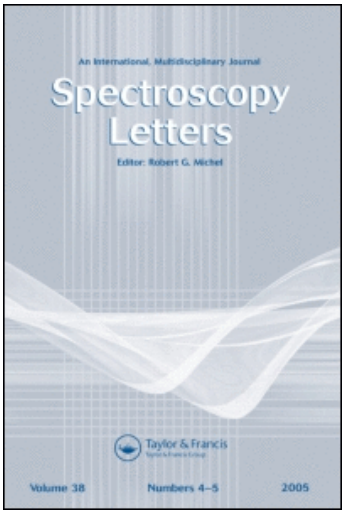

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ABSTRACT A simple, convenient, sensitive, and environmentally friendly analysis method for carbamate pesticide residues in vegetable samples was proposed by using electrokinetic flow analysis (EFA) with on-line hollow fiber liquid–liquid–liquid microextraction (LLLME) and ultraviolet spectrophotometry (UV). On-line LLLME conditions were investigated, including organic solvents, flow rate of sample loading, extraction times and sample volume, and so on. With dodecanol phase immobilized on the hollow fiber, the on-line LLLME unit could be operated for 150 hr. In each regeneration of dodecanol phase on the hollow fiber, the consumption of dodecanol was 50 μL, namely., the consumption rate was 0.33 $\mu L\,h^{-1}$. By introducing 5.0 mL sample solution, the analytical time was 22 min and the enrichment factor of carbaryl achieved 300. The linear calibration range was from 0.0033 to 1.0 $\mu g\,mL^{-1}$ carbaryl, which was used as a converted concentration for total carbamate pesticides. The relative standard deviation (R.S.D.) of peak height was 2.7% (n = 5) and the limit of detection (LOD) was 1 $ng\,mL^{-1}$ carbaryl (K = 3, n = 11).

KEYWORDS carbamate pesticide, electrokinetic flow analysis, green analysis method, hollow fiber, on-line liquid–liquid–liquid microextraction, spectrophotometry, vegetable

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INTRODUCTION

Carbamate pesticides are widely used in agriculture production owing to their shorter persistence in environment and lower toxicity for mammals compared with organochlorine and organophosphorus pesticides. However, the toxicological research indicates that carbamate pesticides

are also acetylcholinesterase inhibitors, and suspected carcinogen and mutagen.[1] Therefore, the development of simple, convenient, sensitive, and green analysis methods for the pesticide residues in farm products is of great importance. High performance liquid chromatography (HPLC) and gas chromatography (GC) are the main analysis techniques for carbamate pesticides[2,3]; however, the former consumes organic solvents and the latter can cause carbamate decomposition due to their thermal lability. Non-chromatographic analysis methods of carbamate pesticides have also been reported, including UV-Vis spectrometry,[4] immunoassay,[5] biosensor[6] and electrochemical detection,[7] and so on.

Sample pretreatment is normally required to clean up matrix and concentrate the pesticide residues in farm products before their determination. Liquid–liquid extraction (LLE)[8] and solid-phase extraction (SPE)[9] are frequently adopted. LLE suffers from time consuming, low extraction efficiency and high consumption of toxic organic solvents. SPE can reduce the solvent consumption compared with LLE. Recently, more attention has been paid to green analytical methods to reduce the negative environment impact of the analytical methodologies.[10–13] In the carbamate pesticide analysis, green sample pretreatments have been developed by avoiding or reducing the use of organic solvents, such as solid-phase microextraction (SPME)[14] and liquid-phase microextraction (LPME).[15] LPME can markedly reduce the solvent consumption and provide high enrichment factor, in which liquid–liquid–liquid microextraction (LLLME) is a simple, convenient, effective, and environmentally friendly pretreatment method for ionizable analytes.[16] Hollow fiber has been adopted to support organic solvents in the aqueous–organic–aqueous extraction, with which solvent consumption is limited and operators can minimize the exposure to toxic solvents. LLLME has been used as an off-line sample pretreatment method for capillary electrophoresis,[17] capillary liquid chromatography (CLC),[16] and HPLC.[18] Mathiasson and co-workers reported an on-line LLLME–CLC method for the determination of bambuterol, but the stability of the solvent phase was 6 h, and the enrichment factor was 54 for 60 min.[19]

Automated and flow-based analysis methods have contributed to green analytical chemistry for reducing the consumption of solvents and reagents adopted in measurement steps.[20] Sequential injection analysis (SIA) is developed by Ruzicka and Marshall[21] with simplicity and versatility, which decreases the consumption of solvents and reagents, and reduces the analytical waste compared with flow injection analysis (FIA) dramatically. Multicommutation with three-way solenoid valves and minipumps as discrete commutation devices controlled by a microcomputer is proposed by Reis et al.[4,22] It provides flexibility and controllability, minimizes the reagent consumption and waste generation, and improves automated spectrometric determination. Porous core electroosmotic pump[23] has exhibited its virtues of simplicity, large flow range, pulseless flow rate, appropriate back pressure, and controllability. Its pump core can be regenerated.[24] However, electrolyte solutions cannot be introduced into the pump core because of the influence on its surface charge density and electroosmotic flow. SIA can overcome this limitation by aspirating solutions into a holding coil to avoid the influence on the pump core. Electrokinetic flow analysis (EFA) is based on the flow analysis techniques of SIA, multicommutation, and electroosmotic pump, and combines with microcolumn chromatography[25] and electrochromatography,[26] and so on, which is developing to be a total analysis system.[27]

In this article, an on-line hollow fiber LLLME–EFA–UV method is proposed to increase the enrichment factor, improve the stability of solvent phase, and reduce the solvent consumption. The on-line LLLME conditions are investigated, including organic solvents, flow rate of sample loading, extraction times, sample volume, and so on. The proposed method is a green analytical method and is applied firstly to determine total carbamate pesticide residues in vegetable samples directly, in which carbaryl concentration is used as a converted one for total carbamate pesticides.

MATERIALS AND METHODS

Reagents and Solutions

The stock concentration of individual carbamates was 100 $\mu g\,mL^{-1}$ in acetone, including carbaryl, propoxur, pirimicarb, metolcarb, carbofuran, isoprocarb, bendiocarb, and fenobucarb (Inst. Agroenvironmental

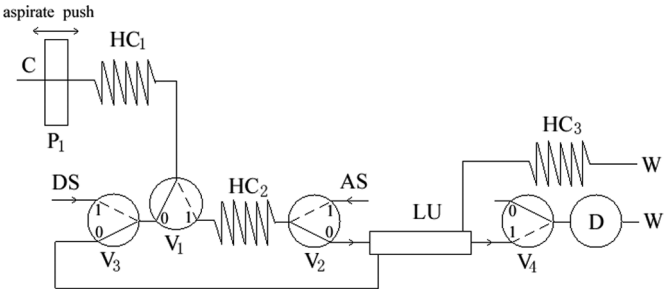

FIGURE 1 **Schematic diagram of on-line hollow fiber LLLME–EFA–UV system for carbamate pesticide analysis. C, carrier; P_1, electroosmotic pump; DS, donor (sample) solution; AS, acceptor (alkaline) solutoion; HC, holding coils; V, solenoid valves; LU, hollow fiber LLLME unit; D, spectrophotometer; W, waste.**

Protection, Ministry of Agric., Tianjin, China). Carbaryl standard solutions with the concentration from 0.0033 to 1.0 $\mu g\,mL^{-1}$ were prepared by diluting the stock solution with tri-distilled water. To calculate the enrichment factor of carbaryl, 1-naphthol solutions, as the hydrolyzed product of carbaryl, were prepared with five concentration levels from 0.72 to 220 $\mu g\,mL^{-1}$ by diluting 250 $\mu g\,mL^{-1}$ stock solution with 0.3 $mol\,L^{-1}$ NaOH and determined without the LLLME pretreatment.

Other reagents were of analytical grade and purchased from Chemical Reagent Ltd. (Shanghai, China). Distilled water was prepared by a tri-distilled water system (SZ-3, Huxi Anal. Instr. Factory, Shanghai). Vegetable samples were purchased from local markets.

Apparatus

The on-line hollow fiber LLLME–EFA–UV system is illustrated in Fig. 1. It consisted of one homemade electroosmotic pump, four solenoid valves (161T031, Nreseach Inc., USA), one homemade hollow fiber LLLME unit and one spectrophotometer (UV9100, Rayleigh Anal. Instr. Ltd., Beijing). The flow components were connected with 0.8 mm i.d. PTFE tube. Holding coils of HC_1 and HC_3 were 3 m, and HC_2 was 30 cm. The electroosmotic pump was operated by changing the polarity and voltage provided by an electrophoretic power supply (DYY-III-4, 20 ~ 1600 V, Liuyi Instr. Factory, Beijing, China). The pump and solenoid valves were controlled by a personal computer with a homemade interface card and a Visual C language program written by our group.[28] The R.S.D. of pump flow rate was 1.7% in 6.5 hr (n = 20) with the pump carrier solution of 0.5 $mmol\,L^{-1}$ hexamethylene tetramine (HMTA). The injection volumes of donor (sample) solution and acceptor (0.3 $mol\,L^{-1}$ NaOH) solution were regulated by the aspirating flow rate of the pump and the switch time of correlative valves. The spectrophotometer was equipped with a 10 mm quartz flow-through cell and the hydrolyzed pesticide products were detected at 245 nm.

The schematic diagram of the on-line hollow fiber LLLME unit is shown in Fig. 2. It consisted of one

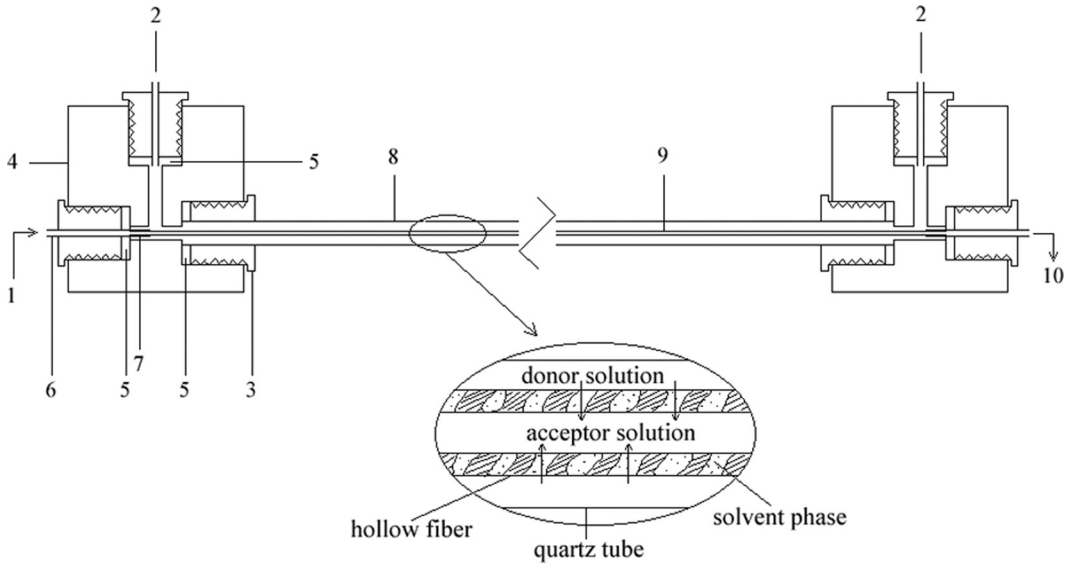

FIGURE 2 **Schematic diagram of hollow fiber LLLME unit. 1, acceptor solution inlet; 2, donor solution; 3, nylon nuts; 4, 1-mm i.d. thru-hole three-way nylon connectors; 5, silicone cushions; 6, fused-silica capillaries; 7, epoxy glue; 8, quartz tube; 9, polypropylene hollow fiber; 10, acceptor solution outlet.**

TABLE 1 Analytical Procedure of LLLME–EFA–UV System

Step	Operation	Flow rate ($mL\,min^{-1}$) pump voltage (V)	Time (s)	Valve position V_1	V_2	V_3	V_4
1	Aspirating 0.3 $mol\,L^{-1}$ NaOH solution to HC_2 through V_2	0.2 (−40)	5	1	1	0	0
2	Pushing NaOH solution from HC_2 to hollow fiber through V_2	0.18 (+40)	5	1	0	0	1
3	Aspirating 1 mL sample solution to HC_1 through V_3 and V_1	2.0 (−380)	30	0	0	1	0
4	Pushing sample solution from HC_1 to donor channel and HC_3 through V_1 and V_3	1.9 (+380)	32	0	1	0	0
5	Aspirating sample solution from HC_3 to donor channel and HC_1	2.0 (−380)	30	0	1	0	0
6	Pushing acceptor solution from hollow fiber to detector	1.9 (+380)	50	1	0	0	1

10 cm × 450 μm i.d. polypropylene hollow fiber with the pore size of 0.45 μm (Inst. Seawater Desalination Multiappl., National Oceanic Admin., Tianjin), one 9 cm × 0.85 mm i.d quartz tube (Qijing Electr. Mach. Ltd., Hefei, China), and two three-way nylon connectors with 1 mm i.d. flow path. The hollow fiber was washed in acetone by an ultrasonic bath (S-2200, 120 W, 35 kHz, J & L Ltd., Shanghai) and dried in air. Two ends of the hollow fiber were inserted into two fused-silica capillaries of 1.5 cm × 530 μm i.d. and fixed by epoxy glue. By inserting the capillaries through the quartz tube and into two connectors, the quartz tube, two capillaries, and the donor and acceptor solution inlet and outlet tubes were fixed on the connectors by fittings. The annular space between the inside of the quartz tube and the outside of the capillaries and hollow fiber was the donor channel with an approximate volume of 40 μL, and the flow path inside hollow fiber was the acceptor channel with a volume of 10 μL. The waste generated in each determination was aqueous solution and contained 5.0 mL sample matrix solution and 1.6 mL carrier solution.

To prepare the solvent phase, the hollow fiber was filled with dodecanol through both the donor and acceptor channels and washed with distilled water to remove the excessive dodecanol. The fiber could be regenerated by ethanol washing and dodecanol immobilizing after the analytical response was reduced to 90% of original one. 2 mL ethanol and 50 μL dodecanol were consumed in the regeneration of solvent phase.

Analytical Procedure

The analytical procedure of the on-line hollow fiber LLLME–EFA–UV method is given in Table 1. Carbamate pesticides in a sample solution were extracted into the dodecanol phase immobilized on the hollow fiber and back-extracted into the alkaline solution inside the hollow fiber with their hydrolyzed products. The extraction was performed for 7 times through the donor solution channel during Step 4 and 5, and 5.0-mL sample solution was introduced into the EFA system by circulating 5 times from Step 3 to 5.

When the room temperature was lower than 20°C, the LLLME unit should be warmed by a 60-W incandescent lamp within 5 cm, and HC_1 and HC_3 were dipped into 30°C water in a water bath to avoid dodecanol concretion.

Sample Preparation

Vegetable samples were wiped off dust and cut into pieces about 1 cm. 12.50 g sample was weighted in a 100 mL bottle, added 25 mL buffer solution containing 83.8 $mmol\,L^{-1}$ Na_2HPO_4 and 23.4 $mmol\,L^{-1}$ $KH_2\,PO_4$ (pH 7.5), blended by a vortex mixer for 2 min, kept for 3 min, and filtered with a 0.22-μm nylon membrane.[29] The treated sample solution was analyzed within 1 hr.

RESULTS AND DISCUSSION

The influence of analytical parameters on the enrichment factor were investigated, including

organic solvents, flow rate of sample loading, extraction times through the donor solution channel sample volume, and so on. The enrichment factor is defined as the ratio of the final analyte concentration in the acceptor solution to the initial one in the sample solution. A concentrated alkaline acceptor solution can reduce the boundary resistance of phenols (hydrolyzed products of the carbamates) between the acceptor solution and solvent phase, speed up the transfer, and enhance the extraction efficiency.[30] However, it had better be lower than 0.4 mol L^{-1} NaOH to reduce Schlieren effect.[31] In this work, 10 μL 0.3 mol L^{-1} NaOH was chosen as the acceptor solution. The baseline shift resulting from Schlieren effect was corrected by deducting the absorbance of the blank solution of 0.3 mol L^{-1} NaOH at 245 nm.

Organic Solvents

Stable and nontoxic organic solvents immobilized on the hollow fiber are preferable in the LLLME method. In this experiment, 1-octanol and dodecanol were investigated for the on-line LLLME–EFA–UV method. According to the experimental results, 1-octanol was markedly removed from the hollow fiber in 40 min. However, a long-term stability of 150 hr was obtained with dodecanol phase. During the regeneration of dodecanol phase, 2 mL ethanol was used to wash the LLLME unit and 50 μL dodecanol was introduced to form the solvent phase immobilized on the fiber. It was found that the consumption rate of ethanol and dodecanol was 13 and 0.33 μL h^{-1}, respectively. For the excellent stability and low toxicity of dodecanol, it was chosen as the organic solvent phase immobilized on the hollow fiber in this work.

Flow Rate of Sample Loading and Extraction Times

An appropriate flow rate of sample loading and optimal extraction times through the donor solution channel not only can improve the enrichment factor, but also reduce the analytical time. Therefore, two variables should be taken into account comprehensively. The influence of the flow rate and extraction times on the enrichment factor and analytical time is listed in Table 2. The flow rate was examined from 0.5 to 4.0 mL min^{-1} with 4.0 mL 0.70 μg mL^{-1} carbaryl. Under the maximal enrichment factors of 230–241, when the flow rate was enhanced from 0.5 to 2.0 mL min^{-1} and the extraction times increased from 3 to 7 for each sample solution of 1.0 mL, the analytical time could be reduced from 35 to 18 min. However, with the flow rate higher than 2.0 mL min^{-1} and the extraction times more than 7, the analytical time could not be reduced further and kept at 18 min. The experimental results indicated that the concentration efficiency could not be enhanced with the flow rate higher than 2.0 mL min^{-1}. Thus, the flow rate of 2.0 mL min^{-1} and extraction times of 7 were selected in the on-line LLLME pretreatment.

TABLE 2 **Influence of Flow Rate in Sample Loading and Extraction Times Through Donor Channel on Enrichment Factor and Analytical Time**

Sample flow rate (mL min^{-1})	Extraction times	Enrichment factor	RSD of enrichment factor, (%, N = 3)	Analytical time (min)
0.5	3	241	1.3	35
1.0	5	230	3.3	26
2.0	7	235	2.3	18
3.0	11	239	2.6	18
4.0	15	232	3.2	18

Sample Volume

To limit the flow resistance, 1.0 mL sample solution was aspirated into the holding coil (HC_1) each time in Step 3. If the volume more than 1.0 mL was required, the circulation from Step 3 to 5 should be performed. The effect of sample volume on peak height was investigated from 2 to 12 mL. In accordance with the experimental results, the peak height of absorbance was enhanced rapidly by increasing the sample volume from 2.0 to 10 mL, and then changed slowly with the volume higher than 10 mL. It implied that the sample volume of 10 mL was the breakthrough volume of the on-line LLLME unit. Although the enrichment factor of 10 mL sample volume could achieve 600, the analytical time was prolonged to 43 min. After considering both enrichment factor and analytical time, the sample volume of 5.0 mL was chosen in this work, with which the enrichment factor was 300 and analytical time was 22 min.

Interference

The interference of benzoic acid, phenol, and aniline was examined due to the possibility of their co-extraction and detection. It was reported that the cold alkaline solution neither hydrolyzed carbamate pesticides nor extracted their hydrolyzed products, but it could extract acidic interfering components.[32] To eliminate the interference of benzoic acid, 1.0-mL sample solution at 30°C was on-line treated with 1.0 mL, 0.5 mol L^{-1} NaOH cold solution by the LLLME–FEA system revised, in which the sample and alkaline solutions were oppositely delivered back and forth 4 times through the donor and acceptor solution channel at 0.5 mL min^{-1} by two electroosmotic pumps, respectively. If the sample volume was more than 1 mL, the treatment procedure should be repeated.

Then the treated sample solution was collected and adjusted to pH 4.0 with 1.0 mol L^{-1} HCl. Aniline changed into aniline hydrochloride and could not be extracted into the acceptor solution.

With the relative difference of peak height lower than 5%, 9.0 μg mL^{-1} benzoic acid, 6.0 μg mL^{-1} phenol, and 500 μg mL^{-1} aniline did not interfere with the determination of 0.70 μg mL^{-1} carbaryl by using the interference elimination method mentioned earlier. As a consequence, the clean-up method can eliminate the interference of aniline and benzoic acid except phenol.

Quantitative Characteristic and Real Sample Analysis

For the proposed method, linear concentration range, precision (R.S.D.), limit of detection (LOD), and recovery were examined with the standard solutions of carbaryl. The R.S.D. of peak height achieved 2.7% with five individual analyses. The linear concentration range was from 0.0033 to 1.0 μg mL^{-1}, and the linear regressive equation was $y = -1.21 \times 10^{-3} + 1.07x$ with the regressive coefficient of 0.999, in which y was peak height in AU and x was the analyte concentration in μg mL^{-1}. The LOD defined as 3-fold standard deviation of blank absorbance (N = 11) was 1 ng mL^{-1} carbaryl or 2 μg kg^{-1} carbaryl for vegetable samples. The LOD value of the proposed method for carbaryl was comparable to those by LPME-HPLC (0.42 ng mL^{-1})[15] and SPME-HPLC (0.44–0.67 ng mL^{-1}).[14]

TABLE 3 Concentrations of Other Carbamate Pesticides Converted to Carbaryl Concentration

Other pesticides (μg mL^{-1})	Carbaryl (μg mL^{-1})	RSD (%, N = 3)
Propoxur 0.500	0.475	3.1
Pirimicarb 0.500	0.379	4.3
Metolcarb 0.500	0.480	2.9
Carbofuran 0.500	0.391	3.7
Isoprocarb 0.500	0.488	2.5
Bendiocarb 0.500	0.520	3.1
Fenobucarb 0.500	0.450	1.0

The proposed method has been applied to the determination of total carbamate pesticide residues in vegetable samples with the converted concentration of carbaryl. In the conversion, the absorbance of 0.500 μg mL^{-1} each carbamate pesticide was determined and converted to the carbaryl concentration with the linear regressive equation of carbaryl, as listed in Table 3. The concentrations of the carbamate pesticide residues in five vegetable samples were lower than the limit of quantitative analysis, namely, 3-times LOD, and much lower than the maximum residue limits (MRLs) of vegetable samples established by the European Union (EU).[33]

The real samples were then spiked with 0.070 and 0.70 μg mL^{-1} carbaryl, respectively, and the recoveries of carbaryl were in the range of 89.2–108% (N = 3).

CONCLUSIONS

The on-line hollow fiber LLLME–EFA–UV method was applied to the determination of total carbamate pesticide residues in vegetable samples satisfactorily. For the analysis of carbamate pesticide residues in 5.0 mL sample solution, the enrichment factor of 300 was obtained and the analytical time was 22 min. With dodecanol as the organic solvent phase immobilized on the hollow fiber, the working time of the solvent phase achieved 150 hr. The proposed method was simple, convenient, sensitive, and environmentally friendly. The portable total analysis system of EFA is our due course.

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