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샴요의 이학석사 학위논문일 인준함
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## Abstract

# Headspace Microextraction Using A Single Bubble Coupled With Capillary Electrophoresis

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Headspace (HS) extraction is a sample pretreatment technique for volatile and semi-volatile organic compounds in a complex matrix. Recently, in-tube microextraction (ITME) coupled with capillary electrophoresis (CE) using an acceptor plug placed at the capillary inlet was developed as a simple but powerful HS extraction method. Here, we present single bubble microextraction (SBME) using a bubble hanging to the capillary inlet immersed in a sample donor solution as a HS of submicroliter volume (~200 nL). The analytes evaporated to the bubble were extracted into the acceptor phase through the capillary opening, then electrophoresis of the enriched extract was carried out. Since the bubble volume was much smaller than conventional HS volume (~1 mL), it was filled with the evaporated analytes rapidly and the analytes could be enriched much faster compared to conventional HS-ITME. Owing to the high surface-to-volume ratio of the

single bubble, 5-min SBME yielded the EF values similar to those of 10-min HS-ITME. When 5-min SBME at room temperature was applied to a tap water, the EFs of 2,4,6-TCP, 2,3,6-TCP and 2,6-DCP were 53, 46 and 51, respectively, and the limits of quantification obtained by monitoring the absorbance at 214 nm were about 12 ppb, much lower than 200 ppb, the World Health Organization guideline for the maximum permissible concentration of 2,4,6-TCP in drinking water.

**Keywords**:

Capillary electrophoresis (CE) / Chlorophenols (CPs) / Headspace extraction (HS) / Single bubble microextraction (SBME) / In-tube microextraction (ITME).

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# 1 INTRODUCTION

Headspace (HS)-*sampling* is a convenient way of selectively collecting compounds evaporated from a condensed-phase sample, first explored in the 1930s [1, 2]. For an additional selectivity and preconcentration power, HS-*extraction* using a solid or liquid acceptor phase was developed [3, 4]. Over the years, HS-extraction has evolved to miniaturized, economic, more efficient, and greener HS-solid phase microextraction (SPME) and HS-liquid phase microextraction (LPME). Currently, both modes are widely used [5-10]. In most cases, HS-SPME is carried out using an acceptor-coated fiber [11, 12] and HS-LPME is done using a single acceptor drop hanging to a syringe needle tip, which is called single drop microextraction (SDME) [13, 14]. Both of HS-SPME and HS-SDME are coupled with various analytical instruments, such as GC and LC, but usually in off-line modes.

HS-SDME was first coupled off-line with CE [15] in 2005 and a number of similar off-line HS-SDME-CE applications have been employed in many fields [16-18]. In-line coupled HS-SDME-CE was then implemented by hanging an acceptor drop at the inlet of a separation capillary placed in the HS by using a homemade CE setup [19, 20], and then it was made more convenient by using a commercial CE instrument [21]. Nevertheless, the efficiency was still limited by the instability of the hanging drop, especially for long extraction times and elevated temperatures. Recently, these shortcomings were resolved with a novel and quite simple technique, in-tube microextraction (ITME), using a liquid plug in the capillary inlet as an acceptor phase instead of a hanging acceptor drop [22, 23]. In HS-ITME-CE,

the capillary inlet containing the acceptor plug was simply placed in the HS above a sample solution. Since the acceptor phase was well protected by the capillary, a long extraction at a temperature as high as 90°C could be performed with ease [22, 24]. However, for a short extraction time, the extraction efficiency of HS-SDME or HS-ITME was not maximized because it takes some time for the analytes to fill the HS and reach saturated concentrations, especially for compounds of low volatility [25-27].

In this report, a novel HS-extraction method of single bubble microextraction (SBME) coupled with CE is presented. A single air bubble attached to the inlet of a capillary immersed in a sample solution is used as a micro-HS inside the solution. Since the surface-to-volume ratio of the submicroliter bubble is much higher than that of a conventional HS of a few milliliters or more, the analyte concentrations in the bubble can be replenished much more rapidly for short extraction times. SBME demonstrated higher sample enrichments than HS-ITME for a short HS extraction of chlorophenols in water.

# 2 THEORY

In HS extraction of an acidic analyte, an acidic donor phase (1) promoting the unprotonated neutral form of the analyte and a basic acceptor phase (2) capturing the evaporated analyte in the HS (h) as the deprotonated ionic form are preferred. The sample enrichment factor (EF) after extraction time, *t*, is defined as the ratio of the analyte concentration in the acceptor phase, $C_2(t)$, to the initial concentration in the donor phase, $C_1(0)$: $\mathrm{EF}(t) \equiv C_2(t)/C_1(0)$. At equilibrium, using the distribution coefficients, $D_1 = C_h(\infty)/C_1(\infty)$, and $D_2 = C_h(\infty)/C_2(\infty)$, the EF is given by

$$\mathrm{EF}_{eq} = \frac{C_2(\infty)}{C_1(0)} = \frac{1}{(D_2/D_1) + D_2(V_h/V_1) + (V_2/V_1)} \qquad (1)$$

For an acidic analyte HA of p$K_a$, assuming that only the neutral form can evaporate, the distribution coefficients are given by [28]

$$D_1 \approx \frac{[\mathrm{HA}]_h}{[\mathrm{HA}]_1 + [\mathrm{A}^-]_1} = K_H \frac{[\mathrm{H}^+]_1}{[\mathrm{H}^+]_1 + K_a}, \text{ and}$$

$$D_2 \approx \frac{[\mathrm{HA}]_h}{[\mathrm{HA}]_2 + [\mathrm{A}^-]_2} = K_H \frac{[\mathrm{H}^+]_2}{[\mathrm{H}^+]_2 + K_a}, \qquad (2)$$

with the Henry constant defined as $K_H = [\mathrm{HA}]_h / [\mathrm{HA}]_{1 \text{ or } 2}$. Under the condition of fixed total volume of the extraction system $V_{total}$ (= $V_1 + V_h + V_2$), the maximum $\mathrm{EF}_{eq}$ can be obtained when the sample donor phase volume is half of the total volume; $V_1 = (1/2)V_{total}$ [19].

For the three acidic chlorophenols in this report, 2,4,6-TCP (p$K_a$ 5.80), 2,3,6-TCP (p$K_a$ 6.23), and 2,6-DCP (p$K_a$ 6.79), $\mathrm{EF}_{eq}$ can be increased by lowering the donor phase pH below and raising the acceptor pH above their

p$K_a$ values. If chlorophenols are extracted, for example, from a 1-mL acidic donor phase of pH 3 to a 10-nL basic acceptor phase of pH 13 through an HS of 1 mL, then $[H^+]_1 >> K_a >> [H^+]_2$. Using the Henry constants of $1.72 \times 10^{-4}$, $9.40 \times 10^{-6}$, and $1.09 \times 10^{-4}$ respectively for 2,4,6-TCP, 2,3,6-TCP, and 2,6-DCP [29], Eq. (1) can be approximated as

$$\mathrm{EF}_{\mathrm{eq}} \approx V_1/V_2 = 10^5, \tag{3}$$

by neglecting the first and second terms in the denominator, which are much smaller than the third term. Note that this large EF value is obtained only when an equilibrium is established and $C_1(0)$ is low enough to avoid disturbing the acceptor pH even after being enriched $10^5$ times in the acceptor phase. In practice, however, extractions are carried out for a relatively short time before reaching equilibrium. Therefore, the equilibrium discussion serves only as a guideline, and kinetic effects govern the actual extraction performance.

The kinetics of HS extraction is mainly governed by the two processes: Evaporation from the donor phase to the HS and the back-extraction from the HS to the acceptor phase [3, 4]. The capacity ratios between the acceptor phase and the HS defined as [25]

$$\mathrm{R} \equiv \frac{C_2(\infty)V_2}{C_{\mathrm{h}}(\infty)V_{\mathrm{h}}} = \frac{1}{D_2}\frac{V_2}{V_{\mathrm{h}}} \approx \frac{[\mathrm{H}^+]_2 + K_{\mathrm{a}}}{K_{\mathrm{H}}[\mathrm{H}^+]_2}\frac{V_2}{V_{\mathrm{h}}} \tag{4}$$

for both HS-ITME ($V_2/V_{\mathrm{h}} \sim 10^{-5}$) and SBME ($V_2/V_{\mathrm{h}} \sim 10^{-1}$) in this report, are quite large ($R_{\mathrm{ITME}} \sim 10^6$, $R_{\mathrm{SBME}} \sim 10^{10}$) due to the extremely small value of $D_2$. Thus, the first process of analyte evaporation from the donor

phase to the HS should be considered as a bottleneck. Since the back extraction from the HS into the acceptor plug through the small capillary opening can also be a bottleneck, the overall kinetics is expected to be quite complicated [30]. The evaporation rate constant $k$ of the evaporation rate

$$\left.\frac{\mathrm{d}C_\mathrm{h}}{\mathrm{d}t}\right|_{\text{evaporation}} = k\,\{C_\mathrm{h}(\infty)^{\infty} - C_\mathrm{h}(t)\} \tag{5}$$

is given as [31]

$$k \propto \frac{A_\mathrm{h}}{V_\mathrm{h}}, \tag{6}$$

where $A_\mathrm{h}$ is the interfacial area between the donor phase and the HS. Therefore, due to the higher surface-to-volume ratio, $A_\mathrm{h}/V_\mathrm{h}$, the evaporation rate constant of SBME should be much higher than that of HS-ITME, and the micro-HS of SBME of about 200 nL is expected to be replenished much more quickly than the HS of ITME of about 1 mL. Thus, it is expected that the EF values obtained from SBME should be higher than those from HS-ITME, especially for a short extraction time.

# 3 EXPERIMENTAL SECTION

## 3.1 Reagents

2,4,6-Trichlorophenol (TCP), 2,3,6-TCP, 2,6-dichlorohenol (DCP), sodium tetraborate decahydrate, potassium phosphate mono basic, and nitric acid were purchased from Sigma-Aldrich (St Louis, MO, USA). HPLC-grade methanol, hydrochloric acid, and sodium hydroxide were from Daejung (Siheung, Korea). Phosphoric acid was from Fluka (Buchs, Switzerland). Boric acid was from Merck (Darmstadt, Germany). Water was purified with a LabTower EDI water purification unit (Thermo Scientific, Langenselbold, Germany).

Individual 40 mM stock solutions of each chlorophenol were prepared in methanol in vials with an aluminum crimp cap and septum (Scilab, Seoul, Korea), and stored in the dark at 4℃ for up to 24 h to prevent the loss of analytes. Standard mixture solutions for CE were prepared by diluting the stock solutions with a run buffer, immediately before a run. Standard solutions for HS-ITME and SBME were prepared in two steps; a 100 $\mu$M working solution was first prepared by diluting the stock solutions with 10 mM NaOH in a 2-mL screw-capped plastic tube, sealed with a paraffin film, and stored in the dark at 4℃ for no longer than 5 h, and then a proper amount of the working solution was transferred to a 2-mL clear sample vial, diluted with a donor phase acidic solvent, capped with a rubber vial cap (#144648, Beckman, Fullerton, CA, USA), and sealed with household wrap, immediately before a run. A 240 mM sodium borate buffer of pH 9.2 prepared by titrating 60 mM sodium tetraborate decahydrate with 500 mM boric acid was used as a run buffer for CE [21, 22]. The run buffer solution was filtered

through a 0.45-$\mu$m PTFE-H syringe filter (Hyundai Micro, Seoul, Korea) and degassed by sonicating for 20 min.

## 3.2 CE

CE analysis was carried out using a P/ACE MDQ CE system (Beckman). A new bare fused silica capillary (Molex, Lisle, Illinois, USA) with id of 50 $\mu$m, od of 360 $\mu$m, and length of 60 cm (50 cm to the detector) was conditioned with 1 M HCl for 30 min, 1 M NaOH for 30 min, water for 30 min, and run buffer for 1 h at a backpressure of 50 psi. Before each CE run, the capillary was rinsed with 1 M HCl for 3 min, 1 M NaOH for 3 min, water for 3 min, and run buffer for 5 min at a backpressure of 50 psi. The sample was injected hydrodynamically at 0.5 psi for 5 s. Electrophoresis was performed by applying a normal potential of 20 kV across the capillary at 25℃ and monitoring the absorbance at 214 nm.

## 3.3 HS-ITME-CE

Fig. 1A shows the schematics of HS-ITME. A 2-mL sample vial was filled with 1.1 mL of standard sample solution freshly prepared as described above and sealed with wrap. The extraction temperature was controlled by thermostating the sample vial with a jacketed beaker connected to a water bath (LAUDA, Baden-Württemberg, Germany). Before each HS-ITME-CE run, the capillary was conditioned with the same rinsing protocol described above, and then an acceptor plug was injected hydrodynamically into the capillary inlet. Without removing the wrap, the capillary inlet tip was inserted into the sample vial and placed in the HS above the aqueous donor solution and the capillary outlet was immersed in a run buffer vial to prevent injecting air into the capillary tube during dynamic extraction. Then, HS-ITME was

carried out for a desired time. For dynamic extraction, a small portion (~4 nL) of the acceptor plug was pushed out of the capillary by applying a backpressure of 0.4 psi for 5 s and then returned back to the capillary during a dwell time. For this reason, pre-injection of the HS phase [22] was not employed since mixing of the analytes in the acceptor phase was already enhanced through the dynamic movement of the acceptor plug. After extraction, the inlet was transferred into a run buffer vial and electrophoresis was carried out.

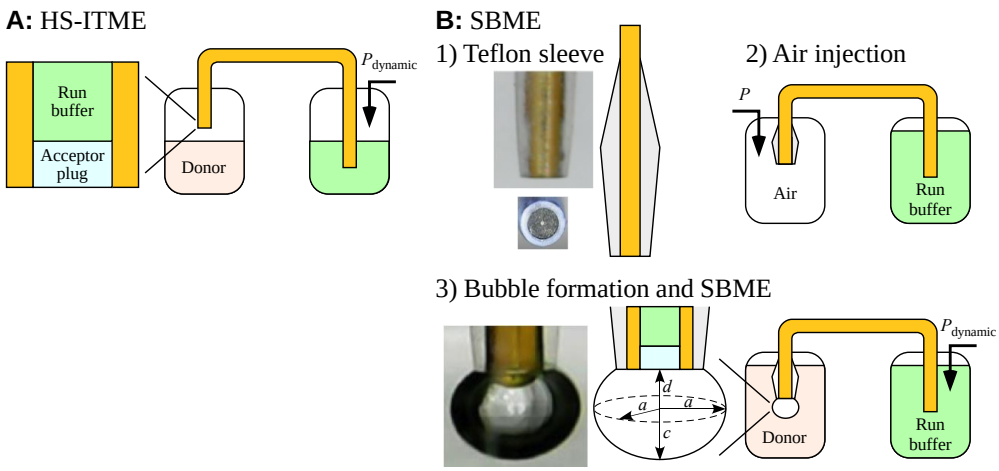


**Figure 1.** Schematics of (A) HS-ITME process and (B) SBME process; 1) Teflon sleeve installation; pictures of the assembly and inlet surface are shown, 2) air injection applying pressure *P*, and 3) single bubble formation and SBME with dynamic extraction applying $P_{dynamic}$; an image of a single bubble attached to a capillary of 360 $\mu$m od and its oblate spheroid model are shown.

## 3.4 SBME-CE

Fig. 1B shows the procedures of SBME-CE. 1) First, a Teflon sleeve was put over the capillary inlet to hold a single bubble of submicroliter volume stably at the capillary tip. The sleeve was made of a 12-mm long polytetrafluoroethylene tube with 300 $\mu$m id and 800 $\mu$m od (Cole-Parmer, Chicago, Illinois, USA). As shown in Fig. 1B1, the tube was tapered by polishing with a sandpaper in order to hold a small bubble and avoid dislocation during abrupt movements through the vial caps. It was important for bubble stability to polish the sleeve tip surface flat. After the capillary cartridge was installed in the CE instrument, the sleeve was manually press-fitted to be flush with the capillary inlet tip. Before each run, the capillary was rinsed with the same rinsing protocol as above, and a basic acceptor plug was injected into the capillary hydrodynamically. 2) Then, the inlet was moved to an empty vial, and a forward pressure of 5 psi was applied to introduce air into the capillary (Fig. 1B2). 3) Without removing the wrap, the capillary inlet was inserted into a sample vial containing 1.8 mL of an acidified sample donor solution, for which the temperature was controlled as in HS-ITME, and a backpressure of 4 psi was applied to drive the air out and form a bubble of submicroliter volume at the capillary inlet tip immersed in the sample solution while keeping the outlet immersed in a run buffer solution. After the bubble was formed, a backpressure of 1 psi was applied to align the acceptor plug at flush with the capillary inlet tip (Fig. 1B3). Then, HS-ITME was performed between the micro-HS (bubble) and the acceptor plug inside the capillary inlet. Dynamic extraction was also implemented in the same way as in HS-

ITME. After a desired extraction time, both the capillary inlet and outlet were placed in the run buffer vials, and electrophoresis was carried out.

The volume injected into a capillary by applying pressure is commonly estimated using the Poiseuille equation [32]. However, due to the limited precision for pressure control by the CE instrument and the surface tension of the bubble, the actual bubble volume could not be controlled or estimated reliably. Instead, the volume, $V_h$, of the single bubble depicted in Fig. 1B3, was estimated by modeling the bubble as an oblate spheroid with the cap portion removed. Then the bubble volume is given as

$$V_h = \frac{\pi}{3} a^2 c \left\{ 2 + 3\left(\frac{d}{c}\right) - \left(\frac{d}{c}\right)^3 \right\}. \quad (7)$$

The surface area of the bubble, $A_h$, was also evaluated by subtracting the cap surface area from the total surface area of the oblate spheroid. The surface area of an oblate spheroid is given by [33]

$$A_{\text{oblate}} = 2\pi a^2 + \frac{\pi c^2}{e} \ln\left(\frac{1+e}{1-e}\right), \quad (8)$$

with $e = \sqrt{1 - c^2/a^2}$. The surface area of an ellipsoidal cap was evaluated by using Keisan Online Calculator (https://keisan.casio.com/exec/system/1311572253).

# 4 RESULTS AND DISCUSSION

## 4.1 HS-ITME

### 4.1.1 Optimization

**Donor phase:** In HS-extraction of an acidic analyte, it is desirable that the acidic sample donor solvent does not interfere with the extraction process; for example, acidic components of the evaporated solvent may reduce the buffer capacity of the basic acceptor phase, hampering deprotonation of the acidic analytes. In previous HS-ITME, the donor pH was varied from 1.5 to 4 by controlling the HCl concentration, and pH 3 was chosen as optimal [22]. In this work, further optimization was conducted by comparing 1 mM $HNO_3$ (pH 3), 1, 10, and 50 mM $H_3PO_4$ (pH 2.2–2.3), and 10 mM potassium phosphate buffer (pH 3) as a donor solvent. Fig. 2A compares the EFs obtained from 10-min HS-ITME-CE at 25°C of 1 $\mu$M chlorophenols using the run buffer as an acceptor phase. Based on EF values, reproducibilities, and convenience, 10 mM $H_3PO_4$ was chosen as optimal.

**Acceptor phase:** The driving force in HS-ITME is the high affinity of acidic chlorophenols for the basic acceptor phase dissolving the chlorophenols in their deprotonated forms. It is thus required that the acceptor phase has a buffer capacity sufficiently large enough to maintain its extraction power, even for a sample in a complicated matrix, but without harming the quality of the subsequent electrophoretic separation. Fig. 2B compares the EFs obtained from 10-min HS-ITME of 1 $\mu$M chlorophenols in 10 mM $H_3PO_4$ with various acceptor phases, such as run buffer, 600 mM borate buffer (pH 10.5), and 10, 100, and 200 mM NaOH. For convenience, 240 mM borate run buffer (pH 9.2) can be used as the acceptor phase [21,22]. Although

the 600 mM borate buffer of pH 10.5 had a much larger buffer capacity than the run buffer, the peak destacking caused by the high sample conductivity lowered the EFs calculated from peak heights. Higher EFs were obtained with the acceptor phases of NaOH, partially due to the stacking effect. The EFs increased as the NaOH concentration was increased from 10 to 100 mM but decreased slightly at 200 mM, probably due to the destacking effect. Thus, 100 mM NaOH was chosen. When the acceptor plug volume was varied from 5 to 30 nL, insignificant changes in EFs were observed.

**Dynamic extraction:** In HS-ITME, the analytes in the HS are transferred to the acceptor plug only through the small opening of a capillary and accumulated at the entrance. With dynamic extraction of pushing the acceptor plug out of and back into the capillary, the mass transfer between the HS and acceptor plug can be enhanced to yield higher EFs [34-36]. When the dwell times between consecutive backpressure applications used to push out the acceptor plug were varied from 0.5 to 5 min, the maximum increase was observed at 1 min (Fig. 2C). Thus, the application of a backpressure of 0.4 psi for 5 s and a dwell time of 1 min were chosen; the acceptor plug volume was also reoptimized, and 30 nL was chosen for the small RSDs (Fig. 2D).

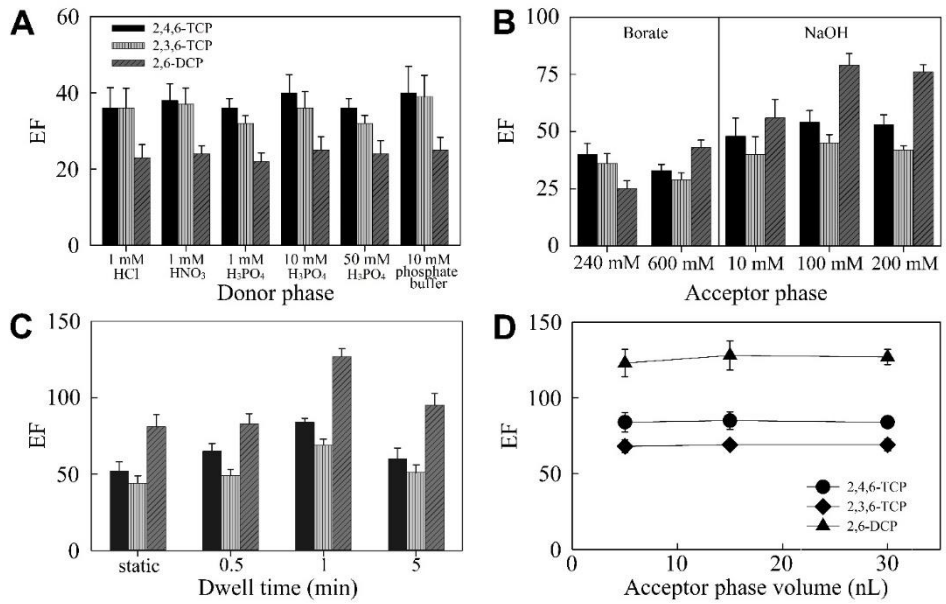


**Figure 2**. Optimization of HS-ITME. EFs obtained using (A) various donor phases with the run buffer as an acceptor phase, (B) various acceptor phases with 10 mM $H_3PO_4$ as a donor phase, (C) dynamic extraction with dwell times of 0.5, 1, and 5 min with a donor phase of 10 mM $H_3PO_4$ and an acceptor phase of 100 mM NaOH, and (D) 5, 15, and 30 nL of 100 mM NaOH as acceptor phase with 10 mM $H_3PO_4$ donor phase and dynamic extraction of backpressure 0.4 psi for 5 s with dwell time of 1 min. Extraction: 10 min, 25℃. Analytes: 1 $\mu$M of three chlorophenols. Bare fused silica capillary: 50 $\mu$m id, 360 $\mu$m od, 60 cm (effective length 50 cm). Separation: +20 kV, 214 nm, 25℃. Run buffer: 240 mM sodium borate buffer of pH 9.2. The EF values were calculated by comparing the peak heights.

**Extraction time:** The optimized conditions were then applied for HS-ITME, and the extraction times were varied from 2 to 45 min (Figs. 4A–C). The EFs were slowly increased when the extraction time was varied from 2 to 5 min and then significantly increased for 5 to 20 min. Although high EFs

were achieved for extraction times beyond 10 min, the RSDs obtained for 10 min of extraction were smaller; thus, this was chosen for HS-ITME.

### 4.1.2 Analytical performance

After optimization, HS-ITME was applied to a 10-min extraction of 1 $\mu$M chlorophenols in a standard sample solution, and the results are summarized in Table 1. The EFs were 84 ± 3, 69 ± 4, and 127 ± 5, for 2,4,6-TCP, 2,3,6-TCP and 2,6-DCP, respectively with the RSDs of migration times ranging from 0.6 to 0.7% and peak heights ranging from 3.0 to 5.8% ($n$ = 4). The LODs and LOQs estimated from the peak heights were in the range of 1.9–2.7 ppb and 6.2–9.1 ppb, respectively.

**Table 1.** HS-ITME analytical performance for standard samples

| Analyte | EF ± SD ($n$ = 4) | %RSD | | LOD (ppb) | LOQ (ppb) |
|---|---|---|---|---|---|
| | | MT | PH | | |
| 2,4,6-TCP | 84 ± 3 | 0.6 | 3.0 | 2.5 | 8.3 |
| 2,3,6-TCP | 69 ± 4 | 0.7 | 5.8 | 2.7 | 9.1 |
| 2,6-DCP | 127 ± 5 | 0.7 | 3.9 | 1.9 | 6.2 |

Donor: 1 $\mu$M analytes in standard solution with 10 mM $H_3PO_4$, 1.1 mL. Acceptor: 100 mM NaOH, 30 nL. Extraction: 10 min, 25℃. Dynamic extraction: back pressure of 0.4 psi for 5 s and dwell time of 1 min. Bare fused silica capillary: 50 cm (effective length)/60 cm. Run buffer: 240 mM sodium borate buffer of pH 9.2. Seperation: +20 kV, 25℃, 214 nm.

MT; migration time, PH; peak height

## 4.2 SBME

### 4.2.1 Optimization

SBME is a combination of micro-HS (single bubble) extraction and ITME, and its optimization was focused on the single bubble formation and the extraction time. Other the optimized conditions for HS-ITME, as described above, were adopted for SBME.

**Single bubble formation:** SBME starts with the formation of a bubble stably attached to a capillary inlet tip immersed in a sample solution. As in SDME-CE [37], the bubble stability could be improved by coating the capillary inlet end surface. However, the unwanted coating of the capillary inner wall near the inlet caused a poor reproducibility in the bubble formation. When a strong cleaning solution, such as 1 M KOH in methanol, was applied to remove the coating on the inner wall, the capillary became fragile after a few runs. Moreover, the bubble attachment to the coating on the small capillary end surface was insufficient to hold the bubble stably. To resolve this problem, a tapered Teflon tube, as shown in Fig. 1B1, was sleeved over the capillary inlet. Its hydrophobic and larger end surface area provided sufficient support for a stable bubble. The tapered shape of the Teflon sleeve ensured trouble-free insertion through the wrap over the sample vials of the commercial CE instrument.

**Sample volume:** When the sample vial was completely filled with a sample solution to prevent the loss to the HS above sample, it was difficult to form a stable bubble due to the increased pressure in the sample vial. Hence, the 2-mL sample vial was filled with 1.8 mL of sample solution to enable easy formation of a bubble.

**Bubble volume:** The effect of bubble volume, thus the surface-to-volume ratio effect, was investigated by varying the volume from 100 to 700 nL for 5-min dynamic SBME of 2 $\mu$M chlorophenols in 10 mM $H_3PO_4$ to the 30-nL acceptor plug of 100 mM NaOH. With a smaller bubble volume, thus the larger surface-to-volume ratio, the EF values were higher (Fig. 3). Although the EFs obtained from a 100-nL bubble was the largest, it was difficult to form a small bubble reproducibly and to place it at the center of the sleeve in line with the capillary opening. For convenience and reproducibility, thus, a bubble volume of 200 nL was chosen.

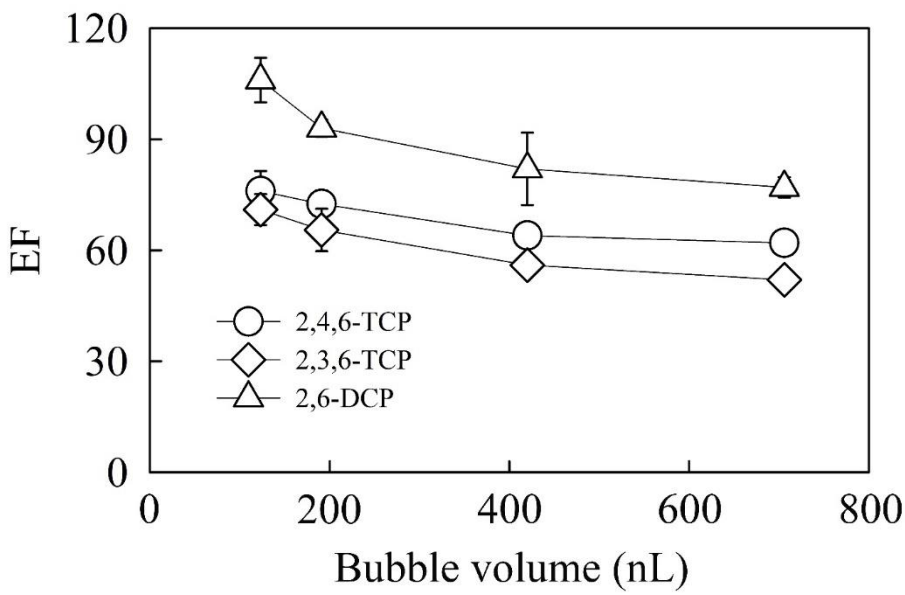


**Figure 3**. EFs from 5-min SBME *vs*. bubble volume. Donor phase: 2 $\mu$M analytes in 10 mM $H_3PO_4$, 1.8 mL. Acceptor phase: 100 mM NaOH, 30 nL.

**Extraction time:** When the extraction times were varied from 2 to 45 min, the EF values from SBME increased rapidly from 2 to 5 min and then less rapidly aftterwards (Figs. 4A–C). The EFs from 5-min SBME were

similar to those from 10-min HS-ITME; thus, 5 min was chosen as the optimal time for SBME.

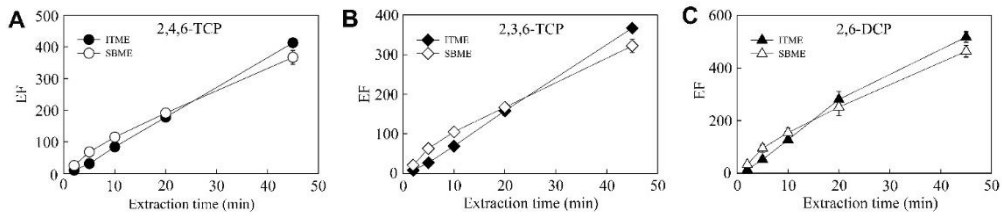


**Figure 4.** EF *vs*. extraction time for HS-ITME (closed symbols) and SBME (open symbols) for (A) 2,4,6-TCP, (B) 2,3,6-TCP, and (C) 2,6-DCP. Donor phase: 1–2 $\mu$M of three chlorophenols in 10 mM $H_3PO_4$. Acceptor phase: 100 mM NaOH, 30 nL. Others as in Fig. 2.

### 4.2.2 Analytical performance

**Standard sample:** Electropherograms from CE of 10 ppm and SBME-CE of 200 ppb standard samples are shown in Fig. 5A and B, respectively. The performance of the optimized 5-min SBME is summarized in Table 2. The method obtained EFs of 61 ± 4, 56 ± 4 and 87 ± 7 for 2,4,6-TCP, 2,3,6-TCP and 2,6-DCP, respectively ($n$ = 3) with RSDs of the migration times and peak heights, 0.7–0.9% and 6–10%, respectively. The LODs were 2.0–2.5 ppb and the LOQs were 6.6–8.4 ppb. The calibration curves showed good linearity in the range of 20–4000 ppb ($r^2$ = 0.9998), 20–5000 ppb ($r^2$ = 0.9996), and 20–4000 ppb ($r^2$ = 0.9980) for 2,4,6-TCP, 2,3,6-TCP, and 2,6-DCP, respectively.

**Table 2**. SBME-CE analytical performance for standard samples

| Analyte | EF ± SD ($n$ = 3) | %RSD | | LOD (ppb) | LOQ (ppb) | Linear range (ppb) | Linearity ($r^2$) | Calibration curve |
|---|---|---|---|---|---|---|---|---|
| | | MT | PH | | | | | |
| 2,4,6-TCP | 61 ± 4 | 0.9 | 6 | 2.5 | 8.2 | 20–4000 | 0.9998 | $y = 12.5x - 83.1$ |
| 2,3,6-TCP | 56 ± 4 | 0.7 | 7 | 2.5 | 8.4 | 20–5000 | 0.9996 | $y = 12.3x + 41.6$ |
| 2,6-DCP | 87 ± 7 | 0.7 | 9 | 2.0 | 6.6 | 20–4000 | 0.9980 | $y = 15.6x + 113.6$ |

The LODs, RSDs of MTs and PHs were evaluated with 200 ppb. $x$; Concentration (ppb), $y$; PH ($\mu$AU·s).

Donor phase: 200 ppb chlorophenols in 10 mM $H_3PO_4$, 1.8 mL. Bubble volume (HS): ~200 nL. Extraction: 5 min. Others as in Table 1.

**Tap water sample:** The electropherogram for SBME-CE of spiked tap water sample was presented in Fig. 5C. For tap water samples spiked with 200 ppb chlorophenols, smaller EFs of 53 ± 5, 46 ± 3, and 51 ± 6 ($n$ = 3) were obtained for 2,4,6-TCP, 2,3,6-TCP, and 2,6-DCP, respectively. The LODs were 3.5–3.7 ppb and LOQs were 11.6–12.4. Since there were significant differences between EFs, LODs and LOQs obtained for standard samples and tap water samples, it was necessary for quantification to address the sample matrix effects. One simple method is single-point standard addition by spiking of the unknown sample with a concentrated standard sample of small volume [38]. A 1.8-mL tap water sample containing 40 ppb chlorophenols (Sample 1) was used as a model real sample of unknown matrix effect. Applying the calibration curves for standard samples from 5-min SBME-CE, the concentrations of 2,4,6-TCP, 2,3,6-TCP, and 2,6-DCP in Sample 1 were estimated as 39, 28, and 13 ppb, respectively. Then, again using the calibration curves for standard samples, another 1.8-mL tap water sample of

40 ppb chlorophenols spiked to 80 ppb with a 20-ppm standard sample (Sample 2) was analyzed to yield the concentrations of 73, 57, and 31 ppb for 2,4,6-TCP, 2,3,6-TCP, and 2,6-DCP, respectively. Thus, the corrected concentration in Sample 1 and spike recovery were 38 (85%), 44 (73%), and 46 ppb (45%), respectively. These differences clearly show that the matrix effects should be considered for HS extraction of chlorophenols in tap water.

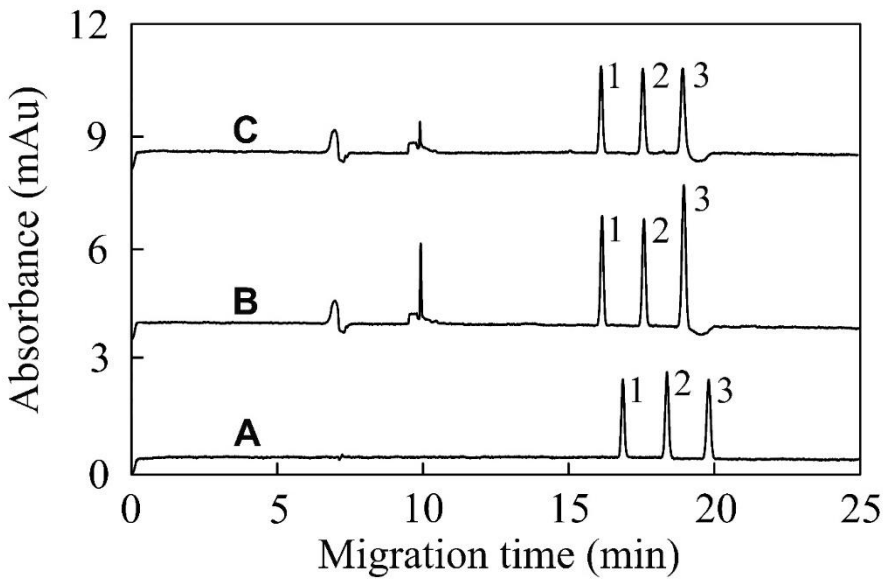


**Figure 5**. Electropherograms from (A) CE of 10 ppm CPs and (B) SBME-CE of 200 ppb CPs in standard sample solutions, (C) SBME-CE of 200 ppb CPs in tap water. Peaks: (1) 2,4,6-TCP, (2) 2,3,6-TCP, and (3) 2,6-DCP. Note that the migration times from SBME-CE are shorter than those from CE due to the acceptor plug.

## 4.3 HS-ITME *vs*. SBME

When the EF values from SBME and HS-ITME in Fig. 4 are presented as EF ratios between SBME and HS-ITME as a function of extraction time (Fig. 6A), a clear trend is observable; the shorter extraction time, the higher the EF ratios. It indicates that the smaller single bubble of a higher surface-to-volume ratio, $A_h/V_h$, was filled up with evaporated chlorophenols much faster than the larger HS of ITME of a much lower ratio, and the evaporation into the HS was a bottleneck process in the beginning of HS extraction (see Eq. 6). However, the EF ratios decreased to 1 in about 20 min, and even somewhat lower than 1 in 45 min. It indicates that the micro-HS of SBME and the HS of ITME became saturated with evaporated chlorophenols and the back-extraction into the acceptor phase through the small capillary opening became a bottleneck process. Since the capacity ratios between the acceptor phase and the HS in Eq. (4) were larger for SBME than HS-ITME, the steady state concentrations, resulting from the competition of the evaporation from the donor phase and back-extraction into the acceptor phase, would be higher for HS-ITME having a much larger reservoir of evaporated chlorophenols compared to SBME. Notice that the EFs from 5-min SBME and HS-ITME plotted together as a function of $A_h/V_h$ showed linear relationships ($r^2$ = 0.9267–0.9649), as in Fig. 6B. Theoretical understanding of these linear relationships would be an interesting subject.

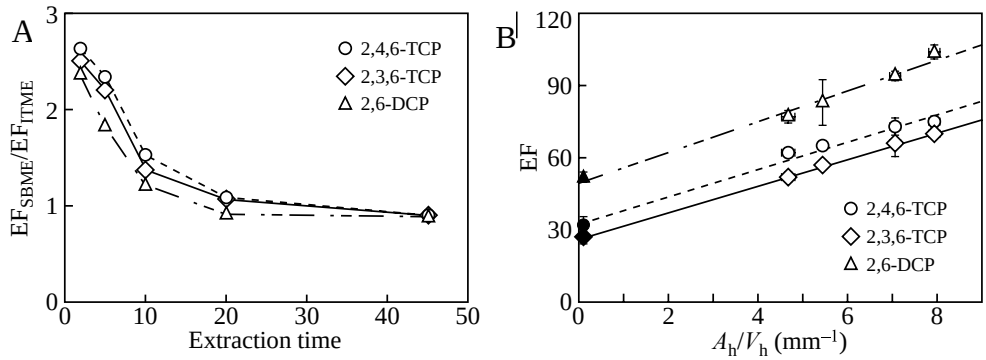


**Figure 6**. (A) Ratios of EF from SBME and EF from HS-ITME. (B) EFs *vs.* $A_h/V_h$. Linear regression curves: 2,4,6-TCP; $y = 5.66x + 32.5$ ($r^2 = 0.9595$), 2,3,6-TCP; $y = 5.50x + 26.3$ ($r^2 = 0.9649$), 2,6-DCP; $y = 6.39x + 49.6$ ($r^2 = 0.9267$)

# 5 CONCLUSIONS

A novel SBME-CE was demonstrated for the determination of chlorophenols in tap water. SBME uses a submicroliter bubble as a micro-HS and extracts the analytes evaporated into the bubble in the same manner as HS-ITME. Since the micro-HS of SBME with a higher surface-to-volume ratio was filled with the evaporated chlorophenols faster than the HS of ITME, higher EFs were obtained from SBME for a short extraction time. 5-min SBME yielded sample enrichments similar to 10-min HS-ITME. Since it is not necessary for SBME to have a sealed container to contain the HS and wait for the HS equilibration, SBME will provide a much faster analysis with fewer preparation requirements.

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# 국문 초록

상공(HS) 추출은 복잡한 매트릭스에 존재하는 휘발성 및 반 휘발성 유기 화합물에 대한 시료 전처리 기법이다. 최근 모세관 내에 위치한 받개층을 활용하여 관내 미세 농축(ITME)을 수행하고, 모세관 전기영동(CE)과 연동한 기법은 매우 간단하면서도 강력한 상공 추출 방법으로 개발되었다. 본 연구에서는, 주개층 용액에 담궈진 모세관 주입부에 형성된 방울로 마이크로리터 이하 부피(~200 nL)의 미세 상공을 활용하여 농축한 단일 방울 미세 추출법 (SBME)를 수행하였다. 방울으로 증발된 분석물질들은 관내에 위치한 받개층에 농축되며, 이후 농축물에 대해 전기 영동하였다. 방울로 형성한 상공의 부피가 기존 상공 부피(~1 mL)보다 훨씬 작기 때문에, 주개층에서 증발된 분석 물질은 신속히 상공으로 이동하며, 따라서 기존 HS-ITME 기법에 비해 훨씬 빠르게 농축 가능하다. 단일 기포의 높은 표면적 대 부피 비율로 인해 5 분동안 수행한 SBME 는 10 분 수행한 HS-ITME 와 유사한 EF 값을 나타내었다. 상온에서 수돗물에 대해 5 분동안 SBME 시, 2,4,6-TCP, 2,3,6-TCP 및 2,6-DCP 의 EF 는 각각 53, 46 및 51 이었으며, 214 nm 파장에서 흡광도를 관찰한 정량 한계는 약 12 ppb 로, 이는 세계 보건 기구 (WHO)에서 정한 2,4,6-TCP 의 음용수 최대 허용 농도치인 200 ppb 보다 훨씬 낮았다.