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Determination of Phenolic Compounds in Wastewater by Liquid-Phase Microextraction Coupled with Gas Chromatography

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Abstract

Liquid-phase microextraction (LPME) coupled with gas chromatography–flame ionization detection is applied to the analysis of phenolic compounds (phenol, *o*-cresol, *m*-cresol, 2,4-dimethylphenol, 2,3- dimethylphenol, and 3,4-dimethylphenol) in water samples. Experimental parameters affecting the extraction efficiency (including extraction solvent and drop volume, stirring rate, extraction time, temperature, salt concentration, and pH) are investigated and optimized. The developed protocol yields a good linear calibration curve from 5 or 20 to 10000 μg/L for the target analytes. The limits of detection are in the range of 0.94 to 1.97 μg/L, and the relative standard deviation is below 9.37%. The established method is applied to determine the phenolic pollutants in real wastewater samples from a coking plant. The recoveries of the phenolic compounds studied are from 92% to 102%, suggesting the feasibility of the LPME method for the determination of the phenolic compounds in wastewater.

Introduction

Phenolic compounds are some of the most important contaminants present in the environment as a result of various processes, such as the production of plastics, dyes, pesticides, paper, and petrochemical products (1–4). They are often found in waters (3–5), soils (6), and sediments (7). Because of their toxicity, phenols are included on the lists of priority pollutants in many countries and are required to be determined. The phenol index number in the China National Standard method includes all watersteam distilled phenolic compounds, which are photometrically detected after derivatization with 4-aminoantipyrine. This time-consuming method only obtains the total content of phenols and is unable to evaluate the exact amount of individual phenols. Currently, analysis of phenolic compounds is frequently based on liquid–liquid extraction (LLE), solid-phase extraction, and steam-distillation extraction, followed by gas chromatography (GC) or high-performance liquid chromatography (HPLC) (8–12). These methods are time-consuming and need a large amount of organic solvent. Solid-phase microextraction (SPME), a rapid and solvent-free extraction preconcentration technique, has been developed for the analysis of phenolic compounds in water, soil, wine, and food (13–15). However, SPME fibers are expensive and have a limited lifetime. The partial loss of the stationary phase, which coeluted with the target analytes, possibly results in the lack of precision of the peaks (16).

Recently, liquid-phase microextraction (LPME) was developed as a solvent-minimized pretreatment technique, which is fast, simple, and inexpensive. It is based on the distribution of the analytes between a microdrop of organic solvent at the tip of a microsyringe needle and an aqueous sample solution. First, the organic solvent drop is exposed to the sample solution, and then target analytes are extracted from the sample matrix into the drop. After equilibrium is reached, the drop containing the concentrated analyte is transferred to the GC or HPLC for further analysis. This novel technique eliminates the disadvantages of conventional extraction methods, such as time-consuming operation and using a specialized apparatus and large amounts of organic solvent. It also combines extraction, concentration, and sample introduction into one step. Until recently, LPME has been successfully applied for the determination of alcohols (17), nitroaromatics explosives (18), chlorobenzenes (19), drugs (20,21), and volatile organic compounds (22–24), in addition to being used for the screening of pesticides in water samples (25–28).

The purpose of this work is to develop a rapid and effective method for the determination of phenolic compounds in wastewater matrices by combining LPME with GC–flame ionization detection (FID). Parameters affecting the extraction of analytes, including organic solvent, organic drop volume, stirring rate, extraction time, extraction temperature, pH, and ionic strength were optimized. The linearity, detection limits, and precision of the method were evaluated. Finally, the proposed method was applied to the analysis of real wastewater samples.

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Experimental

Reagents

Phenol, o-cresol, m-cresol, 2,4-dimethylphenol, 2,3-dimethylphenol, and 3,4-dimethylphenol (> 99%) were purchased from Sigma (St. Louis, MO). The standard mixtures of six phenols were prepared by dissolving 10 mg of each compound in methanol in a 10-mL volumetric flask. The standard solution was stored at 4°C. Sodium chloride, hexane, tetrachloromethane, toluene, and xylene were all of analytical grade, and n-amyl acetate was of HPLC grade. The water used was purified by a WYQ sub-boiling distilling water purification system (Changsha, P.R. China).

Instrument

The determination of phenolic compounds was carried out with a Hewlett Packard 5890 GC–FID (Palo Alto, CA). The separation was performed on a fused-silica capillary column (DB-1701, 30 m × 0.25 mm × 0.25 µm). The carrier gas was nitrogen at a flow-rate of 1.5 mL/min. The injector and detector temperatures were 250°C and 300°C, respectively. The GC oven temperature was programmed as follows: initial temperature 130°C, held for 2 min; increased to 150°C at a rate of 4°C/min and held for 1 min. The inlet was operated in split mode with a split ratio of 20:1. Peak identification was made by the comparison of retention time with the corresponding standard. An 85-2 magnetic stirrer (Shanghai Sile Appliance Factory, Shanghai, P.R. China) was employed for stirring the sample during extraction.

LPME procedures

A 10-mL vial with a stir bar was placed on a magnetic stirrer. LPME was performed with a commercially available 10-µL GC microsyringe (Shanghai Gaoge Industrial and Trading, Shanghai, P.R. China). The microsyringe was fixed above the extraction vial with a clamp. After the needle passed through the septum, the needle tip was immersed into the 5 mL sample solution and was stored at the same height in order to obtain a good reproduction level. Then 3.0 µL of the extraction solvent was extruded from the needle and suspended at the needle tip for extraction. During the extraction, the solution was stirred at 400 rpm. After extracting for a prescribed period of time, the drop was retracted into the microsyringe, which was removed from the sample vial. The needle tip was carefully cleaned to remove any possible water contamination. The extraction solvent with the extracted analytes was injected into the GC inlet for analysis.

Sampling

Water samples (before and after biochemical treatment) from a wastewater treatment plant of a coking factory were collected, acidified to pH 1.5 with hydrochloric acid, and stored in 500-mL amber glasses. The contents of the phenols were analyzed by the proposed method. The water samples were stored at 4°C and analyzed within three days of sampling. Preliminary tests indicated that the pollutant concentrations in the water before biochemical treatment were very high. Therefore, it was necessary to dilute it in linear range for the quantitation of phenols.

Results and Discussion

Optimization of LPME

Extraction solvent

The selection of an appropriate extraction solvent was of major importance for the optimization of the LPME process, which was dependent on the chemical nature of the target analytes. Two requirements were considered when selecting a solvent. First, the solvent had to be immiscible with water. Second, the organic solvent had to have excellent chromatographic behavior (29). On the basis of these considerations, hexane, tetrachloromethane, toluene, xylene, and n-amyl acetate were tested. As shown in Figure 1, the results indicated that n-amyl acetate had the best extraction efficiency. Therefore, n-amyl acetate was chosen as the extraction solvent for this study.

Organic drop volume

The effect of microdrop volume on the extraction efficiencies was examined in the range of 1.5 to 3.5 µL. The relations between the volume of the organic solvent and chromatography

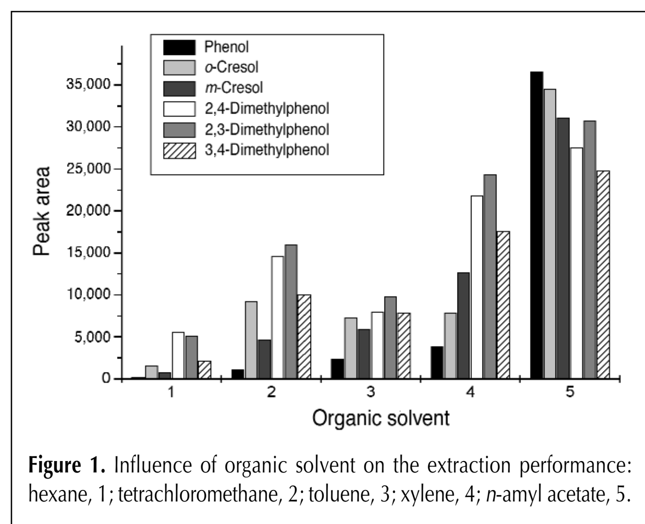

Figure 1. Influence of organic solvent on the extraction performance: hexane, 1; tetrachloromethane, 2; toluene, 3; xylene, 4; *n*-amyl acetate, 5.

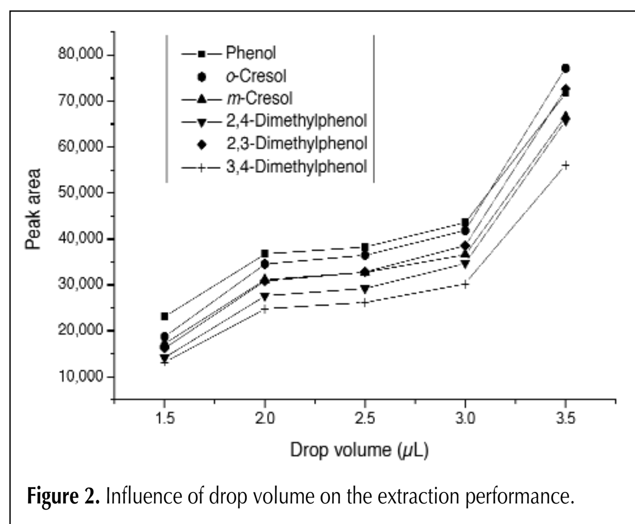

Figure 2. Influence of drop volume on the extraction performance.

peak areas can be seen in Figure 2. The results showed that extraction efficiencies were enhanced by increasing the microdrop volume up to 3.5 µL. When drop size exceeded 3.5 µL, the n-amyl acetate drop became instable and apt to fall from the tip of the syringe. In order to obtain good sensitivity and precision, a drop volume of 3.0 µL was selected for subsequent experiments.

Stirring rate

Sample stirring was beneficial to enhance extraction efficiency and reduce the thermodynamic equilibrium time. Because the solvent drop was directly exposed to the aqueous phase, a fast stirring rate usually resulted in drop displacement or drop dissolution. The response signal of GC was examined at several stirring rates ranging from 100 to 500 rpm. As shown in Figure 3, the results confirmed that agitation of the sample greatly enhanced the extraction efficiency, and the maximum responses of compounds were found at 400 rpm. Thus, 400 rpm was used for all subsequent experiments.

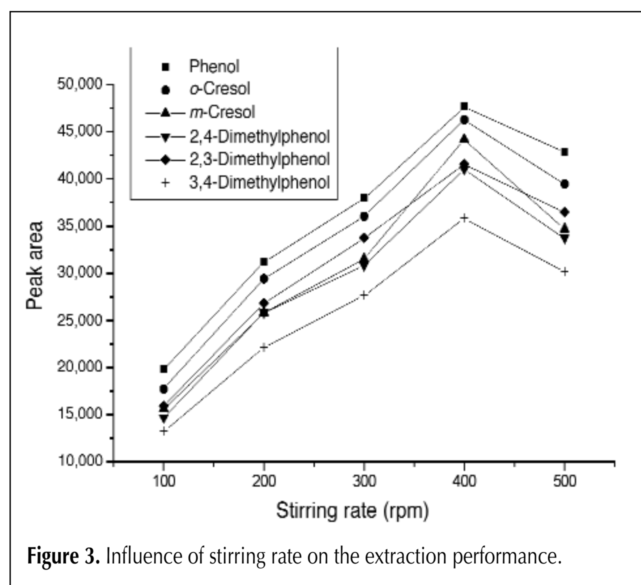

Figure 3. Influence of stirring rate on the extraction performance.

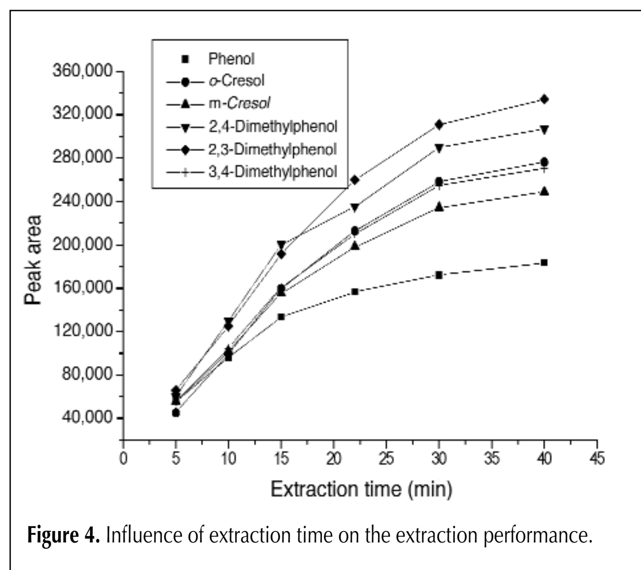

Figure 4. Influence of extraction time on the extraction performance.

Extraction time

Because LPME is an equilibrium extraction method, the maximum amount of analytes could be extracted when equilibrium was established. The extraction time profiles were studied by monitoring the peak area shift and increasing the time intervals from 5 to 40 min. As shown in Figure 4, extraction efficiencies increased when the extraction time was extended. After 30 min, the extraction system was basically at a steady state and did not have a dramatic increase with additional extraction time. Moreover, a long extraction time may have resulted in organic drop dissolution in water and, consequently, resulted in poor sensitivity and precision. From a comprehensive view, 15 min was appropriate as an extraction time for subsequent experiments.

Extraction temperature

The effect of temperature on the extraction efficiency was

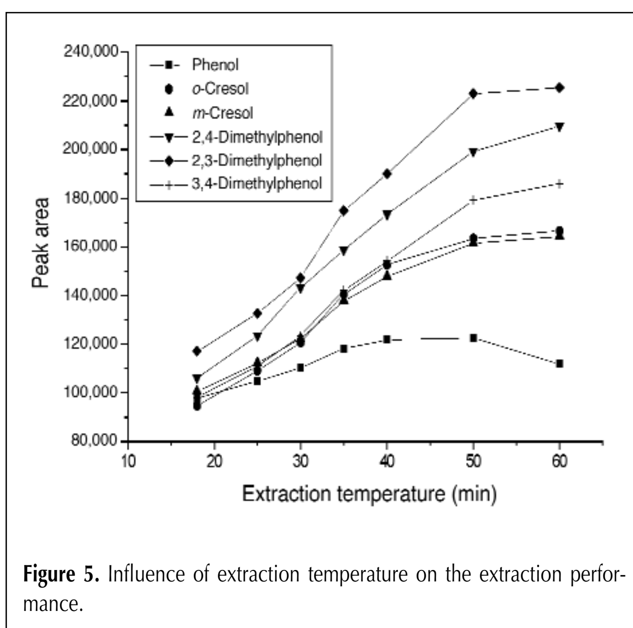

Figure 5. Influence of extraction temperature on the extraction performance.

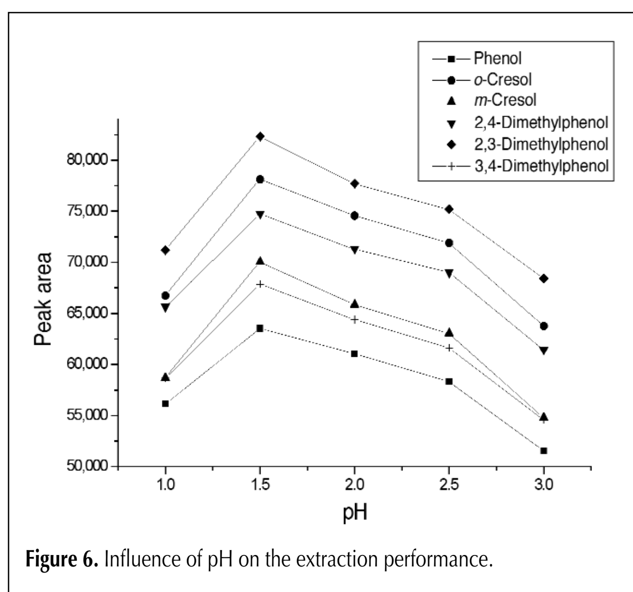

Figure 6. Influence of pH on the extraction performance.

investigated at seven different extraction temperatures (18°C, 25°C, 30°C, 35°C, 40°C, 50°C, and 60°C), and the results are shown in Figure 5. The results revealed that increasing the extraction temperature improved the extraction yield because the higher temperature increased the mobility of the molecules and shortened the time of equilibrium established. However, the higher extraction temperature may have also caused more organic drop to be dissolved in water. Therefore, the extraction temperature of 50°C was most favored in this study.

Effect of salt and pH

The ionic strength of the solution had a great effect upon extraction efficiency in LLE and SPME because of the salting-out effect (30). Nevertheless, in LPME, researchers obtained results contrary to what was expected (31,32). This experiment was conducted to evaluate the effect of sodium chloride (NaCl) (salt) addition on the extraction efficiency by increasing the concentration of NaCl from 0% to 35% (w/v). The results revealed that the peak areas decreased when NaCl concentrations were increased. Hence, no salt addition was performed in the subsequent experiments.

The form of phenols (polar species) was greatly influenced by the level of pH in the solution. The influence of pH on the extraction efficiency was evaluated within the range of 1 to 3, and the results are provided in Figure 6. As demonstrated, pH had a large effect on the LPME selectivity and sensitivity for phenols. Because pH 1.5 caused the highest peak area response, it was selected as the optimum condition.

Table I. Analytical Characteristics of the LPME Procedure for Phenolic Compounds

Compounds	Linear range (μg/L)	*R*	RSD (%)	LODs (μg/L)
Phenol	5.0–10000.0	0.9999	1.65	1.38
o-Cresol	5.0–10000.0	0.9996	3.58	1.97
m-Cresol	5.0–10000.0	0.9997	0.96	1.34
2,4-Dimethylphenol	20.0–10000.0	0.9991	4.16	1.30
2,3-Dimethylphenol	20.0–10000.0	0.9993	4.82	0.94
3,4-Dimethylphenol	5.0–10000.0	0.9998	4.58	1.14

Performance of the method

Under the optimum conditions, the linear range, precision [relative standard deviation (RSD)] and limits of detection (LODs) of the method for all target compounds were evaluated, and the results are given in Table I. As can be seen, good linearity was observed for all compounds over 3-to-4 orders of magnitude (R = 0.9991–0.9999). The LOD, calculated by consecutively diluting the solution, were in the range from 0.94 μg/L for 2,3-dimethylphenol to 1.97 μg/L for o-cresol. The RSD was less than 4.82% in the 1 mg/L spiked working solution through six repeating experiments.

Table II. Analytical Results (mg/L) of Phenolic Cmpounds in Wastewater Samples

Compounds	Concentrations (mg/L) Untreated water	Treated water
Phenol	213.23	0.048
o-Cresol	23.05	0.072
m-Cresol	59.11	0.019
2,4-Dimethylphenol	4.52	0.037
2,3-Dimethylphenol	5.42	0.004
3,4-Dimethylphenol	1.51	ND*

*ND = Not detected.

Application to real samples

Real water samples, before biochemical treatment (untreated water) and after biochemical treatment (treated water), were collected from the wastewater treatment plant in a coking factory. The analysis of real water samples often included problems caused by a high molecular weight matrix and other organic or inorganic components. The effects of such interferences were compensated for by the use of the standard addition method. Table II shows the results for the real water

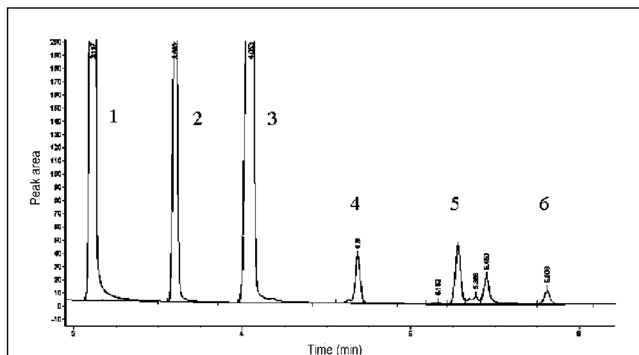

Figure 7. Chromatogram of extraction untreated wastewater sample. Peak numbers are: phenol, 1; *o*-cresol, 2; *m*-cresol, 3; 2,4-dimethylphenol, 4; 2,3-dimethylphenol, 5; 3,4-dimethylphenol, 6.

Table III. Recovery and Repeatability in Spiked Treated Water

Compounds	Concentrations (mg/L) Added	Found	Recovery (%)	RSD (%)
Phenol	0.51	0.49	96	3.17
o-Cresol	0.56	0.54	96	4.95
m-Cresol	0.48	0.44	92	3.71
2,4-Dimethylphenol	0.54	0.55	102	9.37
2,3- Dimethylphenol	0.57	0.56	98	5.80
3,4-Dimethylphenol	0.49	0.45	92	5.55

determination. The compounds (phenol, o-cresol, m-cresol, 2,4-dimethylphenol, 2,3-dimethylphenol, and 3,4-dimethylphenol) were all detected in untreated wastewater, and their concentrations were very high. However, the pollutant 3,4-dimethylphenol was not detected in treated water, and the concentrations of the others were lower. Figure 7 shows the chromatogram for the LPME of an untreated water sample.

Recovery testing was carried out with a 0.5 mg/L phenols mixture spiked to a wastewater sample. As shown in Table III, recoveries were from 92% to 102% with a RSD less than 10%, indicating the feasibility of the LPME method for determining the phenolic compounds in wastewater.

Conclusion

This study evaluated the LPME–GC method for the determination of the phenolic compounds in water samples. The optimized factors of extraction performance were obtained. The established method was applied to determine the pollutant concentration in real wastewater samples, contaminated with phenols. The recoveries of those compounds studied in wastewater were from 92% to 102%. Practical applicability demonstrated the method was feasible for the qualitative and quantitative analysis of phenolic compounds in wastewater samples.

Acknowledgments

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