatment steps for further improvement of the HS-NDME-CE performance.

For the HS extraction of an acidic analyte, HA, it is desirable for the acceptor phase to be of high pH so that the analyte is kept in the ionized form of A^-. This feature makes the HS-NDME operation even simpler; the separation capillary filled with a basic run buffer is simply placed in the HS above the sample donor solution. However, for a sample in a complicated matrix such as red wine, an acceptor phase with a higher buffer capacity should be used (see Section 3.4).

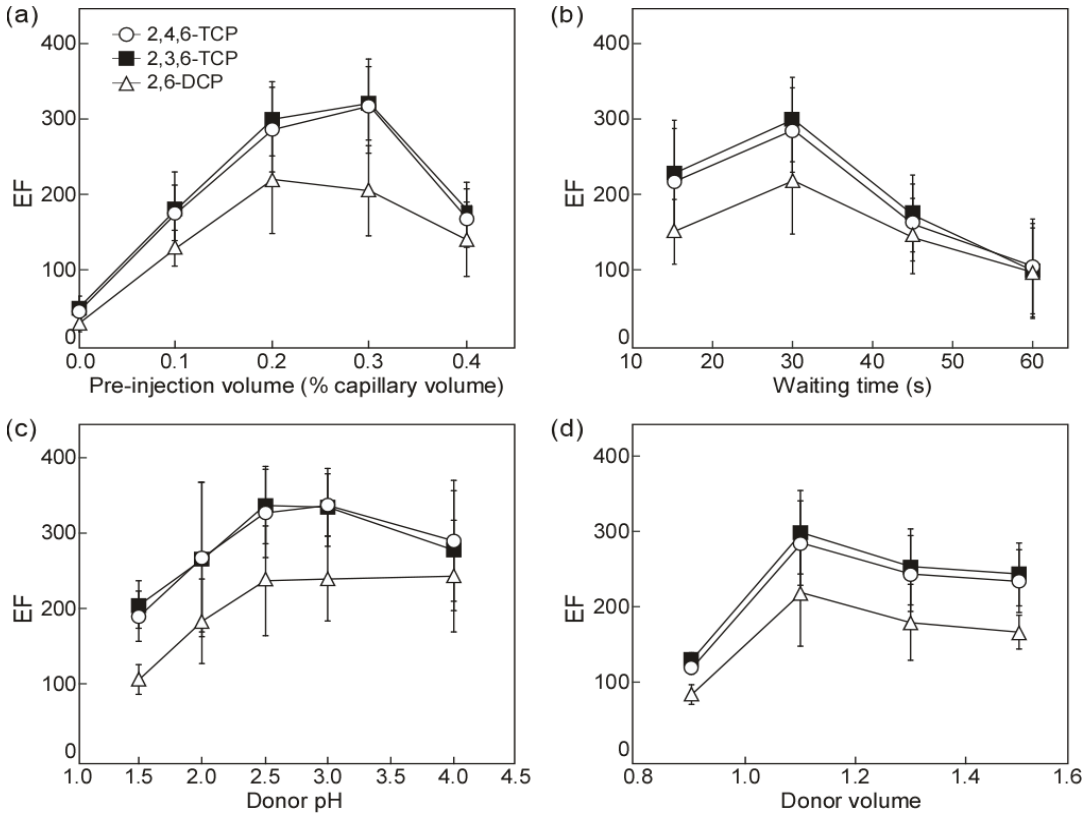

Figure 2. Optimization of HS-NDME conditions; (a) Pre-injection volume, (b) Waiting time, (c) Donor pH, and (d) Donor volume. Acceptor; 240 mM borate buffer of pH 9.2. Donor; 1 μM analytes in 3 mM HCl. Extraction; 60°C, 5 min. Bare fused silica capillary; 50 μm ID, 60 cm (effective length 50 cm). Absorbance; 214 nm. Separation voltage; 20 kV. Run buffer; 240 mM borate buffer of pH 9.2.

3.1.3 Extraction temperature and time

A general means of increasing the extraction efficiency in a HS extraction is to increase the extraction temperature and time.[45] In HS-SDME, the instability of a drop hanging at the capillary inlet made it difficult to carry out the extraction at a high temperature for an elongated time.[44] However, as shown in Fig. 3, HS-NDME could be carried out for a much longer time at a higher temperature as compared to HS-SDME, owing to the absence of a hanging drop. Increasing the extraction temperature from 60 to 80℃, the EF values obtained with 5 min HS-NDME were increased from 170 to 510, from 180 to 650, and from 130 to 300 for 2,4,6-TCP, 2,3,6-TCP, and 2,6-DCP, respectively. Increasing the extraction time from 5 to 45 min, the EFs from HS-NDME at 60℃ were increased from 170 to 1020, from 180 to 1480, and from 130 to 660, respectively. Since the reproducibility worsened with an increase in the extraction temperature and time, we selected 80℃ and 15 min as the optimal temperature and time, respectively. Fig. 4 shows an electropherogram of HS-NDME-CE under the optimum extraction condition. The EFs of 2,4,6-TCP, 2,3,6-TCP, and 2,6-DCP were 850, 1100, and 450, respectively.

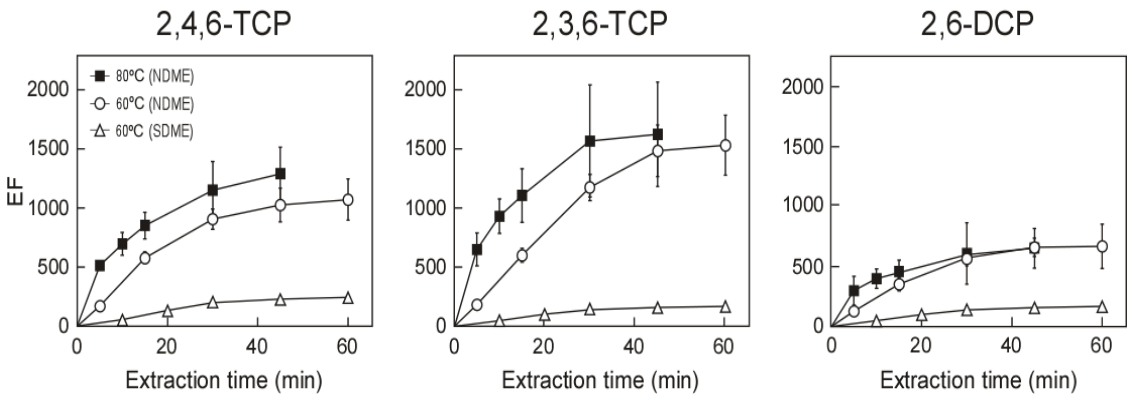

Figure 3. EFs of HS-SDME *vs*. the extraction time and temperature. Other HS-NDME and CE conditions as in Fig. 2.

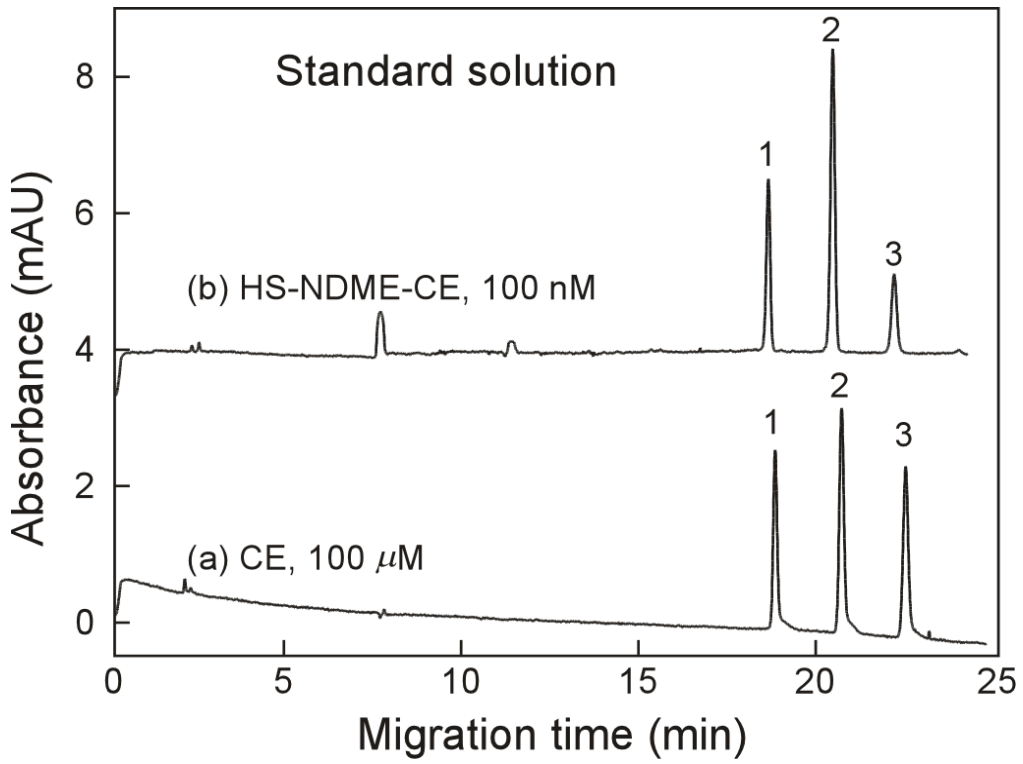

Figure 4. Chlorophenols in standard solutions. Electropherograms of (a) CE and (b) HS-NDME-CE. Donor; 100 nM analytes in 1 mM HCl, 1100 μL. Acceptor; 240 mM borate buffer of pH 9.2. Extraction; 80°C, 15 min. Other HS-NDME and CE conditions as in Fig. 2. Peak identification: 1) 2,4,6-TCP, 2) 2,3,6-TCP, and 3) 2,6-DCP.

3.2 Analytical performance

The LODs (*S/N* = 3) and linear dynamic ranges of the analytes estimated with the peak areas were 3-5 nM and 10-2000 nM, respectively. The RSDs of the migration times and peak areas were 0.88-0.99% and 18-25%, respectively. When 2,3,6-TCP was used as an internal standard, the RSDs of the migration times and peak areas were reduced to 0.06-0.07% and 10% for 2,4,6-TCP and 2,6-DCP, respectively. Table 1 lists the analytical performance of HS-NDME-CE.

Table 1. HS-NDME performance

Analyte	EF	%RSD (w IS, n = 4)		LOD (nM, S/N = 3)	Linear range (nM)	Linearity (r^2)	Calibration curve y; peak area (μAU·s) x; concentration (nM)
		Migration time	Peak area				
2,4,6-TCP	850	0.06	10	4	10-2000	0.9992	$y = 173.967x - 905.793$
2,3,6-TCP	1100	IS	IS	3	10-2000	0.9931	$y = 332.947x - 5601.531$
2,6-DCP	450	0.07	10	5	20-2000	0.9999	$y = 120.469x + 495.534$

Donor phase; 1 mM HCl, 1100 μL. Acceptor phase; 240 mM borate buffer of pH 9.2. Extraction; 80℃, 15 min.

3.3 HS-NDME vs. HS-SDME

In HS extraction, analytes transfer from a donor solution to the HS and then from the HS to an acceptor phase. If the extraction time t is short, the EF in HS extraction increases with t in proportion to the surface-to-volume ratio of the acceptor phase:[44]

$$\mathrm{EF}(t) \propto (A_a/V_a)t \quad (1)$$

where A_a and V_a are the surface area and the volume of the acceptor phase, respectively. In previous HS-SDME, if the acceptor phase is approximated as a half-sphere of radius r with a uniform distribution of the extracted analytes, the EFs should be proportional to $1/r$, that is, to $(1/V_a)^{1/3}$. Although a smaller drop resulted in higher EFs, the stability and reproducibility problems of the acceptor drop in HS-SDME made it difficult to use a drop of less than 300 nL, and the optimal acceptor drop volume was 700 nL.[44] To obtain EFs similar to those for HS-NDME under the optimal condition of 80℃ for 15 min, the acceptor drop in HS-SDME would have been reduced to about 60 nL under the optimal condition of 60℃ for 30 min, which would have been quite difficult. In HS-NDME, if the acceptor phase in a capillary is approximated as a cylinder of length L with a uniform distribution of the extracts, then the EF becomes proportional to $1/L$. The acceptor cylinder length L can be approximated as the length of a sample plug due to the longitudinal diffusion in one dimension:[46]

$$L \approx \sqrt{2Dt} \quad (2)$$

where D is the diffusion coefficient of the analyte in the acceptor phase. According to the Stokes-Einstein equation, the diffusion coefficient is proportional to the ratio of the

absolute temperature, T, and the dynamic viscosity of the medium, η:[47]

$$D \propto \frac{T}{\eta} \quad (3)$$

Thus, the diffusion coefficient at 80℃ is about three times larger than the value at 25℃ approximating the acceptor phase as pure water.[48] L is estimated to be about 2 mm for the extraction time of 15 min and the effective acceptor phase volume as about 4 nL. Although the amounts of analytes being extracted through the small opening of the capillary in HS-NDME are small, the EF values governed by the concentrations of the extracts are high due to the small acceptor volume. Moreover, all of the extracted analytes are already injected into the capillary and used in the analysis, while only a small portion of the extracts is injected and used in actual analysis in other extraction schemes. Methodologically, HS-NDME is a very effective extraction scheme combining sample preconcentrations before and during injection.

3.4 Chlorophenols in red wine

The applicability of HS-NDME was evaluated by analyzing a red wine sample spiked with the three aforementioned chlorophenols. Wine has a complicated sample matrix and consequently is a very difficult sample to analyze.[49] When the acidic chlorophenols in a wine sample were extracted with HS-NDME using the 240 mM run buffer as an acceptor, the EFs of 2,4,6-TCP, 2,3,6-TCP, and 2,6-DCP were 18, 25, and 23, respectively. These EF values were about thirty six times lower than those obtained for chlorophenols in a standard solution as a number of acidic compounds in the wine matrix disturbed the pH of the acceptor phase of a small volume. When the acceptor phase was changed from the run buffer in the capillary (240 mM borate buffer of pH 9.2) to 12 nL of 600 mM borate buffer of pH 9.2, the EFs of 2,4,6-TCP, 2,3,6-TCP, and 2,6-DCP were increased about 30% to 23, 34, and 33, respectively. Then, while fixing the borate buffer concentration at the solubility maximum of 600 mM,[50] the pH and volume of the acceptor phase were varied. When the acceptor phase pH was increased to 10.5, the EFs of 2,4,6-TCP, 2,3,6-TCP, and 2,6-DCP increased to 39, 41, and 43, respectively. When the acceptor phase volume was increased from 12 to 30 nL, the EFs increased to 190, to 180, and 210, respectively. The optimal acceptor phase was thus set to 30 nL of 600 mM borate buffer of pH 10.5. The LODs (S/N = 3) of 2,4,6-TCP, 2,3,6-TCP, and 2,6-DCP in a red wine sample were 15, 27, and 25 nM, respectively. By increasing the buffer capacity of the acceptor phase, the EFs and LODs obtained with HS-NDME for the three chlorophenols in a red wine sample were improved by about 10 times. Fig. 5(a) shows an electropherogram for a wine sample

spiked with 100 μM chlorophenols without HS-NDME, exhibiting the complex nature of the wine matrix. Fig. 5(b) shows an electropherogram for a wine sample spiked with 5 μM chlorophenols with HS-NDME for 15 min at 80°C. This figure clearly demonstrates the sample cleanup capability of HS-NDME, as well as the sample enrichment. Table 2 summarizes the HS-NDME results for red wine samples spiked with chlorophenols.

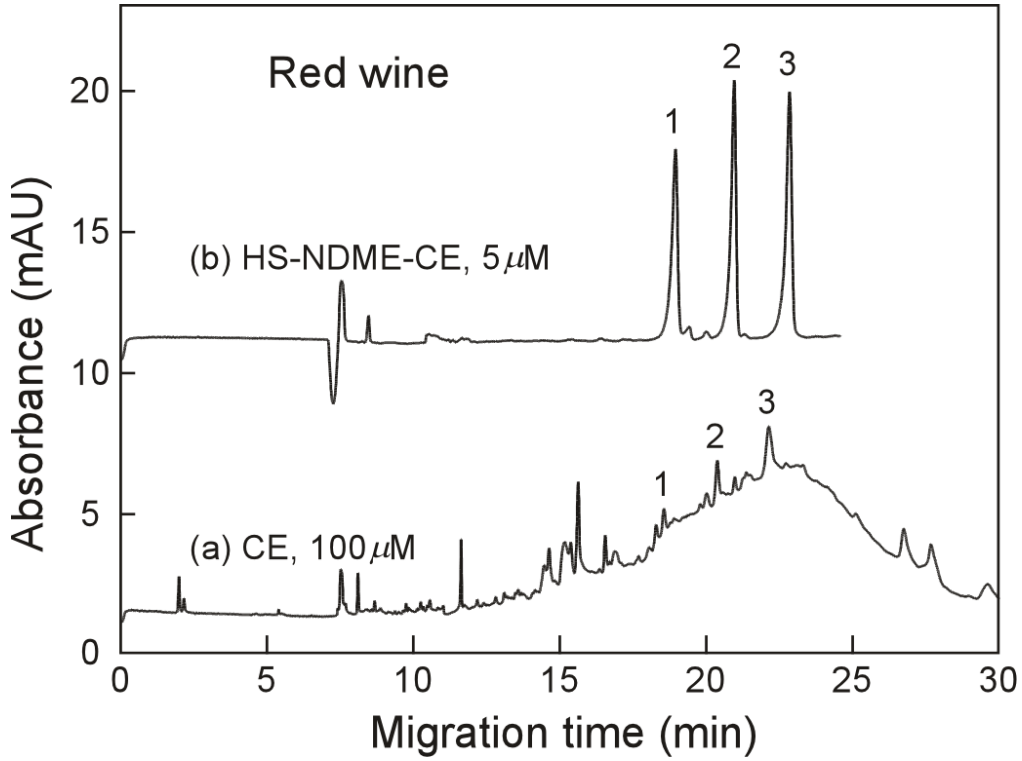

Figure 5. Chlorophenols in red wine. (a) CE of a wine sample spiked with 100 μM chlorophenols. (b) HS-NDME of a wine sample spiked with 5 μM chlorophenols. Donor; 5 μM analytes in wine, 1100 μL. Acceptor; 30 nL of 600 mM borate buffer of pH 10.5. Extraction; 80°C, 15 min. Other HS-NDME and CE conditions as in Fig. 2. Peak identification: 1) 2,4,6-TCP, 2) 2,3,6-TCP, and 3) 2,6-DCP.

Table 2. HS-NDME of chlorophenols in red wine

Analyte	%RSD (w IS, n = 4)		LOD (nM, S/N = 3)	Linearity (r^2)[a)]	Calibration curve[a)] y; peak area (μAU·s) x; concentration (μM)
	Migration time	Peak area			
2,4,6-TCP	0.20	5	15	0.9969	$y = 39097x + 302.4$
2,3,6-TCP	IS	IS	27	0.9923	$y = 57601x - 3137.4$
2,6-DCP	0.15	12	25	0.9840	$y = 27781x + 4739.1$

[a)] The linearity was evaluated using 0.05, 0.1, 1.5, 2.0, and 3.0 μM chlorophenols in red wine.

4 CONCLUSIONS

HS-NDME in-line coupled with CE has been presented for an analysis of chlorophenols in an aqueous sample. NDME uses the liquid in the separation capillary as an acceptor phase and thus utilizes all of the extracts for the subsequent CE analysis, as they are already injected. By eliminating the hanging drop from SDME, NDME opens a new era for sample preparation in CE. It offers a quite powerful but extremely easy method readily usable by anyone without special equipment or training. In addition to HS-NDME-CE, direct immersion-NDME-CE for liquid phase microextraction is in progress.

REFERENCES

(1) Whang, C. W.; Pawliszyn, *J. Anal. Commun.* **1998,** *35*, 353-356.

(2) Pedersen-Bjergaard, S.; Rasmussen, K. E. *Anal. Chem.* **1999,** *71*, 2650-2656.

(3) Breadmore, M. C. *J. Chromatogr. A* **2011,** *1218*, 1347-1352.

(4) Pantuckova, P.; Kuban, P.; Bocek, P. *Electrophoresis* **2013,** *34*, 289-296.

(5) Chien, R. L.; Burgi, D. S. *J. Chromatogr.* **1991,** *559*, 141-152.

(6) Burgi, D. S. *Anal. Chem.* **1993,** *65*, 3726-3729.

(7) Quirino, J. P.; Terabe, S. *Science* **1998,** *282*, 465-468.

(8) Krivankova, L.; Pantuckova, P.; Bocek, P. *J. Chromatogr. A* **1999,** *838*, 55-70.

(9) Britz-McKibbin, P.; Chen, D. D. Y. *Anal. Chem.* **2000,** *72*, 1242-1252.

(10) Kim, B.; Chung, D. S. *Electrophoresis* **2002,** *23*, 49-55.

(11) Chun, M. S.; Chung, D. S. *Anal. Chim. Acta* **2003,** *491*, 173-179.

(12) Breadmore, M. C.; Dawod, M.; Quirino, J. P. *Electrophoresis* **2011,** *32*, 127-148.

(13) Britz-McKibbin, P.; Otsuka, K.; Terabe, S. *Anal. Chem.* **2002,** *74*, 3736-3743.

(14) Hirokawa Takeshi, O. H., Gas Bohuslav *Electrophoresis* **2003,** *24*, 498-503.

(15) Dawod, M.; Breadmore, M. C.; Gujit, R. M.; Haddad, P. R. *J. Chromatogr. A* **2009,** *1216*, 3380-3386.

(16) Dawod, M.; Breadmore, M. C.; Guijt, R. M.; Haddad, P. R. *Electrophoresis* **2010,** *31*, 1184-1193.

(17) Dasgupta, P. K.; Kar, S. *Anal. Chem.* **1995,** *67*, 3853-3860.

(18) Liu, H. H.; Dasgupta, P. K. *Anal. Chem.* **1996,** *68*, 1817-1821.

(19) Pedersen-Bjergaard, S.; Rasmussen, K. E. *Electrophoresis* **2000,** *21*, 579-585.

(20) Halvorsen, T. G.; Pedersen-Bjergaard, S.; Rasmussen, K. E. *J. Chromatogr. A* **2001,** *909*, 87-93.

(21) Rasmussen, K. E.; Pedersen-Biergaard, S. *Trends Anal. Chem.* **2004,** *23*, 1-10.

(22) Xie, H. Y.; He, Y. Z. *Trends Anal. Chem.* **2010,** *29*, 629-635.

(23) Choi, K.; Kim, Y.; Chung, D. S. *Anal. Chem.* **2004,** *76*, 855-858.

(24) Choi, K.; Kim, S. J.; Jin, Y. G.; Jang, Y. O.; Kim, J. S.; Chung, D. S. *Anal. Chem.* **2009,** *81*, 225-230.

(25) Choi, K.; Kim, J.; Chung, D. S. *Bioanalysis* **2011,** *3*, 799-815.

(26) Psillakis, E.; Kalogerakis, N. *Trends Anal. Chem.* **2002,** *21*, 53-63.

(27) Kataoka, H. *Anal. Bioanal. Chem.* **2010,** *396*, 339-364.

(28) Kim, J.; Chun, M. S.; Choi, K.; Chung, D. S. *Electrophoresis* **2009,** *30*, 1046-1051.

(29) Park, Y. K.; Choi, K.; Ahmed, A. Y. B. H.; ALOthman, Z. A.; Chung, D. S. *J. Chromatogr. A* **2010,** *1217*, 3357-3361.

(30) Liang, G.; Choi, K.; Badjah Hadj Ahmed, A. Y.; ZA, A. L.; Chung, D. S. *Anal. Chim. Acta* **2010,** *677*, 37-42.

(31) Choi, J.; Choi, K.; Kim, J.; Ahmed, A. Y.; Al-Othman, Z. A.; Chung, D. S. *J. Chromatogr. A* **2011,** *1218*, 7227-7233.

(32) Gao, W. H.; Chen, G. P.; Chen, Y. W.; Zhang, X. S.; Yin, Y. G.; Hu, Z. D. *J. Chromatogr. B* **2011,** *879*, 291-295.

(33) Gao, W. H.; Chen, G. P.; Chen, T. F.; Zhang, X. S.; Chen, Y. W.; Hu, Z. D. *Talanta* **2011,** *83*, 1673-1679.

(34) Cheng, K.; Choi, K.; Kim, J.; Sung, I. H.; Chung, D. S. *Microchem. J.* **2013,** *106*, 220-225.

(35) Liang, G.; Choi, K.; Chung, D. S. *Electrophoresis* **2009,** *30*, 1953-1957.

(36) Choi, K.; Jin, Y. G.; Chung, D. S. *J. Chromatogr. A* **2009,** *1216*, 6466-6470.

(37) Gao, W. H.; Chen, G. P.; Chen, Y. W.; Li, N. N.; Chen, T. F.; Hu, Z. D. *J. Chromatogr. A* **2011,** *1218*, 5712-5717.

(38) Zhang, J.; Su, T.; Lee, H. K. *Anal. Chem.* **2005,** *77*, 1988-1992.

(39) Jermak, S.; Pranaityte, B.; Padarauskas, A. *Electrophoresis* **2006,** *27*, 4538-4544.

(40) Pranaityte, B.; Jermak, S.; Naujalis, E.; Padarauskas, A. *Microchem. J.*

2007, *86*, 48-52.

(41) Jermak, S.; Pranaityte, B.; Padarauskas, A. *J. Chromatogr. A* **2007,** *1148*, 123-127.

(42) Xie, H. Y.; He, Y. Z.; Gan, W. E.; Yu, C. Z.; Han, F.; Ling, D. S. *J. Chromatogr. A* **2010,** *1217*, 1203-1207.

(43) Ling, D. S.; Xie, H. Y.; He, Y. Z.; Gan, W. E.; Gao, Y. *J. Chromatogr. A* **2010,** *1217*, 7807-7811.

(44) Park, S. T.; Kim, J.; Choi, K.; Lee, H. R.; Chung, D. S. *Electrophoresis* **2012,** *33*, 2961-2968.

(45) Ai, J. *Anal. Chem.* **1997,** *69*, 3260-3266.

(46) Vandeemter, J. J.; Zuiderweg, F. J.; Klinkenberg, A. *Chem. Eng. Sci.* **1956,** *5*, 271-289.

(47) Edward, J. T. *J. Chem. Educ.* **1970,** *47*, 261-270.

(48) Krynicki, K.; Green, C. D.; Sawyer, D. W. *Faraday Discuss.* **1978,** *66*, 199-208.

(49) Riu, M.; Mestres, M.; Busto, O.; Guasch, J. *J. Chromatogr. A* **2002,** *977*, 1-8.

(50) Reijenga, J. C.; Verheggen, T. P. E. M.; Martens, J. H. P. A.; Everaerts, F. M. *J. Chromatogr. A* **1996,** *744*, 147-153.

국문 초록

액체상 미세추출법 (LPME)에서, 높은 농축계수 (EF)는 받개층의 부피가 작을 때 얻어진다. 미세방울 미세추출법 (SDME)는 분리 모세관의 입구에 받개층으로 방울을 매달아 CE와 연동할 수 있다. 방울의 표면적 대 부피가 작을수록 짧은 시간 안에 높은 EF를 얻을 수 있다. SDME에서 실질적인 문제는 방울을 모세관의 입구에 어떻게 안전하게 유지하는 가이다. 여기에서 우리는 모세관의 입구에 방울을 형성하지 않고, 받개층으로 모세관 안에 액체를 사용하는 참신하면서도 매우 간단한 비 방울 미세추출법 (NDME)를 제안한다. 첫 번째로, NDME를 상공 (HS) 추출법과 결합하였다. 단순하게 산성의 주개 용액 위의 HS에 염기성의 분리 완충 용액으로 채워진 모세관을 놓음으로써, 휘발성의 산성 분석물질들이 모세관 안에 받개층으로 추출되었다. 추출 후, 모세관 안에 추출물질들의 전기영동이 수행되었다. 받개층의 튼튼한 성질 때문에, HS-SDME보다 HS-NDME의 추출 온도와 시간의 범위를 확장할 수 있었다. 80° C, 15분의 HS-NDME 최적 조건에서, 표준 용액으로 클로로페놀에 대한 EF는 1100까지였다. 레드 와인과 같은 복잡한 샘플의 경우, 증가된 완충 용량을 가진 받개층을 사용하여 약 20 nM의 검출 감도를 얻었다. HS-NDME-CE는 전 과정이 상용화된 모세관 전기영동 장치에서 제공되는 프로그램을 사용하여 자동으로 수행되었다.

주요어: 모세관 전기영동, 상공-비 방울 미세추출법, 시료 전처리

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