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Application of cloud point methodology to the determination of nitroanilines in natural water

Huiqi Wang*, Haochen Jiang, Naizhong Song*, Xiaoting Liu*, and Qiong Jia*,†**

*College of Chemistry, Jilin University, Changchun 130012, P. R. China
**College of Life Science, Jilin University, Changchun 130012, P. R. China
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Abstract–We developed a methodology based on cloud point extraction (CPE) for the determination of three nitroanilines, *p*-nitroaniline (PNAL), *m*-nitroaniline (MNAL), and *o*-nitroaniline (ONAL). The CPE methodology was coupled with high performance liquid chromatography determinations. Tergitol 15-S-7, an environmentally friendly nonionic surfactant, was employed as the surfactant. To obtain the optimum extraction efficiency, several experimental factors were investigated, including sample pH, salt concentration, surfactant concentration, equilibration temperature, and incubation time. Under the optimized experimental conditions, the linear ranges were 0.06-1, 0.03-1, and 0.01-1 μg mL^{-1} for PNAL, MNAL, and ONAL, respectively. The limits of detection (LOD) for PNAL, MNAL, and ONAL were 0.013, 0.005, and 0.002 μg mL^{-1} with the intraday and interday relative standard deviations less than 3.1% and 6.5%. The present method proved to be simple, green, rapid, sensitive, and competitive when used for the determination of nitroanilines in water samples, and the accuracy was assessed through recovery experiments.

Keywords: Cloud Point Extraction, Nitroanilines, High Performance Liquid Chromatography, Tergitol 15-S-7

INTRODUCTION

During the past decades, separation and preconcentration methods have attracted great attention. Among the various methods, cloud point extraction (CPE) is a preconcentration method based on the phase separation that occurs in aqueous solutions of nonionic surfactant when heated above the cloud point temperature (CPT) [1,2]. Most of the early CPE applications were focused on the determination of metal ions, in which the hydrophobic complexes containing the metal ions and organic ligand were extracted into the micelles of surfactants [3]. Recently, CPE has been extensively applied for the determination of organic compounds. Compared with the traditional liquid-liquid extraction, CPE replaces the usual toxic organic solvents by low amounts of surfactants with low toxicities. In addition, non-flammable and low volatility is also consistent of the principles of "green chemistry" [4].

Nitroanilines are an important kind of chemical raw material, mainly used as intermediate of azo dye, engineering plastics, pharmaceuticals, and agrochemicals. Their toxicity is stronger than anilines and can cause blood poisoning, skin eczema, and dermatitis disease. Therefore, nitroanilines are regarded as one of the priority pollutants in water pollution control. Various methods have been developed for the determination of nitroanilines, including spectrophotometry [5,6], fluorescent sensor [7,8], electrochemistry [9-12], and high performance liquid chromatography (HPLC) [13,14]. However, direct determinations of nitroanilines in complex samples are often difficult because of the matrix effect, *e.g.*, in wastewaters [14] and hair dye samples [15]. During the past decades, many papers have been reported for the separation and preconcentration of nitroanilines, such as adsorption [14,16-22], liquid-phase microextraction [13], and polymer monolith microextraction [15]. These methods have their own advantages or disadvantages. In 2007, Niazi et al. [6] developed a cloud point extraction method for the preconcentration of *m*-nitroaniline, *o*-nitroaniline and *p*-nitroaniline with polyethylene glycol *tert*-octylphenyl ether (Triton X-100) as the surfactant. By coupling with spectrophotometric determinations, the resolution of mixtures of the nitroaniline isomers was performed and applied to the determination of the isomers in synthetic and real matrix samples.

HPLC plays an important role as an instrument for the determination of organic compounds. Various literatures have been reported about the combination of HPLC and CPE [23-26]. As is well known, UV is the most commonly used detector in HPLC determinations. Unfortunately, many surfactants have UV absorbance, which may interfere with HPLC determination of the analytes. In our previous work [27,28], Tergitol 15-S-7, a secondary ethoxylated alcohol, was used as the surfactant in the determination of phenolic compounds, carbamate pesticides, and phthalates. Tergitol 15-S-7 has several advantages [29] including low cloud-point temperature (about 38 °C at a concentration of 1 wt%), no absorption in the UV ranges, and being more environmentally friendly than other conventional surfactants with aromatic rings.

As continuous work in our lab, the present study was focused on the CPE method for the preconcentration of nitroanilines coupled with HPLC determinations. After investigating the experimental parameters, the method was applied to the determination of nitroanilines in water samples.

EXPERIMENTAL

1. Reagents and Materials

Tergitol 15-S-7 was supplied by Sigma-Aldrich (USA) and used without further purification. Various concentrations (v/v) of aque-

†To whom correspondence should be addressed.
E-mail: jiaqiong@jlu.edu.cn

ous surfactant solutions were prepared by dissolving appropriate amounts of surfactants with ultrapure water.

p-Nitroaniline, *m*-nitroaniline, and *o*-nitroaniline (hereafter abbreviated as PNAL, MNAL, and ONAL) were purchased from Aladdin Reagent (Shanghai, China). Each compound was prepared in methanol to prepare the stock standard solution with the concentration of 1,000 μg mL^{-1}. The stock solutions and diluted standard solutions were stored in glass volumetric flasks in the dark at 4 °C. The standard working solutions were daily prepared by appropriate dilution from the individual stock solutions. pH values of the solutions were adjusted using the addition of H_2SO_4 or NaOH solutions. Before HPLC analysis, all solvents and solutions were filtered through a Millipore filter (pore size 0.45 μm). Ultrapure water was prepared with a Milli-Q SP system (Millipore, Milford, MA, USA). All reagents were of HPLC or analytical reagent grade. Real water samples were obtained from lake, river, and industrial water (Changchun, China). All the water samples were filtrated with a microporous membrane (0.45 μm).

2. Instrumentation

An Agilent 1100 series HPLC system was used for the chromatographic analysis with a quaternary pump and degasser, a heated column compartment, a multiple wavelength detector, and an LC workstation. An AichromBond-AQC18 (4.6 mm×250 mm, 5 μm) column was used for analysis. A phenomenex C18 guard column (4.0 mm×3.0 mm) from Phenomenex, Torrance, CA was used to protect the column. The optimized mobile phase was methanol-H_2O (65 : 35, v/v) with the flow rate of 0.6 mL min^{-1}. The column temperature was set at 30 °C. The DAD wavelength was 254 nm.

An ultrasonic apparatus (CQ25-12) from Ningbo Scientz Biotechnology Co., Ltd. (Zhejiang, China) was used for degassing the mobile phase and homogenizing samples. A THZ-82 thermostated bath (Tianjin North China Experimental Instrument Co., Ltd., China) was used to obtain cloud point preconcentration at a proper temperature. A PB-10 pH meter was used for pH measurements (Sartorius AG, Germany). An LD5-2A centrifuge (Jingli Centrifuge Co. Ltd., Beijing, China) was employed for speeding up the phase separation process.

3. Cloud Point Extraction Process

For the preconcentration of the three target analytes, Tergetol 15-S-7 (2%, v/v) and Na_2SO_4 (0.7 mol L^{-1}) were added to the aqueous sample (15 mL) in a glass centrifuge tube. The mixture was kept for 15 min in the thermostatic bath at 40 °C after being sonicated for 10 min. Then the phase separation was accelerated by centrifugation for 5 min at 3,000 rpm. There was a very clear dividing line between the top surfactant-rich phase and the bottom excess aqueous phase after cooled in an ice-bath for 5 min. 50 μL surfactant-rich phase was moved to a sample vial with a pipette and diluted with 50 μL methanol for the subsequent HPLC determinations. Under this case, the enrichment factor could be obtained as 150 for the three anilines.

RESULTS AND DISCUSSION

1. Effect of Sample pH

As a factor which may affect the extraction efficiency, the pH dependencies were studied over the pH range of 3.0-9.0. Results showed that sample pH did not have significant effects on the extraction efficiency. This may be explained with the pK_a values of the nitroanilines. pK_a values of PNAL, MNAL, and ONAL are 1.01, 2.46, and −1.01, respectively. Therefore, pH in the range of 3.0-9.0 did not have significant influence on the CPE process. Effects of pH on the CPE process were also studied in previous reports. Niazi et al. [6] used Triton X-100 as the surfactant for the preconcentration of the three nitroanilines combined with simultaneous spectrophotometric determinations. Although it was claimed that the maximum extraction efficiency was obtained at pH range of 4.0-8.0, the pH dependencies were not very significant. In the present work, we made no pH adjustments.

2. Effect of Na_2SO_4 Concentration

As another parameter, salt concentration can affect the critical micelle concentration. Non-ionic surfactant is easy to separate from the solution, resulting in the decrease of cloud point [30]. Sulfate may reduce the cloud point greatly, while sodium ion is one of the few cations which do not improve the cloud point. Therefore, Na_2SO_4 was selected in the following experiments. To investigate the effect of salts, Na_2SO_4 with the concentration ranging from 0.3 to 0.8 mol L^{-1} was employed. Results are indicated in Fig. 1(a). The extraction efficiency of three nitroanilines increased when the concentration of Na_2SO_4 ranged from 0.3 to 0.7 mol L^{-1} while decreased at higher Na_2SO_4 concentrations. Finally, 0.7 mol L^{-1} was selected for

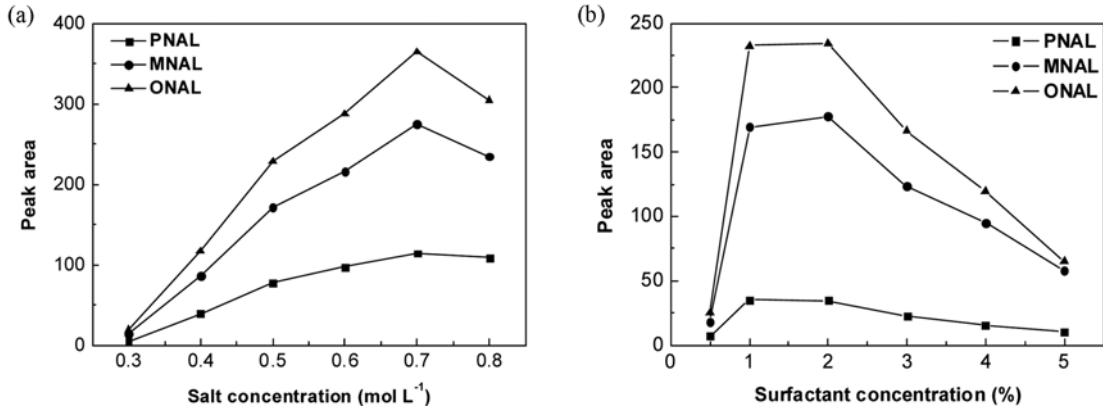

Fig. 1. Effect of salt and surfactant concentration on extraction efficiency. The concentration of nitroanilines was 2.0 μg mL^{-1}. Extraction conditions and HPLC conditions were outlined in "Experimental." (a) Salt concentration and (b) surfactant concentration.

the following experiments.

3. Effect of Surfactant Concentration

When the initial surfactant concentration is above the critical micelle concentration, much of the micellar solution forms a strong ability on the solubilization of hydrophobic compounds. When other conditions were fixed, the effect of the surfactant concentration was investigated in the range of 0.5-5% (v/v). Results are shown in Fig. 1(b). With the increase of the surfactant amount, the peak area increased at first but then decreased. In fact, although the extraction rate of the nitroanilines increased with increasing surfactant amount, the enrichment factor decreased [31]. A possible explanation may be that the increase of the condensed phase volume reduces the solute concentration. However, when the surfactant concentration is too low, it will be difficult for the formation of the surfactant phase. In addition, too small volume of the surfactant phase will lead to the difficulty for operation. Considering the extraction efficiency of the three nitroanilines, 2% (v/v) of Tergitol 15-S-7 was chosen.

4. Effect of Equilibrium Temperature

The equilibration temperature was investigated to obtain the best extraction efficiency. Theoretically, the optimal equilibrium temperature of the extraction is usually 15-20 °C higher than the cloud point temperature of surfactant. Tergitol 15-S-7 can be usually performed at around 25 °C. As shown in Fig. 2(a), the peak areas of the three nitroanilines did not change much among 35-40 °C. Finally, 35 °C was selected for the following experiments.

5. Effect of Incubation Time

The incubation time also affects the preconcentration. The CPE efficiency depends on the time that the analytes interact with micelles and get into the core. Therefore, it is necessary to study the extraction behavior when the incubation time varies. The effect of incubation time was investigated within the range of 5-35 min. Results are shown in Fig. 2(b). The peak areas of three nitroanilines increased significantly within the range of 5-15 min and remained almost constant in the range of 15-30 min, after which the peak areas decreased. Similar results were reported by some authors in the CPE processes for phthalate esters [32], Sudan dyes [33], aesculin/aesculetin [34], and osthole/imperatorin [35]. So far, it is not very clear why such a phenomenon exists. In Fernandez et al.'s work [36], oligoethylene glycol monoalkyl ether (Genapol X-080) and polyoxyethylene-10-cetyl ether (Brij 56) were employed for the CPE process of polychlorinated dibenzofurans. The authors regarded the different equilibration time dependencies as being due to the structure of the molecules (different position of chlorine atoms) which generate different interactions between the analyte and the surfactant micelles. In the present study, 15 min was selected as the incubation time.

6. Analytical Feature and Method Validation

The analytical performance was evaluated under the above selected experimental conditions of CPE and HPLC, including linearity, limit of detection (LOD), and limit of quantitation (LOQ). Results are shown in Table 1. Good linearities of the three nitroanilines were obtained with correlation coefficients (R^2) in the range of 0.9989-0.9992. LOD and LOQ were defined by the S/N set at 3 and 10, respectively. Results are also summarized in Table 1. In addition, intraday and interday relative standard deviations (RSD) were studied. Intraday RSD values were investigated by performing seven extractions of independently prepared samples within one day. The interday precision data were obtained among seven days in a run. The intraday and interday precisions RSD values of the relative peak areas were determined as lower than 3.1% and 6.5%, respectively, implying the satisfactory repeatability of the present strategy.

To illustrate the advantages of the present CPE method, a comparative study with other reported sample preparation procedures was performed (Table 2). The LOD of this method was comparable to the LOD values reported in other literatures. In addition, the

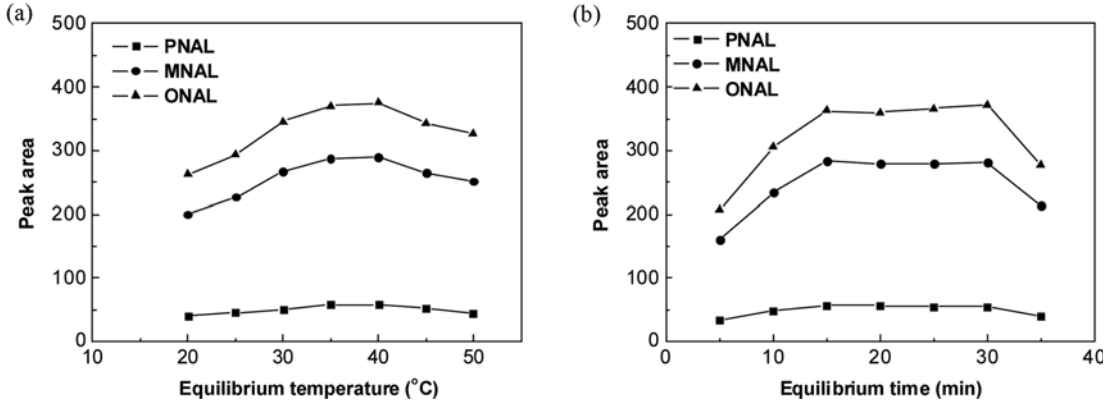

Fig. 2. Effect of equilibrium temperature and time on extraction efficiency. The concentration of nitroanilines was 2.0 μg mL^{-1}. Extraction conditions and HPLC conditions were outlined in "Experimental." (a) Equilibrium temperature and (b) equilibrium time.

Table 1. Features of the CPE method (n=5)

Nitroanilines	Linear range (μg mL^{-1})	R^2	LOD (μg mL^{-1})	LOQ (μg mL^{-1})	Intra-day repeatability (%)	Inter-day repeatability (%)
PNAL	0.06-1	0.9989	0.013	0.045	2.8	6.5
MNAL	0.03-1	0.9990	0.005	0.018	3.1	5.3
ONAL	0.01-1	0.9992	0.002	0.007	2.3	4.9

Table 2. Comparison of different sample preconcentration and detection methods for the determination of PNAL, MNAL, and ONAL

Detection method	Preconcentration method	Limit of detection	Real sample	Ref.
Fluorescent sensor	Solid phase extraction	6.38×10^{-7} mol L^{-1}	Water samples	7
HPLC	Liquid-liquid-liquid microextraction	1 g L^{-1}	Environmental water samples	13
HPLC	Solid phase extraction	-	Acidic wastewater, printing and dyeing wastewater samples	14
HPLC	Polymer monolithic extraction	0.008-0.018 g L^{-1}	Hair dye samples	15
HPLC	Soxhlet extraction	3.073-6.217 μg kg^{-1}	Soil samples	38
Kinetic fluorimetric method	-	10.08 μg mL^{-1}	Water, soil, air samples	37
Modified glassy carbon electrode	-	8.0×10^{-9} mol L^{-1}	Lake water samples	9
Spectrophotometry	CPE	0.05-0.08 μg mL^{-1}	-	6
HPLC	CPE	0.002-0.013 μg mL^{-1}	Water samples	Present method

Table 3. Recovery and detection of three nitroanilines in water samples

			PNAL	MNAL	ONAL
Water sample 1	Found (μg mL^{-1})		<LOD	<LOD	0.61
	Recovery (%)	1 μg mL^{-1} spiked	84.1	105.2	91.2
		2 μg mL^{-1} spiked	100.5	98.2	80.5
Water sample 2	Found (μg mL^{-1})		<LOD	0.36	0.22
	Recovery (%)	1 μg mL^{-1} spiked	98.5	103.5	97.4
		2 μg mL^{-1} spiked	99.8	105.7	106.5
Water sample 3	Found (μg mL^{-1})		<LOD	<LOD	<LOD
	Recovery (%)	1 μg mL^{-1} spiked	84.6	106.7	108.4
		2 μg mL^{-1} spiked	87.5	95.6	88.9
Water sample 4	Found (μg mL^{-1})		<LOD	0.24	0.23
	Recovery (%)	1 μg mL^{-1} spiked	87.3	98.9	87.2
		2 μg mL^{-1} spiked	87.5	91.2	82.5
Water sample 5	Found (μg mL^{-1})		<LOD	<LOD	<LOD
	Recovery (%)	1 μg mL^{-1} spiked	93.6	110.5	103.8
		2 μg mL^{-1} spiked	91.7	96.5	92.2
Water sample 6	Found (μg mL^{-1})		<LOD	<LOD	<LOD
	Recovery (%)	1 μg mL^{-1} spiked	88.8	94.9	92.4
		2 μg mL^{-1} spiked	104.3	102.6	98.9
Water sample 7	Found (μg mL^{-1})		<LOD	0.57	<LOD
	Recovery (%)	1 μg mL^{-1} spiked	93.9	107.3	96.8
		2 μg mL^{-1} spiked	92.3	85.9	99.4
Water sample 8	Found (μg mL^{-1})		<LOD	<LOD	<LOD
	Recovery (%)	1 μg mL^{-1} spiked	80.5	95.5	88.7
		2 μg mL^{-1} spiked	109.3	108.9	104.5
Water sample 9	Found (μg mL^{-1})		<LOD	<LOD	<LOD
	Recovery (%)	1 μg mL^{-1} spiked	94.4	102.1	94.9
		2 μg mL^{-1} spiked	107.1	105.9	100.5

present CPE method was sensitive, rapid, and little consumption of organic reagents.

7. Analysis of Real Samples

Nitroanilines are well-known pollutants in water environments that can easily enter human bodies to be mutagenic and carcinogenic. Therefore, the determination of nitroanilines in water samples is of great importance. In the present study, to test and verify the reliability of the method, the process was used in nine water samples for the determination of three nitroanilines. Real water samples were obtained from lake, river, and industrial water (Changchun, China). All the real samples were spiked with the three nitroaniline standard solutions at two concentration levels to evaluate the matrix effects. Typical chromatograms are shown in Fig. 3. Recovery data are listed in Table 3, indicating that the recoveries for the three analytes are in the range of 80.5-109.3%. It demonstrated that the method is effective and accurate for determination of three nitroa-

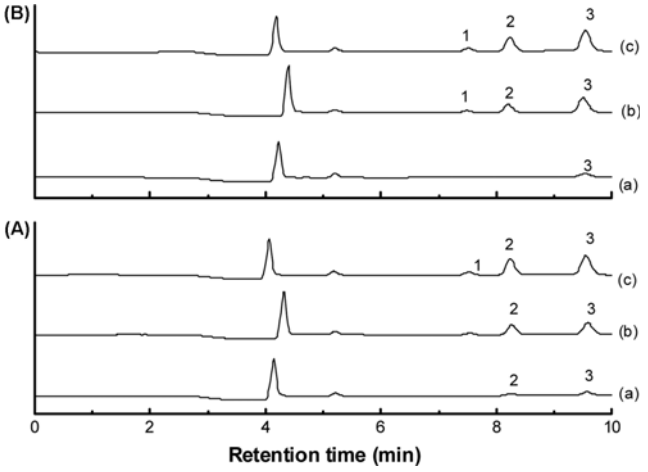

Fig. 3. Chromatograms of water samples with the CPE-HPLC-DAD method. (A) Sample 1 (Lake water sample); (B) Sample 2 (industrial water sample); (a) blank water sample; (b) and (c) water sample spiked with 1 μg mL^{-1} and 2 μg mL^{-1}; Peaks: 1--PNAL; 2--MNAL; 3--ONAL.

nilines in water samples.

CONCLUSIONS

Although CPE belongs to the field of liquid-liquid extraction, CPE avoids employing the usual toxic organic solvents. In this study, a promising analytical method for the determination of three nitroanilines (PNAL, MNAL, ONAL) was built by coupling CPE with HPLC-DAD. We investigated the CPE process in many details and obtained the optimized experimental conditions as follows: salt concentration of 0.7 mol L^{-1} (Na_2SO_4), surfactant concentration of 2% (v/v, Tergitol 15-S-7), equilibrium temperature of 35 °C, and incubation time of 15 min. The present CPE-HPLC-DAD strategy provided itself as an environmentally friendly, sensitive, inexpensive, simple, and rapid method for the determination of nitroanilines.

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