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Novel and simple headspace in-tube microextraction coupled with capillary electrophoresis

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ABSTRACT

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 stable. Here, we present novel but extremely simple in-tube microextraction (ITME) using the liquid inside the capillary as an acceptor phase, without forming a drop at the capillary tip. As a first example, ITME 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-ITME 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-ITME condition of 80 °C for 15 min and the limits of detections (LODs) obtained by monitoring the absorbance at 214 nm were about 4 nM. The whole procedures of HS-ITME-CE were carried out automatically using built-in programs of a commercial CE instrument.

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, in-tube microextraction (ITME) eliminating the hanging drop from SDME. A liquid plug inside the separation capillary,

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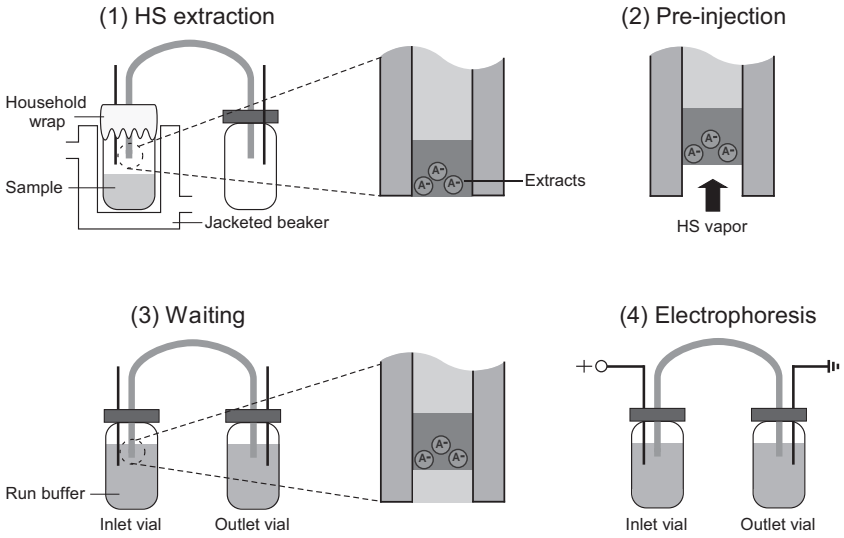

Fig. 1. HS-ITME procedures: (1) HS extraction, (2) pre-injection, (3) waiting, and (4) electrophoresis.

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 ITME 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, ITME-CE was applied to HS extraction. In HS-ITME-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 °C, the EFs obtained for chlorophenols in an aqueous acidic donor phase were up to 1100. The limits of detection (LODs: $S/N = 3$) were about 4 nM with UV absorbance detection. HS-ITME-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

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 °C 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-IT-LPME 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 °C.

2.3. HS-ITME-CE

Fig. 1 shows the HS-ITME-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