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Solvent-bar microextraction of herbicides combined with non-aqueous field-amplified sample injection capillary electrophoresis

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ABSTRACT

Solvent-bar microextraction (SBME) based on two-phase (water-to-organic) extraction was for the first time used as the sample pretreatment method for the non-aqueous capillary electrophoresis (NACE) of herbicides of environmental concern. Due to the compatibility of the extractant organic solvent and the NACE separation system, the extract could be introduced directly to the CE system after SBME. Through investigations of the effect of sample pH, extraction time, agitation speed and salt addition on extraction efficiency, the most suitable extraction conditions were determined: sample solution at a pH of 1, without added salt, and stirring at 700 revolutions per minute for 30 min. SBME as applied here was also compared with single-drop microextraction and hollow fiber-protected liquid-phase microextraction. SBME showed the highest extraction efficiency. In addition, field-amplified sample injection with pre-introduced organic solvent plug removal using the electroosmotic flow as a pump (FAEP) was used to enhance the sensitivity further in NACE. Based on studies of the effect of different organic solvents, different lengths of the organic plugs and different volumes of sample injection on stacking efficiency under the most suitable separation conditions, methanol was found to be the most efficient solvent for on-line preconcentration. Combined with SBME, FAEP-NACE achieved limits of detection of between 0.08 ng/mL and 0.14 ng/mL for the studied analytes. This preconcentration approach for NACE was demonstrated to be amenable to aqueous environmental samples by applying it to spiked river water.

1. Introduction

Capillary electrophoresis (CE) is a popular separation technique for charged molecules. However, because of the short detection optical length and low sample loading, it has some limitations in terms of sensitivity, which restricts its use for a wide range of applications. Much effort has been devoted to address this problem. Sample pretreatment and/or on-line preconcentration represent some approaches to increase the sensitivity of CE.

Since low volume sample (normally nanoliter) is injected in CE, liquid-phase microextraction (LPME) is suitable to be a sample pretreatment method for CE due to its low volume acceptor phase requirement. However, considering the compatibility of the final extract and the CE separation system, most work is focused on three-phase (water-to-organic-to-water) LPME [1–10], rather than two-phase (water-to-organic) LPME since, in the latter, the extract is organic and is therefore not suitable for direct CE analysis. In the three-phase LPME, the sample (donor) aqueous phase is generally adjusted by acid or base to provide a pH environment that is conducive for analytes to remain uncharged. The pores of the hollow fiber are impregnated with a suitable water-immiscible organic solvent, into which mass transfer of the analyte occurs from the sample solution. The hollow fiber channel is filled with an aqueous acceptor phase, whose pH range is opposite to that of the sample donor phase; analytes can be easily transferred from the intermediary organic phase to the acceptor phase. Thus, the latter phase (final extract) can be directly analyzed by reversed-phase high performance liquid chromatography (HPLC) or CE.

It would be useful to have a more direct approach to combine LPME and CE. Two-phase liquid–liquid semi-microextraction has been attempted prior to CE. Zhan et al. [11] reported a strategy to couple this extraction method with CE based on a water-insoluble sample matrix, ethyl acetate, which can be quickly decomposed on-column to ethanol and acetic acid by catalysis using a strong base. However, it is not a true miniaturized extraction method.

One mode of LPME, single-drop microextraction (SDME) normally makes use of an unprotected single drop of organic solvent for extraction based on two-phase extraction. However, recently,

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Chung's [9] and Chen's [10] groups developed on-line three-phase SDME with CE by using the separation capillary as the "drop" holder directly. Combined with the on-line preconcentration, large-volume sample injection and sweeping, satisfactory enrichment factors could be obtained. The delicate operation of this on-line three-phase SDME was required. In an alternative approach, headspace (two-phase) SDME using an aqueous drop as an acceptor phase, integrated with CE separation, has been reported [12,13]. However, this procedure is only applicable to volatile ionic analytes. There is still a need to develop suitable miniaturized sample pretreatment methodologies before CE separation, to extract and concentrate target analytes and improve sensitivity.

In addition to the above sample pretreatment methods (mainly in off-line format), emphasis has been placed on on-line preconcentration methods, especially on electrophoretic strategies because of their easy operation and satisfactory enrichment. These techniques include field-amplified sample stacking, transient isotachophoresis (ITP), sweeping and dynamic pH junction, among others [14–17].

Compared to traditional aqueous CE, non-aqueous capillary electrophoresis (NACE) has several advantages, such as reduction in Joule heating effect, decrease in wall adsorption effects and improvement of selectivity. Besides, it is especially beneficial for the separation of hydrophobic compounds that are not easily dissolved in water. NACE is complementary to aqueous CE and has attracted considerable attention [18–20]. However, some problems associated with organic solvents occur in this type of electrophoresis. For example, most organic solvents have stronger ultraviolet (UV) absorption than water, leading to higher UV background noise in NACE than that in aqueous CE. Consequently, sensitivity may be compromised; this limits the applicability of NACE. Although other sensitive detection techniques can be coupled to it, e.g. NACE has been demonstrated to be suitable for mass spectrometric (MS) detection because of the compatibility of a non-aqueous system, there is still the difficulty of integration and high expense of a mass spectrometer to contend with. It is therefore important to develop sample pretreatment and/or preconcentration methods to increase sensitivity in organic media. On-line preconcentration strategies have been developed to address the problem. However, due to the very low conductivity of non-aqueous media, a simple or direct high field injection method cannot be satisfactorily used for this purpose [21]. Hitherto, there are few reports on on-line preconcentration techniques in NACE [21–30].

Large-volume sample stacking (LVSS) injection using the electroosmotic flow (EOF) pump has been employed to stack trace amounts of negatively charged species [22–27], such as phenolic compounds, aromatic sulfonates, benzoic acid and parabens. The sample in low conductivity solvent is injected under negative voltage, while EOF acts as a pump to remove the sample matrix. In this case, the limits of detection (LODs) of the studied analytes can reach μg/L levels. For basic compounds, quinolizidine alkaloids, the same strategy was applied but with positive voltage, and LODs between 0.0210 ng/mL and 0.0446 ng/mL were achieved with NACE–MS analysis [28]. Tsai et al. [29,30] designed "ultrahigh conductivity zone" and "low temperature bath" systems to obtain large-volume sample injection in NACE. The former system achieved on-line stacking by insertion of an "ultrahigh conductivity zone" between the sample zone and background solution. Due to the sudden increase in conductivity, the analytes would accumulate within a particular segment of the capillary. The low temperature bath procedure was also based on a similar principle but it was achieved by lowering the temperature to obtain high conductivity. By this means, for the model compound, 3,4-methylenedioxymethamphetamine, enhancement could reach several hundred-fold. Additionally, transient ITP [21] has been used in NACE to improve LODs and to reverse the deleterious effects of salts in the sample.

In the present work, we combine off-line sample pretreatment and on-line preconcentration stacking methods to enhance NACE sensitivity, using several commonly used herbicides as test analytes. There are several novelties relating to this approach presented in this report:

(I) NACE could separate the model phenoxy acid herbicides without additives in the buffer, just by changing the organic solvents and electrolytes.
(II) A two-phase LPME approach (solvent-bar microextraction, SBME) was directly combined with NACE.
(III) SBME that has previously been shown to be an effective LPME procedure [31] was, for the first time, employed as the sample preparation method prior to NACE analysis.
(IV) Field-amplified sample injection and pre-introduced organic solvent plug removal with the EOF as a pump (FAEP) was effected for NACE as on-line preconcentration method.

The performance of the SBME was compared with SDME [31] and HF/LPME [32] in the present study.

2. Experimental

2.1. Reagents and materials

The Accurel Q3/2 polypropylene hollow fiber membrane (600-μm I.D., 200-μm wall thickness, 0.2-μm pore size) used in this work was bought from Membrana (Wuppertal, Germany). HPLC-grade methanol and acetonitrile, sodium hydroxide, hydrogen chloride, sodium chloride, 1-octanol, acetic acid and ammonium acetate were purchased from Merck (Darmstadt, Germany). 2,4-Dichlorophenoxy acetic acid (2,4-D) and 2,4-dichlorobenzoic acid (2,4-DCBA) were supplied by Fluka (Buchs, Switzerland); 4-amino-3,5,6-trichloropicolinic acid (Picloram), 3,5-dichlorobenzoic acid (3,5-DCBA) and 2-(2,4,5-trichlorophenoxy) propionic acid (Fenoprop) were purchased from Aldrich (Milwaukee, WI, USA); and 2-(2,4-dichlorophenoxy) propionic acid (Dichlorprop) was obtained from TCI (Tokyo, Japan).

All of the standards were prepared in methanol separately as 1000 μg/mL stock solutions and then diluted to give working solutions at different concentrations. All the analytes were diluted with methanol to 20 μg/mL for experiments relating to the optimization of NACE conditions, and to 100 ng/mL for experiments to determine the stacking conditions. They were diluted to 50 ng/mL with ultrapure water for determining the most suitable SBME conditions, unless otherwise stated. Water samples were collected from a local river.

2.2. Instrumental

The CE experiments were performed on a HP^{3D} CE system (Hewlett-Packard, Waldbronn, Germany) equipped with on-column diode array detection system. A 51-cm-long, 50-μm-i.d. bare fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) having a detector window at 42.3 cm from the inlet, was used for separation. Before use, each capillary was pretreated with 1 M sodium hydroxide for 2 h, water for 10 min, 1 M hydrochloric acid for 2 h and then water and methanol for 10 min, in that order. Before use, the above pretreated capillary was flushed with the solution buffer for 30 min. Wavelengths in the range of 190–400 nm were scanned for the detection of herbicides and 240 nm was chosen for the subsequent detection. Data were collected by HP^{3D} ChemStation software. The LODs were calculated according to peak height at a signal-to-noise (S/N) ratio of 3.

The apparent pH of the buffer was measured using a pH meter, calibrated with aqueous standard buffer solutions. Before use, the buffer was degassed in an ultrasonic bath (Midmark, Versailles, OH, USA) for 5 min and filtered through a membrane of 0.25-μm pore size.

2.3. Preconcentration procedure

FAEP was carried out as follows: pure organic solvent or mixture of organic solvents was first hydrodynamically injected into the capillary at a constant pressure (e.g. 50 mbar) for different durations. A high electric field at the injection point was established because of the lower conductivity of the solvent phase than that of the separation buffer [33]. Following this, the inlet of the capillary was placed in the sample reservoir. Electrokinetic injection (−10 kV) was used to introduce a considerable amount of the herbicides into the capillary in their ionic forms over different durations. Meantime, the EOF pump removed most of the pre-introduced organic solvent plug out of the capillary via the inlet, due to the oppositely moving direction of the EOF and the analytes. The voltage was then turned off and the inlet end of the capillary was returned to the buffer reservoir. Finally, a negative voltage (−30 kV) was applied for the separation.

Fresh analyte samples and running buffer were used for each injection. After each run, the capillary was flushed with buffer for 3 min.

2.4. SBME procedure

SBME is based on a "solvent bar", a sealed hollow fiber membrane in a tube configuration, containing organic solvent (acceptor phase) inside its channel. Extraction was carried out according to our previous work [31]. Briefly, the hollow fiber membrane was cut manually and carefully into 2.0-cm lengths. The segments were ultrasonically cleaned in acetone and dried in air before use. One end of the membrane was heat-sealed (with a conventional plastic bag sealing device). A 10-μL aliquot of 1-octanol was withdrawn into the microsyringe with a cone needle tip. The needle tip was inserted into the open end of the hollow fiber membrane and the organic solvent was infused into the fiber channel. The fiber was then immersed in the organic solvent for 20 s for impregnation of the porous wall. After impregnation, the membrane was carefully removed from the needle, and its open end was heat-sealed. The "solvent bar" was then placed in the sample solution for extraction. After extraction for a prescribed time under different stirring speeds, the solvent bar was taken out and one end of the fiber was trimmed off. The analyte-enriched solvent was withdrawn into the syringe. Finally, the extract was diluted with methanol (2×) (see below for an explanation of this), transferred to the sample vial and directly introduced into the CE system for analysis.

3. Results and discussion

3.1. Optimization of NACE separation conditions

To achieve baseline separation of various herbicides, different approaches in CE have been employed, such as micellar electrokinetic chromatography (MEKC) using surfactant micelles [34], MEKC using surfactant micelles and modified with cyclodextrins [35], and buffer pH manipulation [36]. Compared to aqueous CE, an advantage of NACE is the improvement in selectivity [37]. Since the "levelling effect", which is defined as a solvent's ability to level the effect of a strong base or acid in it, is sometimes not obvious in non-aqueous system, the difference between two closely related structures is normally larger than that in aqueous media. In addition, in NACE, selectivity and resolution can be controlled simply by changing the organic solvent or varying the composition of a mixture of organic solvents [18,19]. Hence, due to the availability of a variety of organic solvents, the choice of buffer is very wide, under the condition that the solubility of electrolytes is satisfied.

Methanol and acetonitrile were the solvents investigated in this work. They are the most commonly used solvents in NACE. Additionally, they have good miscibility with other solvents, allowing them to be compatible with various sample pretreatment methods.

Generally, it is difficult to separate strong acids on a bare fused-silica capillary, because the direction of the EOF and that of the electrophoretic mobilities of the analytes are normally opposite to each other, a condition defined as "counter-EOF" mode. The difference between their magnitudes is not sufficient to achieve separation. However, at certain pH values, acids can be ionized to some degree while the EOF is suppressed. As a result, the analytes have sufficient electrophoretic mobilities to overcome the EOF. Separations can be therefore realized in the counter-EOF mode [38]. It is a direct separation approach without the need of any additive in the buffer. In addition, as described below, separation could be achieved under negative voltage NACE, with counter-EOF mode, to facilitate sample stacking.

Based on the above discussion, acidic or near acidic buffers were studied for the separation of the target herbicide acids in the counter-EOF mode. The appropriate buffer served to suppress the EOF while providing conditions under which the analytes could still be ionized. Acetic acid and ammonium acetate were chosen as electrolytes, which are usually used in NACE. When the concentration of acetic acid was fixed at 1 M while that of ammonium acetate was varied from 50 mM to 20 mM in a 75% methanol–25% acetonitrile system, the migration time of analytes was prolonged. It may be a result of the decreasing apparent pH of buffer from 5.21 to 5.01 and then to 4.79 when ammonium acetate concentration decreased from 50 mM to 25 mM and then to 20 mM. Resolution was enhanced with decreasing ammonium acetate concentration. However, this led to peak tailing. Hence, a compromised concentration of 25 mM ammonium acetate was selected.

When 1 M acetic acid–25 mM ammonium acetate was used as the electrolyte with different proportions of acetonitrile in methanol, with increasing acetonitrile proportion, migration time shortened correspondingly. This may be attributed to two factors. On the one hand, the viscosity of the system increased with increasing acetonitrile proportion. On the other, the apparent pH of the system also increased, corresponding to the increased proportion of acetonitrile. The apparent pH values were from 4.75 to 5.01 to 5.25 and then to 5.30 as the proportion of acetonitrile increased from 0% to 25% to 50% and then to 75%, respectively. Both of the above factors might have led to simultaneous increase in the EOF and electrophoretic mobilities of the test analytes. However, the magnitude of the increase may be different. The ultimate result depended on the mutual impact of these two factors. Given the phenomenon as observed here, it may be that increase in mobilities of analytes was larger than that of the EOF. Therefore, the apparent analyte mobilities, which are the sum of the EOF and electrophoretic mobilities, were increased. As a result, separation was faster. Another factor to be noted is that the ionization ability of the individual analyte may be exhibited quite differently in the presence of different components/proportions of organic solvents. The change of the ionization state of the analytes is complex and difficult to predict in a non-aqueous system.

Meanwhile, the resolution was reduced with the increasing proportion of acetonitrile. In a pure methanol system, 2,4-D and Fenoprop can be baseline-separated while in the presence of 75% acetonitrile, 2,4-DCBA and Fenoprop comigrated. Pure acetonitrile was not investigated because of the limited solubility of the electrolyte in it. Considering the need for satisfactory resolution and simplicity, pure methanol was considered acceptable.

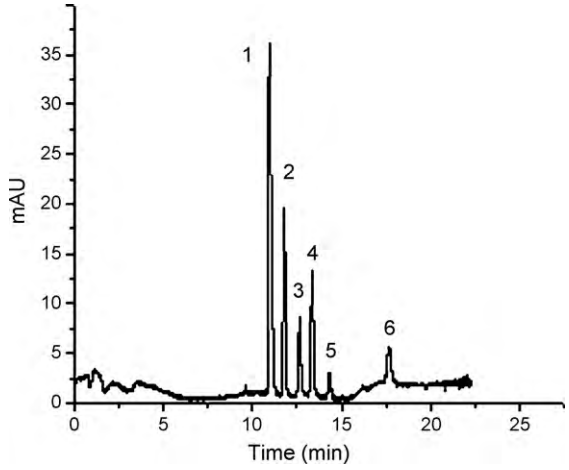

Fig. 1. An electropherogram of acidic herbicides. Conditions: buffer: 25 mM ammonium acetate–1 M acetic acid in methanol; separation voltage: −30 kV; sample: 20 μg/mL; sample injection: −5 kV, 10 s. Peaks: (1) Picloram; (2) 2,4-DCBA; (3) Fenoprop; (4) 2,4-D; (5) Dichlorprop; and (6) 3, 5-DCBA.

Based on the above observations, 25 mM ammonium acetate and 1 M acetic acid in methanol were selected for NACE separation. An electropherogram of all the analytes under this condition is shown in Fig. 1. This optimized NACE separation condition was employed in the following experiments.

3.2. Preconcentration procedure

In our previous work [36], a method termed field-amplified injection and water removal using the EOF pump (FAEP) was successfully developed for the CE determination of phenoxy acid herbicides. A non-ionic hydroxylic polymer, hydroxyethylcellulose, was employed to suppress the EOF and enhance the separation. The water plug introduced before the sample injection helped to maintain field enhancement and acted as a reservoir to hold the sample. The presence of the water plug also improved reproducibility. A 3000-fold preconcentration factor was obtained in that work, as compared with the conventional CE injection mode. Herein, we developed a similar strategy, but based on the introduction of organic solvent plug before the sample injection, into NACE to achieve on-line preconcentration and enhance sensitivity.

3.2.1. Effect of sample injection volume on stacking efficiency

The effect of sample injection volume on stacking efficiency was investigated through different sample injection durations (from 15 s to 120 s) at constant voltage (−10 kV) with a 2 s pre-introduced methanol plug at 50 mbar. The peak area was proportional to the sample injection time from 10 s to 60 s for all the test analytes ($R > 0.9900$, results not shown). However, when the sample injection time was more than 60 s, the peaks broadened gradually with further increase in injection duration. The peak heights also did not show an obvious increasing trend. It was possible that the analytes passed through the boundary of the pre-introduced organic solvent plug and running buffer, dispersing into the buffer, since the field enhancement was not that high in NACE due to the small conductivity in the separation zone. In addition, diffusion of the samples might also have been possible when longer injection durations were involved. As a result, stacking of the analytes was incomplete and separation efficiency was compromised. Based on this observation, a sample injection time of 60 s at −10 kV was chosen.

3.2.2. Effect of different organic solvents as pre-introduced plugs on stacking efficiency

A short plug of water or mixture of water and organic solvent can be introduced before sample injection in aqueous CE to enhance sensitivity [14,33,39]. Since the choice of organic solvents is relatively unlimited, and different organic solvents have dissimilar chemical and physical properties, introduction of different organic solvents prior to sample injection in NACE is supposed to have variable influence on the subsequent stacking process. This pre-introduced plug should be compatible with the separation buffer, to avoid potential precipitation of the analytes because of solubility problems. Here, methanol, acetonitrile and mixture of them were investigated as pre-introduced plugs.

The present procedure was also compared with large-volume sample injection (LVSI), which was carried out without a pre-introduced plug, as shown in Fig. 2(a). Since the directions of electrophoretic mobilities and the EOF are opposite to each other, under negative voltage, LSVI can be realized easily without reversing the voltage. Compared to the normal sample injection, the sensitivity related to LVSI was obviously enhanced, with LODs decreasing by 1–2 orders of magnitude for the individual analytes.

However, in the presence of the pre-introduced organic solvent plugs (the pressure was fixed at 50 mbar and introduction duration at 2 s), whether methanol, acetonitrile, or combination of them was used, all showed enhanced CE peak intensities compared to LVSI, as illustrated in Fig. 2(b)–(d). This result obviously indicated that stacking occurred no matter which kind of organic solvent was used. Since the conductivity of organic solvents was lower than that of the running buffer, a higher electric field could always be established. Thus, any analyte was assumed to have a higher electrophoretic velocity. This implied that greater amounts of analytes could be injected when subjected to the same sample injection duration as LVSI. Meanwhile, once they reached the boundary of the organic plug and buffer, the analytes would slow down owing to the sudden increase in ionic concentration of the buffer, leading to the stacking. After the sample matrix and organic plug were pumped out by the EOF, the separation began.

However, different organic plugs have significantly different impacts on stacking efficiency. From Fig. 2, it can be clearly seen that methanol plug exhibits the highest stacking efficiency amongst the three types of plugs studied. In the case of methanol, LODs can be decreased further by more than one order of magnitude compared to LVSI. The possible reason is due to the difference in ε/η ratios of different organic solvents. Methanol has the lowest ε/η ratio (60.0 cP^{-1}) and acetonitrile the highest (110.0 cP^{-1}), while mixtures of them should have ratios in between these two extremes [40]. Therefore, stacking efficiency decreased from the use of methanol to the mixture, and then to acetonitrile under the same condition.

3.2.3. Effect of pre-introduced organic solvent plug lengths on stacking efficiency

The effect of organic solvent plug (methanol) lengths was investigated for different injection durations at a constant pressure (50 mbar), from 0.5 s to 60 s. A long injection duration represents a long plug length.

Fig. 3 shows the relationship between the duration of methanol injection and peak areas of the analytical signals. The latter increased with increasing duration from 1 s to 10 s, and then decreased with further increase in the duration from 15 s to 30 s. When plug injection duration was more than 45 s, the CE peak areas decreased gradually, with a reduction in separation efficiency. A possible reason may be that organic plug provided a higher electric field because of its lower conductivity, which facilitated sample stacking. However, with a further increase in the pre-introduced plug length, a longer time would be needed to pump the plug out

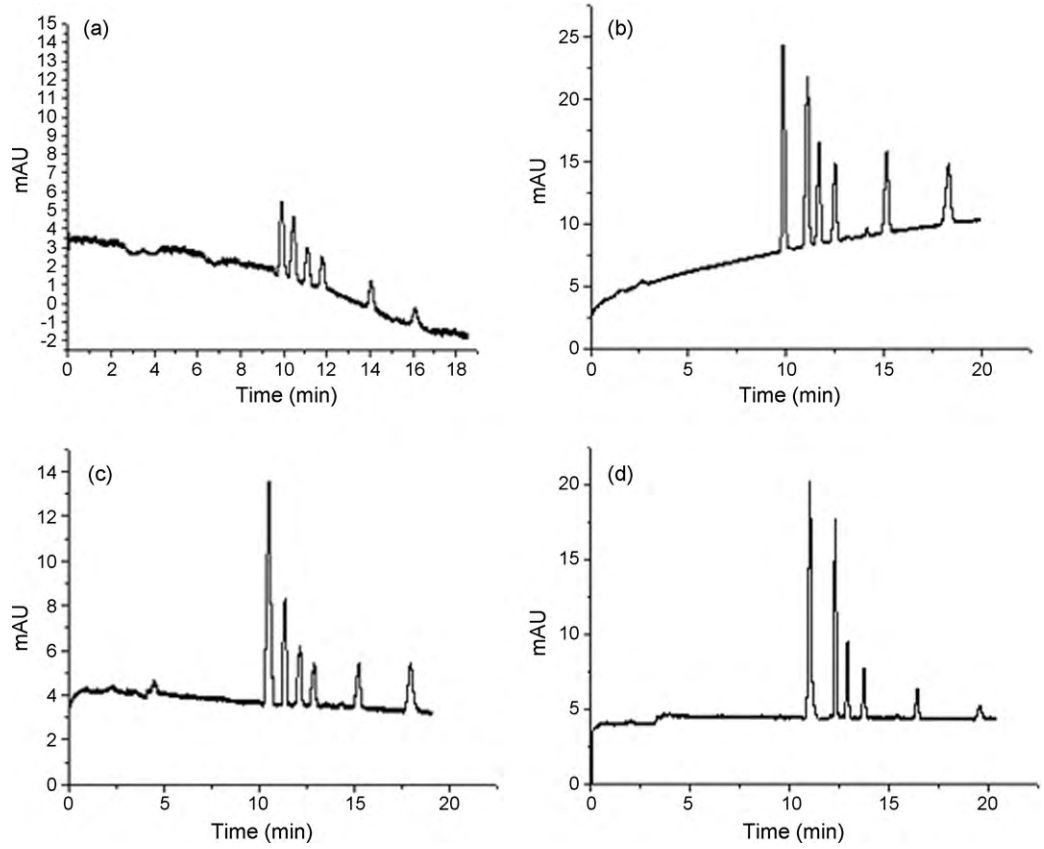

Fig. 2. Electropherograms with different pre-introduced organic plugs. (a) LVSI, sample injection: −10 kV, 60 s; (b)–(d): different pre-introduced organic solvents (50 mbar, 2 s) followed by sample injection (−10 kV, 60 s). (b) Methanol; (c) acetonitrile; (d) methanol:acetonitrile (1:1, v:v). Other separation conditions as in Fig. 1.

of the capillary before separation began. Thus, the sample plug conceivably began to undergo diffusion during stacking. Consequently, the peaks would broaden gradually, resulting in reduced stacking efficiency.

In addition, stacking in non-aqueous media may differ from that in aqueous medium. In an aqueous system using an EOF pump to remove the pre-introduced plug, the long plug can be applied both to provide proper field amplification and to act as a medium to hold the injected anions [26,36]. However, when a long plug of organic solvent was introduced in NACE, stacking was severely compromised and the current was easily disrupted.

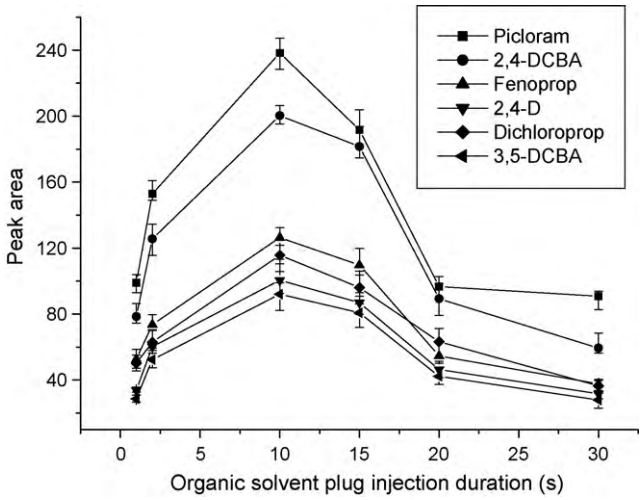

Fig. 3. Methanol injection duration versus peak area.

3.3. Optimization of SBME

SBME was originally introduced by Jiang and Lee [31]. In this method, the extracting organic solvent was confined within a short length of a polypropylene hollow fiber, sealed at both ends. This solvent bar can be directly placed in the sample solution and subjected to agitation during extraction. Due to the stirring and the random tumbling of the solvent bar, mass transfer between aqueous phase and organic phase is facilitated, thus resulting in high extraction efficiency. In the case of pentachlorobenzene and hexachlorobenzene, used as model compounds, which were determined by gas chromatography-electron capture detection, enrichment factors of up to ~110-and ~70-fold respectively could be achieved. In addition, owing to the protection offered by the hollow fiber, the procedure can be directly used for "dirty" samples, such as soil slurries, thus eliminating matrix interferences effectively. This approach was demonstrated to have higher extraction efficiency than that of hollow fiber (HF)–LPME and SDME. Other independent reports have demonstrated the effectiveness of SBME [41,42]. Very recently, we employed a silica monolith as the solvent holder in another SBME approach [43].

In this work, SBME using a polypropylene hollow fiber as the solvent holder, was chosen as the sample pretreatment procedure prior to NACE. This was the first time two-phase (water-to-organic phase) LPME was directly used for CE because of the compatibility of the NACE separation system with the organic nature of the final extract.

3.3.1. Selection of organic solvent for extraction

To choose a suitable organic solvent for SBME, some factors should be considered. First of all, the test analytes should have good solubility in the organic solvent to ensure high enrichment.

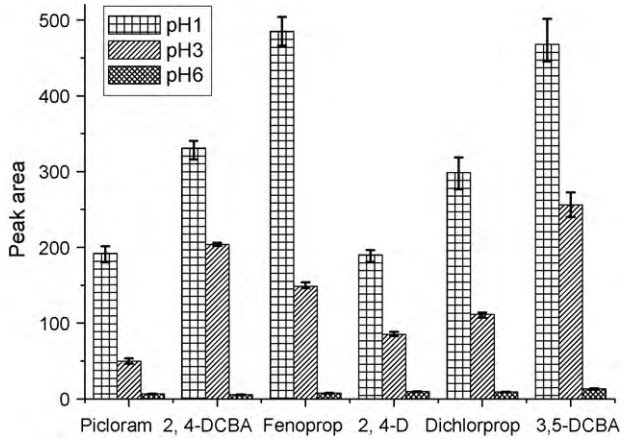

Fig. 4. Influence of sample pH on SBME.

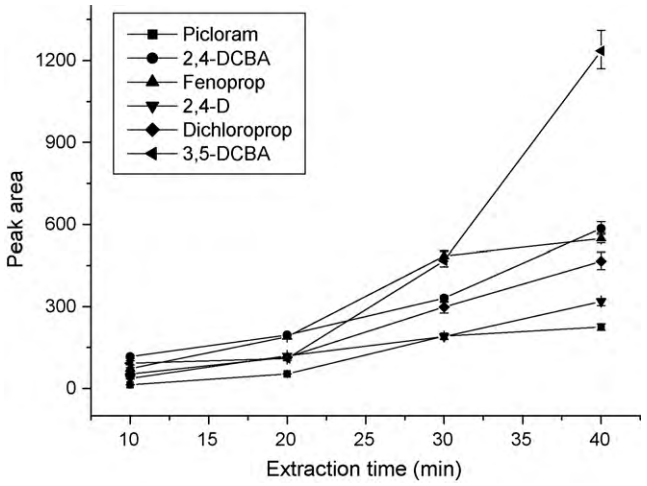

Fig. 5. SBME time profile.

Obviously, the solvent should also be immiscible with water. Additionally, the solvent should preferably possess a low vapor pressure to prevent loss during stirring, especially in this study, because the solvent "bar" is exposed to air to retrieve the extract after extraction. Based on these considerations and referring to our earlier work [44], 1-octanol was selected.

Commonly used water-immiscible solvents cannot maintain a sufficiently high current along the capillary due to their poor electrical conductivity [11]. In addition, 1-octanol has high viscosity. Thus, in the case of 1-octanol as sample matrix, less analytes will be introduced into the capillary. Increasing the injection time may be a solution to introduce more analytes. However, if the injection duration is prolonged, peak broadening will result. Therefore, to address this issue, the extract was diluted 2× with methanol to ensure reasonable electrical conductivity of the sample matrix, whilst at the same time does not reduce the concentration of the analytes by any appreciable degree.

3.3.2. Effect of sample solution pH

SBME efficiency of the herbicides is influenced by their ionization, due to their acidities. The pH values of sample solutions of between 1 and 6 were studied for their effect on extraction. As shown in Fig. 4, the highest CE peak responses were obtained when the pH value was about 1. At this pH, the studied analytes, whose pK_a values are between 2 and 5, existed in their neutral forms and therefore their extraction into the organic phase was facilitated.

3.3.3. Extraction time

Mass transfer is a time-dependent process. A series of exposure time was investigated by extracting from an aqueous solution at pH of 1, containing 50 ng/mL of each analyte at a stirring speed of 700 revolutions per minute (rpm). Fig. 5 showed that CE peak responses increased quickly within 40 min of extraction time. As reported previously [31], SBME is an equilibrium rather than exhaustive extraction process. Hence, when the extraction time was increased to the equilibrium point gradually, the extraction efficiency also mirrored this trend. The enhancement of the extraction of 3,5-DCBA after 30 min relative to the other analytes was observed. It is not clear why this was so. A possible explanation may be that with the increasing amount of the other compounds in the acceptor phase, the solubility of 3,5-DCBA increased. To avoid loss of solvent due to prolonged exposure beyond 30 min, and also to permit a practical implementation of the technique, 30 min was selected as extraction time. It was felt that an extraction time of >30 min would be undesirable for practical applications.

3.3.4. Stirring speed

Like all other modes of LPME, in SBME, the rate at which extraction equilibrium is reached can be enhanced by increasing the sample stirring speed. In our experiments, the stirring speed was investigated from 400 to 700 rpm for 30-min extraction. The relationship between peak area and stirring speed is shown in Fig. 6. Since the solvent bar circumgyrated and tumbled freely in the stirred aqueous solution, with increasing stirring speeds, the peak areas increased correspondingly. In this manner, thermodynamic equilibrium could be achieved completely and quickly. When the stirring speed was increased to 800 rpm, however, solvent loss from the hollow fiber was observed, probably due to mechanical reasons. Therefore, 700 rpm was selected as the most suitable stirring speed.

3.3.5. Effect of salt addition on SBME

The effect of the addition of salt to the sample solutions was also investigated. The influence of salt in the sample on microextraction, including LPME, efficiency, has been widely discussed, and observations have been conflicting [45]. Salting-out (increasing the extraction efficiency by the addition of salt), salting-in (decreasing the extraction efficiency by the addition of salt) or no effects have all been reported. In our study, the salt effect was investigated in the presence of 50–250 mg/mL sodium chloride. No significant change in the CE peak responses was observed in the presence of different concentrations of sodium chloride (results not shown). This

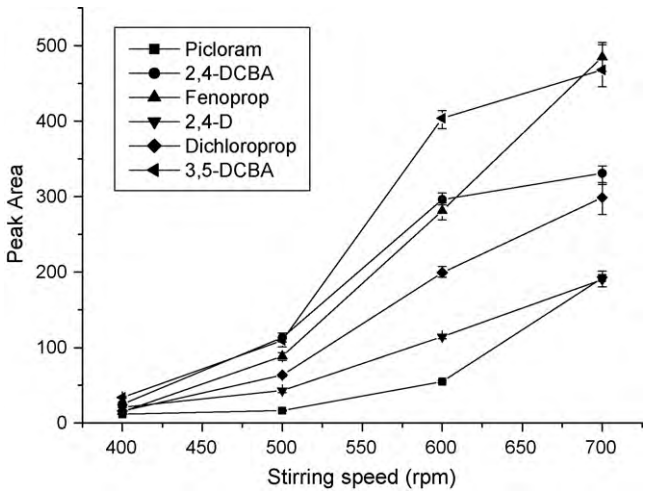

Fig. 6. Effect of stirring speed on SBME.

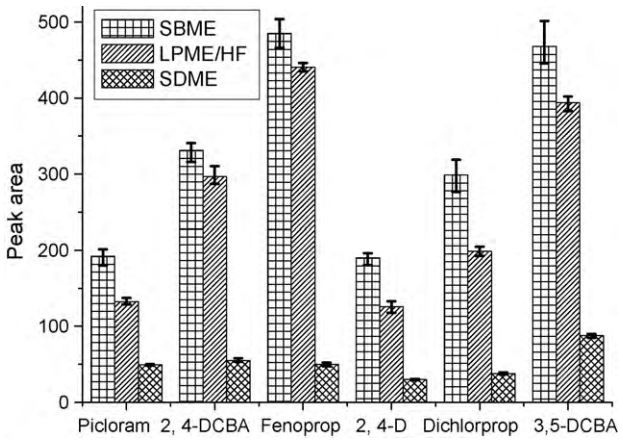

Fig. 7. Comparison amongst SBME, HF–LPME and SDME.

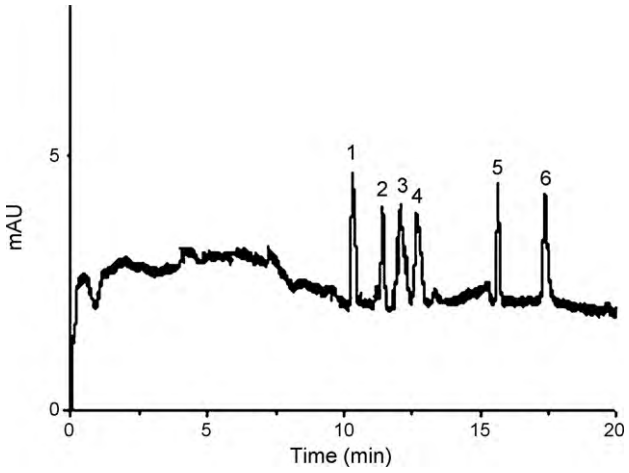

Fig. 8. An electropherogram of an extract after SBME of river water spiked with herbicides (5 ng/mL). Methanol plug: 50 mbar, 10 s; sample injection: −10 kV, 60 s. Peak identities as in Fig. 1.

was in agreement with our previous work [44], as well as several other previous microextraction reports. It is not at all clear why the salting-out effect does not appear to influence LPME significantly although it does so generally to conventional liquid–liquid extraction.

3.4. Comparison of extraction efficiency amongst HF/LPME, SBME and SDME

HF–LPME [46] uses a hollow fiber membrane to hold and stabilize the organic solvent. When the fiber is immersed in the sample solution, the target analytes are extracted into the organic phase through the wall pores of the fiber. In SDME [47], extraction takes place between the aqueous phase and a water-immiscible solvent droplet, with the droplet directly immersed in or held (for headspace mode) above the sample solution.

Identical conditions under which SBME, HF–LPME and SDME were compared included the follows: stirring speed of 700 rpm (400 rpm for SDME, since stirring at a higher rate caused the organic drop to be dislodged) for 30 min, sample solution adjusted to a pH of 1, and no salt addition. Fig. 7 compares the CE peak responses amongst these three modes of microextraction. It can be seen that SBME exhibited higher extraction efficiency than that achieved by SDME and HF–LPME. As discussed above, a leading contributory factor to this result could be the highly efficient contact of the sample solution with the organic extractant phase caused by the free tumbling of the solvent bar in SBME.

3.5. Validation

Table 1 lists the regression data and LODs of analytes generated by combining SBME and FAEP under the optimized conditions. The linearity of FAEP and SBME calibration plots was investigated over a concentration range of 0.5 ng/mL and 100 ng/mL. All the tested herbicides exhibited good linearity with good squared regression coefficients, greater than 0.9960. The LODs of these compounds, calculated at S/N = 3, were in the low ng/mL levels (0.08–0.14 ng/mL).

Table 1
Regression data and LODs of analytes combining FAEP and SBME.

Analyte	Linear range (ng/mL)	r^2	LOD (ng/mL)	RSD (n = 5)
Picloram	0.5–100	0.9996	0.10	6.09
2,4-DCBA	0.5–100	0.9993	0.10	9.57
Fenoprop	0.5–100	0.9994	0.12	9.57
2,4-D	0.5–100	0.9992	0.08	10.8
Dichlorprop	0.5–100	0.9998	0.14	10.3
3,5-DCBA	0.5–100	0.9968	0.12	7.15

The reproducibility of the peak areas was studied for five replicate experiments for an aqueous sample containing 50 ng/mL of herbicides. The RSDs for six tested herbicides were between 6.09% and 10.8%.

However, none of the target herbicides could be found in river water under the extraction conditions developed in this work (this is not surprising since the herbicides are banned from local use). Thus, river water spiked with the herbicides (at 5 ng/mL levels) was subjected to the developed procedure. Fig. 8 shows an electropherogram of an extract of spiked river water after SBME followed by FAEP-NACE. It may be observed that the electropherogram is clear of interfering substances, indicating the effective cleanup afforded by the SBME approach. However, relative recoveries, defined as the analyte peak area ratios relating to the river water and the pure water after the SBSE-FAEP procedure, for Picloram (53.4%), 2,4-DCBA (55.7%), Fenoprop (47.2%), 2,4-D (59.1%), Dichlorprop (62.2%) and 3,5-DCBA (48.5%) were determined. This indicated that the sample matrix had some influence on the SBSE-FAEP procedure, but the overall analysis was not affected.

4. Conclusions

In this study, a combination of a two-phase liquid-phase microextraction, solvent-bar microextraction (SBME), and field-amplified sample injection and organic solvent removal with the electroosmotic flow as a pump (FAEP) in non-aqueous capillary electrophoresis (NACE) to extract, preconcentrate and determine herbicides was successfully carried out. The analytes were separated by adjusting the background solvents and electrolytes, due to the opposite directions of their electrophoretic mobilities and EOF. As an on-line preconcentration method, FAEP was used in NACE. In this method, a short plug of organic solvent was pre-introduced followed by large-volume sample injection. Stacking was achieved during sample injection while the sample matrix and organic plug were removed by the EOF. After optimization of the sample injection time, and the nature of the organic plug and length, the best stacking efficiency was obtained under these conditions: methanol introduced at 50 mbar for 10 s followed by sample injection at −10 kV for 60 s. SBME conditions, such as sample pH, stirring speed, extraction time and salt concentration were carefully studied and optimized. With SBME combined with FAEP, also the first ever report of such a combination to the best of our knowledge, the LODs of the analytes considered were determined to be at ng/mL levels. Compared to normal sample injection, the

present method provided high sensitivity, with LODs lower by 3–5 orders of magnitude. The procedure was applied successfully to real river water spiked with the analytes of interest. Although the relative recoveries were not very high, there were no significant differences amongst the different herbicides. Good LODs, acceptable reproducibilities and clean electropherograms were obtained in the analysis of reasonably complex spiked aqueous samples, indicating that the developed method has potential applicability in the determination of these target compounds in genuine samples using matrix-matched standards. This SBME-FAEP-NACE approach may be helpful to overcome the limitation of the high UV background of organic solvents (that leads to sensitivity problems) and to expand the real-world applicability of NACE.

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