



Aqueous two-phase systems: A new approach for the determination of *p*-aminophenol

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ABSTRACT

A new method has been developed for the spectrophotometric determination of *p*-aminophenol (PAP) in water, paracetamol formulations and human urine samples with a recovery rate between 94.9 and 101%. This method exploits an aqueous two-phase system (ATPS) liquid–liquid extraction technique with the reaction of PAP, sodium nitroprusside and hydroxylamine hydrochloride in pH 12.0, which produces the $[\text{Fe}_2(\text{CN})_{10}]^{10-}$ anion complex that spontaneously concentrates in the top phase of the ATPS ($K_{[\text{Fe}_2(\text{CN})_{10}]^{10-}} = 97.7$). The ATPS does not require an organic solvent, which is a safer and cleaner liquid–liquid extraction technique for the determination of PAP. The linear range of detection was from 5.00 to 500 $\mu\text{g kg}^{-1}$ ($R \geq 0.9990$; $n = 8$) with a coefficient of variation of 2.11% ($n = 5$). The method exhibited a detection limit of 2.40 $\mu\text{g kg}^{-1}$ and a quantification limit of 8.00 $\mu\text{g kg}^{-1}$. The ATPS method showed a recovery that ranged between 96.4 and 103% for the determination of PAP in natural water and wastewater samples, which was in excellent agreement with the results of the standard 4-aminoantipyrine method that was performed on the same samples.

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1. Introduction

The disposal of toxic pollutants in water sources can be very harmful to the environment and to human health, and environmental safety and public health concerns have risen over the last few decades. Therefore, the establishment of simple, fast, low-cost, greener, sensitive, and selective analytical methods for the determination of pollutants in water and other matrices is a primary concern of research in environmental chemistry.

The determination of phenol and its derivative compounds (such as aminophenol) is of significant importance because these species are toxic [1,2] and are involved in many industrial processes [3–5]. For example, according to environmental legislation, the maximum concentration limit for the total phenol concentration in bodies of water is between 3.5 and 10 $\mu\text{g L}^{-1}$ [6,7].

p-Aminophenol (PAP) is recognized as a serious environmental pollutant and has been widely used in various fields, such as medicine, sulfur and azo dyes, rubber, feeding stuff, petroleum, and photography [8–10]. Moreover, PAP is used in the production of paracetamol, and PAP can be formed by the degradation of paracetamol and its analogs in the human body. PAP can also be formed

by the degradation of paracetamol during the storage of this analgesic [11]. PAP is toxic and irritable to the eyes, skin and respiratory system, and the target sites of PAP in the body are blood and the kidneys [9,12]. Furthermore, PAP increases the core body temperature in humans and is characterized by a long biological half-life (the time required for half of the PAP to be removed from the body by biophysical and biochemical processes). According to the European Pharmacopoeia [13], 50 $\mu\text{g L}^{-1}$ is the specified limit of PAP in paracetamol drug preparations. However, because PAP is both biochemically and environmentally hazardous, the determination of trace amounts of PAP in matrices such as water, pharmaceutical formulations and human fluids, is important.

Numerous analytical methods have been developed for the analysis of PAP and other phenolic compounds, including gas chromatography (GC) [14–16], liquid chromatography (LC) [17–19], capillary electrophoresis (CE) [20–22], potentiometric titration [23], nuclear magnetic resonance (NMR) [24], enzyme-based assays [25] and electrochemical analysis [20,26,27]. However, these methods require expensive instruments, skilled operators and derivatization of the analytes, and they do not allow for continuous in situ monitoring. Spectrometric analyses have also been applied for the determination of phenols, which have been performed mainly with UV–visible spectrophotometry [28–31].

The most commonly used analytical method (described in the Standard Methods for the Examination of Water and Wastewater

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[32]) is a spectrophotometric method based on the oxidative coupling of phenols with 4-aminoantipyrine (4-AAP). However, this procedure has many disadvantages, including (i) the requirement of large amounts of reagents, especially chloroform, which is toxic and carcinogenic [33]; (ii) difficulty in finding suitable buffers due to the sensitivity of the reaction to variations in pH; and (iii) tedious operation.

A new spectrophotometric method for the determination of phenolic compounds has been reported by Nagaraj et al. [34] and improved by Kang et al. [35]. Based on the reaction between phenol, sodium nitroprusside (NPS) and hydroxylamine hydrochloride (HL) in an alkaline medium, a blue-colored $[\text{Fe}_2(\text{CN})_{10}]^{10-}$ anion is produced. However, this procedure is not suitable for the determination of phenols at concentrations lower than $50.0 \mu\text{g L}^{-1}$ or for analyses in the presence of electrolytes, such as phosphates and sulfates, which introduce errors. Moreover, a distillation step in the preparation of the sample is required.

A convenient approach to address these limitations is to promote the complex formation reaction at the interface between the bottom and top phases of an aqueous two-phase system (ATPS). This reaction avoids salt interference and induces the posterior transfer of the complex to a small volume in the top phase of the ATPS. Within this context, the ATPS method is a safer and cleaner liquid–liquid extraction technique for the determination of PAP. Under specific thermodynamic conditions, an ATPS can be formed by mixtures of aqueous solutions of (i) certain electrolytes and polymers [36], (ii) two types of water-soluble polymers [37] or (iii) two types of salts [38]. These mixtures result in systems that contain two immiscible liquid phases with a polymer-enriched top phase and a salt-enriched bottom phase (or enriched with the second polymer). Moreover, water is the major component of both phases; it provides an environmental friendly extraction solvent that eliminates the need for hazardous solvents. Lastly, the ease of use, low cost, shorter time for phase splitting and recycling of the system components are major advantages of this technique [39].

This work aimed to determine the concentration of PAP in water, paracetamol and human urine using an ATPS that was composed of polyethylene oxide 1500 (PEO1500), salt (Li_2SO_4 , Na_2SO_4 or $\text{K}_2\text{HPO}_4 + \text{KOH}$) and water. The proposed method was based on the reaction between PAP, NPS and HL in an alkaline medium and spectrophotometric detection of the reaction product. Specific parameters, such as the nature of the ATPS electrolyte, ATPS tie-line length (TLL), partitioning behavior and influence of *p*-chlorophenol (PCP) in the analysis, were also investigated.

2. Experimental

2.1. Apparatus

Spectrophotometric measurements were performed with a UV–visible (UV/vis) spectrophotometer (Shimadzu, UV-2550) using quartz microcell with a 1.00 cm optical path length and a total capacity of $450 \mu\text{L}$. The slit width was 2.0 nm. The software used for system control and data acquisition was UVProbe. The pH measurements were performed using a glass electrode combined with a digital pH meter (Digicron Analítica Ltda, Digimed model DM-20). An ultrasonic bath (Ultra-Cleaner, USC 1400) and centrifuge (Thermo Scientific, Heraeus Megafuge 11R) were used to prepare the paracetamol formulations.

2.2. Chemicals

All reagents were of analytical grade quality and were used as received without further purification. Distilled water was used throughout the experiments. Polyethylene oxide (Synth, Diadema,

São Paulo, Brazil) with an average molar mass (M_m) of 1500 g mol^{-1} (denoted as PEO1500) was the polymer used in the experiments, and Li_2SO_4 , Na_2SO_4 , K_2HPO_4 , KOH , NaOH , NPS and HL , were obtained from Vetec (Duque de Caxias, Rio de Janeiro, Brazil). The reagents for the 4-AAP procedure [32] were 4-aminoantipyrine, NH_4OH , $\text{K}_3[\text{Fe}(\text{CN})_6]$ (Merck, Darmstadt, Germany), K_2HPO_4 and KH_2PO_4 (Vetec). *p*-Aminophenol and *p*-chlorophenol (PCP) were purchased from Merck.

2.3. ATPS composition, solutions and standards

The three types of ATPS applied in the development of the procedure were PEO1500 + Li_2SO_4 + water; PEO1500 + Na_2SO_4 + water [36] and PEO1500 + ($\text{K}_2\text{HPO}_4 + \text{KOH}$) + water [40]. 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). The compositions shown in Table 1 are relative to an ATPS that was composed of 2.00 g of the top phase and 10.0 g of the bottom phase, which was used in most assays.

All solutions were prepared with distilled water, and the pH was adjusted to 12.0 with NaOH because this pH was more favorable for the reaction of NPS with PAP. For systems that were composed with the $\text{K}_2\text{HPO}_4 + \text{KOH}$ salts, the adjustment in pH was not necessary because the pH was 12.0 on preparation. Stock solutions of the top and bottom phases were prepared, and the concentrations of PEO1500 and salt in both phases are provided in Table 1. These solutions were used as solvents to prepare the solutions of NPS and HL. The HL working stock solution (259 mg kg^{-1}) was prepared by weighing the appropriate quantity of hydroxylamine hydrochloride in the bottom phase solution using an analytical balance (Shimadzu, AY 220) with an uncertainty of $\pm 0.0001 \text{ g}$. The NPS working stock solution (1.99 g kg^{-1}) was prepared by dissolving the appropriate amount of the salt in the top-phase solution. Working standard solutions of PAP between 0.125 and 50.0 mg kg^{-1} were prepared daily using water as the solvent and the appropriate dilution of a 1.00 g kg^{-1} stock solution. In the 4-AAP method, the solutions and standards were prepared as previously described [32].

2.4. Procedure

Liquid–liquid equilibrium cells were used for the preparation of the ATPS. Working stock solutions of 2.00 g of NPS and 10.00 g of HL were weighed, and 0.200 g of the PAP standard solution was added to the system. A similar technique was adopted in the analysis of the samples. The final composition of PAP in the ATPS was between 2.10 and $833 \mu\text{g kg}^{-1}$, and the concentrations of NPS and HL were 332 mg kg^{-1} and 216 mg kg^{-1} , respectively. The final concentrations of PEO1500 and salt in the ATPS varied depending on the TLL (Table 1). After the components were added together, the biphasic systems were manually stirred for 3 min and allowed to settle for 20 min at $25.0 \pm 0.1 \text{ }^\circ\text{C}$ in a temperature-controlled bath (Microquímica, MQBTC 99-20). Lastly, aliquots of the top phase were collected with a syringe and diluted by a factor of 1.50. UV/vis spectrophotometric measurements were performed at 720.0 nm .

In general, cyan complexes are photosensitive [41]; therefore, the experiments were performed in the absence of light to avoid decomposition of the complex.

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 ATPS was placed in a temperature-controlled bath for 24 h to reach thermodynamic

Table 1
Percent composition (m/m) for PEO1500 (w_p), salt (w_s) and water (w_w) in an aqueous two-phase system (ATPS) at 25.0 °C.

Tie-line length (TLL)	Global			Top phase			Bottom phase		
	w_p	w_s	w_w	w_p	w_s	w_w	w_p	w_s	w_w
PEO1500 + Li ₂ SO ₄ + water									
29.43	11.40	13.83	74.77	34.44	5.30	60.26	6.85	15.54	77.61
34.97	12.00	14.73	73.27	39.18	4.15	56.67	6.60	16.85	76.55
41.71	12.30	16.64	71.06	44.40	3.25	52.35	5.91	19.32	74.77
46.36	12.80	17.62	69.58	48.38	2.58	49.04	5.68	20.63	73.69
51.67	13.50	18.79	67.71	53.26	2.15	44.59	5.60	22.12	72.28
PEO1500 + (K ₂ HPO ₄ + KOH) + water									
24.65	9.38	13.19	77.43	28.25	5.06	66.69	5.61	14.82	79.57
34.45	9.09	16.09	74.82	34.82	3.36	61.82	3.94	18.64	77.42
36.83	8.97	17.71	73.32	35.94	3.05	61.01	3.58	20.64	75.78
46.55	7.00	21.65	71.35	40.68	2.40	56.92	0.26	25.50	74.24
52.30	7.52	24.43	68.05	44.88	1.99	53.13	0.05	28.92	71.03
PEO1500 + Na ₂ SO ₄ + water									
32.51	7.45	13.50	79.05	32.36	2.84	64.8	2.47	15.63	81.90
35.96	7.52	15.17	77.31	34.51	2.16	63.33	2.12	17.77	80.11
40.10	7.87	16.53	75.60	37.94	1.95	60.11	1.86	19.45	78.69

equilibrium. After, aliquots of the top and bottom phases were collected. The parameter $K_{[\text{Fe}_2(\text{CN})_{10}]^{10-}}$ can 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 readings at 720.0 nm of the $[\text{Fe}_2(\text{CN})_{10}]^{10-}$ complex in the top and bottom phases, respectively.

The influence of the TLLs in the partitioning behavior of the anion complex was studied by determining $K_{[\text{Fe}_2(\text{CN})_{10}]^{10-}}$ as a function of the TLL for the ATPS that was composed of PEO1500 + Li₂SO₄ + water.

2.6. Water samples

To determine the applicability of the method in environmental samples, the recovery rates provided by the proposed method were determined in natural river water, industrial water, distilled water, tap water and samples from wastewater-treatment plants (WWTPs). In these samples, selected amounts of PAP were added to the samples without a distillation step. The 4-AAP method [32] was also applied to the same samples for comparison and to determine the accuracy of the newly proposed method.

2.7. Determination of PAP in paracetamol formulations

Paracetamol formulations were obtained from different manufacturers. Ten tablets that contained 750 mg of paracetamol per tablet were finely homogenized and weighed. Each sample was extracted with 25 mL of ethanol for 15 min in an ultrasonic bath. The suspension was centrifuged for 20 min at 3000 rpm to obtain a clear supernatant, and the appropriate amount of PAP was added. The experiments were performed according to the general procedure, and the recovery rate of PAP in the samples was determined. In our previous studies, we observed that the addition of ethanol volumes that were lower than 0.200 g in the system had no influence in the analytical response.

2.8. Analysis of urine samples

The same assays were used for analysis of PAP concentrations in urine samples from healthy volunteers. Urine samples were collected 5 h after the administration of a single 750 mg dose of paracetamol and were stored in plastic containers. The samples were diluted 100-fold with distilled water, and the appropriate concentration of PAP was added. The experiments were performed

according to the general procedure, and the rate of recovery of PAP in the samples was determined.

3. Results and discussion

3.1. Chemical and spectral characteristics

The proposed method is based on the reaction of PAP, NPS and HL according to the mechanism presented by Kang et al. [35]. In an alkaline medium, the phenol group is deprotonated, which makes the benzene ring very reactive. The increased electronic density in the phenolate allowed for an electrophilic reaction to proceed more readily. In the reaction of PAP, the nitroso group (^+NO) of the nitroprusside preferentially attacks the benzene ring in the ortho-position to yield the green-blue $[\text{Fe}_2(\text{CN})_{10}]^{10-}$ anion complex. Following the methods of Kang et al., the oxidation state for iron was confirmed by the hysteresis loop of the solid complex, which showed the presence of weak ferromagnetism in the product. The magnetic susceptibility calculated was 7.3×10^{-5} cgs. In addition, the Mössbauer spectrum determination experiment at room temperature (298 K) showed that the solid product had no diamagnetism [35].

Fig. 1 shows the absorption spectra of the $[\text{Fe}_2(\text{CN})_{10}]^{10-}$ reaction product that was obtained in the top phase of the ATPS. The

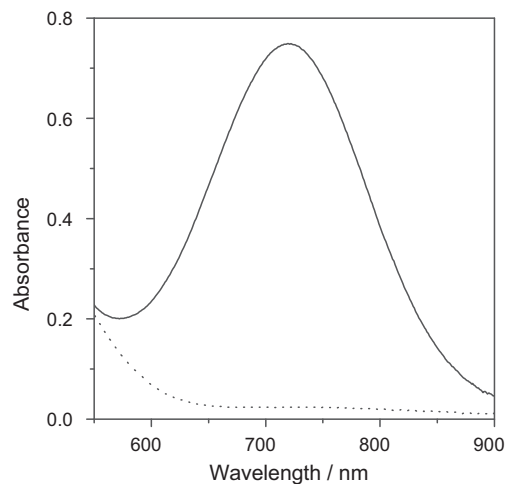


Fig. 1. Absorption spectra of the $[\text{Fe}_2(\text{CN})_{10}]^{10-}$ anion formed by the reaction between NPS, HL and (—) PAP, and (···) a blank assay in the PEO1500/Li₂SO₄ aqueous two-phase system (ATPS) with a tie-line length (TLL) of 41.71% (m/m).

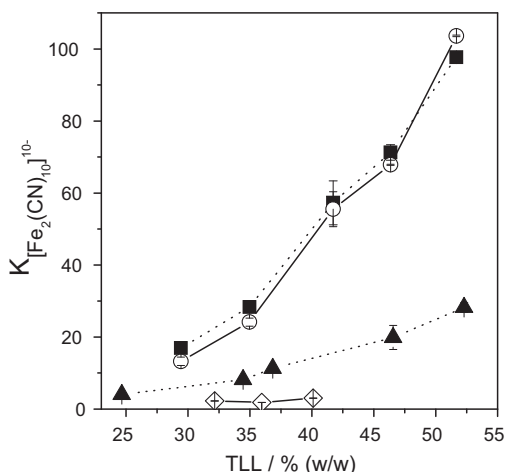


Fig. 2. Partition coefficient ($K_{[\text{Fe}_2(\text{CN})_{10}]^{10-}}$) of the $[\text{Fe}_2(\text{CN})_{10}]^{10-}$ anion in ATPS that was composed of PEO 1500 + salt + water as a function of increasing tie-line length (TLL). (▲) $\text{K}_2\text{HPO}_4 + \text{KOH}$; (◇) Na_2SO_4 ; (■) Li_2SO_4 and (○) Li_2SO_4 in the presence of PCP.

product had a maximum absorption at 720.0 nm, which fortunately was a wavelength with an insignificant blank signal. According to spectra that were obtained in aqueous solutions by Kang et al. [35], the product of the reaction of PAP, NPS and HL exhibits a maximum absorbance at 700 nm. The different environments surrounding the product in the top phase of the ATPS compared to the aqueous solution in previous experiments may account for this shift in the absorption maximum.

3.2. Phase diagrams

All of the phase diagrams have been previously constructed and are reported in the literature [36,40]. After mixing the components at a specific composition (Table 1), the systems spontaneously separated into two isotropic transparent phases with the top and bottom phases enriched in macromolecules and salt, respectively.

3.3. Influence of the tie-line length (TLL) and electrolytes on the partitioning behavior

To improve the sensibility of the proposed method, it was important to determine the partitioning behavior of the $[\text{Fe}_2(\text{CN})_{10}]^{10-}$ anion as a function of the TLL. The increase in the preferential transfer process of the anion complex from the bottom phase to the top phase made it possible to increase the absorbance signal, which decreased the limit of detection. At constant pressure and temperature, the TLL is a thermodynamic parameter of the ATPS that expresses differences in intensive thermodynamic functions between the top and bottom phases [42]. The TLL was expressed as the difference between the polymer and salt concentrations present in the different phases and was commonly used as a variable when determining the processes of solute partitioning. The TLL was calculated by Eq. (2):

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

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 the corresponding salt concentrations of the top and bottom phases.

Fig. 2 shows the partition coefficient ($K_{[\text{Fe}_2(\text{CN})_{10}]^{10-}}$) of the $[\text{Fe}_2(\text{CN})_{10}]^{10-}$ anion plotted against the TLL in an ATPS that was composed of PEO1500 + salt (Li_2SO_4 or $\text{K}_2\text{HPO}_4 + \text{KOH}$ or Na_2SO_4) + water. As shown in Fig. 2, the $[\text{Fe}_2(\text{CN})_{10}]^{10-}$ anion complex has a spontaneous concentration in the polymer-rich phase

that reached a $K_{[\text{Fe}_2(\text{CN})_{10}]^{10-}}$ value of 97.7 for a TLL of 51.67% (m/m) in the PEO1500/ Li_2SO_4 ATPS. The Haynes' model was used to explain the partitioning of $[\text{Fe}_2(\text{CN})_{10}]^{10-}$ to the top phase of the ATPS, which was derived from the Flory-Huggins theory. This model is composed of analytical equations that express the partition coefficient in terms of the enthalpic and entropic contributions.

In the Haynes' model, the entropic contribution to partitioning is described by Eq. (3):

$$\ln K_C = \frac{M_C}{\rho} \left(\frac{n^T}{V^T} - \frac{n^B}{V^B} \right) \quad (3)$$

where M_C is the molar mass of the partitioning solute ($[\text{Fe}_2(\text{CN})_{10}]^{10-}$), n^T and n^B are the total number of molecules in the top and bottom phases, respectively, which result in a parameter called the phase number density when divided by the phase volume, V^T and V^B , and ρ is the number of lattice sites per unit volume.

Based on Eq. (3) and in the absence of enthalpic effects, the transfer process of the anion from the bottom phase to the top phase should lead to a decrease in the entropy of the system. Therefore, for an increase in the system entropy, the $[\text{Fe}_2(\text{CN})_{10}]^{10-}$ anion should concentrate on the bottom phase of the ATPS, which has a higher number density than the polymer-rich phase due to the larger water content in the salt-rich phase, as shown in Table 1. However, Fig. 2 shows that the anion complex preferentially concentrated in the top phase, which indicated that solute partitioning was an enthalpy-driven process.

The contribution of enthalpy in Haynes' model to the partition coefficient is given by Eq. (4):

$$\ln K_C = -\frac{M_C}{RT} \left[\sum_{i=1(i \neq C)}^m (\Phi_i^T - \Phi_i^B) w_{iC} - \sum_{i=1(i \neq C)}^{m-1} \sum_{j=i+1(j \neq C)}^m (\Phi_i^T \Phi_j^T - \Phi_i^B \Phi_j^B) w_{ij} \right] \quad (4)$$

where Φ_i^T and Φ_i^B are the volume fractions of the ATPS-forming compounds on the top and bottom phases, respectively, and w_{ij} is the effective pairwise interchange energy defined as: $w_{ij} = z[\varepsilon_{ij} - (1/2)(\varepsilon_{ii} + \varepsilon_{jj})]$, where z is the number of nearest neighbors and ε_{ij} is the potential energy of an i - j pair.

In Eq. (4), the enthalpic contribution is described by two terms. The first term, $[\sum_{i=1(i \neq C)}^m (\Phi_i^T - \Phi_i^B) w_{iC}]$, is the energetic contribution that is related to the interactions between the $[\text{Fe}_2(\text{CN})_{10}]^{10-}$ anion and the remaining components of the ATPS. Stronger i -complex interactions (ic) lead to more negative w_{iC} values and a more enthalpically favorable transfer of the complex to the i -component-rich phase. Considering that the $[\text{Fe}_2(\text{CN})_{10}]^{10-}$ anions are concentrated in the PEO-rich phase, the enthalpic molecular interaction between the complex molecules and polymer molecules is the driving force of the partitioning behavior.

The second term, $\sum_{i=1(i \neq C)}^{m-1} \sum_{j=i+1(j \neq C)}^m \Phi_i^T \Phi_j^T w_{ij}$, is the self-energy of the top phase and gives the total enthalpy of phase formation (in the absence of the anion complex) divided by the number of lattice sites in the top phase. Insertion of the $[\text{Fe}_2(\text{CN})_{10}]^{10-}$ anion into a phase requires a breaking of interactions between the original components of the phase to create a cavity into which the complex fits. Although this process is dependent on the volume of the solute, da Silva et al. [41] demonstrated that the phase self-energy does not significantly contribute to the partitioning behavior. Consequently, the anion partitioning behavior can be attributed to specific enthalpic interactions between $[\text{Fe}_2(\text{CN})_{10}]^{10-}$ and macromolecules, which are concentrated in

the top phase. The affinity of $[\text{Fe}_2(\text{CN})_{10}]^{10-}$ with the polymer molecules was attributed to acceptor–donor interactions between the anion (mainly the CN site) and macromolecular segments (due to the electronic pair found in the oxygen atom), which changes the CN electronic density [43,44].

The value of $K_{[\text{Fe}_2(\text{CN})_{10}]^{10-}}$ increased as the TLL values became higher (Fig. 2), which was due to an increase in the number of interactions of the complex with polymer molecules such that the first term in Eq. (5) becomes more negative, which increases the $K_{[\text{Fe}_2(\text{CN})_{10}]^{10-}}$ value [45].

The effect of the electrolytes on the partitioning behavior of $[\text{Fe}_2(\text{CN})_{10}]^{10-}$ was investigated (Fig. 2). For similar TLL values, systems that were composed of the Li_2SO_4 electrolyte presented greater values of $K_{[\text{Fe}_2(\text{CN})_{10}]^{10-}}$ compared to ATPSs that were formed by Na_2SO_4 or $\text{K}_2\text{HPO}_4 + \text{KOH}$. This behavior may be explained by the model proposed by da Silva and Loh [46], who investigated the thermodynamics of the formation of ATPS. According to their model, when PEO and salt are mixed, an interaction between the salt cations and the PEO macromolecule forms, which is driven by an increase in entropy due to the release of water molecules. The interaction of the cation–PEO pair is driven by an increase in the system entropy caused by the release of water molecules that formed a sphere of solvation around the macromolecule and the ions. This process continues as more salt is added until the macromolecule becomes saturated with ions and no further gain in entropy is possible. Therefore, phase splitting becomes more favorable. After the phases split, the polymer-rich phase that contains the PEO macromolecules solvated with cations of the salt results in a positively charged polymer surface called a pseudopolycation, which can interact with negatively charged species. Through calorimetric measurements, da Silva and Loh [46] noted that Li^+ cations interact more strongly with the EO segments of the macromolecules than the other cations, indicating that more Li^+ was necessary to saturate the polymer chain. Consequently, in the ATPS composed of Li_2SO_4 , the PEO macromolecule is more positively charged than in systems composed of $\text{K}_2\text{HPO}_4 + \text{KOH}$ or Na_2SO_4 , enabling a stronger electrostatic interaction between the pseudopolycation and the $[\text{Fe}_2(\text{CN})_{10}]^{10-}$ complex.

3.4. Study of the interference

Fig. 2 presents the study of the partitioning behavior of $[\text{Fe}_2(\text{CN})_{10}]^{10-}$ that was produced by the reaction of PAP, NPS and HL in an ATPS that was composed of PEO1500/ Li_2SO_4 in the presence and absence of PCP. The reactivity was determined by the electrophilic or electrophobic properties of the substituents in the benzene ring, which affected the reaction extension and the formation of the $[\text{Fe}_2(\text{CN})_{10}]^{10-}$ complex [35]. Due to the position and electrophilic properties of the chlorinated substituent, the reaction of the proposed method does not take place with PCP. However, its presence does not interfere with the reaction with PAP or the partitioning of the complex to the top phase of the ATPS. A study on the selectivity of the proposed method showed that PCP does not interfere at concentrations less than 7.00 mg kg^{-1} in samples with 1.00 mg kg^{-1} of PAP. The selectivity of this reaction allows for the quantification of certain phenolic compounds in the presence of other nonreactive derivatives.

3.5. Study of the sensitivity of the method

Fig. 3 shows the influence of the TLL on the sensitivity of the method for PAP determination for the three kinds of ATPSs. These data were obtained by plotting analytical curves for all of the TLLs of the ATPSs and comparing the curves to the absorbance values of the PAP standard ($500 \mu\text{g kg}^{-1}$). The PEO1500/ Na_2SO_4 ATPS stud-

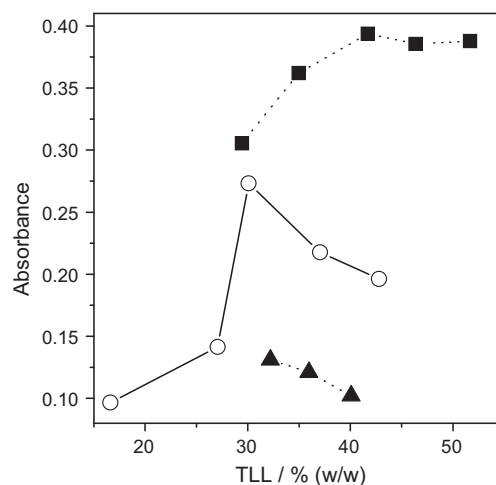


Fig. 3. Effect of tie-line length (TLL) and electrolytes to the determination of the concentration of PAP in an aqueous two-phase system (ATPS) that was composed of PEO1500, salt and water: (■) Li_2SO_4 , (○) $\text{K}_2\text{HPO}_4 + \text{KOH}$ and (▲) Na_2SO_4 .

ies with TLLs greater than 40.10% (m/m) were not practical due to the precipitation of sulfate. The absorbance increased as the TLL increased; however, the signal became constant or decreased at higher TLL values. Increased TLLs cause increased differences between the intensive thermodynamic properties of the top and bottom phases [42]. As a consequence, higher concentrations of $[\text{Fe}_2(\text{CN})_{10}]^{10-}$ were observed in the top phase. This partitioning behavior contributed to the enhanced sensitivity that was observed for the smaller TLL values. However, an increase in the TLL also induced an increase in the salt concentration in the ATPS (Table 1), which interfered with the formation of $[\text{Fe}_2(\text{CN})_{10}]^{10-}$, as previously described by Kang [35]. The decrease of the $[\text{Fe}_2(\text{CN})_{10}]^{10-}$ concentration caused a reduced sensitivity of the method at higher TLL values. Thus, the sensitivity of the method depended on the balance between the formation of $[\text{Fe}_2(\text{CN})_{10}]^{10-}$ and its concentration in the top phase, which depended on the TLL. Because the reaction occurred at the interface in the ATPS method, the interference of ions, such as sulfate and phosphate, is insignificant in comparison to reports by Nagaraj et al. [34] and Kang et al. [35], which indicated that the tolerance limit for sulfate and phosphate was lower than $25,000$ and 5000 mg L^{-1} , respectively.

Fig. 3 shows the effect of the electrolyte on the sensitivity of the method. A TLL value of 41.71% (m/m) using the PEO1500/ Li_2SO_4 ATPS presented the highest response among all of the systems that were studied, which was previously explained by its partitioning behavior in the model presented by da Silva and Loh [46]. Therefore, the PEO1500/ Li_2SO_4 ATPS system was more suitable for applying the proposed method in the determination of trace concentrations of PAP in industrial and environmental water samples.

3.6. Analytical features of the developed procedure

Due to the increased sensitivity (Fig. 3), the ATPS that was composed of PEO1500 + Li_2SO_4 + water was used in these studies. To improve the limits of detection (LOD) and quantification (LOQ), an ATPS with 1.00 g of the top phase and 23.0 g of the bottom phase was used, which offered a higher concentration factor of $[\text{Fe}_2(\text{CN})_{10}]^{10-}$ and a reduction in the range of the analytical curve.

Table 2 shows the main characteristics of the proposed method compared to the standard method [32]. The linear response ($R = 0.9990$, $n = 8$) was within $5.00\text{--}500 \mu\text{g kg}^{-1}$ of PAP, which was suitable for the determination of phenols in water samples, according to the limits established in American and Brazilian legislation [6,7]. The method has an LOD of $2.40 \mu\text{g kg}^{-1}$ and an LOQ of

Table 2

Comparison of the analytical figures of merit of the proposed method (ATPS) with the standard method (4-AAP) [32].

Parameter	Proposed method	4-AAP method [32] (without extraction)
Linear range	5.00–500 $\mu\text{g kg}^{-1}$	1000–5000 $\mu\text{g kg}^{-1}$
R ($n=8$)	0.9990	0.9998
Cell optical path	1.00 cm	1.00 cm
LOD	2.40 $\mu\text{g kg}^{-1}$	100 $\mu\text{g kg}^{-1}$
LOQ	8.00 $\mu\text{g kg}^{-1}$	NA
CV (%) ($n=5$)	2.11	0.350
Waste volume (mL) ^a	24.0	100

^a Estimated per determination; NA means not available.

Table 3

Recoveries (%) of PAP in several water samples ($n=3$).

Water sample	ATPS method	4-AAP method
Distilled	99.8 \pm 3.4	97.9 \pm 0.5
Tap	103 \pm 1	96.4 \pm 0.6
River	102 \pm 3	99.3 \pm 0.6
Industrial	98.0 \pm 0.5	98.0 \pm 0.9
WWTP 1	98.6 \pm 4.8	99.8 \pm 0.6
WWTP 2	101 \pm 4	96.6 \pm 0.6

WWTP: wastewater treatment plant.

8.00 $\mu\text{g kg}^{-1}$. These values were lower than the values that were obtained using the 4-AAP method [32] without extraction. The 4-AAP method provided a lower LOD when three consecutive extractions with chloroform were used. However, this procedure produces large amounts of waste (approximately 550 mL per determination), and the use of chloroform is harmful to the environment and human health.

Moreover, the coefficient of variation (CV) was 2.11%, and the proposed method offered a significant reduction in the volume of waste when compared to the reference method. Therefore, the new procedure is a more environmentally friendly alternative to methods that are currently 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 for the determination of PAP in several sample types.

3.7. Application to real samples

Table 3 shows the results of recovery studies on water samples. The ATPS method was compared to the standard 4-AAP method [32] to determine the accuracy and reliability of the proposed procedure. Studies were performed using natural river water, industrial water, tap water, distilled water and samples from WWTPs. The recovery rates were between 96.4 and 103%, and the results were in excellent agreement with the standard method [32] at the 95% confidence level according to a paired *t*-test.

The proposed method was also used for the determination of PAP in paracetamol pharmaceutical formulations and in human urine samples spiked with approximately 100 $\mu\text{g kg}^{-1}$ of PAP. Table 4 shows the results for two paracetamol formulations and two urine samples. The method was capable of quantifying 100 $\mu\text{g kg}^{-1}$

Table 4

Determination of PAP in pharmaceutical and human urine samples ($n=3$).

Sample	PAP added ($\mu\text{g kg}^{-1}$)	PAP found ($\mu\text{g kg}^{-1}$)	Recovery (%)
Paracetamol A	98.2	97.9	99.7 \pm 0.7
Paracetamol B	97.5	97.3	99.7 \pm 3.9
Urine A	102	96.8	94.9 \pm 3.7
Urine B	96.2	97.1	101 \pm 2

of PAP with recovery rates between 94.9 and 101%. In addition, blanks assays that were analyzed without PAP added in the system resulted in no signal relative to the analyte. Therefore, the present method is able to be used for the quantitative detection of trace PAP in urine and pharmaceutical formulation samples, such as paracetamol tablets, and can be extended to other systems.

4. Conclusions

The experimental results demonstrated, for the first time, the capability and advantages of the proposed ATPS for the determination of PAP in environmental and industrial water, pharmaceutical formulations and human urine samples. The procedure exploits the reaction between PAP, NPS and HL to produce the $[\text{Fe}_2(\text{CN})_{10}]^{10-}$ anion complex, which concentrates in the top phase of the ATPS and is highly sensitive.

The ATPS offers advantageous analytical features in comparison to the standard method [32], including an enhanced sensitivity and linear range as well as a reduced volume of waste and does not use organic solvents. Because the reaction takes place at the interface of the ATPS, the interference of ions, such as sulfate and phosphate, is significantly reduced in comparison to other spectrophotometric methods that employ the same reaction [34,35]. Furthermore, because the presence of PCP in the system has no interference on the reaction of PAP, NPS and HL, the method is suitable to determine the presence of certain phenolic compounds in the presence of other nonreactive derivatives. Preconcentration of the analyte in the polymer-rich phase of the system allowed the method to reach LOD and LOQ values that were suitable for determining the concentration of PAP in water, pharmaceutical formulations and urine with high accuracy, and the reaction was in compliance with the regulatory requirements of environmental agencies [6,7] and pharmacopeia [13].

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