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Capillary Electrochromatography with Liquid Crystal Crown Ether Modified Hybrid Silica Monolith for Analysis of Imidacloprid and Carbendazim in Tomatoes

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This study describes the ability of capillary electrochromatography (CEC) for the determination of imidacloprid and carbendazim in tomato samples. A novel liquid crystal crown ether modified hybrid silica monolithic column was synthesized, characterized and developed as separation column for the first time. Baseline separation of imidacloprid and carbendazim could be achieved using a mobile phase containing 90% (v/v) 20 mmol/L phosphate buffer (pH 7.0) and 10% (v/v) acetonitrile. The matrix matched calibration curves were linear with correlation coefficient $r^2 > 0.9998$ in the range of 0.20-10.00 mg/L. The limits of detection for imidacloprid and carbendazim were 0.061 and 0.15 mg/kg, respectively, which were below the maximum residue limits established by the European Union as well as Codex Alimentarius. Average recoveries for imidacloprid and carbendazim varied from 101.6-108.0% with relative standard deviations lower than 6.3%. This method was applied to the analysis of tomatoes collected from local markets.

Key Words : Capillary electrochromatography, Liquid crystal crown ether modified hybrid silica monolith, Imidacloprid, Carbendazim, Tomato

Introduction

Tomatoes (*Lycopersicon esculentum* L.), one of the most widely grown vegetables in the world, are vulnerable to a wide variety of pests and diseases. To boost agricultural production, chemical pesticides such as imidacloprid and carbendazim were extensively used. However, bioaccumulation in the food chain can occur and they can eventually pose a potential hazard to human health. The maximum residue limits (MRLs) established by European Union[1] and Codex Alimentarius[2] were 0.50 mg/kg both for imidacloprid and for carbendazim in tomatoes. Therefore, the evaluation and monitoring of these pesticides in tomatoes are imperative and momentous.

High performance liquid chromatography (HPLC) coupled with UV or mass spectrometry has been the most frequent technique used for simultaneous determination of imidacloprid and carbendazim in vegetables.[3-6] The relative low peak efficiency afforded by HPLC together with the large amounts of organic solvents used as mobile phases are its main drawbacks.[7] Capillary electrochromatography (CEC) is a promising hybrid micro-separation technique that combines the separation and selectivity potential of HPLC and high efficiency of capillary electrophoresis. Research effort devoted to the applications of CEC for biochemical, food, pharmaceutical and environmental analysis is considerable.[8] However, few reports concerning the utility of CEC in analysis of pesticide residues in agricultural products have been published.[9-13]

Hybrid silica monolith has emerged as an attractive alternative to polymer-based[14,15] and silica-based monolith[16-18] due to its excellent solvent tolerance ability and mechanical stability. Hybrid silica monolith with vinyl,[19] allyl,[20] phenyl,[21] octyl[22] and amino groups[23] has been documented. However, further surface modification via these groups has aroused scant attention. Supramolecules such as calixcrown ether[19] and liquid crystal crown ether (LCCE)[24] as the bonded phase of hybrid silica monolith exhibit superior performance due to their host-guest recognition.

In this contribution, the implementation of a new method for the determination of carbendazim and imidacloprid in tomatoes by CEC with a liquid crystal crown ether, (4-(ω-undecenyl-1-lyoxy)-4'-(4'-carboxylbenzo 15-crown-5)-biphenyl, modified hybrid silica monolith was demonstrated. The validated analytical method was applied to tomato samples purchased from local markets.

Experimental

Apparatus and Chemicals. All CEC experiments were carried out on a Beckman P/ACE™ MDQ instrument (Beckman Coulter, Fullerton, CA, USA) equipped with a diode array detector (DAD) (190-600 nm). Fused-silica capillaries (75 mm i.d. × 375 mm o.d.) were purchased from Xinnuo Photoconductive Fiber Factory (Hebei, China). Scanning electron microscopy (SEM) images were taken by a JSM-6390LV Scanning electron microscope (JEOL, Japan). IKA® T-25 Digital High-Speed Homogenizer Systems (Staufen, Germany) was used for homogenizing samples. Laborota 4010 digital Rotary evaporator (Heidolph, Schwabach,

Germany) and OA-SYS Nitrogen Evaporators (Organomation Associates, Inc, Berlin, MA, USA) were used for concentration. NH_2 (500 mg, 6 mL), Florisil (500 mg, 6 mL) and C18 (500 mg, 6 mL) cartridges obtained from Agela Technologies Inc. (Tianjin, China) were used for solid phase extraction.

LCCE (4-(ω-undecenyl-1-lyoxy)-4'-(4'-carboxylbenzo 15-crown-5)-biphenyl was supplied by Prof. Zaifu Huang (Department of Chemistry, Wuhan University, Wuhan, China). The synthesis procedure and characterization data of the LCCE was shown in Reference 25. Tetramethoxysilane (TMOS) and vinyltrimethoxysilane (VTMS) were supplied by the Chemical Plant of Wuhan University (Wuhan, China). PEG (Mw = 10000), urea and 2,2'-azoisobutyronitrile (AIBN) were obtained from Sinopharm Chemical Reagent (Shanghai, China). HPLC grade acetonitrile, methanol, acetone and toluene were purchased from Tedia (Fairfield, USA). Imidacloprid with purity of 99.0% was supplied by Bayer CropScience China Co. Ltd (Beijing, China), and carbendazim with purity of 98.5% was purchased from Hubei Sanonda (Hubei, China). Stock solutions of 1000 $mg \cdot L^{-1}$ for each pesticide were prepared in methanol and stored at 4 °C. Standard working solutions were prepared by an appropriate dilution of the stock solutions with methanol.

Preparation of the Monolithic Column. Hybrid silica monolithic column labeled as TMOS-VTMS column was prepared according to the previously described procedure.[19] This column was then filled with a dehydrated toluene solution containing 5% (w/v) LCCE and 0.5% (w/v) AIBN. With both ends sealed, it was heated at 70 °C for 6 h and subsequently washed with methanol and water. The resultant monolithic column was designated as LCCE-TMOS-VTMS column. Figure 1(b) shows the schematic of the monolithic column.

CEC Procedures. The total length and effective length of the monolithic column were 40.2 cm and 30.0 cm respectively. Samples were injected electrokinetically at 5 kV for 3 s. The separation was performed at an applied voltage of 15 kV at 25 °C. The DAD detection was conducted at 270 nm for imidacloprid and 286 nm for carbendazim. An equal pressure of 50 psi was applied at both ends of the capillary.

Sample Preparation. Approximately 2000 g of tomato samples was weighed, chopped, and homogenized in a blender. A 25.00 g portion of homogenized sample was accurately weighed into a centrifuge tube and mixed with 50 mL acetonitrile. The mixture was homogenized using a high-speed homogenizer for 2 min at 13500 rpm, then added 7.0 g sodium chloride and homogenized for 2.0 min again. The mixture was centrifuged for 5 min at 3500 rpm. Then a 30 mL aliquot of the supernatant was transferred to a pear shaped flask and evaporated to dryness at 50 °C using a rotary evaporator. The acetonitrile extract was redissolved with 2 mL methanol-dichloromethane (5:95, v/v).

NH_2 cartridges were used for solid-phase extraction. The extract was percolated through the preconditioned cartridge at a flow rate of 1 mL/min. The retained analytes were eluted with 8 mL of methanol-dichloromethane (5:95, v/v). The eluate was collected, dried under a gentle stream of nitrogen at 50 °C. The residue was reconstituted with 250 μL methanol. Finally, the solution was vigorously shaken on a vortex mixer and filtered through a 0.22 μm microfilter prior to CEC analysis.

Results and Discussion

Monolith Characterization. The SEM image of LCCE-TMOS-VTMS column is shown in Figure 1(a). As seen, the formed hybrid silica monolith was well attached to the inner wall of the capillary, and no crack and shrinkage were observed.

FTIR spectra of LCCE-TMOS-VTMS showed typical bands of the stretching vibration of aromatic ring and C=O at 1580 cm^{-1} and 1700 cm^{-1} respectively, and the disappearance of the stretching vibration of C=C at 1620 cm^{-1}, which confirmed that liquid crystal crown ether was covalently bonded to the hybrid silica monolith.

Quantification of electroosmotic flow (EOF), such as the dependence of EOF on pH of the buffer could be used to assess the chemical modification process of monolithic column.[26] Figure 2 plots EOF at the pH ranging from 5.5 to 9.0 for LCCE-TMOS-VTMS column and TMOS-VTMS column, respectively. The EOF for both columns increased with an increase in pH due to ionization of silanol groups. However, lower EOF for LCCE-modified column than hybrid silica monolithic column was observed. This phenomenon was probably due to the fact that the silanol groups were shielded by LCCE moiety, which further indicated the successful attachment of LCCE to the capillary inner surface.

Optimization of CEC Separation. In this study, the influences of pH, acetonitrile content and buffer concentration on the separation performance in LCCE-TMOS-VTMS column were investigated. As shown in Figure 3, an increase

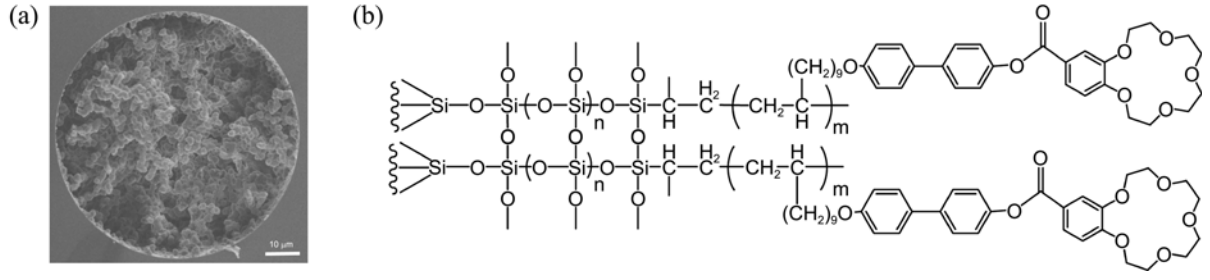

Figure 1. The scanning electron microscope image (a) and schematic (b) of LCCE-TMOS-VTMS column.

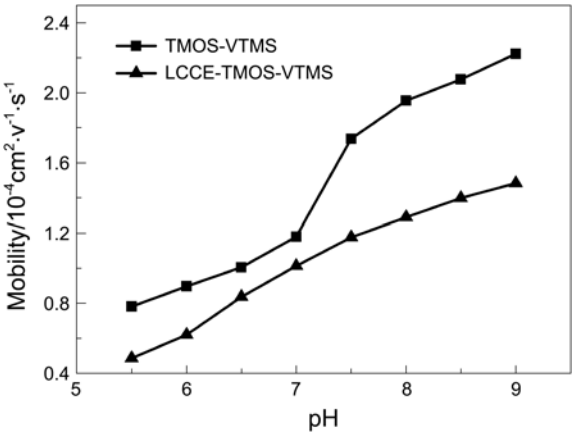

Figure 2. Effect of pH on EOF on the TMOS-VTMS and LCCE-TMOS-VTMS column. Buffer: 20 mmol·L^{-1} phosphate buffer; EOF marker, thiourea; detection wavelength, 214 nm; applied voltage, 15 kV.

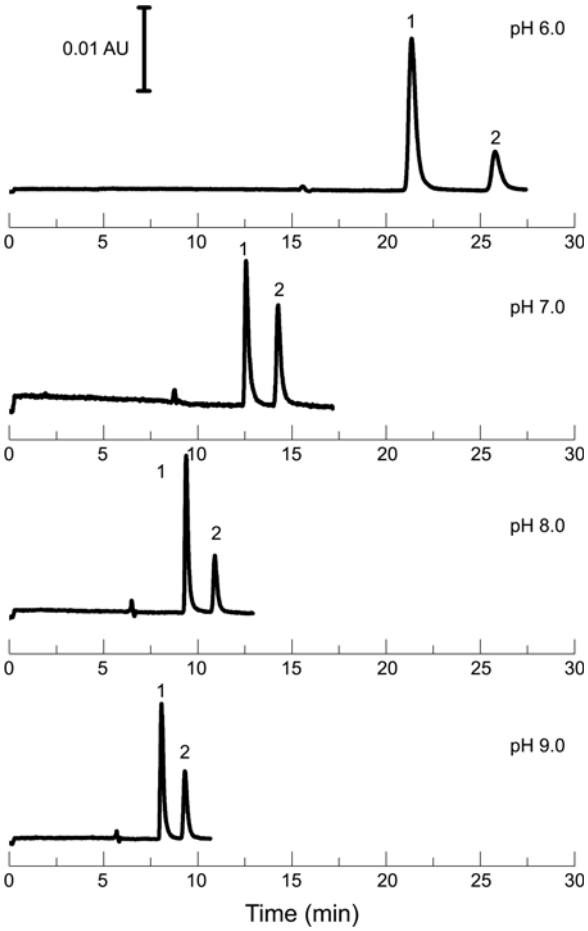

Figure 3. Effect of pH of running buffer on resolution. Mobile phase, 20 mmol·L^{-1} phosphate buffer-acetonitrile (90:10, v/v); detection wavelength, 270 nm; applied voltage, 15 kV. Peak identification: 1, carbendazim; 2, imidacloprid.

in pH could shorten the analysis time but deteriorate the separation performance. To take into account both resolution and analysis time, pH 7.0 was selected as the optimum pH condition.

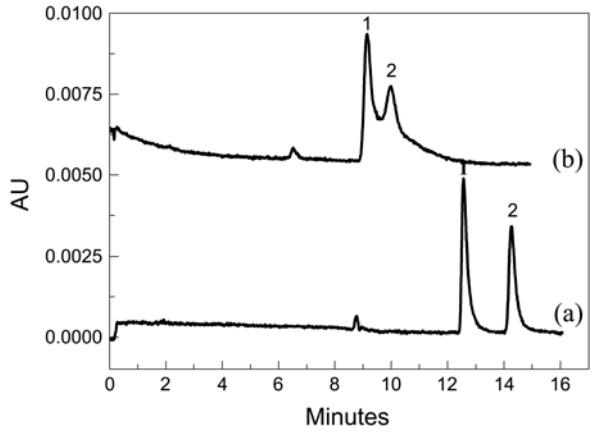

Figure 4. Electrochromatograms of carbendazim and imidacloprid obtained on LCCE-TMOS-VTMS column (a) and TMOS-VTMS column (b). Mobile phase, 20 mmol·L^{-1} phosphate buffer (pH 7.0) - acetonitrile (90: 10, v/v). Other conditions are the same as in Fig. 3. Peak identification: 1, carbendazim; 2, imidacloprid.

Acetonitrile with various volume fractions (5-25%) in mobile phase was studied. At acetonitrile content below 10%, a decrease in acetonitrile content contributed to longer migration time. Further increase in acetonitrile content led to the loss of selectivity and efficiency. As a consequence, 10% acetonitrile addition was chosen for subsequent work.

Enhancing buffer concentration from 10 to 50 mmol/L prolonged the migration time of pesticides. Buffer concentration lower than 20 mmol/L could worsen resolution, whereas higher buffer concentration could cause peak broadening. 20 mmol/L of phosphate buffer was thus selected as running buffer in mobile phase.

Column Performance. The performance of the LCCE-TMOS-VTMS column was compared with TMOS-VTMS column under the optimum conditions. Imidacloprid and carbendazim were baseline-separated within 15 min in LCCE-TMOS-VTMS column with acceptable peak symmetry (Figure 4(a)). However, they were coeluted in TMOS-VTMS column under the same conditions (Figure 4(b)). Different combining power of hydrogen bonds between crown ether and the two analytes could be responsible for their retention behavior. Besides, the rod-like shape and ordered arrangement of the liquid crystal might in part explain their separation. It can be concluded that it was the synergic effect between crown ether and liquid crystal moieties that improved the separation and retention behavior.[24]

Optimization of Sample Preparation. For the determination of pesticide residues in food matrices, the choice of extraction solvent is of significant importance. Typical solvents have been acetone, ethyl acetate and acetonitrile. Acetonitrile extracts of vegetables contain fewer interfering substances than the corresponding ethyl acetate and acetone extracts. Furthermore, acetonitrile can be separated fairly easily from water by salting out,[27] and therefore acetonitrile was adopted as the extraction solvent in this experiment.

In the clean-up procedure, the feasibility of different sorbents for the clean-up of tomato samples was investigated.

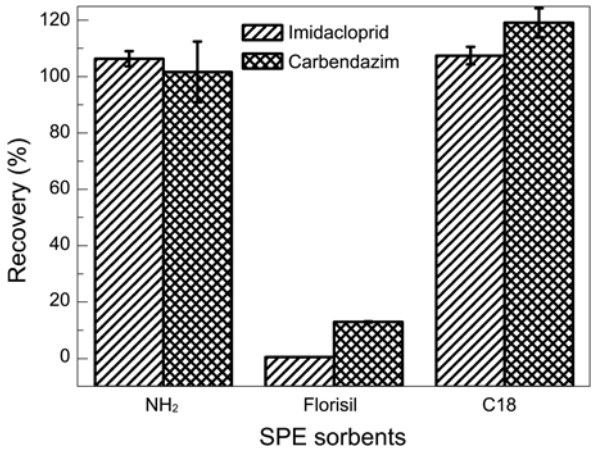

Figure 5. Recoveries of imidacloprid and carbendazim from spiked tomato samples with different SPE cartridges.

Figure 5 shows the mean recoveries for Florisil, NH_2 and C18 sorbents respectively. In case of Florisil, pesticides were greatly absorbed by magnesium silicate particles, and therefore the recoveries of pesticides were less than 20%. For C18 test, the endogenous interfering substances in tomato matrix could not be effectively eliminated. The best cleanup effect and recoveries for imidacloprid and carbendazim were achieved with NH_2 sorbents. Consequently, NH_2 cartridge was selected for sample clean-up.

Analytical Performance. Due to the presence of many endogenous compounds in complex matrices like vegetables that could influence the chromatographic signal of the selected pesticides, the matrix matched calibration is proposed for the quantification of imidacloprid and carbendazim in tomatoes in this work. The matrix matched calibration curves were linear with correlation coefficients (r^2) higher than 0.9998 as shown in Table 1. The precision of the proposed method was assessed in terms of intraday and interday analysis. Figures of merit are summarized in Table 1. Intraday RSDs were found to be lower than 2.3% for retention time, and 7.6% for peak area. Interday RSDs on five consecutive days were lower than 2.9% for retention time, and 9.4% for peak area. The limits of detection (LODs), calculated as the lowest spiked concentration that yielded a signal-to-noise ratio equal to 3, were 0.061 and 0.15 $mg \cdot kg^{-1}$ for imidacloprid and carbendazim respectively, which were verified by the analysis of tomatoes fortified at the LODs levels. These values are below the maximum residue limits (MRLs) for imidacloprid and carbendazim in tomatoes established by Codex alimentarius and European Union.

Table 2. Results for the determination of imidacloprid and carbendazim in tomatoes ($n = 5$)

Pesticide	Spiked level (mg/kg)	Found (mg/kg)	Recovery (%)	RSD (%)
Imidacloprid	0	nd[a]		
	0.50	0.53	105.0	5.9
	1.0	1.06	106.3	5.4
Carbendazim	0	nd[a]		
	0.50	0.54	108.0	6.3
	1.0	1.02	101.6	0.90

[a]nd: not detected.

It is well known that CEC suffers from its relatively poor concentration sensitivity because of the short optical path length and the limited sample volume for on-column photometric detection. However, the detection limits obtained in this contribution allow for the application to real samples. On the other hand, the CEC method shows several advantages compared to other developed procedures: (i) high selectivity of monolithic column, (ii) satisfactory resolution, and (iii) lower operational cost with regard to columns and solvents.

Application to Real Samples. In order to validate the applicability of the proposed method, the determination of imidacloprid and carbendazim in tomatoes bought in local markets was fulfilled. Reproducibility and recovery experi-

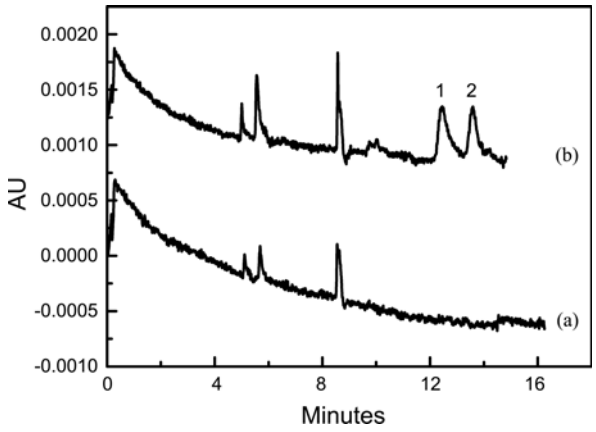

Figure 6. Electrochromatograms of carbendazim and imidacloprid in non-spiked tomato samples (a) and spiked tomato samples (b). Mobile phase, 20 $mmol \cdot L^{-1}$ phosphate buffer (pH 7.0)-acetonitrile (90:10, v/v). Other conditions are the same as in Fig. 3. Peak identification: 1, carbendazim; 2, imidacloprid.

Table 1. The analytical characteristics of the proposed method

Pesticides	Linear regression data			LOD (mg/kg)	Intraday precision, RSD (%), $n = 5$		Interday precision, RSD (%), $n = 5$	
	Regression equation	Test range (μg/mL)	r^2		t_R	Peak area	t_R	Peak area
Carbendazim	$y = 19810x - 4605.5$	0.50-10	0.9998	0.061	2.3	7.6	2.9	4.2
Imidacloprid	$y = 25523x - 3403.3$	0.20-10	0.9999	0.15	2.0	5.2	2.9	9.4

ments were carried out at two concentration levels of 0.50 and 1.0 $mg \cdot kg^{-1}$ for each pesticide. Table 2 lists the experimental results and no imidacloprid and carbendazim were found in tested tomatoes. The reproducibility of the method was obtained with relative standard deviations ranging from 0.9 to 6.3% and average recoveries were between 101.6 and 108.0%, demonstrating that the proposed method was a reliable technique for analysis of imidacloprid and carbendazim in tomatoes. A clean electrochromatogram is depicted in Figure 6.

Conclusions

Using a liquid crystal crown ether modified hybrid silica monolithic column, a simple and reliable CEC method for the determination of imidacloprid and carbendazim in tomatoes was developed. The bonded liquid crystal crown ether enabled the hybrid silica monolith to exhibit excellent performance toward imidacloprid and carbendazim. Applied to the analysis of tomato samples, the LODs for imidacloprid and carbendazim were 0.061-0.15 mg/kg and average recoveries were 101.6-108.0% with RSDs less than 6.3%. The results show the potential of CEC as a routine analytical technique for monitoring pesticide residues in complex samples.

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