



## A green and sensitive method to determine phenols in water and wastewater samples using an aqueous two-phase system

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### ABSTRACT

A greener and more sensitive spectrophotometric procedure has been developed for the determination of phenol and *o*-cresol that exploits an aqueous two-phase system (ATPS) using a liquid–liquid extraction technique. An ATPS is formed mostly by water and does not require organic solvent. Other ATPS components used in this study were the polymer, polyethylene oxide, and some salts (i.e., Li<sub>2</sub>SO<sub>4</sub>, Na<sub>2</sub>SO<sub>4</sub> or K<sub>2</sub>HPO<sub>4</sub> + KOH). The method is based on the reaction between phenol, sodium nitroprusside (NPS) and hydroxylamine hydrochloride (HL) in an alkaline medium (pH 12.0), producing the complex anion [Fe<sub>2</sub>(CN)<sub>10</sub>]<sup>10-</sup> that spontaneously concentrates in the top phase of the system. The linear range was 1.50–500 μg kg<sup>-1</sup> ( $R \geq 0.9997$ ;  $n = 8$ ) with coefficients of variation equal to 0.38% for phenol and 0.30% for *o*-cresol ( $n = 5$ ). The method yielded limits of detection (LODs) of 1.27 and 1.88 μg kg<sup>-1</sup> and limits of quantification (LOQs) of 4.22 and 6.28 μg kg<sup>-1</sup> for phenol and *o*-cresol, respectively. Recoveries between 95.7% and 107% were obtained for the determination of phenol in natural water and wastewater samples. In addition, excellent agreement was observed between this new ATPS method and the standard 4-aminoantipyrine (4-AAP) method.

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### 1. Introduction

The restricted availability of unpolluted freshwater sources coupled with the high costs of obtaining potable water has caused increasing awareness about the use of water sources. Thus, there is a constant search for inexpensive and greener analytical methods that can provide reliable results for monitoring water pollutants such as phenolic compounds.

Phenol and its derivatives (e.g., cresol) are extremely harmful to the environment and human health [1,2] due to their high toxicity. As a result, they have been included on lists of high priority pollutants in several countries [2,3]. American and Brazilian environmental legislation established limits of 10.0 μg L<sup>-1</sup> of total phenols permitted in bodies of water [4,5].

Several analytical procedures with different systems of detection were developed to determine phenolic compounds, including gas chromatography (GC) [6–9], liquid chromatography (LC) [10–13], capillary electrophoresis (CE) [14–16], voltammetry [17–19] and amperometric analysis [20–22]. However, these meth-

ods do not allow *in situ* continuous monitoring, are costly to implement, demand skilled operators and require derivatization of analytes. Spectrophotometric analysis is applied in the determination of phenols [23,24], exploiting mainly UV–vis detection [25–27].

The most commonly used analytical methods (e.g., those described in the Standard Methods for the Examination of Water and Wastewater [28]) are spectrophotometric methods based on the oxidative coupling of phenols with 4-aminoantipyrine (4-AAP). However, this procedure has many disadvantages, including (i) employment of large amounts of reagents, especially chloroform, which is toxic and carcinogenic [29]; (ii) difficulty in finding suitable buffers, due to the reactive sensitivity to pH variations; and (iii) tedious operations such as a distillation step required for some sample analysis.

An interesting spectrophotometric method for the determination of phenolic compounds has been reported by Nagaraj et al. [30] and improved by Kang et al. [31]. It is based on the reaction between phenol, sodium nitroprusside (NPS) and hydroxylamine hydrochloride (HL) in an alkaline medium, producing the blue-colored complex anion [Fe<sub>2</sub>(CN)<sub>10</sub>]<sup>10-</sup>. However, this procedure is not yet suitable for the determination of phenols at concentrations lower than 50.0 μg L<sup>-1</sup> and for analyses in the presence of elec-

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trolites such as phosphates and sulfates, which promote serious errors. Moreover, a previous distillation step for sample preparation is required.

A convenient approach to address these limitations is to promote the complex formation reaction at the interface between the bottom and top phase of an aqueous two-phase system (ATPS). This reaction avoids salt interference and the posterior transfer of the complex to a small volume of the ATPS top phase. Within this context, ATPS is a strategic liquid–liquid extraction technique for phenol determination. Under specific thermodynamic conditions, an ATPS can be formed by the mixtures of aqueous solutions of (i) certain inorganic salts and a polymer [32], (ii) two types of water-soluble polymers [33] or (iii) two types of salts [34]. The resulting systems are two immiscible liquid phases with a polymer-enriched top phase (or enriched with a salt) and a salt-enriched bottom phase (or enriched with the second polymer or salt). Moreover, water is the major component of both phases, thus providing an extraction technology that eliminates the use of hazardous solvents. Finally, ease of use, low costs, shorter times for phase splitting and recycling of the system components are advantages of the technique [35]. ATPS methodologies have already been applied successfully in the separation of different ions [35–38].

The aim of this work was to develop a novel, greener and more sensitive procedure for determining phenol and *o*-cresol compounds. The proposed method is based on the reaction between phenolic compounds, NPS and HL in ATPSs composed of polyethylene oxide 1500 (PEO1500) + salt ( $\text{Li}_2\text{SO}_4$ ,  $\text{Na}_2\text{SO}_4$  or  $\text{K}_2\text{HPO}_4 + \text{KOH}$ ) + water, exploiting spectrophotometric detection of reaction products. Parameters, such as ATPS electrolyte nature, pH, reagent concentrations, sample stirring time and tie-line length (TLL) were also optimized for the method.

## 2. Experimental

### 2.1. Apparatus

Spectrophotometric measurements were performed with a UV-visible (UV/vis) spectrophotometer (Shimadzu UV-2550) with quartz microcells with a 1.00-cm optical path length and total capacity of 450  $\mu\text{L}$ . The slit width was kept at 2.0 nm. The software used for system control and data acquisition was UVProbe. The pH measurements were collected with a combined glass electrode using a digital pH meter (Digimed model DM-20, Digicron Analítica Ltda, São Paulo, Brazil).

### 2.2. Chemicals

All reagents were of analytical grade quality and used as received without further purification. Distilled water was used throughout the experiments. The polymer was polyethylene oxide (Synth, Diadema, São Paulo, Brazil) with an average molar mass ( $M_m$ ) of 1500  $\text{g mol}^{-1}$  (denoted as PEO1500). The chemicals in the proposed method,  $\text{Li}_2\text{SO}_4$ ,  $\text{Na}_2\text{SO}_4$ ,  $\text{K}_2\text{HPO}_4$ ,  $\text{KOH}$ ,  $\text{NaOH}$ , NPS and HL, were obtained from Vetec (Duque de Caxias, Rio de Janeiro, Brazil). The reagents used in the 4-AAP procedure [28] were 4-aminoantipyrine,  $\text{NH}_4\text{OH}$ ,  $\text{K}_3[\text{Fe}(\text{CN})_6]$  (Merck, Darmstadt, Germany),  $\text{K}_2\text{HPO}_4$  and  $\text{KH}_2\text{PO}_4$  (Vetec). Phenol and *o*-cresol were purchased from Sigma–Aldrich (Milwaukee, WI, USA).

### 2.3. ATPS composition, solutions and standards

The three types of ATPS applied in the development of the procedure were PEO1500 +  $\text{Li}_2\text{SO}_4$  + water; PEO1500 +  $\text{Na}_2\text{SO}_4$  + water [32] and PEO1500 + ( $\text{K}_2\text{HPO}_4 + \text{KOH}$ ) + water. For each system at least three different compositions were studied to verify the effect

of the TLL in the partitioning behavior of the  $[\text{Fe}_2(\text{CN})_{10}]^{10-}$  complex (Table 1). Moreover, global compositions shown in Table 1 are relative to an ATPS composed of 2.00 g of top phase and 10.0 g of bottom phase, which were used in most assays.

All solutions were prepared with distilled water. In the ATPS method, the pH of the water was adjusted to 12.0 with  $\text{NaOH}$  because this pH is more favorable to the complete reaction of NPS with phenol compounds. For systems composed of the salts  $\text{K}_2\text{HPO}_4 + \text{KOH}$ , correction was not necessary because the pH was 12. Stock solutions of top and bottom phases were prepared in distilled water (pH 12.0). PEO1500 and salt concentrations in the phase stock solutions are provided in Table 1. These solutions were used as solvent to prepare all solutions of phenols, NPS and HL. Working standard solutions of phenol within 5.00 and 2000  $\mu\text{g kg}^{-1}$  were prepared daily by using the bottom phase as a solvent and the appropriate dilution of a 1.00  $\text{g kg}^{-1}$  stock solution. HL working stock solutions (518  $\text{mg kg}^{-1}$ ) were prepared by weighing the appropriate quantity of hydroxylamine hydrochloride in the bottom phase using an analytical balance (Shimadzu, AY 220) with an uncertainty of  $\pm 0.0001$  g. NPS working stock solutions (1992  $\text{mg kg}^{-1}$ ) were obtained by dissolving the appropriate amount of salt in the top phase. In the 4-AAP method, the solutions and standards were prepared as described in the literature [28].

### 2.4. Procedure

Liquid–liquid equilibrium cells were used to prepare the ATPSs. Working stock solutions of 5.000 g of phenol, 2.000 g of NPS and 5.000 g of HL were weighed. In spectroscopic measurement reference cell were carried out with the top or bottom phase stock solutions containing NPS and HL, respectively. Thus, a final composition of phenol within 2.50 and 1000  $\mu\text{g kg}^{-1}$  in the bottom phase was obtained, and the global concentrations of NPS and HL were 332.0 and 216  $\text{mg kg}^{-1}$ , respectively. The final concentrations of PEO1500 and salt in the ATPS were depended on the TLL studied (Table 1). Subsequently, the biphasic systems were manually stirred for 3 min and allowed to settle for 20 min at  $25.0 \pm 0.1$  °C in a temperature-controlled bath (Microquímica, MQBTC 99-20). Finally, aliquots of the top phase were collected with a syringe and diluted by a factor of 1.50. UV/vis spectrophotometric measurements were carried out at 722.0 nm for phenol and 717.5 nm for *o*-cresol.

### 2.5. Partitioning experiments

To determine the  $[\text{Fe}_2(\text{CN})_{10}]^{10-}$  complex partition coefficient ( $K_{[\text{Fe}_2(\text{CN})_{10}]^{10-}}$ ) between the two phases, the types of ATPS studied were kept for 24 h in a temperature-controlled bath to reach thermodynamic equilibrium. Subsequently, aliquots of top and bottom phases were collected. The parameter  $K_{[\text{Fe}_2(\text{CN})_{10}]^{10-}}$  can therefore be defined as

$$K_{[\text{Fe}_2(\text{CN})_{10}]^{10-}} = \frac{A_T}{A_B} \quad (1)$$

where  $A_T$  and  $A_B$  are the absorbance (at 722.0 or 717.5 nm) of the  $[\text{Fe}_2(\text{CN})_{10}]^{10-}$  complex in the top and bottom phases, respectively.

The influence of TLLs in the partitioning behavior of the complex anion was studied by determining  $K$  as a function of the two different TLL values for each ATPS.

### 2.6. Environmental samples

A distilled sample of furnace coke effluent was used to determine the applicability of the method in environmental samples. To determine the concentration of phenol in these samples, without

**Table 1**  
Percent composition (w/w) for PEO1500 ( $w_p$ ), salt ( $w_s$ ) and water aqueous two-phase system (ATPS) at 25.0 °C.

Tie-line length (TLL)	Global		Top phase		Bottom phase	
	$w_p$	$w_s$	$w_p$	$w_s$	$w_p$	$w_s$
<i>PEO1500 + Li<sub>2</sub>SO<sub>4</sub> + water</i>						
29.43	11.4	13.83	34.44	5.30	6.85	15.54
34.97	12.0	14.73	39.18	4.15	6.60	16.85
41.71	12.3	16.64	44.40	3.25	5.91	19.32
46.36	12.8	17.62	48.38	2.58	5.68	20.63
51.67	13.5	18.79	53.26	2.15	5.60	22.12
<i>PEO1500 + (K<sub>2</sub>HPO<sub>4</sub> + KOH) + water</i>						
16.61	9.31	14.83	20.60	6.82	7.05	16.43
27.06	9.01	18.09	27.36	4.99	5.34	20.71
30.09	9.75	18.79	30.35	4.50	5.63	21.65
37.05	8.87	21.15	34.32	3.68	3.78	24.65
42.80	8.66	23.31	38.13	3.23	2.76	27.33
<i>PEO1500 + Na<sub>2</sub>SO<sub>4</sub> + water</i>						
32.51	7.45	13.50	32.36	2.84	2.47	15.63
35.96	7.52	15.17	34.51	2.16	2.12	17.77
40.10	7.87	16.53	37.94	1.95	1.86	19.45

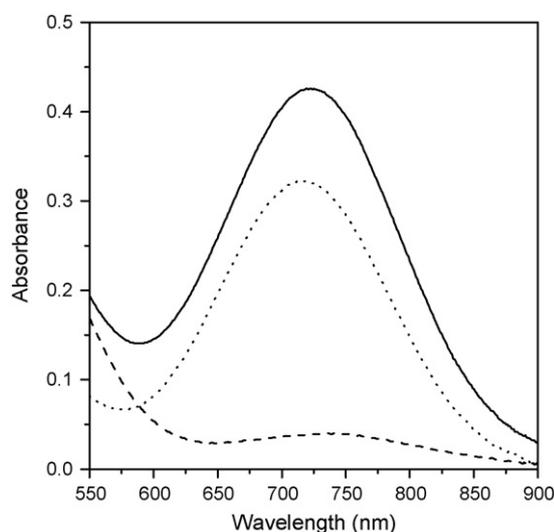
matrix effects, the Standard Addition Method was performed by adding 0.250 g of sample to each ATPS standard. The recovery rate provided by the proposed method was also determined in samples such as natural river water, industrial water, distilled water and samples from wastewater treatment plants (WWTPs). In these cases, samples without the distillation step were used as the bottom phase of the ATPS, to which appropriate amounts of phenol were added. The 4-AAP method [28] was also applied to the same samples for comparison and to check the accuracy of the proposed method.

### 3. Results and discussion

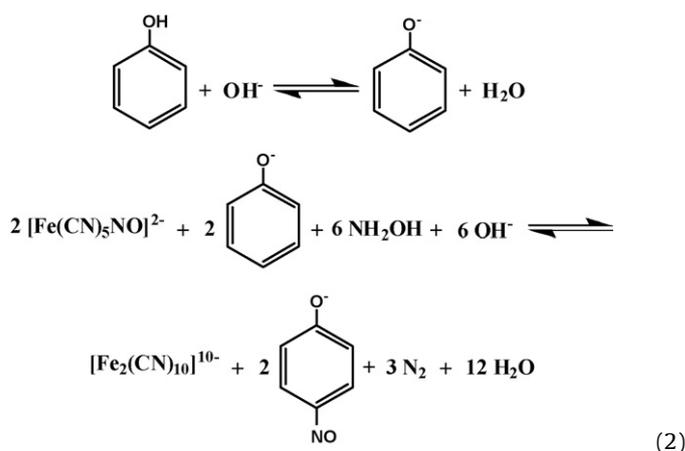
#### 3.1. Chemical aspects

The procedure is based on an aromatic electrophilic reaction, which is represented by Eq. (2). In strongly alkaline mediums, phenol is converted to phenolate. Thus, the electrophilic reaction can take place more easily because the benzene ring becomes very active due to the presence of the negative oxygen ( $O^-$ ). According to theoretical calculations, the sites that the nitroso group ( $+NO$ ) of

the NPS attacks the phenolate molecule at *para*- or *ortho*-positions. The electrophilic or electrophobic properties of the substituents in the benzene ring affect the reaction extension, and consequently the sensitivity of the method [31]. Fig. 1 shows the absorption spectra of the reaction product ( $[Fe_2(CN)_{10}]^{10-}$ ) obtained in the ATPS top phase. The complex chemical structure was determined by Kang et al. [31]. The maximum absorbance is at 722.0 nm for phenol and 717.5 nm for *o*-cresol. Blank signals are insignificant in the wavelength chosen for taking measurements. Moreover, the spectra show (Fig. 1) that the procedure is more sensitive for phenol than *o*-cresol, which confirms that substituents present on the benzene ring affect the properties of the compounds and the formation of the  $[Fe_2(CN)_{10}]^{10-}$  complex.



**Fig. 1.** Absorption spectra of the  $[Fe_2(CN)_{10}]^{10-}$  anion formed by the reaction system between NPS, HL and (—) phenol or (···) *o*-cresol, and (---) blank assay spectrum in the PEO1500/Li<sub>2</sub>SO<sub>4</sub> aqueous two-phase system (ATPS) with a tie-line length (TLL) = 41.71% (w/w).



#### 3.2. Optimization of method

Method optimization was done to improve the sensitivity for low phenol concentrations. An experiment was carried out using Central Composite Rotatable Design (CCRD) in Response Surface Methodology (RSM) to evaluate the significance level of the factors. Five factors were investigated: system pH; NPS concentration; HL concentration; sample stirring time and phase splitting time. After exposure to conditions determined by the CCRD, analytical responses were evaluated by spectrophotometric measurements. Only significant coefficients at 5% probability were considered in the model using a Student's *t*-test. Table 2 shows the evaluated range and the optimum values for each variable.

**Table 2**  
System optimization for the proposed aqueous two-phase system (ATPS) method using a standard of 600  $\mu\text{g kg}^{-1}$  phenol in the bottom phase.

Parameter	Evaluated range	Selected value
pH	8.00–12.0	12.0
NPS concentration ( $\text{mg kg}^{-1}$ )	140.0–670.0	332.0
HL concentration ( $\text{mg kg}^{-1}$ )	110.0–385.0	216.0
Stirring time (min)	1–5	3
Phase splitting time (min)	20–60	20

NPS = sodium nitroprusside; HL = hydroxylamine hydrochloride.

### 3.3. Stability of the $[\text{Fe}_2(\text{CN})_{10}]^{10-}$ anion in ATPS

In general, cyan complexes are photosensitive, so experiments were performed in the absence of light to avoid complex decomposition. Fig. 2 shows the stability of the  $[\text{Fe}_2(\text{CN})_{10}]^{10-}$  anion complex. Stability was studied through UV/vis absorption spectra of the top phase. After a period of 75 h, no change in the UV/vis spectra was observed, and the absorbance at 722.0 nm for phenol or 717.5 nm for *o*-cresol remained constant over 75 h.

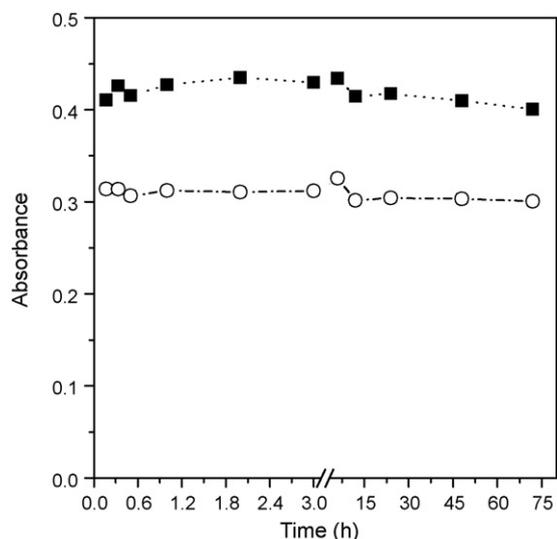
### 3.4. Partition coefficients and effects of the tie-line length (TLL)

The TLL is a thermodynamic parameter that expresses the difference in intensive thermodynamic functions between the top and bottom phases at a constant pressure and temperature [39]. The TLL is expressed by the difference between polymer and salt concentrations present in the different phases. Generally, this parameter is a determinant in the partitioning process of some solutes and is calculated with the following equation:

$$\text{TLL} = [(C_p^T - C_p^B)^2 + (C_s^T - C_s^B)^2]^{1/2} \quad (3)$$

where  $C_p^T$  and  $C_p^B$  are the polymer concentrations in the top and bottom phases, respectively, and  $C_s^T$  and  $C_s^B$  are applied in the same way to the salt.

Table 3 shows the values of  $K_{[\text{Fe}_2(\text{CN})_{10}]^{10-}}$  for two different TLL values for all ATPSs studied. Similar values of TLL were chosen to determine the influence of electrolytes in the partitioning behavior of the complex. The  $[\text{Fe}_2(\text{CN})_{10}]^{10-}$  anion shows a preferential concentration in the polymer-rich phase, reaching  $K_{[\text{Fe}_2(\text{CN})_{10}]^{10-}}$  values as high as 66.2 for a TLL of 41.71% (w/w) of the ATPS



**Fig. 2.** Stability of the anion  $[\text{Fe}_2(\text{CN})_{10}]^{10-}$  formed by the reaction between NPS, HL and (■) phenol or (○) *o*-cresol in the PEO1500/ $\text{Li}_2\text{SO}_4$  aqueous two-phase system (ATPS) with a tie-line length (TLL) = 41.71% (w/w).

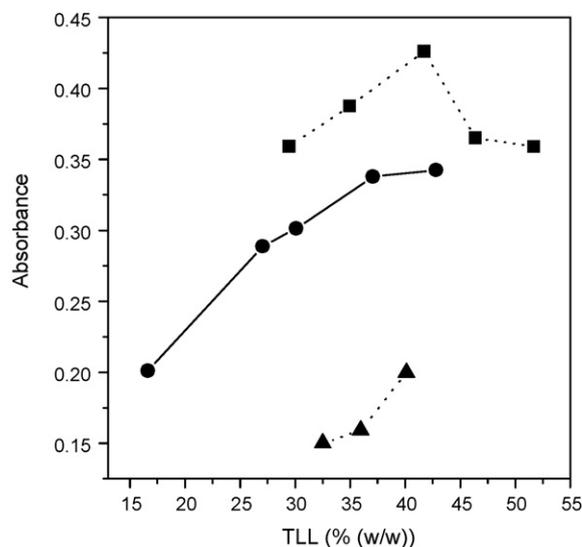
**Table 3**  
Partition coefficient values of the  $[\text{Fe}_2(\text{CN})_{10}]^{10-}$  anion complex for different aqueous two-phase system (ATPS) with different tie-line lengths (TLLs).

ATPS	TLL (% (w/w))	$K_{[\text{Fe}_2(\text{CN})_{10}]^{10-}}$	
		Phenol	<i>o</i> -Cresol
PEO1500 + $\text{Li}_2\text{SO}_4$	29.43	17.8	56.6
	41.71	66.2	60.2
PEO1500 + ( $\text{K}_2\text{HPO}_4$ + KOH)	30.09	13.7	27.3
	42.80	32.4	36.6
PEO1500 + $\text{Na}_2\text{SO}_4$	32.51	3.86	6.30
	40.10	7.52	8.88

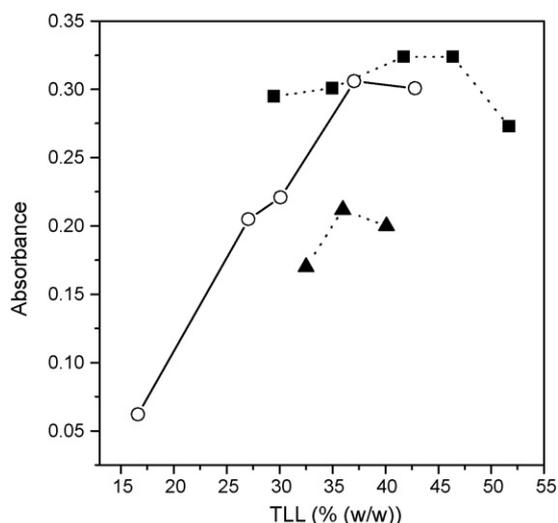
PEO1500 +  $\text{Li}_2\text{SO}_4$  + water, which shows the best responses for both phenol and *o*-cresol. Moreover, the value of  $K_{[\text{Fe}_2(\text{CN})_{10}]^{10-}}$  increases as the TLL increases because more variable intensive thermodynamic difference between top and bottom phases properties (i.e., salt and polymer compositions) is associated with increasing TLL values [40]. As a consequence, higher concentrations of  $[\text{Fe}_2(\text{CN})_{10}]^{10-}$  anion are observed in the top phase [41].

Figs. 3 and 4 show the influence of the TLL on the sensitivity of the method for phenol and *o*-cresol determination for the three kinds of ATPS. These data were obtained by plotting analytical curves for all the TLLs of the ATPSs and then comparing the absorbance values of the same phenol standard (600  $\mu\text{g kg}^{-1}$ ). ATPS PEO1500/ $\text{Na}_2\text{SO}_4$  studies in TLLs >40.10% (w/w) were not practical due to the precipitation of sulfate. Absorbance increases as the TLL increases, but for higher TLL values the signal becomes constant or even decreases. A possible reason for this behavior is that the increase in TLL also induces an ATPS salt concentration increase (Table 1), which interferes with the formation of  $[\text{Fe}_2(\text{CN})_{10}]^{10-}$  anion, as described by Kang et al. [31]. Thus, the sensitivity of the method depends on the competition between forming  $[\text{Fe}_2(\text{CN})_{10}]^{10-}$  anions and its concentration in the top phase, which in turn depends on the TLL. Since the reaction process occurs at interfaces using an ATPS method, it is interesting to note that the interference of ions, such as sulfate and phosphate, is insignificant compared to reports by Nagaraj et al. [30] and Kang et al. [31], indicating that the tolerance limit for sulfate and phosphate is lower than 25,000 and 5000  $\text{mg L}^{-1}$ , respectively.

The effects of electrolytes on the determination of phenol are also shown in Figs. 3 and 4. A TLL value of 41.71% (w/w) using



**Fig. 3.** Effect of tie-line length (TLL) and electrolytes on the phenol determination in an aqueous two-phase system (ATPS) composed of PEO1500, salt and water: (■)  $\text{Li}_2\text{SO}_4$ , (○)  $\text{K}_2\text{HPO}_4$  + KOH and (▲)  $\text{Na}_2\text{SO}_4$ .



**Fig. 4.** Effect of tie-line length (TLL) and electrolyte on the *o*-cresol determination in an aqueous two-phase system (ATPS) composed of PEO1500, salt and water: (■)  $\text{Li}_2\text{SO}_4$ , (○)  $\text{K}_2\text{HPO}_4 + \text{KOH}$  and (▲)  $\text{Na}_2\text{SO}_4$ .

PEO1500/ $\text{Li}_2\text{SO}_4$  ATPS presented the highest response among all the systems studied. This behavior may be explained by the model proposed by da Silva and Loh [42]. Through calorimetric measurements, it was suggested that the trend in the effectiveness of a salt to induce ATPS formation is associated with cation–polymer interactions. According to this model, when salts and polymers are mixed, cations of the salt interact with the ethylene oxide PEO groups, releasing water molecules that solvate the polymer in a process driven by an entropy increase. This cation binding continues as more electrolytes are added until saturation is reached and after which no further gain in entropy is possible, and phase splitting becomes more favorable. In da Silva and Loh's study [42], it was proposed that a higher amount of the  $\text{Li}^+$  cation is necessary to energetically saturate the polymer chain compared to  $\text{K}^+$  and  $\text{Na}^+$  cations. Thus, in the ATPS composed of  $\text{Li}_2\text{SO}_4$ , the macromolecule of PEO is more positively charged than the systems composed of  $\text{K}_2\text{HPO}_4 + \text{KOH}$  or  $\text{Na}_2\text{SO}_4$ , which enables a stronger electrostatic interaction between the PEO segments and the  $[\text{Fe}_2(\text{CN})_{10}]^{10-}$  complex. Consequently, higher partitioning of the complex anion to the PEO-rich phase occurs. Therefore, the PEO1500/ $\text{Li}_2\text{SO}_4$  ATPS system is more suitable for applying the proposed method in the determination of trace concentrations of phenols in environmental samples.

### 3.5. Analytical figures of merit

Under optimum experimental conditions (Table 2), additional experiments were performed to establish the analytical features of

**Table 4**  
Analytical features of different UV/vis spectrophotometric procedures for phenol determinations.

Parameter	Proposed method		4-AAP method [28] (without extraction)	Literature method [31]
	Phenol	<i>o</i> -Cresol		
Linear range	1.50–500 $\mu\text{g kg}^{-1}$		1000–5000 $\mu\text{g L}^{-1}$	50–5000 $\mu\text{g L}^{-1}$
$R$ ( $n=8$ )	0.9999	0.9997	0.9998	0.9998
Cell optical path	1.00 cm		1.00 cm	2.00 cm
LOD ( $\mu\text{g kg}^{-1}$ )	1.27	1.88	100	50
LOQ ( $\mu\text{g kg}^{-1}$ )	4.22	6.28	NA	NA
CV (%) ( $n=5$ )	0.38	0.30	0.35	1.3
Waste volume (mL) <sup>a</sup>	24.0		100	50.0

NA = mean not available; LOD = limit of detection; LOQ = limit of quantification; CV = coefficients of variation.

<sup>a</sup> Estimated per determination.

**Table 5**  
Recoveries of phenol and *o*-cresol in water samples ( $n=3$ ).

Sample	ATPS method recovery (%)		4-AAP method recovery (%)	
	Phenol	<i>o</i> -Cresol	Phenol	<i>o</i> -Cresol
Distilled water	97.6 ± 1.6	99.4 ± 1.5	97.8 ± 0.1	98.6 ± 0.2
River water	107 ± 0	108 ± 0.1	104 ± 1	105 ± 1
Industrial water	98.0 ± 1.5	100 ± 3	95.8 ± 0.3	95.8 ± 0.3
WWTP water 1	100 ± 2	98.5 ± 1.0	101 ± 0	96.2 ± 0.2
WWTP water 2	95.7 ± 0.5	96.4 ± 0.3	99.2 ± 0.6	99.2 ± 0.6

ATPS = aqueous two-phase system; 4-AAP = 4-aminoantipyrine; WWTP = wastewater treatment plant.

the procedure. The PEO1500/ $\text{Li}_2\text{SO}_4$  ATPS system was used in these studies due to its higher sensitivity (Figs. 3 and 4).

Table 4 shows the main characteristics of the proposed method compared to the standard method [28] and described by Kang et al. [31]. To increase the limits of detection (LOD) and limits of quantification (LOQ), ATPS with 1.0000 g of top phase and 23.0000 g of bottom phase were used, offering a higher concentration factor of  $[\text{Fe}_2(\text{CN})_{10}]^{10-}$  anion and consequently a reduction in the range of the analytical curve. The linear responses ( $R \geq 0.9997$ ) were within 1.00–500  $\mu\text{g kg}^{-1}$  phenol, which is suitable for the determination of phenols in water samples according to the limits established by the American and Brazilian legislation [4,5]. The method achieves LODs of 1.27 and 1.88  $\mu\text{g kg}^{-1}$ , and LOQs of 4.22 and 6.28  $\mu\text{g kg}^{-1}$  for phenol and *o*-cresol, respectively. These values are lower than those obtained using the 4-AAP method [28] or the reaction between phenol, NPS and HL [31] without extraction. However, it is also notable that spectrometric UV/vis methods that provide the lowest LOD involve the use of an optical path length of 100 cm [27] or three consecutive extractions with chloroform [28]. Nevertheless, the employment of chloroform is harmful to the environment and human health.

The coefficients of variation (CV) were 0.38% for phenol and 0.30% for *o*-cresol. The proposed method offers a significant reduction in the volume of waste compared to the reference method. Hence, the new procedures are a suitable and environmentally friendly alternative to methods available in the literature that employ hazardous solvents, such as chloroform, or generate large amounts of waste. Furthermore, these results support the feasibility of applying the proposed method to the determination of phenol in environmental samples.

### 3.6. Analysis of environmental samples

Table 5 shows the results of recovery studies on water samples. The ATPS method was compared with the standard 4-AAP method [28] to check the accuracy and reliability of the proposed procedures. Studies were carried out using natural river water, industrial water, distilled water and samples from WWTPs. Recovery rates between 95.7% and 107% were obtained, and the results

had excellent agreement with the standard method [28] at the 95% confidence level according to a paired *t*-test [43]. Moreover, concentrations of total phenols in furnace coke effluent without spiked phenol were determined. This sample was previously distilled and diluted according to the linear range of the method. The concentration values of total phenols found in the sample with the ATPS and 4-AAP methods were 815.7 and 781.7 mg kg<sup>-1</sup>, respectively, which correspond to a relative error of 4.35% between both methods.

#### 4. Conclusions

The analytical utility of an ATPS method for determining trace phenols in water and wastewater was demonstrated for the first time by exploiting the reaction between phenol, NPS and HL. This reaction produced the [Fe<sub>2</sub>(CN)<sub>10</sub>]<sup>10-</sup> anion complex, which concentrated in the top phase of the ATPSs studied. The proposed method offers clearly advantageous analytical features in comparison to procedures involving the same reaction [31] or with a standard method [28], including an enhanced sensitivity and linear range as well as reduced waste volumes and no organic solvents. Pre-concentrations of analyte in the polymer-rich phase of the system allow the method to reach LOD and LOQ values that are suitable for determining total phenols in natural water and wastewater with high accuracy and in compliance with the regulatory requirements of environmental health agencies.

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#### References

- [1] K.P. Kringstad, E. Lindstrom, *Sci. Technol.* 18 (1984) 236A.
- [2] <http://www.epa.gov> (accessed in May 2009).
- [3] D. Puig, D. Barceló, *Trends Anal. Chem.* 15 (1996) 362.
- [4] J. Švitel, S. Miertuš, *Environ. Sci. Technol.* 32 (1998) 828.
- [5] <http://www.mma.gov.br> (accessed in May 2009).
- [6] S. Vichi, A. Romero, J. Tous, E.L. Tamames, S. Buxaderas, *J. Chromatogr. A* 1211 (2008) 1.
- [7] X. Chen, T. Zhang, P. Liang, Y. Li, *Microchim. Acta* 155 (2006) 415.
- [8] B.N. Estevinho, I. Martins, N. Ratola, A. Alves, L. Santos, *J. Hazard. Mater.* 143 (2007) 535–540.
- [9] J. Rolfes, J.T. Andersson, *Anal. Chem.* 73 (2001) 3073.
- [10] B. Bennett, B.F.J. Bowler, S.R. Larter, *Anal. Chem.* 68 (1996) 3697.
- [11] H.M. Oliveira, M.A. Segundo, J.L.F.C. Lima, V. Cerdà, *Talanta* 77 (2009) 1466.
- [12] J. Meyer, A. Liesener, S. Gtz, H. Hayen, U. Karst, *Anal. Chem.* 75 (2003) 922.
- [13] C. Terashima, T.N. Rao, B.V. Sarada, D.A. Tryk, A. Fujishima, *Anal. Chem.* 74 (2002) 895.
- [14] W. Wei, X.B. Yin, X.W. He, *J. Chromatogr. A* 1202 (2008) 212.
- [15] Y. Ding, C.D. Garcia, *Analyst* 131 (2006) 208.
- [16] K. Tsukagoshi, T. Kameda, M. Yamamoto, R. Nakajima, *J. Chromatogr. A* 978 (2002) 213.
- [17] J.M. Gutiérrez, A. Gutiérrez, F. Céspedes, M. del Valle, R. Muñoz, *Talanta* 76 (2008) 373.
- [18] K.S. Khachatryan, S.V. Smirnova, I.I. Torocheshnikova, N.V. Shvedene, A.A. Formanovsky, I.V. Pletnev, *Anal. Bioanal. Chem.* 381 (2005) 464.
- [19] M.A. El Mhammedi, M. Achak, M. Bakasse, A. Chtaini, *J. Hazard. Mater.* 163 (2009) 323.
- [20] S. Korkut, B. Keskinler, E. Erhan, *Talanta* 76 (2008) 1147.
- [21] J. Kochana, A. Gala, A. Parczewski, J. Adamski, *Anal. Bioanal. Chem.* 391 (2008) 1275.
- [22] W.J.R. Santos, P.R. Lima, C.R.T. Tarley, L.T. Kubota, *Anal. Bioanal. Chem.* 389 (2007) 1919.
- [23] M. Shaghghi, J.L. Manzoori, A. Jouyban, *Food Chem.* 108 (2008) 695.
- [24] W. Cao, X. Mu, J. Yang, W. Shi, Y. Zheng, *Spectrochim. Acta A* 66 (2007) 58.
- [25] J. Vuković, S. Matsuoka, K. Yoshimura, V. Grdinić, R.J. Grubešić, *Microchim. Acta* 159 (2007) 277.
- [26] M. Manera, M. Miró, J.M. Estela, V. Cerdà, *Anal. Chim. Acta* 582 (2007) 41.
- [27] K.O. Lupetti, F.R.P. Rocha, O. Fatibello-Filho, *Talanta* 62 (2004) 463.
- [28] L.S. Clesceri, A.E. Greenberg, A.D. Eaton, *Standard Methods for the Examination of Water and Wastewater*, twentieth ed., American Public Health Association, Washington, DC, 1999.
- [29] M.C.H. da Silva, L.H.M. da Silva, F.J. Paggioli, *Anal. Sci.* 21 (2005) 933.
- [30] P. Nagaraj, J.M. Bhandari, B.N. Achar, *Indian. J. Chem.* 32A (1993) 641.
- [31] C. Kang, Y. Wang, R. Li, Y. Du, J. Li, B. Zhang, L. Zhou, Y. Du, *Microchem. J.* 64 (2000) 161.
- [32] J.P. Martins, P.C. Carvalho, L.H.M. da Silva, J.S.R. Coimbra, M.C.H. da Silva, G.D. Rodrigues, L.A. Minim, *J. Chem. Eng. Data* 53 (2008) 238.
- [33] X. Zeng, K. Osseo-Asare, *Colloids Surf. A: Physicochem. Eng. Aspects* 226 (2003) 45.
- [34] N.J. Bridges, K.E. Gutowski, R.D. Rogers, *Green Chem.* 9 (2007) 177.
- [35] G.D. Rodrigues, M.D.H. da Silva, L.H.M. da Silva, F.J. Paggioli, L.A. Minim, J.S.R. Coimbra, *Sep. Purif. Technol.* 62 (2008) 687.
- [36] L.H.M. da Silva, M.C.H. da Silva, J.A. Júnior, J.P. Martins, J.S.R. Coimbra, L.A. Minim, *Sep. Purif. Technol.* 60 (2008) 103.
- [37] A.E. Visser, S.T. Griffin, D.H. Hartman, R.D. Rogers, *J. Chromatogr. B* 743 (2000) 107.
- [38] L. Bulgariu, D. Bulgariu, *J. Chromatogr. A* 1196 (2008) 117.
- [39] L. Capezio, D. Romanini, G.A. Pico, B. Nerli, *J. Chromatogr. B* 819 (2005) 25.
- [40] L.H.M. da Silva, M.C.H. da Silva, R.A.N. de Aquino, K.R. Francisco, M.V.C. Cardoso, L.A. Minim, J.S.R. Coimbra, *J. Phys. Chem. B* 110 (2006) 23540.
- [41] L.H.M. da Silva, M.C.H. da Silva, K.R. Francisco, M.V.C. Cardoso, L.A. Minim, J.S.R. Coimbra, *J. Phys. Chem. B* 112 (2008) 11669.
- [42] L.H.M. da Silva, W. Loh, *J. Phys. Chem. B* 104 (2000) 10069.
- [43] J.C. Miller, J.N. Miller, *Statistics for Analytical Chemistry*, fourth ed., Prentice Hall, 2000.