# Pressurized capillary electrochromatography with indirect amperometric detection for analysis of organophosphorus pesticide residues

Weimin Wu,[a] Yimin Wu,[a] Minmin Zheng,[a] Liuming Yang,[a] Xiaoping Wu,*[a] Xucong Lin[a] and Zenghong Xie*[ab]

*Received 18th February 2010, Accepted 21st May 2010*
**DOI: 10.1039/c0an00101e**

A new analytical method, pressurized capillary electrochromatography with indirect amperometric detection, has been developed for the determination of some non-electroactive organophosphorus pesticides (OPPs). When 0.1 mmol $L^{-1}$ of 3,4-dihydroxybenzylamine (DHBA) was added to the mobile phase containing 50% v/v of ACN and 50% v/v of MES buffer (10 mmol $L^{-1}$, pH 5.5), and +0.9 V (*vs.* Ag/AgCl) of working potential were used, maximal signal levels of analytes could be achieved. A separation voltage of +10 kV, a column pressure of 7.0 MPa and a pump flow rate of 0.05 mL $min^{-1}$ were selected as the other optimal conditions for separation of six OPPs, namely, dimethoate, methyl parathion, ethyl parathion, chlorpyrifos, chlorpyrifos-methyl, trichlorfon. The OPPs could be separated within 15 min and determined with the detection limits ranging from 0.008 to 0.2 mg/kg. Combining with a solid phase extraction procedure, mean recoveries between 78.9 and 87.2% for vegetable samples and from 81.4 to 98.6% for fruit samples were obtained.



## 1. Introduction

Extensive use of organophosphorus pesticides (OPPs) in agriculture has caused serious environmental and food safety problems.[1] During the last decade, increasing evidence showed that some OPPs not only cause obvious ill-health, but also damage the human endocrine system.[2,3] As a result, chlorpyrifos, dimethoate and trichlorfon have been listed as potential endocrine disrupters by the German Federal Environmental Agency,[4] and ethyl parathion is also classified as a suspected endocrine disrupting chemical.[5] Maximal residue limits (MRLs) for pesticides have been established by the United Nation's Food and Agriculture Organization and the World Health Organization[6] over a variety of foods. The Agricultural Ministry of China has also set an MRL of 1.0 mg/kg for trichlorfon, 1.0 mg/kg for dimethoate and 0.2 mg/kg for ethyl parathion in cabbages. To ensure food safety, it is necessary to develop simple and effective analytical methods for the rapid assay and quantitation of OPP residues in foods.

Biosensors based on acetylcholine esterase (AChE) inhibition have been widely used for the detection of OPPs. Such biosensors give a sum parameter of AChE inhibition without any qualitative or quantitative information on the individual analytes.[7] Typical instrumental analytical methods for OPP residues are gas chromatography (GC), liquid chromatography (LC) and capillary electrophoresis (CE).[8] It should be taken into account that GC and LC have been applied to pesticide-residue analysis since the mid-1970s, whereas applications of CE to pesticide-residue analysis did not begin until the early 1990s. The analysis by GC usually requires an additional step of derivatization, and the analysis by LC consumes considerable amounts of organic solvents. CE and capillary electrochromatography (CEC)[9] offer high separation efficiency, fast analysis, low consumable expenses and ease of operation, and have become attractive techniques for the determination of pesticide residues in food matrices.[10,11] However, CE is not a suitable separation method for neutral OPPs, and the extensive application of pure CEC has been hampered by insufficient robustness and reproducibility, mostly due to bubble formation in capillary columns during separation.[12,13] Although application of gas pressure is popular in commercial CEC instruments, gas pressures of up to 150 psi are simply not enough to wet the column packed with micron-sized particles and prevent bubbles. Recently, a hybrid technique known as pressurized capillary electrochromatography (pCEC),[14,15] which coupled a micro-HPLC pump to the inlet end of the capillary column to minimize bubble formation, has been successfully applied to the analysis of carbamate insecticides and pyrethroid pesticide residues in vegetables by UV absorption detection.[16,17] By applying a supplementary pump pressure, the analysis of analytes by pCEC can be speeded up to some extent.[34] Meanwhile, several attempts have been made to develop more selective and sensitive detection strategies for pCEC.

UV is the most widely used detection mode for OPPs, however, it suffers from low detection sensitivity due to a minor sample volume and limited optical path length for on-capillary UV photometric detection. As a more sensitive on-line hyphenated detection technique of pCEC, amperometric detection (AD)[18,19] promises higher detection capability, simplicity and low cost. Although direct AD is a sensitive means for solute monitoring, many solutes are not electroactive and cannot be detected by this approach.[20] To circumvent this limitation, sample derivatization and hydrolysis methods have been applied in non-electroactive objects to obtain electroactive groups for AD.[21,22] However, several drawbacks are identified in the application of these approaches to real sample analysis. The derivatization procedure often results in multiple products which are not beneficial to the separation of target compounds, and derivatization is usually difficult with very small volume samples.[23] The pre-column hydrolysis method is time-consuming.[24]

[a]*College of Chemistry and Chemical Engineering, Fuzhou University, 350108, China. E-mail: wapple@fzu.edu.cn*
[b]*Xiamen Huaxia Vocational College, Xiamen, 361024, China. E-mail: zhxie@fzu.edu.cn; Fax: +86-591-22866131; Tel: +86-591-22866131*

An indirect AD method, based on the displacement of an electroactive molecule added to the run buffer (electrophore) by the non-electroactive solute, is an alternative method for sensitive detection of non-electroactive compounds.[25] The indirect AD method has been successfully coupled with CE and LC.[25,26] To the best of our knowledge, there are no published reports about the combination of indirect AD with CEC or pCEC.

In this work, endocrine disrupting OPPs, including chlorpyrifos, dimethoate, trichlorfon and ethyl parathion, with chlorpyrifos-methyl and methyl parathion, were selected as target analytes. The aim of this research is to verify the feasibility and applicability of pCEC with an indirect AD method, and therefore to develop an effective approach for the rapid separation and indirect determination of non-electroactive OPP residues in fruits and vegetables.

## 2 Experimental

### 2.1 Chemicals and reagents

Methyl parathion, ethyl parathion standards were supplied by National Pesticide Quality Inspection Center (Beijing, China). Chlorpyrifos, chlorpyrifos-methyl, dimethoate, and trichlorfon standards were supplied by Sigma-Aldrich (St. Louis, MO, USA). The chemical structures and molecular weights of these OPPs are listed in Table 1.

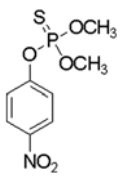
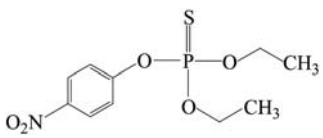
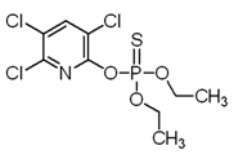
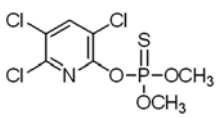
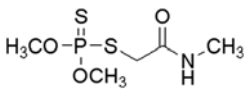
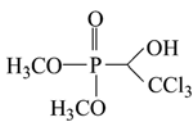
Standard stock solutions of OPPs were prepared in acetone at a concentration of 1 mg $mL^{-1}$, and were stored in glass stoppered bottles at 4 °C in a refrigerator. Working solutions of OPPs at various concentrations were prepared daily by appropriate dilution of aliquots of the stock solution in the mobile phase.

3,4-Dihydroxybenzylamine (DHBA) was purchased from Acros (New Jersey, USA). 2-Morpholinoethanesulfonic acid (MES) was obtained from Alfa Aesar (Ward Hill, MA, USA). HPLC-grade acetonitrile (ACN) was purchased from Chemical Industry Co., Ltd., Yuwang Branch (Shangdong, China). Analytical grade acetone was purchased from Shanghai Reagent Factory (Shanghai, China). Highly pure deionized water was prepared by using a Millipore Milli-Q purification system (Milford, MA, USA).

### 2.2 Apparatus

A pCEC system (Trisep-2100, Unimicro Technologies, Pleasanton, CA, USA) coupled with end-column AD was employed in

**Table 1** Chemical structure of studied organophosphorus pesticides

| Analytes | Chemical structures | Molecular weight |
|---|---|---|
| Methyl parathion |  | 263.21 |
| Ethyl parathion |  | 291.3 |
| Chlorpyrifos |  | 350.5 |
| Chlorpyrifos-methyl |  | 322.5 |
| Dimethoate |  | 229.3 |
| Trichlorfon |  | 257.5 |

the experiments. The details of this system have been described in previous works.[18] A pre-aligned electrochemical cell,[27,28] consisting of three electrodes (300 μm diameter carbon disc working electrode, platinum auxiliary electrode and Ag/AgCl reference electrode), was used in combination with an LC-3D potentiostat AD (BAS, West Lafayette, IN). Data collection was performed using a chromatographic workstation (Qianpu Software Co., Ltd., Shanghai, China). The separation column was a home-made 50 μm inner diameter monolithic column bonded with octadecyl ligands and sulfonate groups.[29] Cyclic voltammetric experiments were performed on a CHI 660 electrochemistry workstation (Shanghai CH instruments, China). Sep-Pak C18 (6cc, 500 mg) solid-phase extraction (SPE) cartridges were purchased from Waters (Elstree, Herts, UK).

### 2.3 Sample preparation

The samples analyzed, *i.e.*, cabbage, white radish, grape, pear, and orange, were obtained from a local market. All the samples were taken in accordance with the guidelines of the European Union Directive 79/700/CEE.[30] The sample weighed at least 1 kg and consisted of at least 10 individual fruits or vegetables.

A representative portion of sample (25 g of whole fruit or vegetable) was chopped and homogenized. Sample extraction was performed according to the criterion established by Chinese Ministry of Agriculture for OPPs in vegetables and fruits. Firstly, 50.0 mL acetone was added to the 25 g of sample, and the mixture was shaken for 1 min with a mechanical shaker in order to extract the OPPs. Then the homogenate was filtered and transferred into a 100 mL separating funnel (*ca.* 5–7 g NaCl was placed inside in advance). After shaking for *ca.* 2–3 min and allowing the funnel to stand for 10 min, the upper organic phase was collected.

For clean-up, the extraction solutes were introduced into the $C_{18}$ SPE cartridge that had been conditioned with 5.0 mL of hexane, and then 5.0 mL of acetone was added to the cartridge and the sample was allowed to elute dropwise by applying a slight vacuum. The eluent was collected in a graduated conical tube (15 mL) and dried slowly under a stream of nitrogen evaporating at 50 °C. Finally, the dry extract was redissolved in 1.0 mL of methanol and filtered with a 0.22 μm membrane filter before pCEC analysis.

### 2.4 Analysis procedure

A certain amount of DHBA was added into the mixture of equivalent volume of ACN and MES buffer (10 mmol $L^{-1}$, pH 5.5). This mobile phase was degassed in an ultrasonic bath for 20 min before use. The pump flow rate was set at 0.05 mL $min^{-1}$, and 1000 psi back pressure valve was used to achieve 7.0 MPa of column pressure. The capillary column was conditioned with the mobile phase for 1 h.

Prior to use, the surface of the carbon disc working electrode was polished on a polishing cloth with alumina powder ($\alpha$-$Al_2O_3$, 0.05 μm, Buehler, USA), and then ultrasonically cleaned for 1 min. The three-electrode system was fixed in the electrochemistry detection cell, and the detection potential was set at 0.9 V (*vs.* Ag/AgCl). The capillary column and carbon disc electrode were aligned in a straight line, and adjusted in a wall-jet configuration. The separation voltage was increased gradually from 0 to +10 kV and then operated at +10 kV. When a stable baseline was obtained, electrochromatographic experiments can be carried out. The electrochromatogram peaks were identified by comparison of retention time with OPP reference standards.

## 3 Results and discussion

### 3.1 Electrochemical properties of selected organophosphorus pesticides

Cyclic voltammetry tests showed that no oxidation peaks or reduction peaks were observed for target pesticides except for methyl-parathion, indicating that most of them (ethyl parathion, chlorpyrifos, chlorpyrifos-methyl, dimethoate and trichlorfon) are non-electroactive. When using phosphate buffer solution (10 mmol $L^{-1}$, pH 5.5) as the background electrolyte, methyl-parathion has an oxidation peak at the peak potential of −0.6 V (*vs.* Ag/AgCl).

Using 50% v/v ACN and 50% v/v MES (10 mmol $L^{-1}$, pH 5.5) as the mobile phase without any electroactive additive, the mixture of six OPPs (100 μg $mL^{-1}$) was analyzed by pCEC with direct AD. Only the solvent peak was observed in the electrochromatograms. No positive peaks of corresponding analytes were obtained, which further confirmed the non-electroactive properties of the OPPs. Electroactive methyl parathion has no response on AD. The possible reason maybe affected by the unavoidable interference of oxygen in the electrochemistry detection cell. It is clear that these selected OPPs cannot be directly detected by AD.

### 3.2 Selection of electroactive additive in mobile phase

An indirect electrochemical detection mode was introduced for the analysis of non-electroactive OPPs. The most important factor in designing the indirect AD method is the selection of the electroactive mobile phase additive, which should not react with analytes, and be able to generate a steady-state background current. A few electroactive chemicals have been used as mobile phase additives in LC,[26] or as a buffer additive in CE,[31] including hydroquinone, uric acid, ferrocene carboxylic acid and DHBA.

A certain concentration of hydroquinone or ferrocene carboxylic acid was added to the mobile phase respectively. Although these two additives can generate a relatively high background current, there were no expected negative peaks of OPPs, indicating that neither of them is an effective additive suitable for pCEC with an indirect AD method.

The cyclic voltammogram of DHBA recorded with the carbon disc electrode in the mobile phase is presented in Fig. 1, which showed quasi-reversible redox peaks at $E$(pa) of 550 mV and $E$(pc) of 160 mV. When using DHBA as the mobile phase additive for indirect pCEC–AD analysis of OPPs, a constant background current was obtained at a working potential of 0.9 V (*vs.* Ag/AgCl), and the corresponding negative peak of each pesticide could be observed. DHBA is chosen as the effective additive for pCEC with the indirect AD method.

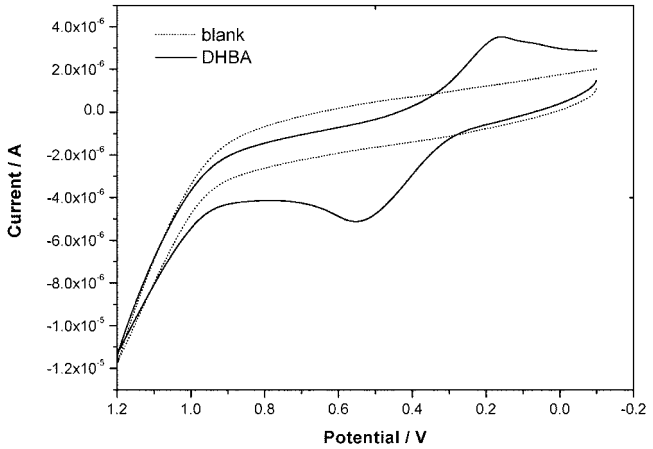



**Fig. 1** Cyclic voltammograms (scan rate, 50 mV/s at 25 °C) recorded in 50% v/v ACN and 50% v/v MES (10 mmol $L^{-1}$, pH 5.5) with carbon disc electrode. Solid line: in the presence of 0.1 mmol $L^{-1}$ DHBA; dotted line: in the absence of DHBA.

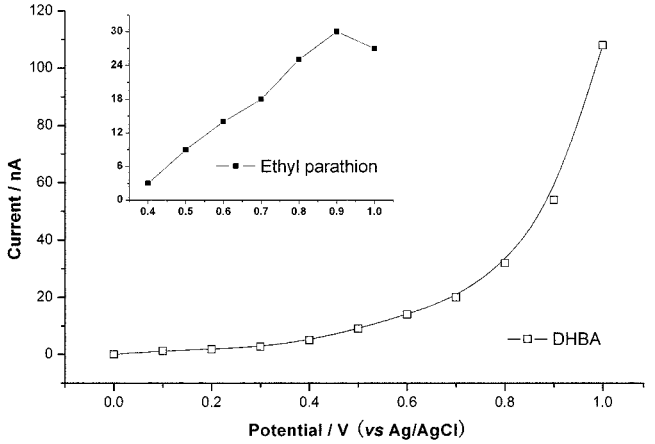



**Fig. 2** Hydrodynamic voltammograms (HDVs) for 0.1 mmol $L^{-1}$ DHBA in mobile phase and ethyl parathion (50 μg $mL^{-1}$). The response of ethyl parathion was defined as the absolute difference of baseline current and peak current. Working electrode: 0.3 mm diameter carbon disc electrode; pCEC mobile phase: 50% v/v ACN and 50% v/v MES (10 mmol $L^{-1}$, pH 5.5); separation voltage: +10 kV; column pressure: 7.0 MPa.

### 3.3 Effect of concentration of DHBA

In the indirect AD method, a certain concentration of electroactive additive in the mobile phase could produce a steady background current. When non-electroactive mixture flows through the column driven by the mobile phase, separation is reached gradually by the different zones formed. In each sample zone, the concentration of electroactive additive reduces in varying degrees. When these sample zones reached the surface of the working electrode in turn, a transitory decrease in the background current level will be observed at the detector and negative peaks are thereby produced.[32] An anodic background current, produced by DHBA in the mobile phase, is dependent on the concentration of DHBA and the applied potential. In pCEC with indirect AD, the effect of concentration of DHBA and the working potential on the background current and response of OPPs were investigated respectively.

Different concentrations of DHBA, namely, 0.01 mmol $L^{-1}$, 0.05 mmol $L^{-1}$, 0.1 mmol $L^{-1}$ and 0.2 mmol $L^{-1}$, were added to the mobile phase respectively. The working potential was set at 0.55 V. The background current increased with increase of DHBA concentration. When the DHBA concentration was 0.2 mmol $L^{-1}$, the system noise level became noticeable, which went against the obtainment of lowest detection limits. Relatively stable and sensitive detection signals were achieved when the concentration of DHBA in mobile phase was 0.1 mmol $L^{-1}$.

### 3.4 Effect of working potential

Using a mobile phase consisting of 50% v/v ACN and 50% v/v MES buffer (10 mmol $L^{-1}$, pH 5.5) and containing 0.1 mmol $L^{-1}$ DHBA, the effect of the working potential on the background current was investigated. Fig. 2 shows that the background current (recorded as the response height of the baseline) increases with an increase of the working potential from 0 to 1.0 V (*vs.* Ag/AgCl). When the potential was greater than 0.7 V, the background current increased more rapidly. In the potential range from 0.4 to 0.9 V, the peak height of ethyl parathion increased with increasing working potential. The relationship between the peak heights of the other five OPPs and the working potential were similar. However, the baseline noise became noticeable when the working potential was over 1.0 V. Consequently, a working potential of 0.9 V was chosen for obtaining a relatively steady baseline and a maximum signal-to-noise ratio of analytes.

### 3.5 Optimization of pCEC separation conditions

**3.5.1 Organic modifier concentration.** ACN is usually selected in pCEC as the organic modifier for its low viscosity and a relatively high electro-osmotic flow (EOF) generated.[33] In order to examine the effect of different ACN contents on the separation of OPPs, the ACN content in the mobile phase was varied from 40 to 80% v/v. A decrease of retention time was observed with the increase of ACN content in the mobile phases. The typical reverse-phase retention behavior of non-polar OPP solutes originates from the bonded octadecyl ligands and sulfonate groups on the monolithic column used in the experiment. The lower ACN content would benefit the separation of OPPs, but a longer analysis time was needed. To achieve the best compromise in terms of the resolution and analysis time, 50% v/v of ACN in the mobile phase was selected.

**3.5.2 Buffer concentration and pH value.** In pCEC, a separation current in the capillary column is generated by the voltage applied at both ends of the column. This separation current, which is different from the background current generated by DHBA, is easy to interfere with the detection signal of AD. A zwitterionic buffer of MES with low conductivity and low separation current was chosen as a suitable composition of the mobile phase for more reproducible electrochromatography. Varying the MES concentration from 10 to 50 mmol $L^{-1}$ had little effect on the separation of the OPPs. Finally, 10 mmol $L^{-1}$ MES was chosen due to the lower separation current and stable detection signals.

Considering the effective buffer range of MES (5.5–6.7), the pH value of MES buffer (5.5, 6.0, and 6.7) in the mobile phase

was compared. Results showed that the elution order of six analytes remained unchanged with the increase of pH, but the retention time reduced a little accordingly due to the higher EOF velocity. As the sulfonate groups in the monolithic stationary phase provided the negative charge necessary for generating the EOF, a higher pH would benefit the dissociation of the sulfonate group and therefore stimulate a stronger EOF. When the buffer pH was 5.5, a lower EOF and better resolution of the OPPs could be obtained. So, a pH of 5.5 was selected as the optimal pH value in subsequent experiments.

**3.5.3 Column pressure and separation voltage.** Column pressure is a key factor for pCEC, and is usually used to prevent bubble formation and provide pressurized flow for driving the mobile phase and solutes. A higher column pressure could increase the linear velocity of the OPP analytes and improve the analysis speed. With the pressure increased from 3.6 to 8.7 MPa, a decrease in the retention time of all the analytes and a loss of resolution were observed. Finally, 7.0 MPa of column pressure was selected to achieve the compromise.

To test the effect of another driving force for pCEC separation, a separation voltage of between 0 and +16 kV was set on the capillary column while keeping the column pressure at 7.0 MPa. With increasing separation voltage, the migration time of the six OPPs decreased, and the migration order still remained unchanged. When the voltage was applied at +10 kV, good separation was achieved within 15 min. The separation current in capillary column was 0.9 μA at +10 kV of separation voltage. Upon further increasing the voltage, the higher separation current generated would increase the baseline noise and interfere with the detection signal. So, +10 kV was used as the optimal separation voltage.

When the separation voltage was 0 kV, *i.e.* under the capillary HPLC (cHPLC) mode, a longer analysis time of 28 min was needed. The difference of column efficiency under cHPLC and pCEC modes was also compared in Table 2. In pCEC, the mobile phase is propelled by an EOF of flat plug-like profile and a pressurized flow of parabolic profile as in HPLC,[14] so the column efficiency could be increased with the effect of the electric field strength.

**Table 2** Theoretical plate numbers of OPPs in capillary HPLC and pCEC modes, resolution of OPPs in pCEC mode[a]

| Analytes | Theoretical plate number for cHPLC (N/m) | Theoretical plate number for pCEC (N/m) | Resolution for pCEC |
|---|---|---|---|
| (1) Dimethoate | 6323 | 26 310 | $R_{s(1,2)} = 3.3$ |
| (2) Methyl parathion | 7260 | 34 216 | $R_{s(2,3)} = 2.7$ |
| (3) Ethyl parathion | 16 434 | 61 632 | $R_{s(3,4)} = 5.4$ |
| (4) Chlorpyrifos | 12 711 | 46 322 | $R_{s(4,5)} = 1.2$ |
| (5) Chlorpyrifos-methyl | 13 220 | 54 220 | $R_{s(5,6)} = 10.2$ |
| (6) Trichlorfon | 5266 | 31 461 | |

[a] pCEC conditions: mobile phase: 50% v/v ACN, 50% v/v MES buffer (10 mmol $L^{-1}$, pH 5.5), 0.1 mmol $L^{-1}$ DHBA, separation voltage: +10 kV, column pressure: 7.0 MPa, pump flow rate: 0.05 mL $min^{-1}$, electrode potential: +0.9 V (*vs.* Ag/AgCl); cHPLC conditions are the same as pCEC except that the separation voltage was not applied.

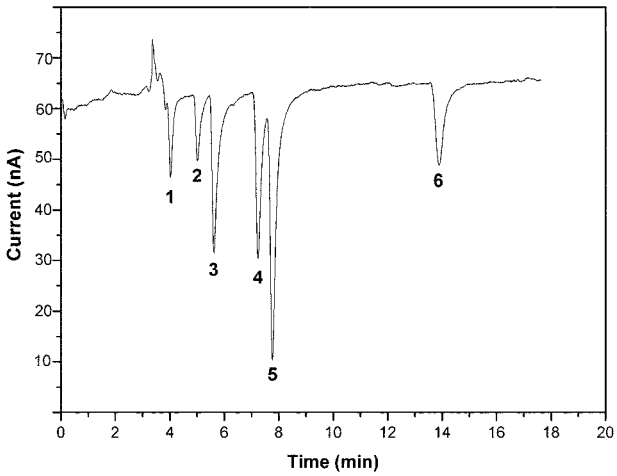



**Fig. 3** Typical pCEC chromatogram of selected OPPs under the optimal separation and detection conditions. Solutes: (1) dimethoate, (2) methyl parathion, (3) ethyl parathion, (4) chlorpyrifos, (5) chlorpyrifos-methyl, (6) trichlorfon (50 μg $mL^{-1}$ of each solute).

After conducting this series of experiments for the analysis of selected OPPs, a mobile phase consisting of 50% v/v ACN, 50% v/v MES buffer (10 mmol $L^{-1}$, pH 5.5) containing 0.1 mmol $L^{-1}$ DHBA, and +10 kV of separation voltage, 7.0 MPa of column pressure, and 0.9 V (*vs.* Ag/AgCl) of working potential of carbon disc electrode, were considered as the optimal separation and determination conditions. The pCEC chromatogram under the optimal conditions is shown in Fig. 3, and the resolutions of the OPPs are listed in Table 2.

### 3.6 Analytical performance

**3.6.1 Precision, linear range and limits of detection.** The precision of this proposed method was evaluated under optimized conditions. Intra-day RSDs were found to be lower than 6.7% for retention time, and 9.2% for peak height. Inter-day RSDs on five different days ranged from 4.5 to 8.3% for retention time and 6.9 to 10.4% for peak height, indicating acceptable reproducibility of this pCEC method. A series of OPPs with concentrations ranging from 0.1 to 1 mg $mL^{-1}$ were tested to determine the calibration parameters in pCEC under the optimized conditions. The results from regression analysis between the peak heights and concentrations are shown in Table 3. Good correlation coefficients for the six analytes with calibration curves covering one or two orders of magnitude were obtained. The instrumental detection limits ($3\sigma/S$, the concentration necessary to yield a net signal equal to three times the standard deviation of the background) are 2.0, 2.5, 0.5, 0.5, 0.2, 2.5 μg $mL^{-1}$ for dimethoate, methyl parathion, ethyl parathion, chlorpyrifos, chlorpyrifos-methyl, and trichlorfon, respectively.

**3.6.2 Analysis of organophosphorus pesticide residues in fruits and vegetables.** The standard addition method was applied to examine the reliability of the proposed method. Three replicate samples of each fruit or vegetable were spiked with OPPs at an 0.8 mg $kg^{-1}$ concentration level, and then prepared and determined by the proposed pCEC–indirect AD method. The pCEC chromatograms of extracts of pear and pear spiked with OPPs

**Table 3** Analytical parameters for the determination of OPPs in standard mixtures and fruit by pCEC–AD[a]

| Analytes | Intra-day RSD for time (%) ($n = 5$) | Intra-day RSD for peak height (%) ($n = 5$) | Linear regression equation[b] | $r$ | Linear range (μg/mL) | LODs for standard solution (μg/mL)[c] | LODs for fruit (mg/kg)[d] |
|---|---|---|---|---|---|---|---|
| Dimethoate | 3.6 | 4.4 | $I = 3.2 \times 10^5 C + 0.23$ | 0.9961 | 10–100 | 2.0 | 0.2 |
| Methyl parathion | 5.3 | 5.2 | $I = 2.1 \times 10^5 C + 0.61$ | 0.9932 | 10–100 | 2.5 | 0.1 |
| Ethyl parathion | 4.2 | 4.7 | $I = 6.4 \times 10^5 C + 0.52$ | 0.9990 | 5–100 | 0.5 | 0.02 |
| Chlorpyrifos | 3.9 | 4.3 | $I = 6.0 \times 10^5 C - 0.12$ | 0.9967 | 5–100 | 0.5 | 0.02 |
| Chlorpyrifos-methyl | 4.1 | 6.8 | $I = 1.1 \times 10^6 C + 0.66$ | 0.9943 | 1–100 | 0.2 | 0.008 |
| Trichlorfon | 6.7 | 9.2 | $I = 3.7 \times 10^5 C + 0.43$ | 0.9926 | 10–50 | 2.5 | 0.1 |

[a] Conditions are identical to Fig. 3. [b] $I$: negative peak height (nA); $C$: concentration (μg mL$^{-1}$). [c] Based on $3\sigma/S$. [d] Tested and calculated by the SPE-pCEC procedure in Section 2.3 and 2.4 (analysis of pear sample).

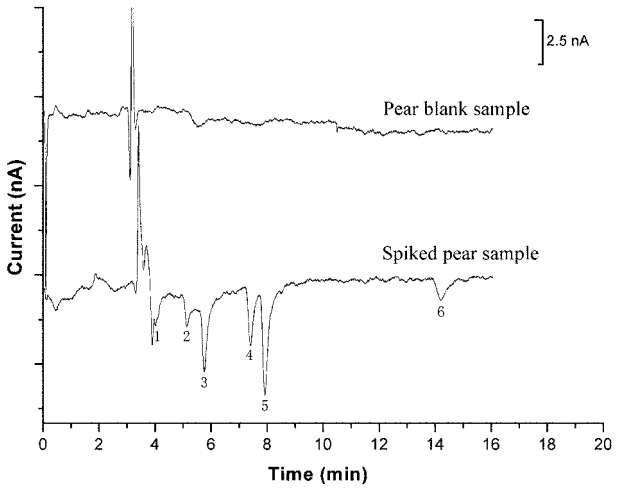



**Fig. 4** pCEC chromatograms of blank pear extract compared with extract of pear spiked with 0.8 μg g$^{-1}$ of dimethoate, methyl parathion, ethyl parathion, chlorpyrifos, chlorpyrifos-methyl, and trichlorfon.

were respectively shown in Fig. 4. No target OPPs existed in blank pear extract. Some impurity peaks would not interfere with the determination of OPPs. As summarized in Table 4, mean recoveries of OPPs in vegetables ranged from 78.9 to 87.2%, and mean recoveries in fruit samples ranged from 81.4 to 98.6%, respectively.

Twenty-five grams of pear sample was spiked at a concentration level the same as the instrumental detection limits, then analyzed by the proposed method. Result shows that the signal-to-noise ratios (SNR) of five pesticides except dimethoate were greater than 3. Then the spike concentration of dimethoate was further increased to achieve 3 × SNR. Method detection limits of 0.2, 0.1, 0.02, 0.02, 0.008 and 0.1 μg g$^{-1}$ for dimethoate, methyl parathion, ethyl parathion, chlorpyrifos, chlorpyrifos-methyl, and trichlorfon were achieved, which were lower than the MRLs set by the Agricultural Ministry of China.

Three vegetable samples (cabbage, white radish, orange) and two fruit samples (grape, pear) were prepared by procedures described in Section 2.3. Commercially available $C_{18}$ SPE columns were used for clean-up, and the proposed indirect AD method was applied to the determination of OPP residues. Non-interfering peaks appeared on the electrochromatograms of real samples, indicating improvement in the elimination of matrix interferences by the SPE step. There were no OPPs found in these vegetable and fruit samples.

## 4. Conclusions

The utility of indirect AD with pCEC for the analysis of non-electroactive OPPs is demonstrated. Under the optimized conditions, six selected OPPs can be separated within 15 min. The method detection limits for pear sample ranged from 0.008 to 0.2 mg/kg. Mean recoveries were higher than 78.9% for vegetables, and 81.4% for fruits. The proposed method is simple, and a less expensive alternative for monitoring OPP residues in foods. In spite of a higher sensitivity still being needed, the

**Table 4** Mean recoveries of six OPPs in vegetable and fruit samples ($n = 3$)[a]

| | Recovery (%)[b] | | | | |
|---|---|---|---|---|---|
| Analytes | Cabbage | White radish | Pear | Orange | Grape |
| Dimethoate | 82.1 ± 6.3 | 79.3 ± 7.2 | 87.5 ± 7.3 | 88.2 ± 8.2 | 89.0 ± 9.9 |
| Methyl parathion | 78.9 ± 5.2 | 82.2 ± 6.7 | 92.3 ± 7.0 | 86.5 ± 6.6 | 93.7 ± 9.3 |
| Ethyl parathion | 83.2 ± 4.8 | 83.6 ± 5.2 | 96.2 ± 6.9 | 86.6 ± 7.2 | 95.2 ± 8.8 |
| Chlorpyrifos | 86.5 ± 4.5 | 84.5 ± 5.2 | 98.4 ± 6.5 | 85.7 ± 6.8 | 97.4 ± 8.3 |
| Chlorpyrifos-methyl | 87.2 ± 4.5 | 80.1 ± 5.4 | 96.6 ± 5.9 | 82.0 ± 6.3 | 98.6 ± 8.0 |
| Trichlorfon | 83.1 ± 4.9 | 80.6 ± 5.8 | 90.1 ± 6.3 | 81.4 ± 7.4 | 90.7 ± 8.7 |

[a] Under the optimal separation and determination conditions of pCEC–AD; OPP concentration added: 0.8 μg g$^{-1}$. [b] Mean ± standard deviation for three determinations.

general applicability of pCEC with AD can be expanded if indirect detection modes are employed.

## Acknowledgements

This project was financially supported by funding from National Natural Science Foundation (20907009, 40976071), Program for New Century Excellent Talents in University of China (NECT-06-0572) and of Fujian Province (HX2006-99), the Key Science & Technology Project of Fujian Province (2008Y0051).

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