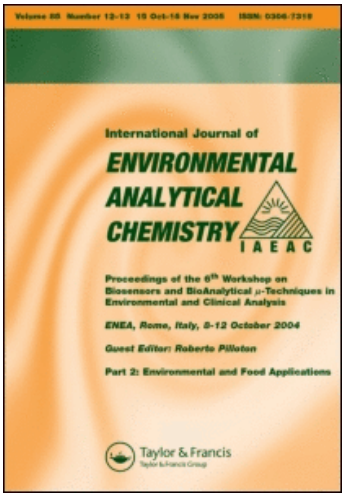

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THE 4-AMINOANTIPYRINE METHOD REVISITED: DETERMINATION OF TRACE PHENOLS BY MICELLAR ASSISTED PRECONCENTRATION

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The traditional method for phenol analysis based on the oxidizing coupling of 4-aminoantipyrine (4-APP) with phenol in alkaline solution is re-evaluated in this study in combination with micellar assisted preconcentration (cloud point extraction). The method employs the conventional reaction pathway while extraction is facilitated by surfactant based precipitation, during which the nonpolar derivative of 4-AAP–phenol is entrapped in the micelles and concentrated into a surfactant-rich phase. The latter is the re-solubilized and the complex is quantified spectrophotometrically in the presence of a surfactant. Compared to the traditional method the modification proposed offers certain analytical advantages like massive analysis of many samples, lower detection limits and shorter time of analysis. The method was applied in various samples of different origin with satisfactory results.

Keywords: Phenol; 4-Aminoantipyrine; Cloud point extraction; Natural waters; Wastewater

INTRODUCTION

Phenols are among the most abundant organic impurities penetrating into the aquatic environment as a result of their use in a large number of processes, including petroleum and paper industry, synthesis of plastics and pharmaceuticals etc. [1,2]. Because most phenolic compounds exhibit a high degree of toxicity, they have been included in the list of high priority pollutants by the US Environmental Protection Agency (EPA) and several other countries [3,4]. The European Union has set the maximum total and individual phenol permitted concentrations in water used for human consumption at 0.5 and 0.1 μg/L, respectively [5]. In this regard, their determination is of great importance and many analytical methods have been developed for that purpose.

Among the most widely applied methods are those based on the chromatographic separation and selective determination of individual phenolic compounds [6–8]. Spectrophotometric methods are also applied for the determination of the sum of phenolic compounds after derivatization reactions with a suitable reagent [9].

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Since 1965, the standard method for the spectrophotometric determination of phenols in natural waters and wastewater has been based on the oxidative coupling of phenol with 4-aminoantipyrine (4-AAP) in alkaline solution to form a dye complex [10]. This method has been studied extensively and its optimum experimental conditions (pH, oxidizing agent, intereferences, etc.), sensitivity and selectivity have been evaluated [11,12]. Despite its recognized advantages, a severe deficiency of the method is its inability to detect low μg/L levels of phenol without prior preconcentration. To enable the determination of phenol at the concentrations usually present in the aquatic environment, a preconcentration step must precede the measurement. Conventional liquid–liquid extraction with chloroform is applied for that purpose [10]. However, this solvent is highly toxic and it is considered as an environmental pollutant [13]. Furthermore, the frequency of using organic solvents is continually declining in analytical chemistry applications as a result of the development of safer methods of extraction [14–17]. In a different perspective the method is time-consuming as different reaction and extraction steps are employed that reduces the manageability of the method for routine analysis, considering also the fact that on-line extraction procedures are not possible with conventional liquid–liquid extraction techniques.

A convenient alternative to most conventional extraction schemes is the use of preconcentration steps based on phase separation by surfactant-based techniques. Micellar systems have generally attracted considerable attention in the last few years as potential extracting media and continue to have a broad appeal for extraction applications [18,19]. Their separation properties are based on the capacity of the micellar entities, having a nonpolar core, to interact with nonpolar species by hydrophobic interactions. During the cloud point separation–precipitation process, these micellar formations aggregate into a surfactant-rich phase within which any bound nonpolar species would concentrate. Although, the possibilities of micellar systems to concentrate and extract organic compounds from water were demonstrated many years ago [18] their analytical utility for phenol preconcentration has been disregarded.

To this effect, the present study is dedicated to a revision of the 4-AAP method by exploiting the analytical merits of the micelle mediated preconcentration (cloud point extraction) procedure. The proposed scheme employs cloud point extraction of the phenol–4-AAP product followed by conventional spectrophotometric determination. From an analytical standpoint, the proposed method offers significant advantages and improvements compared to the traditional and other methods of extraction including shorter time of analysis, lower detection limits and higher sample throughput.

EXPERIMENTAL

Apparatus and Reagents

Normal spectra measurements were obtained with a Shimadzu (UV2100) spectrophotometer using matched quartz cells of 1 cm path length. A Radiometer Copenhagen digital pH-meter type PHM83 was employed for the measurement of the pH value of solutions. Stock solutions of phenol (1 g/L) were prepared by initial dissolution of 0.1 g phenol (Aldrich) in 100 mL H_2O. Working standard solutions were prepared daily by appropriate dilution with distilled water. NH_3/NH_4Cl buffer solutions 0.1 M were used for pH adjustments.

Recommended Procedure

50 mL of sample (or standard solution) is transferred into a scaled vial (1 mL Scale). After the successive additions of 4-AAP and potassium hexacyanoferrate(III) the pH is adjusted to the value of 9 with the aid of a NH_3/NH_4Cl buffer solution. The mixture is vigorously shaken for 30 s and 1.25 g/L Triton X-114 are added. Heating at 60–70°C for 10 min completes the reaction and extraction of the phenol–4-AAP complex. Subsequently, the mixture is centrifuged for 15 min to facilitate phase separation and preconcentration. The vial is then placed in an ice bath for 5 min to increase the viscosity of the surfactant-rich phase and the remaining aqueous solution is discharged by simply inverting the tube. The remaining water is then removed under a gentle stream of nitrogen. The resulting micellar phase is re-solubilized in 1 mL TX-100 solution (4%, w/v) and measurements are performed spectrophotomertically against reagent blank at 480 nm.

RESULTS AND DISCUSSION

In order to establish the setting of optimum conditions a univariate experimental design procedure was performed. All parameters, which can influence the performance of the system, were considered. 10 mL aqueous solutions containing 20 μg/L of phenol were used throughout the optimization.

The effect of pH was the first parameter evaluated for its effect on the analytical signal of phenol. The optimum pH ensuring quantitative reaction and extraction of the phenol–4-AAP complex is known to lie within the pH range of 8–10 [9,10]. Experimental results verified that these pH values produce the best results, which points the fact that in alkaline pH the aggregation of the micelles is not altered which is important for the extraction step. Since strict control of pH is recommended in order to ensure the reproducibility of the analytical procedure an ammonium buffering system of 0.1 M was applied throughout.

The required reagents concentrations necessary to obtain the maximum analytical signal were then evaluated. The concentration of 4-AAP was tested over a fairly wide range (10–200 mg/L). Maximum sensitivity was observed at concentrations above 80 mg/L yielding maximum recovery at 100 mg/L (Fig. 1). The value of 100 mg/L was finally selected as it produces low blank signal.

The effect of several oxidizing agents was also investigated, as it is known to critically control the performance of the phenol–4AAP reaction while salts addition is known to contribute in the alteration of the cloud point temperature and aggregation properties of the surfactants [20]. Potassium peroxomonosulfate, peroxodisulfate, potassium periodate and hexacyanoferrate(III) were investigated for that purpose covering a concentration range between 0.01 and 0.4 g/L. The results reveal that hexacyanoferrate(III) was superior owing to the need for significantly less amount in order to ensure the completion of the reaction within only 5–10 min after its introduction. However, increased blank absorbance was observed especially with increasing concentrations above the range of optimum values (approximately 0.1–0.2 g/L). However, oxidant concentration needs to be maintained in excess to avoid parallel reactions with reducing agents and metallic species [12] present in real samples. Taking the above into consideration a concentration of 0.15 g/L hexacyanoferrate(III) was finally selected.

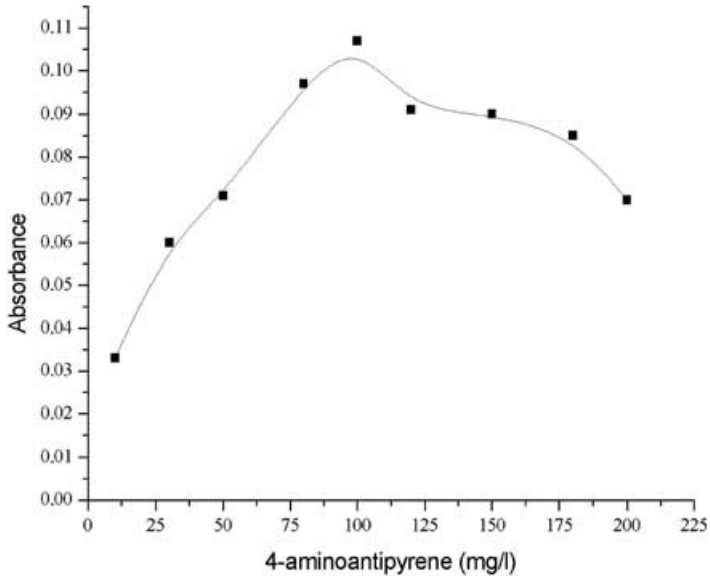

FIGURE 1 Effect of 4-AAP concentration on the reaction and extraction efficiency of phenol. [Phenol] = 20 μg L^{-1}, pH = 9, [Phenol] = 20 μg L^{-1}, [potassium hexacyanoferrate(III)] = 0.1 g L^{-1}, TX-114 = 0.1% (w/v). Solubilization in TX-100 = 0.8% (w/v).

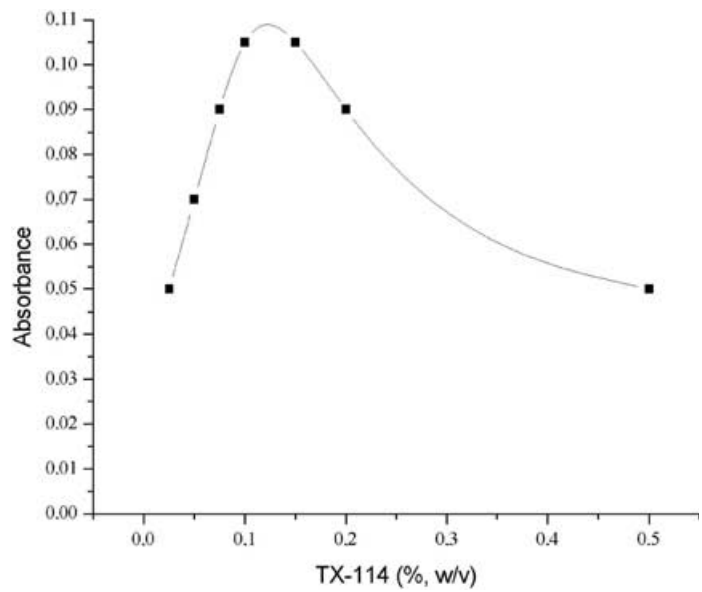

FIGURE 2 Effect of TX-114 concentration on the extraction efficiency of phenol. [Phenol] = 20 μg L^{-1}, pH = 9, [4-AAP] = 100 mg L^{-1}, [potassium hexacyanoferrate (III)] = 0.15 g L^{-1}. Solubilization in TX-100 = 0.8% (w/v).

The amount of TX-114 required for the quantitative extraction of 20 μg/L phenol was then optimized. The results of Fig. 2 show that a concentration of 1.25 g/L (125 μL of 10% w/v solution) is sufficient for the complete extraction of the phenol–4-AAP complex. Larger quantities of the surfactant can be used at the expense of

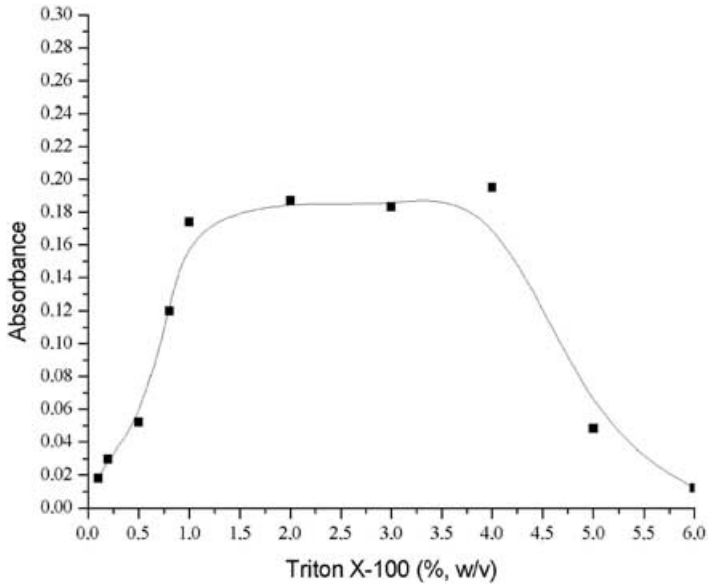

FIGURE 3 Effect of TX-100 concentration on the resolubilization of the 4-AAP–phenol complex from the concentrated surfactant-rich phase. [Phenol] = 20 μg L^{-1}, pH = 9, [4-AAP] = 100 mg L^{-1}, [potassium hexacyanoferrate(III)] = 0.15 g L^{-1}, TX-114 = 0.15% (w/v).

detection limits, as larger volumes reduce the analytical signal, due to the decrease of concentration.

The solubilization of the preconcentrated complex after the completion of the reaction and cloud point extraction procedure was then evaluated. The use of water-miscible organic solvents like ethanol, methanol or acetone seems to deteriorate the analytical signal. To enable an increased sensitivity as well as good solubilization of the final product another surfactant was also decided. TX-100 was chosen due to its high cloud point temperature and high solubilization properties. Various concentrations were tested and the results are depicted in Fig. 3. As we can observe, the signal was increasing up to 4 (%, w/v) and started to decline above this value due to the increase in the viscosity which resulted in too high turbidity (hazy solution).

In concurrence with previous studies [19,21], centrifugation time was not found to pose any significant effects. A centrifugation time of 15 min was applied throughout since analyte extraction was almost quantitative. The time required for the preconcentrated complex to re-solubilize in the TX-100 surfactant medium was also investigated. Depending on the expected amount of phenol in the sample solubilization time was completed within 5–10 min after the initiation of the process. To ensure that the re-solubilization process has been driven into its completion the final extract was gently mixed with a 4% (w/v) solution of TX-100 for 10 min. It is worth mentioning that good solubilization of the derivatization product as well as removal of any remaining water in the final micellar phase is essential to ensure the reproducibility of the results. Oven-drying of the residual water entrapped in the final complex, although possible, may increase the solubilization time and partially prevent the quantitative solubilization of the complex.

Figures of Merit

Under the optimum experimental conditions linear calibration curves in the range of 2–16 μg/L were obtained by preconcentrating 50 mL of sample volume. The quantitation limits (defined as 10 times the signal to noise ratio) are sufficiently low compared to the conventional procedure employing liquid–liquid extraction [9,10]. Further decrease is feasible by preconcentrating larger sample volumes or by diluting the condensed phase to a smaller TX-100 volume. The analytical characteristics of the method are given in Table I.

From an analytical standpoint, the proposed method offers significant advantages and improvements compared to the traditional method. In the first place, the reaction and extraction procedures are now merged in one single process thus reducing the time of analysis and alleviating the possibility of analytical errors as a function of the sequential steps involved in the conventional procedure. Furthermore, the detection limits is lowered 10 times although 10 times less sample volume is used. The use of 500 mL employed in the standard method can bring the quantitation and detection limits of the method about 100 times below the established detection limit [9]. In addition to the above, the presence of surfactant medium in the final condensed phase containing the preconcentrated–extracted species enhances the reproducibility of the analysis [22]. Furthermore, no hazardous solvents are used throughout the overall experimental procedure. Finally, massive analysis of many samples is feasible as the preconcentration step can be applied simultaneously to many samples. Depending on the capacity of the centrifuge 16–40 samples can be prepared in 1 h.

In a different perspective, the modification proposed in this study offers the possibility of using other methods of detection like liquid or gas chromatography provided that a clean-up step of the final surfactant extract precedes the measurement [23].

Interferences

The presence of matrix components such as fulvic and humic acids did not interfere with the measurements, as they are not extracted in the micellar phase due to their polarization at alkaline solutions. Owing to the excess of oxidant in the solution unwanted parallel reactions with certain metals present in real samples are alleviated. Additionally, no adverse effects from several anions (phosphates, nitrates, fluoride, chloride, etc.) were observed as they do not affect the reaction since they are not entrapped in the surfactant assemblies.

TABLE I Analytical features of the method

Parameter	*Phenol*
Phase volume ratio	0.02
Preconcentration factor	50
Extraction concentration factor	~1
LOD[a] (μg/L)	0.5
LOQ[a] (μg/L)	1.6
RSD (%) ($n=4$, C = 5 μg/L)	3.11%
Regression equation	$A = 0.0790\ (\pm 0.0007) + 0.0044\ (\pm 0.0001)\ C$ (C in $\mu g\ L^{-1}$)
Correlation coefficient (r)	0.9994

[a]From 50 mL sample volume.

TABLE II Analysis of real samples and recoveries from fortified samples

Sample	*Measured* ($\mu g\,L^{-1}$)	*Spiked* ($\mu g\,L^{-1}$)	*Found* ($\mu g\,L^{-1}$)	*Recovery* (%)[a]
Lake water[b]	n.d.	5	4.8	96
		10	10.1	101
Settled sewage	1.2	5	6.6	108
		10	11.5	103
Treated wastewater[b]	0.02	5	4.5	90
		10	9.7	97

[a]Estimated according to the IUPAC recommendations; [b]200 mL sample volume was extracted; n.d. – not detected.

Analysis of Real Samples

Method validation and verification was made by analyzing samples of natural waters and wastewater from the Epirous region (North Western Greece). Lake water and wastewater were obtained from Lake Pamvotis and the local wastewater treatment plant, filtered through a Whatman No. 40 filter and acidified with phosphoric acid prior to their storage at 4°C. Whenever possible analysis was undertaken at the day of sampling, due to the low content of the samples in phenol, 100 mL volume was preconcentrated including the volume of the standard solutions used for the calibration curve. As a part of the evaluation study, several samples were spiked with different quantities of phenol. The results gathered in Table II reveal that the proposed method can be reliably used for the determination of phenols in various water matrixes of different and complex origin.

CONCLUSIONS

The analytical utility of cloud point extraction for the determination of trace phenol in natural waters and wastewater was demonstrated for the first time. The method exploits the well-known reaction of phenol with 4-AAP in alkaline conditions. Extraction is facilitated through micelle mediated extraction – preconcentration which significantly enhances the effectiveness of the method in terms of detection limits but also in terms of shorter time of analysis and increased flexibility. Additionally the method raises the possibility of employing the conventional 4-AAP method for the determination of phenol in on-line automated manifolds a possibility, which has received only minor attention. Method validation in real samples revealed that the proposed revision of the traditional method is sufficiently robust for the determination of phenol is water samples at the μg/L levels.

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