



Green speciation of iron using aqueous two-phase system

RAQUEL A. CAMPOS¹, PAMELA R. PATRÍCIO¹, SILVIA JULIANA R. VARGAS¹,
LUIS HENRIQUE M. DA SILVA¹ and MARIA C. HESPANHOL^{1,2}

¹Grupo de Química Verde Coloidal e Macromolecular (QUIVECOM), Departamento de Química, Centro de Ciências Exatas e Tecnológicas, Universidade Federal de Viçosa/UFV, Av. P.H. Rolfs, s/n, 36570-900 Viçosa, MG, Brazil

²Grupo de Análises e Educação para a Sustentabilidade (GAES), Departamento de Química, Centro de Ciências Exatas e Tecnológicas, Universidade Federal de Viçosa/UFV, Av. P.H. Rolfs, s/n, 36570-900 Viçosa, MG, Brazil

Manuscript received on August 13, 2017; accepted for publication on October 29, 2017

ABSTRACT

Fe(II) and Fe(III) have distinct chemical and biological functions. Consequently, it is more important to determine the fraction of both oxidation state that knowing the total iron concentration in a sample. However, green methods for iron speciation are still limited. This work uses aqueous two-phase system, a safe alternative to liquid–liquid extraction, to perform the chemical speciation of iron. This method is based on the reaction of Fe(II) with 1,10-phenanthroline extractant, forming a complex of Fe(II)-phenanthroline that concentrates in the top phase of the system. The Fe(III) specie concentrated in the bottom phase of the system. Iron speciation was affected by the electrolyte nature, macromolecule type, quantity of phenanthroline added, and pH. The system formed by PEO1500 + Na₃C₆H₅O₇ + H₂O at pH 6.00, containing 5.00 mmol kg⁻¹ of phenanthroline, was successfully used to separate the iron species before determination by flame atomic absorption spectrometry. Under these optimal conditions, a separation factor of 233 was obtained between Fe(II) and Fe(III) with extraction percentages of (95.1 ± 1.0)% and (7.68 ± 0.50)%, respectively. The proposed method was successfully applied for iron speciation in water samples, and provided recovery percentages ranging between 90 and 106%.

Key words: aqueous two-phase system, flame atomic absorption spectrometry, iron, speciation, green chemistry.

INTRODUCTION

In nature, the same element can exist in different chemical forms (different isotopic compositions,

oxidation state, or type of binding ligand), and each one can have a different toxicity, mobility, and bioavailability (Safavi et al. 2002, Kiss et al. 2017). Thus, it is more important to determine the concentration of each one rather than to determine the total concentration of an element in a sample.

Iron is an essential element in the biochemistry of organisms, since the simple ones as

Correspondence to: Maria do Carmo Hespagnol
E-mail: mcarmohespagnol@gmail.com

* Contribution to the centenary of the Brazilian Academy of Sciences.



microorganisms to complexes as humans (Vukosav et al. 2012). It has a central hole in chemical and biological functions as phytoplankton growth, respiratory, photosynthetic electron transport chains, synthesis of chlorophyll, and reduction of nitrate and nitrite (Lin and Twining 2012, Vukosav et al. 2012). Iron is generally found as Fe(II) and Fe(III) species and its crucial functions are dependent of the oxidation states (Pohl and Prusisz 2006, Suárez et al. 2015). Fe(II) is essential for the smooth transport and storage of oxygen in animals, while its oxidized form does not bind to oxygen (Safavi et al. 2002). Especially in an aquatic environment, it is important to identify the iron species, since its chemical form influences its bioavailability and the physicochemical properties of other elements and organic substances (Bulgariu and Bulgariu 2010). The oxidation state of iron in an environment can also indicate its electrical potential and microbial activity (Moghadam et al. 2011). Therefore, it is important to determine not only the total iron content, but also the fraction of Fe(II) and Fe(III) species. The tool to identify and/or measure the quantity of one or more individual chemical species in a sample is called “chemical speciation” according to the International Union of Pure and Applied Chemistry (IUPAC) (Templeton et al. 2000).

One of the most widespread analytical techniques for the determination of metals is flame atomic absorption spectrometry (FAAS) because of its cost and ease of operation (Paleologos et al. 2002, Tasev et al. 2006, Bahar and Zakerian 2012). However, FAAS cannot distinguish between the different chemical forms of an element. Therefore, it is necessary a sample preparation step, in which the species are separated, for chemical speciation using FAAS is performed (Tabrizi 2010).

An interesting strategy to separate iron species is a liquid-liquid extraction (LLE) process (Pournaghi-Azar and Fatemi 2000, Tasev et al. 2006, Pepper et al. 2010). This process is based

on the distribution of the analyte between an aqueous phase and an organic phase containing an extractant, such as thiocyanate (Tasev et al. 2006), 1,10-phenanthroline (Tasev et al. 2006), bis (2-ethylhexyl) hydrogen phosphate (Pepper et al. 2010), or 8-hydroxyquinoline (Pournaghi-Azar and Fatemi 2000). A simple procedure, LLE allows the separation of species using different solvents (isobutylmethyl ketone (Tasev et al. 2006), heptane (Pepper et al. 2010), or chloroform (Pournaghi-Azar and Fatemi 2000). However, LLE involves the use of toxic, carcinogenic and/or flammable organic solvents, and typically requires a long time for phase separation, since emulsions can be formed. These disadvantages limit the applications of the solvent extraction (SE) technique, making it necessary to develop new separation procedures that are environment-friendly, safer, faster, and cost-effective.

An environmentally safe and promising alternative for the extraction and/or separation of metals is the use of an aqueous two-phase system (ATPS). The ATPS consists of two immiscible liquid phases, spontaneously formed by combining aqueous solutions of polymer-polymer (Persson et al. 2000), polymer-electrolyte (Patricio, et al. 2011a), ionic liquid-electrolyte (Bridges et al. 2007), or ionic liquid-polymer (Freire et al. 2012) under specific thermodynamic conditions of temperature, pressure, and composition (Freire et al. 2012). Each formed phase is rich in one of the components, but water is the major component in both phases (Martins et al. 2008, De Andrade et al. 2011, De Lemos et al. 2011, Patricio et al. 2011a, Virtuoso et al. 2012). This LLE system has a high separation efficiency and low cost; it is also easy to operate, does not require the use of organic solvents, and some components can be recycled. As a clean and economical alternative, it has been explored for the purification, separation, preconcentration, and/or determination of biomolecules (Reh et al.

2002, Haraguchi et al. 2004, Azevedo et al. 2009), phenolic compounds (Rodrigues et al. 2010, 2011), dyes (Chethana et al. 2007, Mageste et al. 2009, 2012), and metallic ions (Patricio et al. 2011b, De Lemos et al. 2012, Rodrigues et al. 2012, Da Cunha et al. 2016). However, few studies have explored the separation of species using ATPS, some of which include studies on mercury (Roy and Lahiri 2009), chromium (Akama and Sali 2002, Roy and Lahiri 2008, Patricio et al. 2016, Simonova et al. 2016), iodine (Griffin et al. 2004), and iron (Bulgariu and Bulgariu 2010).

Only one study proposes the use of ATPS to prepare samples for the separation of iron species. Bulgariu and Bulgariu (2010), used the poly(ethylene oxide) + $(\text{NH}_4)_2\text{SO}_4$ + H_2O ATPS to promote a selective extraction of Fe(III) in the presence of Fe(II). While Fe(III) was quantitatively extracted to a polymer-rich phase with the help of the thiocyanate extractant, the Fe(II) ions were concentrated in the $(\text{NH}_4)_2\text{SO}_4$ -rich phase. The authors investigated the influence of pH, salt stock solution, and extractant concentration in the ATPS, on the separation of the species. However, they did not evaluate the influence of the system composition, the nature of the ATPS-forming polymer or electrolyte, or especially, the possible interconversion of the iron species in the system.

In the present work, a new green method is proposed for the chemical speciation of iron. This method involves the separation of Fe(III) and Fe(II) using ATPS, followed by determination of the species with the non-selective FAAS technique. While Fe(II) preferentially concentrated in the polymer-rich phase (top phase, TP), with the aid of the 1,10-phenanthroline (phen) extractant, the Fe(III) ions were partitioned to the salt-rich phase (bottom phase, BP). Parameters of the ATPS that could affect the separation of species, such as the nature of the macromolecule (L64 triblock copolymer or PEO1500 polymer), the use of environmentally safer electrolytes ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$ or

Na_2SO_4), the effect of a wide pH range, and the quantity of the extractant added in the system, were thoroughly investigated. The proposed method is green, simple, and allows the speciation of iron using FAAS.

MATERIALS AND METHODS

REAGENTS

All reagents were of analytical grade and used as received without further purification. The materials used were a poly(ethylene oxide)₁₃-poly(propylene oxide)₃₀-poly(ethylene oxide)₁₃ copolymer (L64; Aldrich, Milwaukee, WI), with mean molar mass of 2900 g mol^{-1} and 40% of ethylene oxide, and a poly(ethylene oxide) polymer (PEO1500; Synth, São Paulo, Brazil), with mean molar mass of 1500 g mol^{-1} . The reagents $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$, Na_2SO_4 , H_2SO_4 , and NaOH (Vetec, Rio de Janeiro, Brazil), $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (Synth, São Paulo, Brazil), $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (Ecibra, São Paulo, Brazil), and 1,10-Phenanthroline (Carlo Erba, Val de Reuil, France), were also used. All experiments were performed with deionized water (Millipore Corp., Massachusetts, USA).

EQUIPMENT

The pH measurements were carried out with a pH meter (Metrohm, 827, Herisau, Switzerland) equipped with a combined glass electrode. An analytical balance (Shimadzu, AY 220, São Paulo, Brazil) with an uncertainty of $\pm 0.0001 \text{ g}$ was used to prepare the working solutions and the ATPS. The temperature of the ATPS was controlled by a thermostatic bath (Marconi, MA 184, São Paulo, Brazil) at $(25.0 \pm 0.1) ^\circ\text{C}$. To accelerate phase separation, a centrifuge (Thermo Scientific, Heraeus Megafuge 11R, Osterode, Germany), was used. The concentration of the ions was determined by ultraviolet-visible molecular absorption spectrometry (Shimadzu, 2550, Kyoto, Japan) at 512 nm or by FAAS (Varian,

AA240, Melbourne, Australia) using the following instrumental conditions: a wavelength of 248.3 nm, lamp current of 5.0 mA, spectral resolution of 0.2 nm, and flame composition (air/C₂H₂) of 3.50/1.50 L min⁻¹.

ATPS COMPOSITION

Stock solutions of the macromolecule and electrolyte were prepared using water at a pH of 1.00, 6.00, or 12.0, adjusted with H₂SO₄ (5.37 mol kg⁻¹) or NaOH (3.92 mol kg⁻¹). Stock solutions of the macromolecules (L64 or PEO1500) and the electrolyte (Na₃C₆H₅O₇ or Na₂SO₄) were prepared in suitable concentrations to provide an ATPS with the compositions described in Table I, when these two solutions were mixed.

EXTRACTION PROCEDURE

The phen working solution was obtained by dissolving the reagent into the macromolecule stock solution (L64 or PEO1500) to obtain concentrations of 1.00, 2.00, 5.00, 10.0, and 20.0 mmol kg⁻¹, corresponding to the following phen:Fe molar ratios: 5:1, 10:1, 25:1, 50:1, and 100:1, respectively. Stock solutions of Fe(II) and Fe(III) in water with a concentration of 4.1 mmol kg⁻¹ were also prepared.

The ATPS used to extract the iron species was prepared by mixing 2.00 g of the working solution (extractant in the macromolecule solution) with 2.00 g of the electrolyte stock solution, prepared previous section, in a 10-mL centrifuge tube. This mixture was bubbled with nitrogen to prevent the oxidation of Fe(II) to Fe(III), following which 0.100 g of Fe(II) or Fe(III) stock solution was added to it, forming 0.100 mmol kg⁻¹ of the iron specie in the ATPS.

Following the addition of the iron species, the centrifuge tube was manually stirred for 3 min, centrifuged at 10,000 rpm for 5 min at 25 °C, and left in a thermostatic bath at (25.0 ± 0.1)°C for 10 min to obtain two clear phases. The TP (or BP)

aliquots were collected and appropriately diluted to determine the iron concentration by FAAS. All experiments were performed in triplicate. Eq. (1) was used to calculate the extraction percentage (% E):

$$\%E_{TP} = \frac{(n_{M^{x+}})_{TP}}{(n_{M^{x+}})_{Total}} \times 100 \quad (1a)$$

$$\%E_{BP} = \frac{(n_{M^{x+}})_{BP}}{(n_{M^{x+}})_{Total}} \times 100 \quad (1b)$$

where $(n_{M^{x+}})_{TP}$ is the quantity of metal ions in the TP, $(n_{M^{x+}})_{BP}$ is the quantity of metal ions in the BP, and $(n_{M^{x+}})_{Total}$ is the total quantity of metal ions in the ATPS.

EXTRACTION OF Fe(II) IN THE PRESENCE OF Fe(III)

Fe(II) specie (5.64 mg kg⁻¹) was added to the PEO1500 + Na₃C₆H₅O₇ + H₂O APTS at a pH of 6.00, which contained 5.00 mmol kg⁻¹ of phen and different concentrations of Fe(III) (0.101, 0.507, or 1.01 mmol kg⁻¹). Fe(II) was determined in the two phases of the ATPS by UV-vis spectrophotometry using the 1,10-phenanthroline method (Harvey et al. 1955, Eaton and Franson 2005). For this determination, aliquots of the two phases were collected and diluted in a phen aqueous solution (1.00 g kg⁻¹), and the absorbances were measured at 512 nm.

DETERMINATION OF THE %E OF THE IRON SPECIES BY FAAS

In a centrifuge tube, 2.61 g of an aqueous solution containing only one of the iron species, Fe(II) (8.87 mg kg⁻¹) or Fe(III) (7.94, 17.3, 39.7, or 86.7 mg kg⁻¹), was added to 0.745 g of PEO1500, 0.642 g of Na₃C₆H₅O₇, and 3.60 mg of phen (5.00 mmol kg⁻¹ of phen). This mixture was stirred, centrifuged, and left in a thermostatic bath at the abovementioned temperature, following which an ATPS with 2.00 g of each phase was obtained. Aliquots of the TP and BP were collected and adequately diluted, and their iron concentration was

TABLE I
Concentration of components in the ATPS.

ATPS	overall / % (m/m)		top phase (TP) / % (m/m)		bottom phase (BP) / % (m/m)		Reference
	M	E	M	E	M	E	
L64 + Na ₃ C ₆ H ₅ O ₇ + H ₂ O	22.55	8.46	44.76	3.37	0.33	13.54	(De Andrade et al. 2011)
PEO1500 + Na ₃ C ₆ H ₅ O ₇ + H ₂ O	18.62	13.95	37.06	3.02	0.18	24.88	(Patricio et al. 2011a)
PEO1500 + Na ₂ SO ₄ + H ₂ O	19.90	10.70	37.94	1.95	1.86	19.45	(Martins et al. 2008)

M: macromolecule, E: electrolyte.

measured by FAAS. The %E of the iron species in the TP and BP was calculated using the Eq. (1a) and Eq. (1b), respectively.

DETERMINATION OF IRON SPECIES IN WATER SAMPLES

Firstly, water samples doped with both iron species were prepared. The concentration of Fe(II) was always constant and equal to 8.87 mg kg⁻¹, while the Fe(III) concentration was varied as 7.94, 17.3, 39.7, or 86.7 mg kg⁻¹. Then, 0.642 g of the electrolyte (Na₃C₆H₅O₇), 0.745 g of the polymer (PEO1500), 3.60 mg of the phen, and 2.61 g of the doped water sample were poured into a centrifuge tube. The contents of the tube were stirred and centrifuged; the tube was then left in a thermostatic bath as previously described. The mixture formed an ATPS with 2.00 g of TP and 2.00 g of BP. The TP and BP aliquots were then collected, appropriately diluted, and the total iron concentration in the top and bottom phases (C_{TP}^T and C_{BP}^T) were measured by FAAS.

RESULTS AND DISCUSSION

STABILITY OF THE IRON SPECIES IN THE ATPS

A common problem encountered in the determination of the chemical species is the interconversion between the species. Especially in the case of iron, studies report that oxidation of Fe(II) to Fe(III) can occur in the presence of O₂ (Stumm and Lee 1961, King et al. 1995, Morgan

and Lahav 2007, Jones et al. 2015). Hence, in this work, investigations were carried out to check whether Fe(II) got oxidized in the presence of dissolved oxygen in the ATPS, and if the ATPS could cause an interconversion of the species during the extraction procedure. Extraction studies of the species were carried out, with (by bubbling with nitrogen) and without the removal of oxygen dissolved in the ATPS (Butler et al. 1994). The TP extraction percentage for Fe(II), $\%E_{TP}^{Fe(II)}$, was obtained with the PEO1500+Na₃C₆H₅O₇+H₂O ATPS, at pH of 6.00, and 10.0 mmol kg⁻¹ of phen. The system containing Fe(II) remained in the thermostatic bath at 25 °C, and aliquots of the TP were taken at time intervals of 0, 0.25, 0.5, 2, 3, 4, and 20 h. The aliquots were adequately diluted and their metal concentration was determined by FAAS. In the presence or absence of O₂, $\%E_{TP}^{Fe(II)}$ was found to be (90.6 ± 1.8)% or (91.7 ± 1.8)%, respectively; these values were similar for all investigated time intervals. These results indicate that Fe(II) did not undergo oxidation for at least 20 h. Therefore, it is not necessary to bubble the mixture with nitrogen; this makes the method easier by eliminating the nitrogen bubbling step from the procedure.

EXTRACTION BEHAVIOR OF THE IRON SPECIES IN THE ATPS

The complexation reaction between Fe(II) and 1,10-phenanthroline is well known (Vogel 1981,

Marczenko et al. 2000). Studies report that the iron-phenanthroline complex (Fe(II)-phen) is formed by three molecules of the extractant and an iron atom (Lee et al. 1948). It is also recognized that the amount of extractant added to the ATPS influences the extraction behavior of the species (Patricio et al. 2011b, Da Cunha et al. 2016). Therefore, in this work, the effect of adding phen up to 100 times the molar quantity of iron, was investigated.

Figure 1 shows the %E of the iron species as a function of the phen quantity added to the PEO1500+Na₃C₆H₅O₇+H₂O ATPS at pH of 6.00. In the absence of phen, Fe(II) and Fe(III) ions were practically not extracted for the TP (macromolecule-rich phase); in this situation, $\%E_{TP}^{Fe(II)} = (6.67 \pm 0.60)\%$ and $\%E_{TP}^{Fe(III)} = (6.34 \pm 1.42)\%$. The presence of phen in the system did not influence $\%E_{TP}^{Fe(III)}$; however, $\%E_{TP}^{Fe(II)}$ gradually increased with increasing phen, reaching a maximum $\%E_{TP}^{Fe(II)}$ of $(96.0 \pm 3.9)\%$ in the presence of $10.0 \text{ mmol kg}^{-1}$ of phen.

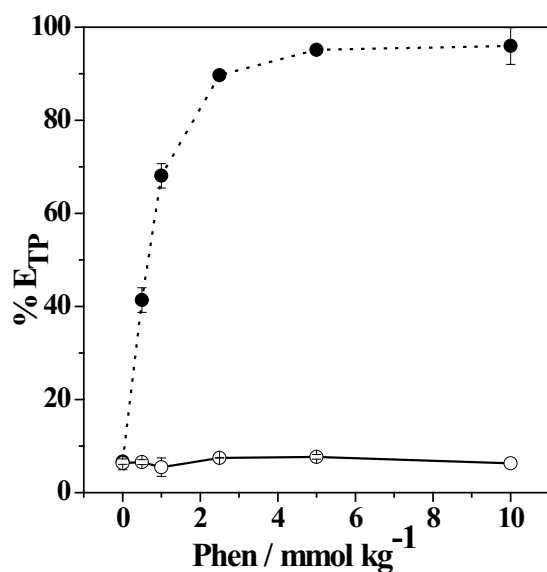
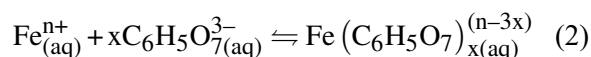


Figure 1 - Effect of 1,10-phenanthroline (phen) quantity added to PEO1500 + Na₃C₆H₅O₇+H₂O ATPS on the %E_{TP} of iron species: (●) Fe(II); (○) Fe(III); [iron] = 0.100 mmol kg⁻¹; pH=6.00; T=25.0 °C; n=3.

The low extraction efficiency of Fe(II) and Fe(III) in the absence of phen is related to the complexation of iron species with citrate anions that are mainly present in the bottom phase of the ATPS. At pH of 6.00 the citrate ions are deprotonated (pK_as=3.128, 4.761, and 6.396 (Harris 2013)); consequently, they are free to interact and form stable complexes with iron (Eq. (2)), for which the formation constants are $\log K_{Fe(II)-Cit} = 15.5$ (Patnaik 2004) and $\log K_{Fe(III)-Cit} = 25.0$ (Patnaik 2004). Thus, the iron species stay primarily in the bottom phase (electrolyte-rich phase).



The addition of phen to the ATPS did not influence the extraction behavior of Fe(III), because Fe(III) interacts more strongly with citrate (present in large quantities in the BP) than with phen (predominantly present in the TP). This agrees with the Fe(III)-cit complex and Fe(III)-phen complex formation constant findings in literature ($\log K_{Fe(III)-Cit} = 25.0 > \log K_{Fe(III)-phen} = 23.5$) (Patnaik 2004). However, the addition of phen to the ATPS affected the extraction of Fe(II). Fe(II) preferentially reacts with the phen at the interface of the ATPS, because the Fe(II)-phen complex formation constant ($\log K_{Fe(II)-phen} = 21.3$) (Patnaik 2004) is greater than that of the Fe(II)-Cit complex ($\log K_{Fe(II)-Cit} = 15.5$) (Patnaik 2004). The Fe(II)-complex then gets transferred to the TP because of the interaction of the Fe(II)-phen complex with the pseudopolycations present in the TP.

In a pioneering study, da Silva and Loh (2000) proposed the existence of pseudopolycations to explain the formation of an ATPS. The authors determined the variation of the interaction energy between the ATPS-forming cations and the ethylene oxide segments of the polymer. They concluded that a biphasic system is generated because of the polymer-ion interaction. This interaction is governed by an increase in the enthalpy and entropy.

When phase separation occurs in the polymer-rich phase, cations are adsorbed on the polymer chain, forming *pseudopolycations*. Hence, during the Fe(II) extraction process, the pseudopolycations attract the Fe(II)-phen complex because of the high negative charge density of the aromatic rings in phen, favoring the extraction of Fe(II) to the macromolecule-rich phase (TP).

With an increase in the quantity of phen in the ATPS, the $\%E_{TP}^{Fe(II)}$ also increases. This is because more complex molecules are formed and transferred to the TP by displacing the equilibrium toward the formation of more Fe(II)-phen complex. Thus, when $10.0 \text{ mmol kg}^{-1}$ of phen is added, $\%E_{TP}^{Fe(II)} = (96.0 \pm 3.9)\%$.

INFLUENCE OF pH ON THE %E OF IRON SPECIES

Another factor that affects the formation of complexes between the iron species and phen, and consequently the preferential partitioning of the complex for one of the ATPS phases, is the pH medium. Hence, the partitioning of Fe(II) and Fe(III) species in the system was studied at pH values of 1.00, 6.00, and 12.0.

Figure 2 shows $\%E_{TP}^{Fe(II)}$ and $\%E_{TP}^{Fe(III)}$ as a function of the phen concentration in the PEO1500+Na₃C₆H₅O₇+H₂O ATPS for different pH values. Fe(III), independent of the quantity of phen or the pH of the medium, was concentrated in the BP of the system, and reached $\%E_{TP}^{Fe(III)}$ equal to (6.64 ± 2.56) , (11.4 ± 1.1) , and $(14.1 \pm 1.0)\%$ on an average, for the pH values of 6.00, 1.00, and 12.0, respectively. At pH of 6.00, as aforementioned, the citrate ions are deprotonated and free to complex the Fe(III), concentrating Fe(III) specie in the BP. Therefore, a pH of 6.00 provided the lowest $\%E_{TP}^{Fe(III)}$. At a pH of 1.0, the citrate ions are protonated, which increases the free-fraction Fe(III), and consequently the $\%E_{TP}^{Fe(III)}$. At a pH of 12.0, $\%E_{TP}^{Fe(III)}$ is slightly higher, because part of the Fe(III) is complexed

with hydroxyl-forming iron anionic aquohydroxy complexes ($Fe(OH)_x^{(3-x)}$) that interact with the pseudopolycations present in the TP of the ATPS.

For all the investigated values of pH, $\%E_{TP}^{Fe(II)}$ increased with an increase in the quantity of phen, reaching a maximum $\%E_{TP}^{Fe(II)}$ of (96.0 ± 3.9) , (86.1 ± 0.6) , and $(85.9 \pm 0.6)\%$ for pH values of 6.00, 1.00, and 12.0, respectively, when $10.0 \text{ mmol kg}^{-1}$ of phen was added.

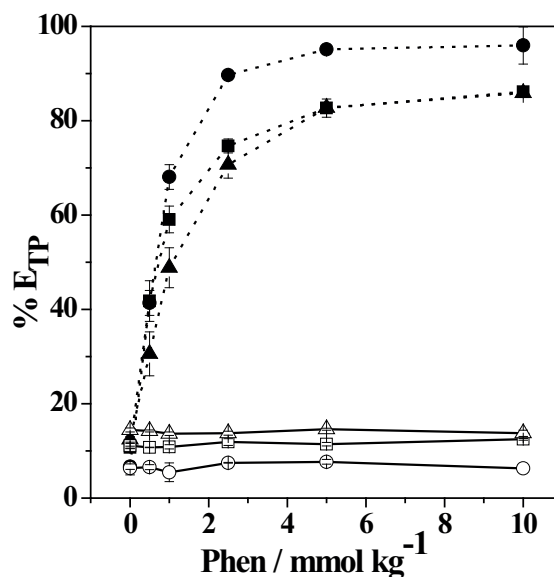


Figure 2 - Effect of pH and 1,10-phenanthroline (phen) quantity added to PEO1500 + Na₃C₆H₅O₇ + H₂O ATPS on the $\%E_{TP}$ of iron species: (\square, \blacksquare) pH 1.00; (\circ, \bullet) pH 6.00; (Δ, \blacktriangle) pH 12.0; (full) Fe(II); (open) Fe(III); [iron]= $0.100 \text{ mmol kg}^{-1}$; T=25.0 °C; n=3.

The Fe(II) ions, at optimal phen concentrations, were quantitatively extracted for all investigated pH values. The highest $\%E_{TP}^{Fe(II)}$ of $(96.0 \pm 3.9)\%$ was obtained at pH of 6.00 in the presence of $10.0 \text{ mmol kg}^{-1}$ of phen. In an acidic medium, protonation of the phen molecules occurs, which reduces the complexing efficiency of phen with Fe(II) and consequently, $\%E_{TP}^{Fe(II)}$. On the other hand, in basic media (pH = 12.0), the formation of Fe(II) aquohydroxy complexes ($Fe(OH)_x^{(2-x)}$) decreases the Fe(II) available amount for complexation with phen. Hence, a medium with a pH of 6.00 was used as the optimal experimental condition for

the development of the analytical method for iron speciation. This result agrees with literature data which affirm that the complex formation reaction between Fe(II) and phen is favored in slightly acidic solutions (Marczenko et al. 2000).

INFLUENCE OF THE NATURE OF THE ATPS-FORMING MACROMOLECULE ON THE %E OF THE IRON SPECIES

Studies have shown that the nature of the ATPS-forming macromolecule influences the extraction of metallic ions (Rodrigues et al. 2013, Patricio et al. 2016). In these works, the most commonly used macromolecules were PEO, L35, and L64. Normally, when a hydrophilic solute is partitioned, the systems containing PEO are more efficient in the extraction, while L35 and L64 are more efficient to extract the hydrophobic solutes. L35 or L64 are triblock copolymers, with a structure of (ethylene oxide)_n-(propylene oxide)_m-(ethylene oxide)_n. Because of this structure, copolymer molecules aggregate above a specific temperature and concentration to form micelles. These micelles have a hydrophobic core (units of propylene oxide, PO) surrounded by a hydrophilic crown (units of ethylene oxide, EO) (Rodrigues et al. 2008, Patricio et al. 2016). Thus, an ATPS with a copolymer-rich phase will be more hydrophobic, while that with an polymer-rich phase will be more hydrophilic (da Silva et al. 2006, 2009, de Lemos et al. 2010, Virtuoso et al. 2012).

Figure 3 shows $\%E_{TP}^{Fe(II)}$ and $\%E_{TP}^{Fe(III)}$ as a function of phen concentration using the PEO1500 + Na₃C₆H₅O₇+H₂O ATPS or the L64+Na₃C₆H₅O₇+H₂O ATPS at pH of 6.00. In both systems, the phen amount added to the ATPS and the structure of the macromolecule had little effect on the Fe(III) extraction. The mean values of $\%E_{TP}^{Fe(III)}$ were (6.64±2.56)% and (14.1±1.0)% for the ATPS containing PEO and L64, respectively. This difference in the $\%E_{TP}^{Fe(III)}$ values is due to a difference in the concentration of Na₃C₆H₅O₇ in the PEO ATPS (24.88% (m/m)) and L64 ATPS

(13.54% (m/m)). The higher the concentration of Na₃C₆H₅O₇ in the system formed by PEO, the more favorable is the formation of stable Fe(III)-citrate complexes. Thus, Fe(III) species concentrate in the BP (electrolyte-rich phase), resulting in a lower $\%E_{TP}^{Fe(III)}$.

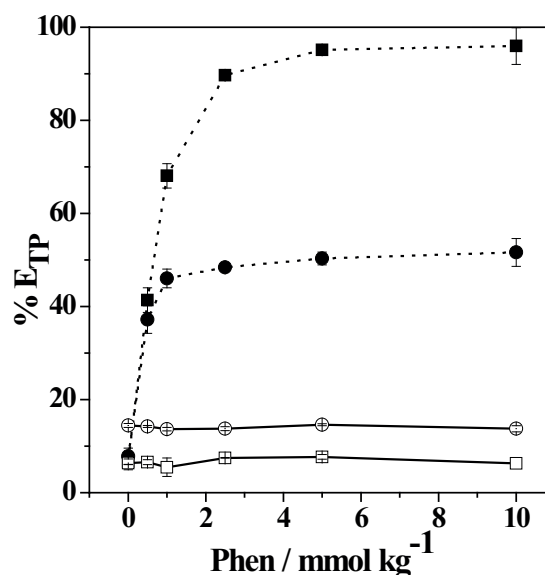


Figure 3 - Effect of macromolecule nature and 1,10-phenantroline (phen) quantity added in the ATPS on the $\%E_{TP}$ of iron species: (■) Fe(II) and (□) Fe(III) at PEO1500+Na₃C₆H₅O₇ + H₂O ATPS; Fe(II) (●) and Fe(III) (○) at L64+Na₃C₆H₅O₇ + H₂O ATPS; [iron]=0.100 mmol kg⁻¹; pH = 6.00; T=25.0 °C; n=3.

The extraction behavior of Fe(II) was dependent on the phen concentration and on the nature of the macromolecule present in the ATPS. There was a marked increase in $\%E_{TP}^{Fe(II)}$ with the increase of the phen amount added to the systems formed by both PEO1500 and L64. An increase in the amount of phen added to the system favors the formation of a higher amount of Fe(II)-phen complexes, which are subsequently transferred to TP (macromolecule-rich phase). However, the maximum $\%E_{TP}^{Fe(II)}$ of (96.0±3.9)% obtained for the ATPS containing PEO1500 was higher than the maximum $\%E_{TP}^{Fe(II)}$ of (51.6±3.0)% obtained in the system constituted by L64. The more efficient extraction in the presence of PEO1500

is related to the structure of the macromolecule. PEO1500 is formed only by EO monomers, while L64 contains EO and PO monomers. Hence, the pseudopolycondensation amount formed in the L64 ATPS is lower than that formed in the PEO1500 ATPS, lowering $\%E_{TP}^{Fe(II)}$ in the L64 ATPS.

INFLUENCE OF THE NATURE OF THE ATPS-FORMING ELECTROLYTE ON THE %E OF THE IRON SPECIES

It is well established in the literature that the type of the ATPS-forming electrolyte influences the partitioning of solutes in the ATPS. Sulfate salts are widely used in metal extraction studies (Rodrigues et al. 2008, 2013, Patricio et al. 2011b), while the use of citrate salts is still rare (Da Cunha et al. 2016, Patricio et al. 2016). The effect of these anions on the separation of the iron species was also investigated.

Figure 4 shows $\%E_{TP}^{Fe(II)}$ and $\%E_{TP}^{Fe(III)}$ as a function of the phen concentration in the systems composed of PEO1500+Na₃C₆H₅O₇+H₂O ATPS or PEO1500+Na₂SO₄+H₂O ATPS at pH of 6.00. In the absence of phen, both iron species concentrated in the BP of the investigated systems. However, with the addition of phen, the Fe(II) species were preferentially partitioned toward the TP of the system, independent of the nature of the ATPS-forming electrolyte. A $\%E_{TP}^{Fe(II)}$ of (96.0±3.9)% was obtained for the ATPS composed of citrate, while a $\%E_{TP}^{Fe(II)}$ of (92.3±1.1)% was obtained for the ATPS composed of sulfate, where both systems were in the presence of 10.0 mmol kg⁻¹ of phen.

The preferential partition of Fe(II) toward TP is due to the stronger interaction of Fe(II) and phen ($\log K_{Fe(II)-phen} = 21.3$) (Patnaik 2004), as compared to the interactions of Fe(II) and citrate ($\log K_{Fe(II)-Cit} = 15.5$) (Patnaik 2004) or Fe(II) and sulfate ($\log K_{Fe(II)-sulf} = 1.0$) (Wells and Salam 1968). Hence, Fe(II) gets extracted to TP, because the Fe(II)-phen complex interacts more strongly

with the macromolecules mainly present in TP due to the presence of pseudopolycondensations.

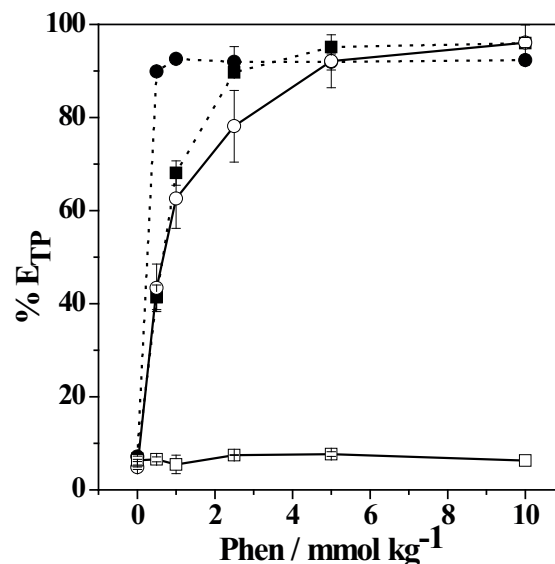


Figure 4 - Effect of electrolyte nature and 1,10-phenanthroline (phen) quantity added to the ATPS on the $\%E_{TP}$ of iron species: (■) Fe(II) and (□) Fe(III) at PEO1500 + Na₃C₆H₅O₇ + H₂O ATPS; (●) Fe(II) and (○) Fe(III) at PEO1500 + Na₂SO₄ + H₂O ATPS; [iron] = 0.100 mmol kg⁻¹; pH = 6.00; T = 25.0 °C; n = 3.

When the PEO1500+Na₂SO₄+H₂O ATPS was used, Fe(III) ions got extracted to TP, at a $\%E_{TP}^{Fe(III)}$ of (96.1±1.1)% in the presence of 10.0 mmol kg⁻¹ of phen. Here, the interaction between Fe(III) and phen is more favored ($\log K_{Fe(III)-phen} = 23.5$) (Patnaik 2004) than the interaction of Fe(III) with the sulfate ($\log K_{Fe(III)-sulfate} = 2.98$) (Patnaik 2004) abundantly present in the BP. On the other hand, when the ATPS containing citrate was used, Fe(III) concentrated in the BP of the ATPS, and showed a $\%E_{TP}^{Fe(III)}$ of (6.64±2.56)%. This occurs because the interaction of Fe(III) with citrate (abundant in BP) is more intense than that of Fe(III) with phen. Hence, the PEO1500+Na₃C₆H₅O₇+H₂O ATPS can be used for iron speciation. In this ATPS, Fe(II) is quantitatively extracted to TP, while Fe(III) is maintained in the BP. Note that it is not necessary to use 10.0 mmol kg⁻¹ of phen to perform the speciation. When 5.00 mmol kg⁻¹ of phen was

added to the ATPS, a high $\%E_{TP}^{Fe(II)}$ of $(95.1 \pm 1.0)\%$, and low $\%E_{TP}^{Fe(III)}$ of $(7.68 \pm 0.50)\%$ were obtained. Hence, the PEO1500 + Na₃C₆H₅O₇+H₂O ATPS at a pH of 6.00 with a phen concentration equal to 5.00 mmol kg⁻¹ was used as the optimal experimental condition to develop an analytical method for iron speciation.

SEPARATION FACTOR OF Fe(II) AND Fe(III) IONS

In a liquid-liquid extraction, it is possible to determine whether there is an effective separation between two species M and N, by determining the separation factor ($S_{M,N}$) (Park and Fray 2009). This factor is expressed in Eq. (3):

$$S_{M,N} = \frac{D_M}{D_N} \quad (3)$$

where D_M is the distribution coefficient for the species M, and D_N is the distribution coefficient for the species N. The distribution coefficient for any species is expressed by Eq. (4):]where D_M is the distribution coefficient for the species M, and D_N is the distribution coefficient for the species N. The distribution coefficient for any species is expressed by Eq. (4):

$$D_M = \frac{\%E_{TP}}{100 - \%E_{TP}} \quad (4)$$

Figure 5 shows $S_{Fe(II),Fe(III)}$ as a function of the quantity of phen added to the PEO1500 + Na₃C₆H₅O₇ + H₂O ATPS at pH of 6.00. In the presence of 10.0 mmol kg⁻¹ of phen, the $\%E_{TP}^{Fe(II)}$ of $(96.0 \pm 3.9)\%$ and $\%E_{TP}^{Fe(III)}$ of $(6.30 \pm 0.01)\%$ resulted in a high $S_{Fe(II),Fe(III)}$ of 357. However, the use of 5.00 mmol kg⁻¹ of phen also provides an expressive $S_{Fe(II),Fe(III)}$ of 233 with $\%E_{TP}^{Fe(II)}$ and $\%E_{TP}^{Fe(III)}$ equal to $(95.1 \pm 1.0)\%$ and $(7.68 \pm 0.50)\%$, respectively. Iron speciation under this experimental condition provides economic and environmental benefits, due to a decrease in the quantity of phen used.

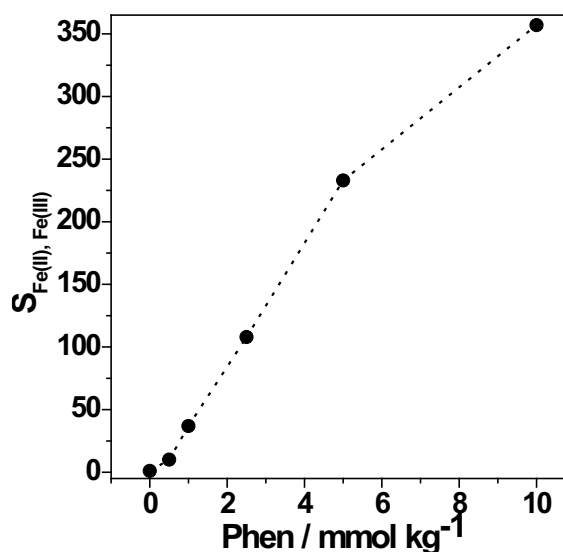


Figure 5 - Separation factor between Fe(II) and Fe(III) ($S_{Fe(II),Fe(III)}$) as a function of the quantity of 1,10-phenanthroline (phen) added in the PEO1500 + Na₃C₆H₅O₇+H₂O ATPS at pH=6.00; T=25.0 °C; n=3.

EXTRACTION OF Fe(II) IN THE PRESENCE OF Fe(III)

In this work iron speciation in the presence of large quantities of Fe(III) was investigated. The extraction of Fe(II) was performed in a system formed by PEO1500+Na₃C₆H₅O₇+H₂O, at a pH of 6.00 and with 5.00 mmol kg⁻¹ of phen, in the presence of different concentrations of Fe(III) (0.101–1.01 mmol kg⁻¹). The determination of Fe(II) in the ATPS phases was performed using the standard 1,10-phenanthroline method (Eaton and Franson 2005). Using the above methodology, $\%E_{TP}^{Fe(II)}$ and the extraction percentage of Fe(II) to BP ($\%E_{BP}^{Fe(II)}$) were obtained. It was observed that when the Fe(III) concentration in the medium increases from 0.101 to 1.01 mmol kg⁻¹, $\%E_{TP}^{Fe(II)}$ slightly varies from (98.4 ± 0.7) to $(101 \pm 1)\%$, while $\%E_{BP}^{Fe(II)}$ slightly varies from $(1.65 \pm 0.67)\%$ to $(0.369 \pm 0.183)\%$. These results demonstrate that it is possible to determine Fe(II) in the presence of Fe(III), since in these conditions, the phen reacts only with Fe(II).

DETERMINATION OF IRON SPECIES IN WATER SAMPLES

Firstly, only Fe(II) or only Fe(III) was added to some water samples. The ATPS formed by PEO1500+Na₃C₆H₅O₇+H₂O at pH of 6.00 containing 5.00 mmol kg⁻¹ of phen was used to separate the iron species in the water samples before the determination of the iron species by the non-selective and low-cost FAAS technique. The percentage extraction of each specie in each ATPS phase was then determined using Eq. (1a) and Eq. (1b). The experiments were repeated for many days and at different concentrations of the iron species to evaluate the variation in the extraction percentages (Table II). In addition, known amounts of Fe(II) and Fe(III) were added to the different water samples, and recovery was determined (Table III). The known concentration of Fe(II) was called C_{Fe(II)}^{S,ad}, while that of Fe(III) was denoted as C_{Fe(III)}^{S,ad}.

The results of Table II show that the varying the concentration of Fe(III) in the ATPS does not affect the extraction percentage of the species. It can be concluded that when an ATPS is used in the extraction, a %E_{TP}^{Fe(II)} of 118%, %E_{BP}^{Fe(II)} of 8.10, %E_{BP}^{Fe(III)} of 120%, and %E_{TP}^{Fe(III)}=14.4% do not vary. Hence, these percentage extraction values were used to determine the concentration of each iron specie in the real samples.

Firstly, the values of %E_{TP}^{Fe(II)}= 118%, %E_{BP}^{Fe(II)} = 8.10%, %E_{BP}^{Fe(III)} = 120%, and %E_{TP}^{Fe(III)} = 14.4% were substituted in the Eq. (5) and Eq. (6). Secondly, the total iron concentration was determined in the TP (C_{TP}^T) and in the BP (C_{BP}^T) of the ATPS by FAAS. Then, the obtained values of C_{TP}^T and C_{BP}^T were substituted in Eq. (5) and Eq. (6). It was then possible to obtain the concentrations of Fe(II) (C_{Fe(II)}^{ATPS}) and Fe(III) (C_{Fe(III)}^{ATPS}) in the ATPS with the water samples. Finally, the values of C_{Fe(II)}^{ATPS} and C_{Fe(III)}^{ATPS} were substituted in Eq. (7)

and Eq. (8) to obtain the concentration of Fe(II) (C_{Fe(II)}^{S,ob}) and Fe(III) (C_{Fe(III)}^{S,ob}) in the water sample.

$$E_{TP}^{Fe(II)} C_{Fe(II)}^{ATPS} + E_{TP}^{Fe(III)} C_{Fe(III)}^{ATPS} = \frac{C_{TP}^T}{2} \quad (5)$$

$$E_{BP}^{Fe(II)} C_{Fe(II)}^{ATPS} + E_{BP}^{Fe(III)} C_{Fe(III)}^{ATPS} = \frac{C_{BP}^T}{2} \quad (6)$$

The terms C_{TP}^T and C_{BP}^T are divided by 2 because the mass of ATPS is 4.00 g, but the masses of TP and BP are 2.00 g each.

$$C_{Fe(II)}^{ATPS} m_{ATPS} = C_{Fe(II)}^{S,ob} m_S \quad (7)$$

$$C_{Fe(III)}^{ATPS} m_{ATPS} = C_{Fe(III)}^{S,ob} m_S \quad (8)$$

where m_S is the mass of the water sample added in the ATPS, and m_{ATPS} is the mass of the ATPS.

The known concentration values of Fe(II) and Fe(III) (C_{Fe(II)}^{S,ad} and C_{Fe(III)}^{S,ad}) were very close to the concentration values obtained (C_{Fe(II)}^{S,ob} and C_{Fe(III)}^{S,ob}) by the proposed method, reaching recoveries ranging from 90% to 106%. These results demonstrate the efficacy of an ATPS for iron speciation and the possibility of using FAAS for the determination of species determination.

Some researchers used UV-Vis spectrophotometry to determine the Fe(II) and Fe(III) species (Jamali et al. 2016, Chen et al. 2015, Suárez et al. 2015, Bazmandegan et al. 2015, Silva et al. 2018), since it is one of the cheapest analytical technique (Silva et al. 2018, Pena-Pereira et al. 2011). However, in the present work was used FAAS because it is necessary less steps to prepare solutions and analytical curve, reducing reagents consume and time spent. In addition, it has other attractive green and useful characteristics as summarized in Table IV. As can be seen, the proposed method has several advantages comparing to other reported methods for speciation of iron species. Unlike the others, it does not require organic solvents, corrosive, reducing or oxidizing agents. In addition, it can be performed without the removal of dissolved oxygen in the ATPS and demanding a short stirring time of 180 s.

TABLE II
Percentage extraction of Fe(II) and Fe(III) using PEO1500+Na₃C₆H₅O₇+H₂O
ATPS at a pH of 6.00 with 5.00 mmol kg⁻¹ of phen.

$C_{Fe(II)}^{S,ad}$ ^a / mg kg ⁻¹	$C_{Fe(III)}^{S,ad}$ ^b / mg kg ⁻¹	$\%E_{TP}^{Fe(II)}$ \pm sd ^c	$\%E_{TP}^{Fe(III)}$ \pm sd ^d	$\%E_{BP}^{Fe(II)}$ \pm sd ^e	$\%E_{BP}^{Fe(III)}$ \pm sd ^f
8.87	0.00	118 \pm 1	-	8.10 \pm 0.90	-
0.00	7.94	-	17.6 \pm 0.2	-	128 \pm 2
0.00	17.3	-	13.9 \pm 0.6	-	115 \pm 1
0.00	39.7	-	14.0 \pm 0.2	-	126 \pm 5
0.00	86.7	-	12.1 \pm 0.1	-	110 \pm 5

^aKnown concentration of Fe(II) in doped samples. ^bKnown concentration of Fe(III) in doped samples. ^cMean extraction percentage of Fe(II) to TP \pm standard deviation, n = 5. ^dMean extraction percentage of Fe(III) to TP \pm standard deviation, n = 5. ^eMean extraction percentage of Fe(II) to BP \pm standard deviation, n = 5. ^fMean extraction percentage of Fe(III) to BP \pm standard deviation, n = 5.

TABLE III
Determination of Fe(II) and Fe(III) concentration in water samples.

$C_{Fe(II)}^{S,ad}$ ^a / mg kg ⁻¹	$C_{Fe(III)}^{S,ad}$ ^b / mg kg ⁻¹	C_{TP}^T ^c / mg kg ⁻¹	C_{BP}^T ^d / mg kg ⁻¹	$C_{Fe(II)}^{S,ob}$ ^e / mg kg ⁻¹	$C_{Fe(III)}^{S,ob}$ ^f / mg kg ⁻¹	(recovery \pm sd) ^g / %	
						Fe(II)	Fe(III)
8.87	7.94	15.6	14.2	8.92	7.91	102 \pm 3	106 \pm 4
8.87	17.3	17.0	27.3	8.93	17.6	101 \pm 4	97.2 \pm 3.5
8.87	39.7	20.6	67.7	8.54	40.5	91.7 \pm 2.3	107 \pm 3
8.87	86.7	26.9	122	8.80	84.0	90.1 \pm 3.2	89.2 \pm 4

^aKnown concentration of Fe(II) in doped samples. ^bKnown concentration of Fe(III) in doped samples. ^cTotal iron concentration determined in the TP. ^dTotal iron concentration determined in the BP. ^eFe(II) concentration obtained by the proposed method. ^fFe(III) concentration obtained by the proposed method. ^gRecovery \pm standard deviation, n = 5.

CONCLUSIONS

An economically viable and environmentally friendly method was proposed for the separation and determination of iron species. This method uses aqueous two-phase systems for the separation of the species, which provides several advantages like the use of non-toxic, biodegradable and recyclable components, ease of operation, low-cost, and rapid phase separation without the formation of stable emulsions. In addition, the non-selective FAAS technique can be used for chemical speciation.

The studies carried out in this work showed that, through a selection of the ATPS-forming macromolecule and electrolyte, and a control of the quantity of phen and the pH of the medium, it is possible to separate Fe(II) and Fe(III) without

interconversion of the species during the separation and determination. The ATPS formed by the PEO1500 macromolecule and the Na₃C₆H₅O₇ electrolyte was found to be most efficient for the iron speciation. For extractant addition, in the absence of phen, the ions concentrated in the bottom phase, giving %E values lower than 15.0%. By the addition of phen, Fe(II) was extracted to the top phase, while Fe(III) was maintained in the bottom phase.

The obtained results can be considered to be extremely satisfactory, because ATPS-FAAS coupling was employed for the first time in chemical speciation. This approach can be considered more environmentally friendly than those resorting to organic solvents and at the same

TABLE IV
Comparison of iron speciation methods

Extractant	Remove dissolved oxygen	Detection technique	Use corrosive agents ^a	Organic solvent	Reaction/stirring time/s	Reduction/oxidation step	References
HDEHP ^b	Yes	ICP-MS	Yes	n-heptane	3600	No	(Pepper et al. 2010)
PAN ^c	No	FAAS	Yes	chloroform	1200	Yes	(Yaman and Kaya 2005)
Phen	No	Spectrophotometer	Yes	None	240	Yes	(Paluch et al. 2017)
Phen	No	Spectrophotometer	Yes	None	10	Yes	(Jamali, et al. 2016)
Phen	No	Spectrophotometer	Yes	None	NS ^d	Yes	(Chen et al. 2015)
Hmpp ^e	No	Spectrophotometer	Yes	None	NS ^d	Yes	(Suárez et al. 2015)
TPTZ ^f	No	Spectrophotometer	Yes	Ethanol	180	Yes	(Bazmandegan et al. 2015)
Br-PADAP ^g	No	Spectrophotometer	Yes	Ethanol	1200	Yes	(Silva et al. 2018)
ANPAP ^h	No	Spectrophotometer	Yes	DMF ⁱ /p-DCB ^j	1200	Yes	(Kassem and Amin 2013)
Phen	No	Spectrophotometer	Yes	None	600	Yes	(Eaton and Franson 2005) ^k
Phen	No	FAAS	No	None	180	No	Present work

^aAcids or bases. ^bbis(2-ethylhexyl) hydrogen phosphate. ^c1-(2-pyridylazo)-2-naphthol. ^dnot specified.

^e3-hydroxy-1(H)-2-methyl-4-pyridinone. ^f2,4,6-tri(2'-pyridyl)-1,3,5-triazine.

^g2-(5-Bromine-2-pyridylazo)-5-diethylaminophenol. ^h2-Amino-4-(3-nitrophenylazo)pyridine-3-ol. ⁱN,N-Dimethylformamide. ^jp-dichlorobenzen. ^kStandard method.

time, expands its realm of application for speciation of other elements.

Furthermore, the synergy arising from the combination of the beneficial characteristics of flame atomic absorption spectrometry, such as attractive cost, simple operation, wide availability in many laboratories, with the advantages of an aqueous two-phase extraction system makes this work an attractive and promising starting point for future studies and developments in chemical speciation.

ACKNOWLEDGMENTS

The authors thank the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Instituto Nacional de Ciências e Tecnologias Analíticas Avançadas (INCTAA) for financial support. R.A.C., P.R.P. and S.J.R.V. acknowledge the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for research studentships. The authors

also acknowledge Prof. Dr. Fábio R. P. Rocha for his critical revision of this manuscript.

REFERENCES

- AKAMA Y AND SALI A. 2002. Extraction mechanism of Cr(VI) on the aqueous two-phase system of tetrabutylammonium bromide and (NH₄)₂SO₄ mixture. *Talanta* 57: 681-686.
- AZEVEDO AM, GOMES AG, ROSA PAJ, FERREIRA IF, PISCO AMMO AND AIRES-BARROS MR. 2009. Partitioning of human antibodies in polyethylene glycol-sodium citrate aqueous two-phase systems. *Sep Purif Technol* 65: 14-21.
- BAHAR S AND ZAKERIAN R. 2012. Speciation of Fe(II) and Fe(III) by using Dispersive Liquid-Liquid Microextraction and Flame Atomic Absorption Spectrometry. *J Braz Chem Soc* 23: 944-950.
- BAZMANDEGAN-SHAMILI A, HAJI-SHABANI AM, DADFARNIA S, SAEIDI M AND ROHANI-MOGHADAM M. 2015. Spectrophotometric determination of iron species using ionic liquid ultrasound assisted dispersive liquid-liquid microextraction. *Turk J Chem* 39: 1059-1068.
- BRIDGES NJ, GUTOWSKI KE AND ROGERS RD. 2007. Investigation of aqueous biphasic systems formed from solutions of chaotropic salts with kosmotropic salts (salt-salt ABS). *Green Chem* 9: 177-183.

- BULGARIU L AND BULGARIU D. 2010. Selective extraction of Fe(III) species in presence of thiocyanate ions using aqueous peg-based two-phase systems. *Analele Universității din Oradea-fascicula Chimie* 17: 3-10.
- BUTLER IB, SCHOONEN MAA AND RICKARD DT. 1994. Removal of dissolved oxygen from water: A comparison of four common techniques. *Talanta* 41: 211-215.
- CHEN SJ, LI N, ZHANG XS, YANG DJ AND JIANG HM. 2015. Online spectrophotometric determination of Fe(II) and Fe(III) by flow injection combined with low pressure ion chromatography. *Spectrochim Acta A* 138: 375-380.
- CHEZHANA S, NAYAK CA AND RAGHAVARAO KSMS. 2007. Aqueous two phase extraction for purification and concentration of betalains. *J. Food Eng* 81: 679-687.
- DA CUNHA RC, PATRICIO PR, VARGAS SJR, DA SILVA LHM AND DA SILVA MCH. 2016. Green recovery of mercury from domestic and industrial waste. *J Hazard Mater* 304: 417-424.
- DA SILVA LHM, DA SILVA MDH, DE SOUSA RDS, MARTINS JP, RODRIGUES GD, COIMBRA JSR AND MINIM LA. 2009. Surface Excess Enthalpy of PEO + Salt + Water and L35 + Salt + Water Aqueous Two-Phase Systems. *J Chem Eng Data* 54: 531-535.
- DA SILVA LHM AND LOH W. 2000. Calorimetric Investigation of the Formation of Aqueous Two-Phase Systems in Ternary Mixtures of Water, Poly(ethylene oxide) and Electrolytes (Or Dextran). *J Phys Chem B* 104: 10069-10073.
- DA SILVA MDH, DA SILVA LHM, AMIM J, GUIMARÃES RO AND MARTINS JP. 2006. Liquid-Liquid Equilibrium of Aqueous Mixture of Triblock Copolymers L35 and F68 with Na₂SO₄, Li₂SO₄, or MgSO₄. *J Chem Eng Data* 51: 2260-2264.
- DE ANDRADE VM, RODRIGUES GD, DE CARVALHO RMM, DA SILVA LHM AND DA SILVA MCH. 2011. Aqueous two-phase systems of copolymer L64 + organic salt + water: Enthalpic L64-salt interaction and Othmer-Tobias, NRTL and UNIFAC thermodynamic modeling. *Chem Eng J* 171: 9-15.
- DE LEMOS LR, PATRICIO PD, RODRIGUES GD, CARVALHO RMM, DA SILVA MCH AND DA SILVA LHM. 2011. Liquid-liquid equilibrium of aqueous two-phase systems composed of poly(ethylene oxide) 1500 and different electrolytes ((NH₄)₂SO₄, ZnSO₄ and K₂HPO₄): Experimental and correlation. *Fluid Phase Equilib* 305: 19-24.
- DE LEMOS LR, SANTOS IJB, RODRIGUES GD, DA SILVA LHM AND DA SILVA MCH. 2012. Copper recovery from ore by liquid-liquid extraction using aqueous two-phase system. *J Hazard Mater* 237-238: 209-214.
- DE LEMOS LR, SANTOS IJB, RODRIGUES GD, FERREIRA GMD, DA SILVA LHM, DA SILVA MDH AND DE CARVALHO RMM. 2010. Phase Compositions of Aqueous Two-Phase Systems Formed by L35 and Salts at Different Temperatures. *J Chem Eng Data* 55: 1193-1199.
- EATON AD AND FRANSON MAH. 2005. Standard methods for the examination of water & wastewater, 21st ed., Washington: American Public Health Association, 1082 p.
- FREIRE MG, PEREIRA JFB, FRANCISCO M, RODRIGUEZ H, REBELO LPN, ROGERS RD AND COUTINHO JAP. 2012. Insight into the Interactions That Control the Phase Behaviour of New Aqueous Biphasic Systems Composed of Polyethylene Glycol Polymers and Ionic Liquids. *Chem-Eur J* 18: 1831-1839.
- GRIFFIN ST, SPEAR SK AND ROGERS RD. 2004. Effects of speciation on partitioning of iodine in aqueous biphasic systems and onto ABEC resins. *J Chromatogr B* 807: 151-156.
- HARAGUCHI LH, MOHAMED RS, LOH W AND PESSOA PA. 2004. Phase equilibrium and insulin partitioning in aqueous two-phase systems containing block copolymers and potassium phosphate. *Fluid Phase Equilib* 215: 1-15.
- HARRIS DC. 2013. Quantitative Chemical Analysis, 8th ed., New York: LTC, 898 p.
- HARVEY AE, SMART JA AND AMIS ES. 1955. Simultaneous spectrophotometric determination of iron(ii) and total iron with 1,10-phenanthroline. *Anal Chem* 27: 26-29.
- JAMALI MR, TAVAKOLI M AND RAHNAMA R. 2016. Development of ionic liquid-based in situ solvent formation microextraction for iron speciation and determination in water and food samples. *J Mol Liq* 216: 666-670.
- JONES AM, GRIFFIN PJ AND WAITE TD. 2015. Ferrous iron oxidation by molecular oxygen under acidic conditions: The effect of citrate, EDTA and fulvic acid. *Geochim Cosmochim Acta* 160: 117-131.
- KASSEM MA AND AMIN AS. 2013. Spectrophotometric determination of iron in environmental and food samples using solid phase extraction. *Food Chem* 141: 1941-1946.
- KING DW, LOUNSBURY HA AND MILLERO FJ. 1995. Rates and mechanism of Fe(II) oxidation at nanomolar total iron concentrations. *Environ Sci Technol* 29: 818-824.
- KISS T, ENYEDY ÉA AND JAKUSCH T. 2017. Development of the application of speciation in chemistry. *Coord Chem Rev* 352: 401-423.
- LEE TS, KOLTHOFF IM AND LEUSSING DL. 1948. REACTION OF FERROUS AND FERRIC IONS WITH 1,10-Phenanthroline. II. Kinetics of Formation and Dissociation of Ferrous Phenanthroline. *J Am Chem Soc* 70: 3596-3600.
- LIN H AND TWINING BS. 2012. Chemical speciation of iron in Antarctic waters surrounding free-drifting icebergs. *Mar Chem* 128: 81-91.
- MAGESTE AB, DE LEMOS LR, FERREIRA GMD, DA SILVA MDH, DA SILVA LHM, BONOMO RCF

- AND MINIM LA. 2009. Aqueous two-phase systems: an efficient, environmentally safe and economically viable method for purification of natural dye carmine. *J Chromatogr A* 1216: 7623-7629.
- MAGESTE AB, SENRA TDA, DA SILVA MCH, BONOMO RCF AND DA SILVA LHM. 2012. Thermodynamics and optimization of norbixin transfer processes in aqueous biphasic systems formed by polymers and organic salts. *Sep Purif Technol* 98: 69-77.
- MARCZENKO Z, BALCERZAK M AND KLOCZKO E. 2000. Separation, Preconcentration and Spectrophotometry in Inorganic Analysis, 1sted, Amsterdam: Elsevier, 528 p.
- MARTINS JP, CARVALHO CD, DA SILVA LHM, COIMBRA JSD, DA SILVA MDH, RODRIGUES GD AND MINIM LA. 2008. Liquid-Liquid Equilibria of an Aqueous Two-Phase System Containing Poly(ethylene) Glycol 1500 and Sulfate Salts at Different Temperatures. *J Chem Eng Data* 53: 238-241.
- MOGHADAM MR, SHABANI AMH AND DADFARNIA S. 2011. Spectrophotometric determination of iron species using a combination of artificial neural networks and dispersive liquid-liquid microextraction based on solidification of floating organic drop. *J Hazard Mater* 197: 176-182.
- MORGAN B AND LAHAV O. 2007. The effect of pH on the kinetics of spontaneous Fe(II) oxidation by O₂ in aqueous solution – basic principles and a simple heuristic description. *Chemosphere* 68: 2080-2084.
- PALEOLOGOS EK, GIOKAS DL, TZOUWARA-KARAYANNI SM AND KARAYANNIS MI. 2002. Micelle mediated methodology for the determination of free and bound iron in wines by flame atomic absorption spectrometry. *Anal Chim Acta* 458: 241-248.
- PALUCH J, KOZAK J, WIECZOREK M, KOZAK M, KOCHANA J, WIDUREK K, KONIECZNA M AND KOŚCIELNIAK P. 2017. Novel approach to two-component speciation analysis. Spectrophotometric flow-based determinations of Fe(II)/Fe(III) and Cr(III)/Cr(VI). *Talanta* 171: 275-282.
- PARK YJ AND FRAY DJ. 2009. Separation of zinc and nickel ions in a strong acid through liquid-liquid extraction. *J Hazard Mater* 163: 259-265.
- PATNAIK P. 2004. *Dean's Analytical Chemistry Handbook*, 2nd ed., New York: McGraw-Hill, 1280 p.
- PATRICIO PD, MAGESTE AB, DE LEMOS LR, DE CARVALHO RMM, DA SILVA LHM AND DA SILVA MCH. 2011a. Phase diagram and thermodynamic modeling of PEO+organic salts+H₂O and PPO+organic salts+H₂O aqueous two-phase systems. *Fluid Phase Equilib* 305: 1-8.
- PATRICIO PD, MESQUITA MC, DA SILVA LHM AND DA SILVA MCH. 2011b. Application of aqueous two-phase systems for the development of a new method of cobalt(II), iron(III) and nickel(II) extraction: a green chemistry approach. *J Hazard Mater* 193: 311-318.
- PATRICIO PR, CUNHA RC, VARGAS SJR, COELHO YL, DA SILVA LHM AND DA SILVA MCH. 2016. Chromium speciation using aqueous biphasic systems: Development and mechanistic aspects. *Sep Purif Technol* 158: 144-154.
- PENA-PEREIRA F, COSTAS-MORA I, ROMERO V, LAVILLA I AND BENDICHO C. 2011. Advances in miniaturized UV-Vis spectrometric systems. *Trac-Trends Anal Chem* 30: 1637-1648.
- PEPPER SE, BORKOWSKI M, RICHMANN MK AND REED DT. 2010. Determination of ferrous and ferric iron in aqueous biological solutions. *Anal Chim Acta* 663: 172-177.
- PERSSON J, KAUL A AND TJERNELD F. 2000. Polymer recycling in aqueous two-phase extractions using thermoseparating ethylene oxide-propylene oxide copolymers. *J Chromatogr B* 743: 115-126.
- POHL P AND PRUSISZ B. 2006. Redox speciation of iron in waters by resin-based column chromatography. *Trac-Trends Anal Chem* 25: 909-916.
- POURNAGHI-AZAR MH AND FATEMI BM. 2000. Simultaneous determination of ferric, ferrous and total iron by extraction differential pulse polarography: application to the speciation of iron in rocks. *Microchem J* 65: 199-207.
- REH G, NERLI B AND PICO G. 2002. Isolation of alpha-1-antitrypsin from human plasma by partitioning in aqueous biphasic systems of polyethyleneglycol-phosphate. *J Chromatogr B* 780: 389-96.
- RODRIGUES GD, DA SILVA MDH, DA SILVA LHM, PAGGIOLI FJ, MINIM LA AND COIMBRA JSD. 2008. Liquid-liquid extraction of metal ions without use of organic solvent. *Sep Purif Technol* 62: 687-693.
- RODRIGUES GD, DE LEMOS LR, DA SILVA LMH AND DA SILVA MCH. 2013. Application of hydrophobic extractant in aqueous two-phase systems for selective extraction of cobalt, nickel and cadmium. *J Chromatogr A* 1279: 13-19.
- RODRIGUES GD, DE LEMOS LR, DA SILVA LHM DA SILVA MDH, MINIM LA AND COIMBRA JSD. 2010. A green and sensitive method to determine phenols in water and wastewater samples using an aqueous two-phase system. *Talanta* 80: 1139-1144.
- RODRIGUES GD, DE LEMOS LR, DA SILVA LHM AND DA SILVA MCH. 2012. Monosegmented Flow Analysis Exploiting Aqueous Two-phase Systems for the Determination of Cobalt. *Anal Sci* 28: 1213-1218.
- RODRIGUES GD, DE LEMOS LR, PATRICIO PD, DA SILVA LHM AND DA SILVA MDH. 2011. Aqueous two-phase systems: a new approach for the determination of p-aminophenol. *J Hazard Mater* 192: 292-298.

- ROY K AND LAHIRI S. 2008. Species dependent radiotracer study of Cr(VI) and Cr(III) using an aqueous biphasic system. *Radiochim Acta* 96: 49-53.
- ROY K AND LAHIRI S. 2009. Extraction of Hg(I), Hg(II) and methylmercury using polyethylene glycol based aqueous biphasic system. *Appl Radiat Isot* 67: 1781-1784.
- SAFAVIA, ABDOLLAHI H AND HORMOZI-NEZHAD MR. 2002. Simultaneous kinetic determination of Fe(III) and Fe(II) by H-point standard addition method. *Talanta* 56: 699-704.
- SIMONOVA TN, DUBROVINA VA AND VISHNIKIN AB. 2016. Speciation of chromium through aqueous two-phase extraction of complexes of Cr(III) with 4-(2-pyridylazo)resorcinol and Cr(VI) with 1,5-diphenylcarbazide. *J Serb Chem Soc* 81: 1-15.
- SILVA AFO, DE CASTRO WV AND DE ANDRADE FP. 2018. Development of spectrophotometric method for iron determination in fortified wheat and maize flours. *Food Chem* 242: 205-210.
- STUMM W AND LEE GF. 1961. Oxygenation of ferrous iron. *Ind Eng Chem* 53: 143-146.
- SUÁREZ R, MESQUITA RBR, RANGEL M, CERDÀ V AND RANGEL AOSS. 2015. Iron speciation by microsequential injection solid phase spectrometry using 3-hydroxy-1(H)-2-methyl-4-pyridinone as chromogenic reagent. *Talanta* 133: 15-20.
- TABRIZI AB. 2010. Development of a dispersive liquid-liquid microextraction method for iron speciation and determination in different water samples. *J Hazard Mater* 183: 688-693.
- TASEV K, KARADJOVA I, ARPADJAN S, CVETKOVIC J AND STAFILOV T. 2006. Liquid/liquid extraction and column solid phase extraction procedures for iron species determination in wines. *Food Control* 17: 484-488.
- TEMPLETON DM, ARIESE F, CORNELIS R, DANIELSSON, LG, MUNTAU H, VAN-LEEUEWEN HP AND LOBINSKI R. 2000. Guidelines for terms related to chemical speciation and fractionation of elements. definitions, structural aspects, and methodological approaches. *Pure Appl Chem* 72: 1453-1470.
- VIRTUOSO LS, VELLO K, DE OLIVEIRA AA, JUNQUEIRA CM, MESQUITA AF, LEMES NHT, DE CARVALHO RMM, DA SILVA MCH AND DA SILVA LHM. 2012. Measurement and Modeling of Phase Equilibrium in Aqueous Two-Phase Systems: L35 plus Sodium Citrate plus Water, L35 Sodium Tartrate plus Water, and L35 plus Sodium Hydrogen Sulfite plus Water at Different Temperatures. *J Chem Eng Data* 57: 462-468.
- VOGEL AI. 1981. *Química analítica qualitativa*, 4th ed., São Paulo: Mestre Jou, 690 p.
- VUKOSAV P, MLAKAR M AND TOMISIC V. 2012. Revision of iron(III)-citrate speciation in aqueous solution. Voltammetric and spectrophotometric studies. *Anal Chim Acta* 745: 85-91.
- WELLS CF AND SALAM MA. 1968. Complex formation between iron(II) and inorganic anions. Part II. The effect of oxyanions on the reaction of iron(II) with hydrogen peroxide. *J Chem Soc A* 2: 308-315.
- YAMAN M AND KAYA G. 2005. Speciation of iron (II) and (III) by using solvent extraction and flame atomic absorption spectrometry. *Anal Chim Acta* 540: 77-81.