http://dx.doi.org/10.5012/bkcs.2014.35.4.1011

Octadecyl-Modified Graphene as an Adsorbent for Hollow Fiber Liquid Phase Microextraction of Chlorophenols from Honey

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Received July 29, 2013, Accepted December 5, 2013

Octadecyl-modified graphene (graphene-C18) was fabricated and used as adsorbent in hollow fiber liquid phase microextraction (HF-LPME) for the first time. The extraction performance of graphene-C18 reinforced HF-LPME was evaluated using chlorophenols as model analytes. The factors affecting the extraction efficiency, such as extraction time, pH of the sample solution, agitation rate, the concentration of graphene-C18 and salt addition were optimized. After the graphene-C18 reinforced HF-LPME of the chlorophenols from honey sample, the analytes were separated and determined by high-performance liquid chromatography. The linearity was observed in the range of 5.0-200.0 ng g^{-1} for 2-chlorophenol and 3-chlorophenol, and 2.0-200.0 ng g^{-1} for 2,3-dichlorophenol and 3,4-dichlorophenol, respectively. The limits of detection (S/N = 3) of the method were lower than 1.5 ng g^{-1}. The recoveries of the method were between 88% and 108%. The method is simple, sensitive and has been resoundingly applied to analysis of chlorophenols in honey samples.

Key Words : Hollow fiber liquid phase microextraction, Graphene-C18, High performance liquid chromatography, Chlorophenols, Honey sample

Introduction

In recent years, people are increasingly worried about the chemical contamination residues in food and environment. Nevertheless, most of them exist in complex sample matrices at trace levels.[1] Therefore, for their effective determination, it is important to choose a proper sample preconcentration technique to efficiently extract and enrich them before instrumental analysis. For sample preconcentration, apart from the conventional liquid–liquid extraction (LLE)[2] and solid-phase extraction (SPE)[3,4] techniques, some novel sample preparation methods, such as solid-phase microextraction (SPME)[5] and liquid phase microextraction (LPME)[6] have been developed.

In 1999, Pedersen-Bjergaard and Rasmussen developed a novel operating mode of LPME, which was named hollow fiber liquid phase microextraction (HF-LPME).[7] In this technique, extraction phase is poured into the lumen of porous hollow fibers made of polypropylene, and the target analytes are extracted from the sample solution through the pore of the fiber into an extraction phase.[8] Since its inception, due to its attractive advantages[9] such as low cost, simplicity, prominent clean-up efficiency and high enrichment factors, HF-LPME has become a promising alternative approach to conventional LLE. It has been successfully applied as a novel preconcentration technique for the analysis of inorganic and organic analytes in complex matrix samples.[10,11]

To further improve the extraction efficiency of HF-LPME, carbon nanotube reinforced hollow fiber microporous membrane liquid phase microextraction has been reported.[12,13] In these works, a porous polypropylene membrane modified with carbon nanotube that assume an analyte trap to preconcentrate the analytes from different samples was used, which bring about a higher selectivity and enrichment for the analytes.

Graphene (G), which is a fascinating and novel carbon nanomaterial, has attracted increasing attention because of its unique properties.[14] Since the large delocalized π-electron system of the G-related materials can form strong hydrophobic and π-stacking interactions with some organic molecules, they could possibly serve as a suitable adsorbent.[15,16] For the past few years, graphene has been used as the adsorbent in solid-phase microextraction (SPME),[17] solid-phase extraction (SPE),[18] and magnetic solid-phase extraction (MSPE).[19,20]

Recently, the modification of graphene with a variety of functional groups has sparked considerable research interest. Compared with unmodified graphene, the alkyl-functionalized graphene showed improved lipophilicity, and enhanced dispersibility and stability in nonpolar solvents.[21] In addition, the introduction of long alkyl chains into graphene resulted in a high adsorption performance to some target analytes.[22]

Chlorophenols (CPs) are widespread environmental pollutants due to their extensive use as preservatives, disinfectants and intermediates in many industries.[23] Although they are generally present in trace level, their carcinogenicity and toxicity may have adverse effects on human beings.[24] They have been classified by the International Agency for Research on Cancer (IARC) as possible carcinogenic agents to humans.[25]

In this work, graphene modified with octadecyle (G-C18) was fabricated and used as the adsorbent in HF-LPME. The performance of the G-C18 reinforced HF-LPME was evalu-

ated for the extraction of some CPs in honey samples prior to their determination by high performance liquid chromatography (HPLC).

Experimental

Chemicals and Materials. Graphite powder (50 meshes) was purchased from the Boaixin Chemical Reagent Company (Baoding, China). Standards of 2-chlorophenol (2-CP), 3-chlorophenol (3-CP), 2,3-dichlorophenol (2,3-DCP) and 3,4-dichlorophenol (3,4-DCP) were purchased from Aladdin-reagent (Shanghai, China). Acetonitrile, acetone, hydrochloric acid (HCl), sodium hydroxide (NaOH), 1-octanol, ethyl acetate, dichloromethane, *n*-hexane and chlorobenzene were purchased from Huaxin Chemical Reagent Company (Baoding, China). Sodium chloride (NaCl) was from Tianjin Fuchen Chemical Reagent Factory (Tianjin, China). The water used throughout the work was double-distilled on a SZ-93 automatic double-distiller purchased from Shanghai Yarong Biochemistry Instrumental Factory (Shanghai, China).

The Accurel Q 3/2 polypropylene hollow fiber membrane (200 μm thick wall, 600 μm inner diameter and 0.2 μm average pore size) was obtained from Membrana GmbH (Wuppertal, Germany).

A mixture stock solution containing each of 2-CP, 3-CP, 2,3-DCP and 3,4-DCP at 20.0 μg mL^{-1} was prepared in methanol. A series of standard solutions were prepared by mixing an appropriate amount of the stock solution with methanol in a 10 mL volumetric flask. All the standard solutions were stored at 4°C and protected from light.

Instruments. HPLC was carried out on a LC-20AT liquid chromatography (Shimadzu, Japan) with two LC- 20AT VP pumps and a SPD-20A UV/vis detector. Chromatographic separations were performed on a Century SIL C18 column (250 mm × 4.6 mm I.D., 5.0 μm) from Dalian Johnsson Separation Science Technology Corporation (Dalian, China). The mobile phase was a mixture of methanol-water (65:35 v/v) at a flow rate of 1 mL min^{-1}. The UV monitoring wavelength was chosen at 280 nm. Figure 1 shows the representative chromatogram of the standard solution of the CPs.

Sample Preparation. Honey sample was purchased from local markets (Baoding, China). 10.0 g of the honey sample was weighed and placed into a 25 mL of graduated glass vial. Double-distilled water was added into the vial to the mark of 15.0 mL for the following HF-LPME extraction.

Preparation of Graphene-C18 Reinforced Hollow Fiber (G-C18 -HF). G-C18 was synthesized in our lab according to the literature method.[21] For the preparation of the acceptor phase, a certain amount of the G-C18 was thoroughly dispersed in 1-octanol by ultrasonication at room temperature for 1.0 h.

The hollow fiber was cut manually into approximately 4 cm long pieces, which were ultrasonically washed with acetone for 5 min to remove the impurities in the fiber, and then dried. Then the fiber was soaked with 1-octanol for 30 s to dip the pores and then cleaned with water under ultrasonication to remove the organic solvent on the surface and the inner wall. 20 μL of 1-octanol containing 2 mg mL^{-1} of G-C18 as the acceptor phase was injected into the lumen of the fiber. Then both sides of the fiber were sealed together with a heated tweezers.

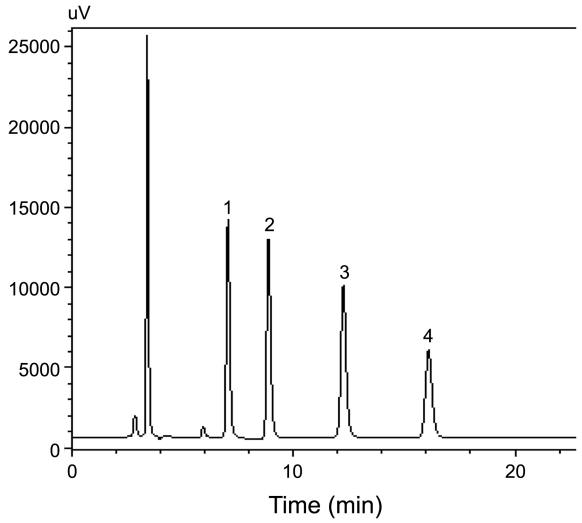

Figure 1. The chromatogram of the standard solution of CPs at each concentration of 20 ng mL^{-1}. Peak identification: 1. 2-CP, 2. 3-CP, 3. 2, 3-DCP, 4. 3, 4-DCP.

HF-LPME Procedure. The prepared hollow fiber was immersed into a 25 mL glass vial containing 15.0 mL of the sample solution, 0.75 g NaCl and 10 μL 1-octanol. The extraction was performed with a magnetic stirring at 800 rpm for 30 min. After the extraction, the fiber was taken out from the vial and transferred into a 500 μL micro-vial. Then the analytes were desorbed from fiber with 100.0 μL methanol under vortexing for 2 min. Finally, an aliquot of 15.0 μL was injected into the HPLC instrument for analysis.

Results and Discussion

In this study, the following several parameters which influence the extraction efficiency were studied and optimized. All the optimization was performed in triplicate by using double-distilled water spiked with the CPs at each concentration 40.0 ng mL^{-1} as the sample.

Effect of the Type and Volume of Extraction Solvent. The polyropylene membrane is hydrophobic in nature, so it is necessary to select an organic solvent which immobilized in the hollow fiber for the enrichment of the analytes. According to our previous experience,[11] 1-octanol, *n*-hexane, dichloromethane, ethyl acetate and chlorobenzene were tested as the candidate solvent. As a result, among the organic solvents tested, 1-octanol revealed the best analytical signals for CPs and therefore was selected.

The literature work[26] showed that when small amount of the organic solvent was added into the sample solution, it could increase the HF-LPME extraction efficiency since the contact area between the extractant and the sample solution would increase. In this study, the addition of different volumes of 1-octanol (5.0, 10.0, 15.0, and 20.0 μL) into the

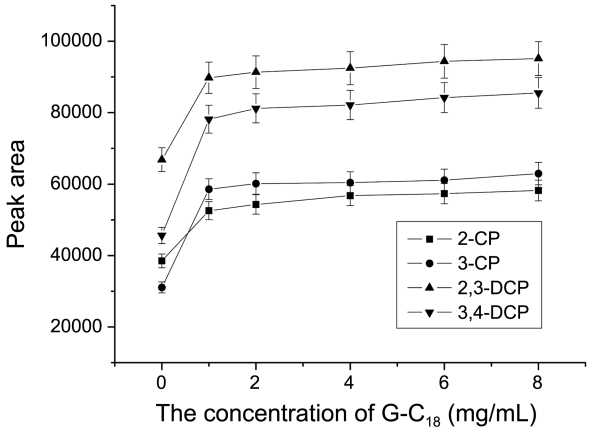

Figure 2. Effect of the concentration of graphene-C18 on the extraction efficiency of the CPs.

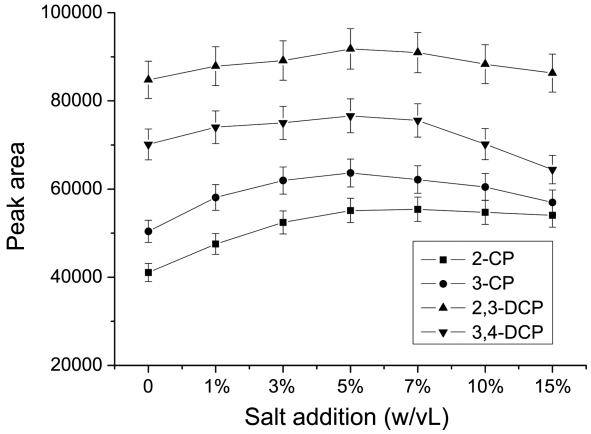

Figure 3. Effect of salt addition on the extraction efficiency of the CPs.

sample solution was studied. The results indicated that the peak areas reached the maximum when 10.0 μL of 1-octanol was added. Therefore, 10.0 μL of 1-octanol was added into the sample solution for the following studies.

Effect of the Concentration of Graphene-C18. In the extraction procedure, acceptor phase and donor phase are in close contact. The adsorption capacity would be affected by the concentration of the graphene-C18 in the acceptor phase. The influence of graphene-C18 concentration was examined from 0 to 8 mg mL^{-1}. As shown in Figure 2, when the concentration of graphene-C18 was increased from 0 to 2 mg mL^{-1}, the peak areas of the analytes were increased and after that remained almost unchanged. Therefore, the concentration of the graphene-C18 in the acceptor phase was selected at 2 mg mL^{-1}.

Effect of the Sample Solution pH. The CPs are ionizable at the pH of the sample solution larger than their corresponding pK_a values, which are between 6.44 and 8.85. Therefore, the pH of the sample solution will play an important role for their extraction. The pH of the sample solution was investigated in the range between 2 and 10, and the peak areas kept almost constant as the pH was increased from 2 to 7, then decreased when the pH was further increased. Based on this result and considering that the pH of the honey sample was about 5, the pH of the sample solution did not need to be adjusted.

Effect of Salt Addition in the Sample Solution. Generally, the addition of salt to the sample can enhance the ionic strength of aqueous solution, which can affect the solubility of the compounds. The effect of salt addition was studied by increasing the NaCl concentration from 0% to 15% (w/v) in sample solutions. Figure 3 showed that the peak areas of the CPs remained nearly constant when the concentration of NaCl was increased from 0% to 5% and the peak areas decreased when the concentration of NaCl was further increased. The results can be explained by the two simultaneously occurring processes: the salting out effect and the electrostatic interactions between polar molecules and salt ions in sample solution. At first, the former process played the primary role. But the salt molecules began to interact with analyte molecules when salt concentration increased further, which straightly lead to the decrease of the signal.[27] On the basis of these results, the concentration of NaCl was chosen as 5% for the subsequent experiments.

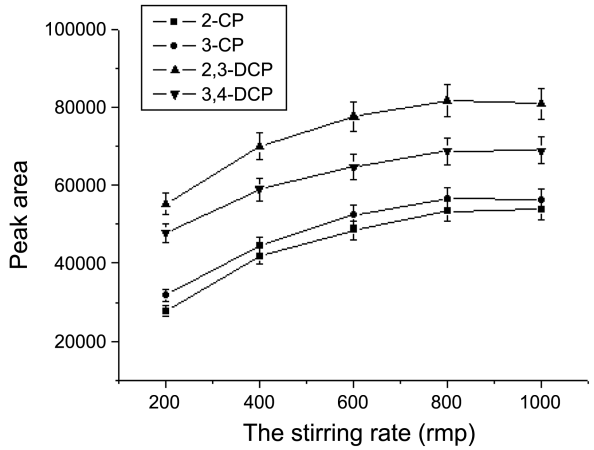

Figure 4. Effect of the stirring rate on the extraction efficiency of the CPs.

Effect of the Stirring Speed. Sample agitation played an important role in enhancing the extraction efficiency. To evaluate the influence of the stirring rate on the extraction of the CPs, the stirring rate in the range of 200-1000 rpm was investigated. The results (Figure 4) indicated that the extraction efficiency of the CPs was increased when the stirring rate was increased from 200 to 800 rpm and then remained almost the same. High stirring rate produced many bubbles on the surface of the hollow fiber, which impeded the transfer of the analytes. Therefore, the stirring rate was selected at 800 rpm for the subsequent experiments.

Effect of Extraction Time. Since HF-LPME is not an exhaustive extraction but a partition process of the analytes between the sample matrix and the extractant, HF-LPME often needs a period of time to reach the extraction equilibrium. In this experiment, for the investigation of the effect of the extraction time, the extraction times were varied in the range from 10 to 60 min. As can be seen from Figure 5, the peak areas of the CPs increased by increasing the extraction

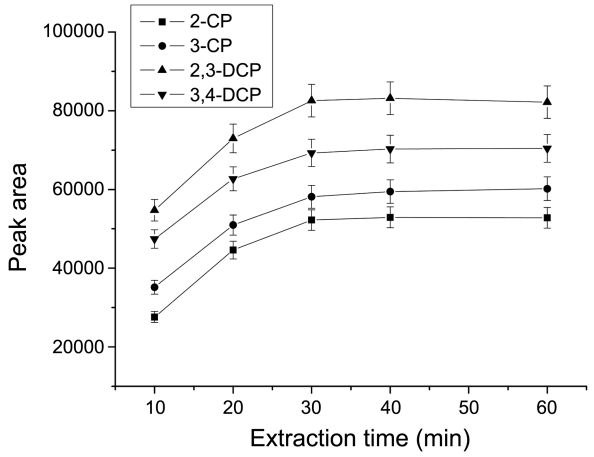

Figure 5. Effect of extraction time on the extraction efficiency of the CPs.

time up to 30 min, and after that, the signal kept almost constant. So, 30 min was selected as the optimum extraction time.

Method Validation. A series of CPs-free honey samples containing each of the CPs at seven concentration levels of 2.0, 5.0, 10.0, 20.0, 50.0, 100.0, and 200.0 ng g^{-1} were prepared to establish the calibration curve. Each concentration level was performed in five replicate extractions. The results are listed in Table 1. Good linearity was observed in the concentration range of 5.0-200.0 ng g^{-1} for 2-CP, 3-CP and 2.0-200 ng g^{-1} for 2,3-DCP, 3,4-DCP, respectively, with the correlation coefficients (r) ranging from 0.9942 to 0.9989. Limits of detection (LODs) at a signal to noise ratio of 3 (S/N = 3) were between 0.5 to 1.0 ng g^{-1}. The reproducibility of the method was tested by five parallel determinations at the concentration of 20.0 ng g^{-1} for each of the CPs under the optimal conditions. The results expressed as the relative standard deviation (RSD) were between 4.2% and 6.8%.

The enrichment factor (EF) was defined as the ratio between the analyte concentration in the desorption solution (100 ìL methanol) and the initial analyte concentration in the sample solution. Under the above optimized experimental conditions, the enrichment factors of this method for 2-CP, 3-CP, 2,3-DCP and 3,4-DCP were 66, 61, 69, and 63, respectively.

Analysis of Real Samples. To investigate the applicability and accuracy of the developed method, the extraction and determination of the four CPs in commercial honey sample were conducted under the optimum conditions. As a result, 3.1 ng g^{-1} of 2,3-DCP and 2.6 ng g^{-1} of 3,4-DCP were found respectively in one of the honey samples. To evaluate the accuracy of the method, the honey sample was spiked with the standards of the CPs to the concentrations of 5.0 and 50.0 ng g^{-1}. The results are shown in Table 2. The recoveries and the RSDs of the four CPs were in the range of 88%-108% and 4.9%-6.9%, respectively, which indicated that the new method was applicable for the analysis of the target analytes in real complex matrix samples like honey. The typical chromatograms of the blank and spiked honey sample were shown in Figure 6.

Table 1. The linear ranges (LR), correlation coefficients (*r*), limits of detection (LODs) and relative standard deviations (RSDs) for honey sample.

CPs	LR[a] (ng g^{-1})	*r*	LODs (ng g^{-1})	RSDs (%) (*n* = 5)
2-CP	5.0-200.0	0.9957	1.5	6.8
3-CP	5.0-200.0	0.9989	1.5	4.2
2,3-DCP	2.0-200.0	0.9942	0.5	5.9
3,4-DCP	2.0-200.0	0.9974	0.5	6.4

LR[a]: linear range.

Table 2. The precision and recoveries of CPs in honey sample

CPs	Spiked (ng mL^{-1})	Honey sample (*n* = 5)		
		Measured (ng mL^{-1})	R[a] (%)	RSD (%)
2-CP	0.0	nd[b]		
	15	14	94	6.9
	50	48	95	6.3
3-CP	0.0	nd[b]		
	15	14	92	5.6
	50	44	88	5.3
2, 3-DCP	0.0	3.1		
	15	18	98	4.9
	50	53	101	5.2
3, 4-DCP	0.0	2.6		
	15	17	92	6.4
	50	57	108	5.9

R[a]: recovery of the method; nd[b]: not detected.

Comparison of the Current G-C18–HF-LPME Method with Other Sample Preparation Methods. For the evaluation of the current method, it was compared with the other reported methods coupled with HPLC, such as ultrasound-assisted headspace liquid-phase microextraction (UAHS-LPME),[28] solid-phase microextraction with micellar desorption (SPME-MD),[29] stir bar sorptive extraction (SBSE),[30]

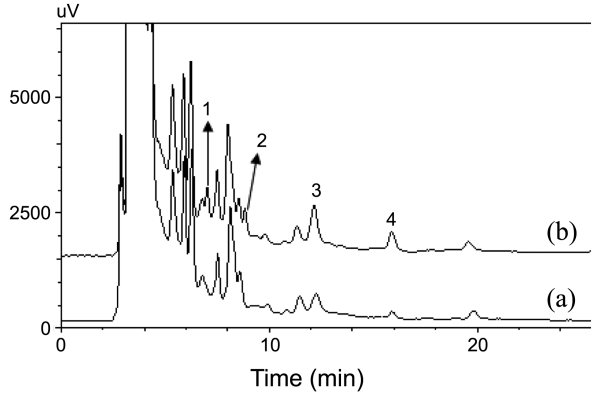

Figure 6. The typical chromatograms of (a) honey sample and (b) honey sample spiked with the CPs at each concentration of 10.0 ng g^{-1}. Peak identification: 1. 2-CP, 2. 3-CP, 3. 2,3-DCP, 4. 3,4-DCP. Detection wavelength: 280 nm.

Table 3. Comparison of the current G-C18–HF-LPME method with other sample preparation methods for the determination of CPs coupled with HPLC

Methods	LRs ($ng\ mL^{-1}$)	LOD ($ng\ mL^{-1}$)	RSD (%)	Samples	References
UAHS-LPME	50-2000	6-23	2.4-4.6	water	28
SPME-MD	1-200	1.1-5.9	6.3-15	water	29
SBSE	5-150	0.72-1.4	< 4.1	water	30
MSPE	1.0-105	0.17-0.22	< 7	water	31
SPE	–	30-80 $ng\ g^{-1}$	< 14	soil	32
UA-HF-LLLME	0.2-250	0.03-0.05	2.6-4.8	water	33
HFSLM	1-200	0.3-0.4	< 8.6	water	34
Three phase HF-LPME	0.45-60	0.14-0.25	< 4.3	water	35
G-C18–HF-LPME	2-200 $ng\ g^{-1}$	0.5-1.5 $ng\ g^{-1}$	< 6.8	honey	This method

magnetic solid-phase extraction (MSPE),[31] SPE,[32] ultrasound-assisted HF-LLLME (UA-HF-LLLME),[33] hollow fiber-based supported liquid membrane (HFSLM)[34] and three phase HF-LPME[35] for the determination of CPs from the viewpoint of linear ranges (LRs), LODs, and RSDs. The comparison results are shown in Table 3. As we all known, the fiber in HF-LPME is disposable, the carry-over problems can be overcame. And it does not need special apparatus and other additional clean-up processes. Moreover, the data illustrate that the G-C18–HF-LPME method has comparable LRs, LODs, and RSDs with those of the other referenced extraction methods. Thus, Hence, these results show that the G-C18–HF-LPME method is indeed a rapid, sensitive and easy to handle technique for the preconcentration of some analytes from complex sample matrices.

Conclusion

In this work, the graphene-C18 reinforced HF-LPME combined with HPLC has been developed for the extraction and determination of some CPs in honey sample. The results indicated that the method had the advantages such as simplicity, low cost, high sensitivity and excellent clean-up efficiency. The method is suitable for the analysis of the CPs in honey samples.

Acknowledgments. This research was financially supported by the National Natural Science Foundation of China (No. 31171698), the Science and Technology Supporting Program of Hebei Province (No. 12396908D), and the Natural Science Foundation of Hebei Province (B2012204028).

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