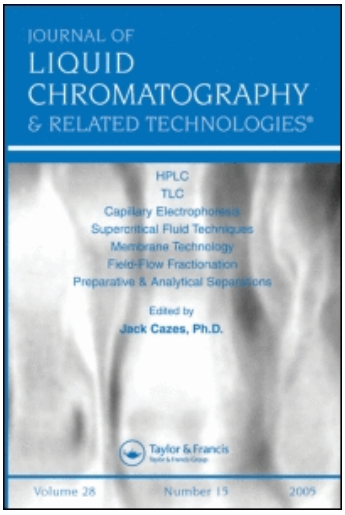

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Y. He[a]; H. K. Lee[a]

[a] Department of Chemistry, National University of Singapore Kent Ridge Crescent, Singapore, Republic of Singapore

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ENRICHMENT OF NITROPHENOLS IN WATER BY OFF- AND ON-COLUMN CONCENTRATION FOR CAPILLARY ELECTROPHORESIS

Y. He, H. K. Lee*

Department of Chemistry
National University of Singapore
Kent Ridge Crescent
Singapore 119260, Republic of Singapore

ABSTRACT

A method based on the combined use of off-column solid phase extraction (SPE) using C_{18} membrane disk and on-column field amplified injection (FAI) was developed to isolate, recover, and concentrate nitrophenols in river water prior to analysis by capillary electrophoresis (CE). The effect of eluent, sample pH, and volume in SPE, sample solvent and injection volume in FAI, and the combination of SPE and FAI was investigated in order to find optimal conditions for overall enrichment. With the use of SPE combined with FAI, extraction time was reduced, most interferences were removed, limit of detection was decreased to 5-10 ppb, and recovery of each nitrophenol was > 80%. Combination of off-column SPE and on-column FAI provides a rapid and selective approach for quantitatively concentrating nitrophenols in river water at ppb level prior to CE analysis.

INTRODUCTION

Nitrophenols, as products of photochemical reaction, have been found in rain, snow, and fog.[1-3] Additionally, nitrophenols, as decomposed products of carbamate and phosphorus pesticides, are present in polluted ground-water and river water.[4-5] Even at low concentration, some nitrophenols pose toxic effects on fish and human beings.[6] Hence, methods for their determination that offer high sensitivity and selectivity are required.

The methods of analysis of nitrophenols include gas chromatography (GC),[7,8] high performance liquid chromatography (HPLC),[9,10] and flow injection.[11] GC involves the use of derivatization and suffers the problem of peak tailing due to the high polarity of nitrophenols, especially dinitrophenols. HPLC is restricted by inadequate separation capacity as compared to capillary GC. In recent years, a number of studies were carried out to investigate the applicability of capillary electrophoresis (CE) to separate various kinds of phenolics.[12-16] In spite of the impressive separation power and short run time, the poor detection limit (ca. 1 μg/mL) of CE with on-column UV detector restricts its applicability to the analysis of phenolics at low concentrations in many aqueous environments.

Two approaches have been developed for enrichment of analytes to enhance the detection capability of CE: Off-column preconcentration by solid phase extraction (SPE)[17-19] and on-column enrichment by field amplified injection (FAI).[20-23] In many cases, SPE is faster, safer, and more efficient than traditional liquid extraction. However, it sometimes suffers the problem of low recovery and long extraction time when high enrichment is required for some compounds. FAI involves the injection of large volumes of sample dissolved in a lower conductivity buffer matrix than those used for CE separation. FAI is suitable for concentrating analytes from relatively clean matrix with low and reproducible ionic strength, but suffers the problems of low concentration factors and poor precision when used for treating a dirty sample with high ionic strength. Moreover, there will be interferences that are often enriched together with the targeted compounds. To address these problems, combined use of SPE and FAI has recently been investigated by a few works in the determination of phenoxy acids, pentachlorophenol, and pesticides.[23-25]

The objective of this study was to investigate the applicability of combined use of off-column SPE and on-column FAI for enrichment of nitrophenols from river water prior to CE analysis. It was expected that the combination would provide reduced analysis time, improved separation selectivity and enhanced detection sensitivity.

EXPERIMENTAL

Apparatus

Solid phase extraction was carried out on a 25-mm glass filtering apparatus (Millipore Bedford, MA, USA). A C_{18} membrane disk (47 mm diameter) was bought from 3M (St. Paul, MN, USA).

Field amplified concentration, separation, and detection of nitrophenols was performed on a PRINCE CE system equipped with the Btuler buffer exchanger (PRINCE Technologies, The Netherlands), with detection by UV at 220 nm on a Lambda 1000 spectrophotometer (Bischoff, Leonberg, Germany). A Chromatopac C-R6A integrator (Shimadzu, Kyoto, Japan) was used for data processing.

The fused-silica capillary used was 60 cm long (effective length 47 cm) with an I.D. of 50 μm. The support buffer was made up by 25 mM sodium borate, 20 mM phosphoric acid and 50 mM sodium dodecyl sulphate (pH 8.0).

Reagents

2-Nitrophenol (2-NP), 3-nitrophenol (3-NP), 4-nitrophenol (4-NP), 2.4-dinitrophenol (2,4-DNP), 2,5-dinitrophenol (2,5-DNP), and 4,6-dinitro-*o*-cresol (2-M-4,6-DNP) were purchased from Fluka (Buchs, Switzerland).

Sodium tetraborate was purchased from Merck (Holtenau, Germany). Phosphoric acid was purchased from Carlo Erba (Milan, Italy). Sodium dodecyl sulphate (SDS) was obtained from Fluka (Buchs, Switzerland).

HPLC-grade tetrahydrofuran and methanol were obtained from J.T. Baker (Phillipsburg, NJ, USA). The water used for the preparation of the sample and buffers were purified by a Milli-Q system (Millipore, Bedford, MA, USA).

Sample Preconcentration

The water sample was taken from the Singapore river by Bukit Timah express way. It was preconcentrated using solid phase extraction. The Empore C_{18} bonded membrane (active diameter 15 mm) was conditioned by passing 5 mL of methanol followed by 10 mL of pure water acidified with 10^{-3}M

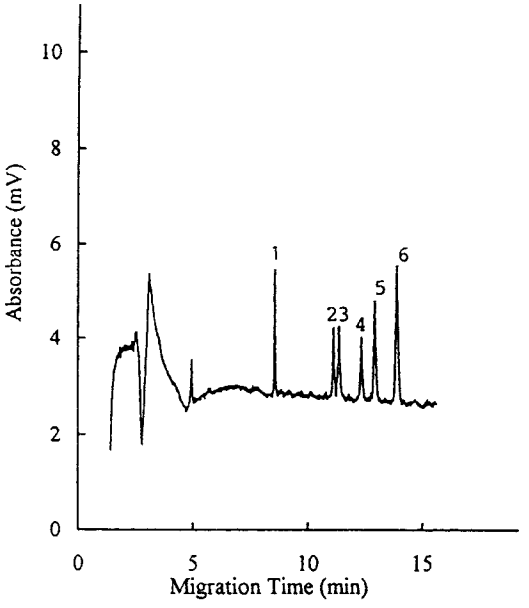


Figure 1. Electropherogram of six nitrophenols. Conditions: Column, 60 cm x 50 μm i.d. bare fused silica tube (effective length 47 cm); buffer, borate (25 mM)-phosphoric acid (20 mM)-50 mM SDS, pH 8.0; applied voltage, 20 kV; detection, UV 220 nm. Peak identities: 1. 3-NP; 2. 2-M-4,6-DNP; 3. 4-NP; 4. 2,5-DNP; 5. 2-NP; 6. 2,4-DNP.

hydrochloric acid. The 15 mL water sample acidified with 10^{-3}M hydrochloric acid was passed through (ca. 3 mL/min) the membrane disk with the aid of vacuum pump. After the sample has completely passed through, the membrane disk was washed with 2 mL of pure water acidified with 10^{-3}M hydrochloric acid, and then air dried for 5 minutes to remove most residual water and hydrochloric acid.

Elution of the sample was then accomplished by passing 0.7 mL of tetrahydrofuran (THF) through the membrane. The eluent should be soaked into the membrane for about 2 minutes before letting it pass.

The eluent, collected in a graduated test tube, was reconstituted with 0.7 mL of 12 mM borate to make up the final sample solution consisting of THF:H_2O = 1:1, 6 mM borate, pH 8.7. This sample solution was then used for on-column field amplified injection (FAI) prior to CE analysis.

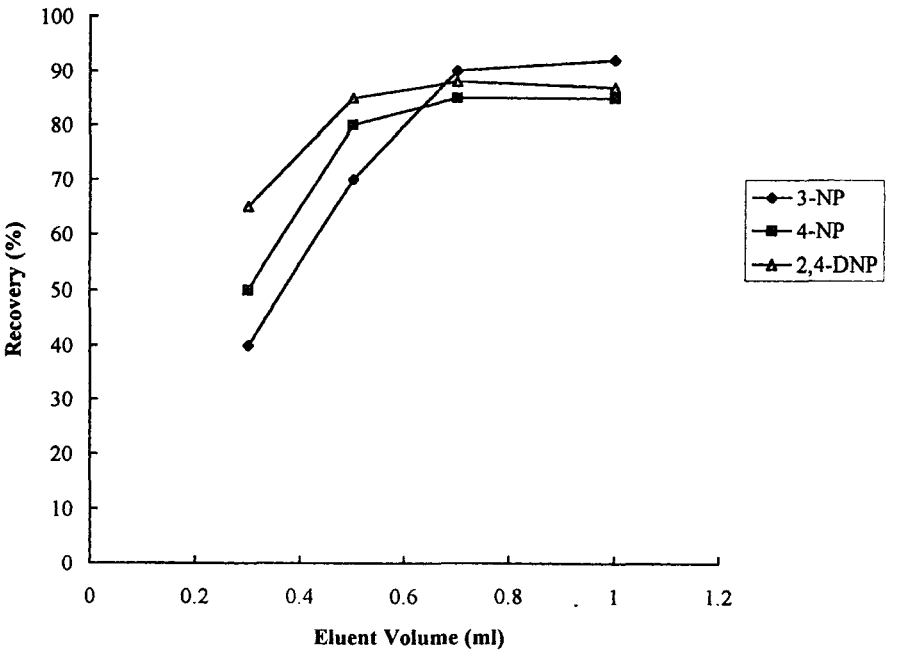


Figure 2. Recovery dependence on eluent volume for 3-NP, 4-NP and 2,4-DNP.

The percentage recoveries of nitrophenols were calculated directly as proportions of sample and standard peak areas. Recoveries were calculated as mean values of two measurements when studying the dependencies of the recovery on eluent volume, sample pH, and sample volume, and as mean values of 8 analytes in reproducibility determinations.

RESULTS AND DISCUSSION

Separation of NPs by MEKC

Experiments were first conducted to develop a separation scheme enabling the complete resolution of six NPs. Phenols can be separated as anions under CZE conditions, or either as anions or neutral molecules under MEKC conditions.[12-16] In our experiments, six NPs were well separated as anions within 15 minutes under MEKC conditions (Figure 1).

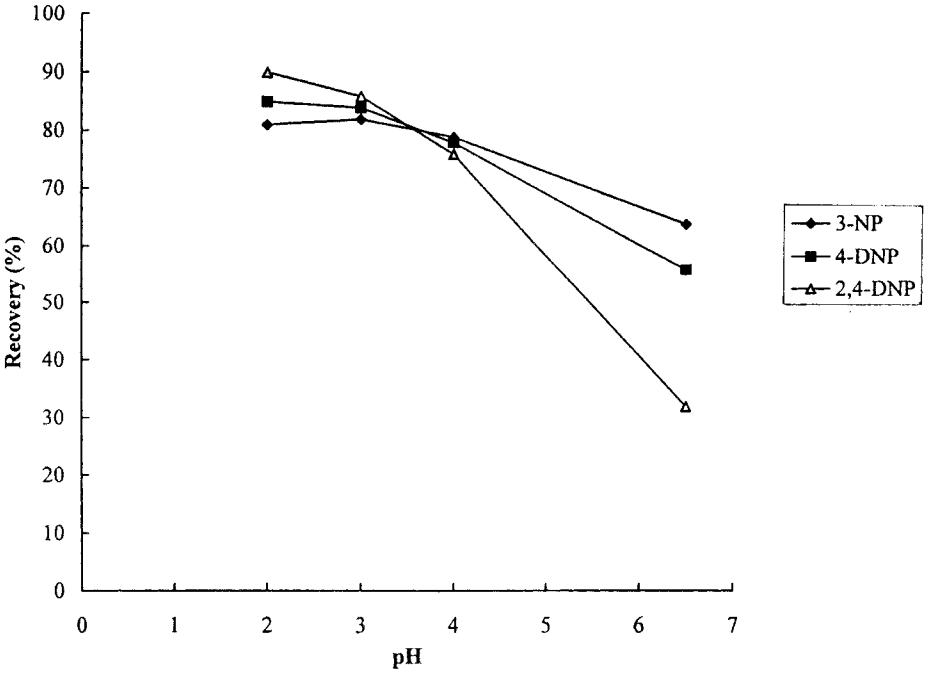


Figure 3. Recovery dependence on sample pH for 3-NP, 4-NP and 2,4-DNP.

Detection was made at UV 220 nm. The limit of detection without preconcentration was about 1 μg/mL, which is far below the required limit in many environmental analyses. Hence, preconcentration of NPs was necessary prior to CE analysis.

Off-column Enrichment of NPs by SPE

A variety of sorbents have been used for enrichment of polar phenolics by SPE.[25-27] Graphitized carbon black (GCB) provides high enrichment of polar phenolics[25,26] but back extraction is required prior to CE analysis.

Cyclohexyl and polystyrene sorbents also provide adequate enrichment, but are not easily available.[27] C_{18}, one of the most commonly used sorbents, has been successfully applied to extract nonpolar and relatively polar compounds.[28-30]

Table 1

Recovery Dependence on Sample Volume for 3-NP, 4NP and 2,4-NP

Sample Volume (mL)	Recovery (%) 3-NP	4-NP	2,4-DNP
10	87	91	85
15	85	91	82
20	74	83	65

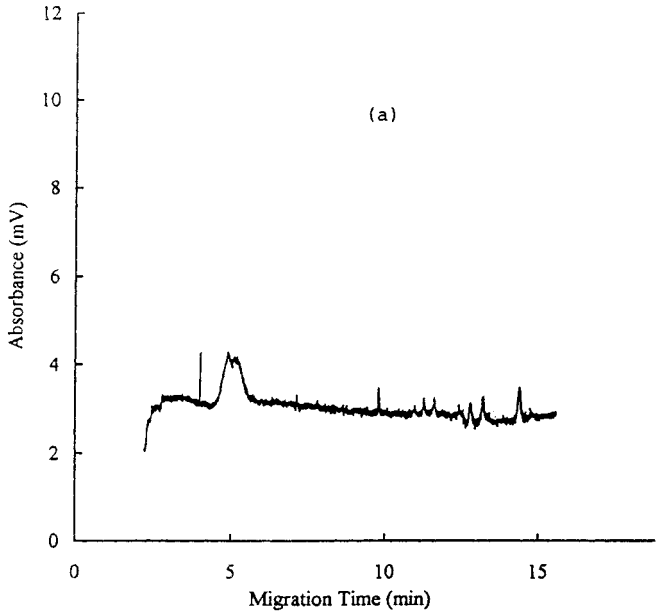
However, C_{18} sorbent suffers the problem of low recoveries and enrichment when it is used for extracting highly polar compounds, such as NPs.[26] Nevertheless, some enrichment of polar phenols by nonpolar C_{18} sorbent can be achieved when extraction conditions are optimised.

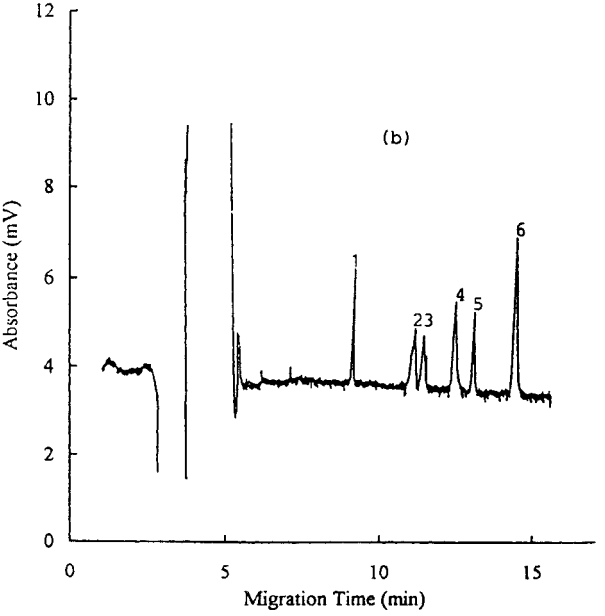
In our experiment, C_{18} bonded membrane was used to extract NPs. Membrane disk offers a competitive alternative for cartridge in SPE. One advantage of membrane is that only small volumes of elution solvent are needed, thanks to small particle diameter (dp = 8 μm) and the negligible dead-volume. Our experiment showed that 0.7 mL tetrahyrofuran (THF) was adequate to quantitatively elute NPs from C_{18} membrane (Figure 2).

In order to achieve efficient retention of NPs, two membranes are stacked together to make up double layers of C_{18} membrane. In this way, recoveries of nitrophenols were found to be improved by 28-37% as compared to those obtained on one membrane.

In addtion, the effect of pH value on recovery was investigated. Since NPs under investigation are weak acids (pKa 4.1-8.2) that can dissociate as anions at high pH values, it is necessary to adjust pH of sample at low value to neutralise nitrophenols, and decrease their polarity, and hence increase their retention. For brevity, three NPs (3-NP, 4-NP and 2,4-DNP) were selected as representatives.

Figure 3 shows that recoveries for NPs were quantitative when pH is below 4. It appeared that pH 3 was optimum for all Nps. With sample pH at 3, and two C_{18} membrane stacked together, we investigated the dependence of recovery on sample volume as shown in Table 1.





The recoveries of NPs decrease rapidly when sample volume exceeds 15 mL, implicating that sample breakthrough occurs. On comparing the results for different NPs, it is obvious that the more polar 2,4-DNP is more weakly retained than 3-NP and 4-NP. In agreement with these results, the increase in recovery with increasing eluent volume is also more significant for 2,4-DNP.

As NPs are weakly retained by the C_{18} membrane disk even at low pH value, the recovery becomes much smaller when a large volume of sample was loaded. Under optimal condition, 15 mL of water sample can be loaded, resulting in a maximum enrichment factor of ca. 20. Hence, it is not practical to obtain high enrichment of NPs by simply increasing sample volume loaded. For further enrichment of NPs, on-column concentration by FAI was investigated.

On-column Concentration of NPs by FAI

On column concentration by FAI involves several techniques, the simplest of which consists in the injection of large volumes of sample without matrix removal. For optimised sample stacking by this technique, the sample solution should meet the following requirements:

1. Conductivity of sample solution is about 10 times less than that of separation buffer so that a compromise between sample stacking and laminar broadening is attained.[20]
2. Conductivity of sample solution is reproducible so that effect of matrix variation is alleviated and good precision is attained.[23]
3. In the case of stacking weak acids (i.e. NPs), pH of sample solution should be high enough for weak acids to be dissociated as anions.[21]

It is obvious that the eluent (THF) used in the SPE cannot be directly used as sample solvent in FAI of NPs. Reconstitution is thus necessary to adjust the conductivity and pH of eluent. Usually, diluted separation buffer is used for reconstitution. In our experiment, however, pure borate buffer was used to reconstitute the eluent, primarily due to the consideration that the pH of sample solution should be high enough for all NPs to be largely or completely dissociated as anions. After reconstitution of the eluent in SPE, sample solution consisted of THF:H_2O = 1:1 with 6 mM sodium borate (pH 8.7).

Figure 4. **(left)** Electropherograms of a 1 ppm mixture of nitrophenols obtained with injection times of (a) 6 and (b) 60 s. In all experiments, the injection pressure was maintained constant at 30 mbar.

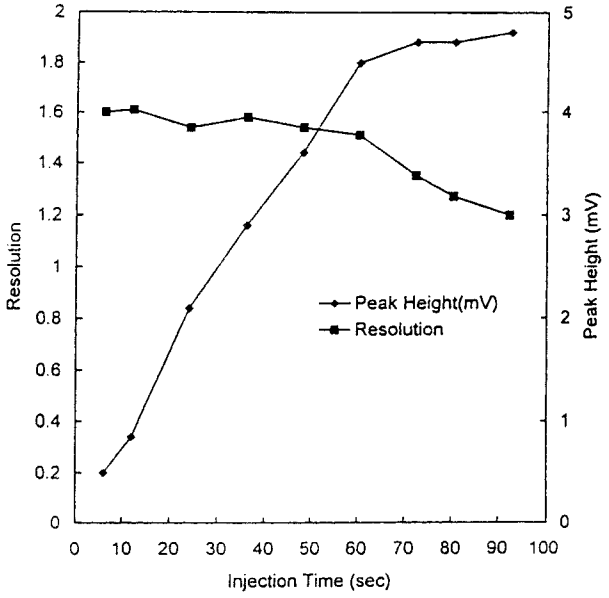


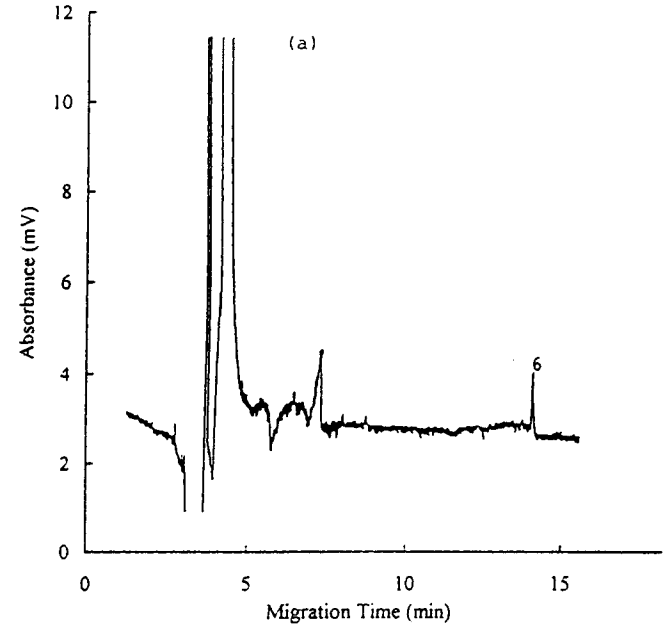
Figure 5. Effect of injection time on resolution of critical pair (peak **2** and **3)** and peak height (peak **6**).

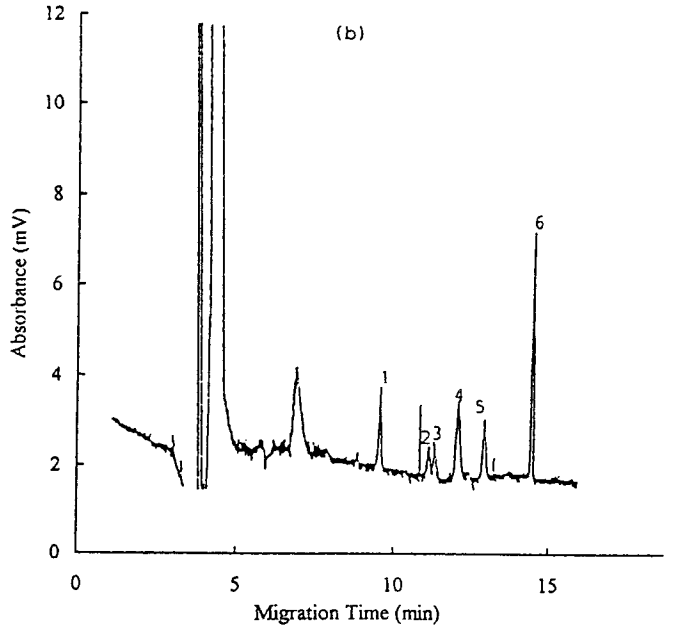
Table 2

Comparison of Detection Limit and Reproducibility in Concentration Between MEKC and MEKC with SPE/FAI

NP (Selected)	MEKC Detection Limit (μg/mL)	MEKC RSD% (n=8)	MEKC with SPE/FAI Detection Limit (ng/mL)	MEKC with SPE/FAI RSD% (n=8)
3-NP	0.6	3.4	5.5	12.5
4-NP	1.0	2.9	10.0	8.7
2,4-NP	1.0	4.6	9.0	9.2

Figure 6. (right) Electropherograms of (a) river water sample extract without fortification and (b) water sample extract fortified at 20 ppb. Separation and detection conditions as in Fig. 1.





Since the conductivity of 60 mM borate buffer is similar to that of the separation buffer, borate (25 mM)-phosphoric acid (20 mM)-SDS (50 mM) (pH 8.0), the conductivity of the sample solution with 6 mM borate is about ten times less than that of the separation buffer. Further, pH of the sample solution at 8.7 guarantees that all NPs (pKa 4.1-8.2) be largely or completely ionised.

With the sample solution as above, sample stacking by FAI was investigated by fixing injection pressure at 30 mbar, and varying injection time at 6 sec increment (Figures 4 and 5). It was found that over a 10-fold gain in peak area was obtained with little loss in resolution of critical pair when injection time is at 60 sec, corresponding to a plug length of 26 mm, and injection volume of 105 nL. The minor variation in enrichment factor (e.g. 11 for 2-NP and 14 for 2,4-DNP) among NPs may be due to their difference in electrophoretic mobility.

The tenfold enrichment factor is comparable to the published data of other reports.[23-25] The enrichment can be further enhanced up to several hundreds fold using more sophisticated methods, such as extremely large volume injection with matrix removal.

However, these techniques are not easily implemented routinely, and often suffer from the problem of poor precision of quantification, especially in real sample analysis.[25]

Combination of Off-column SPE and On-column FAI and Application to River Water Analysis

From the above discussion, we arrive at the optimal conditions for the combined use of off-column SPE and on-column FAI to enrich NPs, as described in the experimental section.

Comparison of MEKC without preconcentration and MEKC with preconcentration by off-column SPE and on-column FAI is shown in Table 2. As can be seen, MEKC with SPE and FAI provides over 100-fold enhancement in detection sensitivity as compared to MEKC alone.

Although precision of quantification was somewhat deteriorated, it compares favourably with those reported by Muller et al who combine SPE and FAI with extremely large volume injection followed by matrix removal.[25] Calibration graphs were constructed in the range of 5 - 100 ppb, showing linearity coefficients (R^2) of 0.993-0.998.

The method was applied to the analysis of nitrophenols in river water. Recoveries of six nitrophenols were in the range of 82-94 %, not affected by the sample concentration in the range from 20-100 ppb. Figure 6 shows the electropherograms of river water sample without fortification and that fortified with 20 ppb of each NP. Comparison of eletropherograms in Fig 6 (a) and (b) indicates that 2,4-DNP (6) may be present in the river water at a concentration of 8.5 ppb. In addition, it is interesting to note that there was little interference caused by other constituents present in the water sample.

This suggests that most interferences have been removed by the combination of off-column SPE and on-column FAI. In fact, enrichment by SPE is based on the difference in polarity between interferences and targeted analytes while enrichment by FAI is based on the difference in charge. Some interferences that can not be removed by SPE may be removed by FAI, and vice versa. Therefore, combination of SPE and FAI may provide improved separation selectivity, especially in the analysis of complex samples.

CONCLUSION

Combination of off-column SPE and on-column FAI provided not only enhanced detection sensitivity but also improved separation selectivity. With the use of on-column concentration by FAI, the limited enrichment of polar nitrophenols by C_{18} sorbent was enhanced. With the use of off-column concentration by SPE, FAI can be used to handle relatively dirty water sample with strong matrix. In addition, total analysis time was reduced due to the use of small sample volume. The method provides a rapid and selective approach for analysis of nitrophenols at ppb levels in natural water.

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