Reagent-free flow-injection amperometric sensor for quantification and speciation of iron for bio-hydrometallurgical applications

Albert Saavedra[1], Edgardo Donati[2] and Eduardo Cortón[1*]

[1] Departamento de Química Biológica, FCEyN – UBA and IQUIBICEN – CONICET. Ciudad Universitaria, Buenos Aires (1428), Argentina.

[2] Centro de Investigación y Desarrollo en Fermentaciones Industriales (CINDEFI - CONICET) and Facultad de Ciencias Exactas, UNLP, 50 y 115, (1900) La Plata, Argentina.

Abstract

Iron ions are monitored in processes such as bio-leaching, bio-oxidation, ferric leaching, passivation control, and others. The role of iron in different hydrometallurgy processes is very important because it affects strongly several industrial ore production, as chalcopyrite cooper extraction. In this work we present an amperometric FIA system that allows rapid quantification and speciation of iron in bio-mining processes. Linearity range, passivation of the working electrode and interferences were studied. We found a useful lineal range from 10 to 1500 mgL^{-1} for each of the ions, with a detection limit (Fe^{2+} and Fe^{3+}) determined at 15±2 mgL^{-1}. High copper concentrations could interfere with the amperometric readings, though not in the copper/iron relationship encountered in this industry. Real samples, including an acid mine drainage sample, and the monitoring of the bio-oxidation kinetics of iron by *Acidithiobacillus ferrooxidans* were also quantified. In all cases, our results were compared with a standard colorimetric method that allows iron speciation (1,10-phenanthroline), showing good agreement between both methods. The electrochemical method presented here allows high sample throughput (ca. 45 samples h^{-1}), fast analysis (ca. 1 min), and reagent free quantification of total iron, ferric and ferrous ions.

Keywords: *Acidithiobacillus ferrooxidans*, Bio-hydrometallurgic, Electrochemistry, Ferric ion, Ferrous ion

*Corresponding author. Address: Intendente Güiraldes 2160, Pabellón II, Piso 4. Universidad de Buenos Aires, (1428) CABA, Argentina. Tel/FAX: INT + 54 11 4576-3342.

E-mail address: eduardo@qb.fcen.uba.ar (E. Cortón)

1.1 Introduction

Iron is one of the most abundant chemical elements in the Earth [1], and plays an important role in the environment, industrial, chemical, biological and human systems [2]. Its diverse role in industry includes use as a supplement in the food industry, in the manufacture of alloys, and hydrometallurgical industries, among others. The most common species of iron are ferric and ferrous ions. Ferrous ions are easily oxidized in presence of oxygen, and are stable at pH $\leq$ 2, while ferric ions are more stable in a wider pH range and oxygen conditions [3].

Iron is present in various types of minerals as oxides (goethite, magnetite, hematite) and sulfides (pyrite, chalcopyrite, arsenopyrite), among others, and is extracted by metallurgical techniques [4]. In ferric leaching and bioleaching-biooxidation processes, metal sulfides are mainly solubilized by ferric ions; in this solubilization the metal of interest (e.g. Cu, Ni, Zn) is liberated from the mineral or eventually exposed to further leaching (e.g. Au). The balance between ferrous and ferric ions is critical because high concentrations of ferric ions can provoke the formation of ferric precipitates (like jarosite) and mineral passivation which could harm the metal recovery [5].

Different microbial groups are involved in the biomining bioleaching process, including microorganisms able to oxidize iron and/or sulfur (*Acidithiobacillus ferrooxidans*, *Leptospirillium ferrooxidans*, *Acidithiobacillus thiooxidans*, *Sulfolobus metallicus*, etc.) [6]. Iron oxidizing organisms get their energy from the oxidation of ferrous ion [7]. Biofilm formation on the mineral is important and it has been shown that the presence of ferric ions stimulate the formation of biofilm (among other factors, as the surface of sulfide minerals), which consists of extracellular polymeric substances (EPS) and microorganisms [7]. The space within the EPS containing the ferric ion complex is where the metal of interest is solubilized

[7]. This space requires special conditions hitherto unknown, such as level of pH, redox potential (Eh), ionic strength, etc. It has been estimated that the iron concentration within the biofilm can be up to 53 gL^{-1}, which can only be kept in solution by complexes of various species that prevent precipitation [7]. The iron oxidizing microorganisms can be inhibited by high ferrous and ferric ion concentrations, limiting the industrial process [8,9].

A straightforward and rapid way to estimate the oxidative capacity of a liquid media is the Eh, which can be easily measured using commercially available platinum band potentiometric electrodes. But this simple method gives no information about the amount of iron present in the sample; just the relationship between ferric and ferrous ions (if iron is the main redox couple in the media) [10]. The Eh, pH, the presence and concentrations of certain inhibitory anions and cations, ferric, ferrous and oxygen concentration, among other factors, can strongly modify the ferrous-oxidizing activity of the microorganisms present in the media. Moreover, if oxygen became depleted, some microorganisms can reverse the process, by using as electron acceptor the ferric species. Although oxygen monitoring (among other chemical and physical characteristics) could be a very interesting analyte in order to manage some bio-mining processes (as for example biomass production), there is no a simple, economic and fast method able to measure this gas in bio-mining conditions (corrosive acidic media). Moreover, given the high atmospheric oxygen concentrations, is not easy to obtain and process a sample without contaminations in industrial conditions; this is especially important if small samples are to be measured. That all make very important, in order to understand and optimize the mining process, to make available a simple method to monitor the iron species in chemical processes such as leaching, bioleaching, biomass production, and other biomining operations.

At present there are several methods of quantifying iron, but few manage to chemically speciate it. The most accepted methods used in the mining environment are the colorimetric methods of 1,10-phenanthroline and sulfosalicylic acid (SSA) [11], the volumetric method of potassium dichromate, and atomic absorption [12]; the latter fails to speciate iron. The two colorimetric methods are recognized as

the standard to measure iron speciation, but require specialized personnel, are time-consuming, and require specialized equipment and reagents. The volumetric method is recognized as inaccurate. As bio-mining is a continuous process, with some operations stretching over several weeks or months, a simple, rapid and continuous method to chemically speciate iron is required.

Electrochemical techniques are recognized as simple, economic and robust with the possibility of automation. Different electrochemical methods for the detection and quantification of iron have been reported previously, but few of them have the ability to chemically speciate this ion. Most of the reported techniques were conducted at pH greater than 3, and would therefore only quantify total iron or ferric ion [13]. AdSV (adsorptive stripping voltammetry) have been proposed as and electrochemical way to iron determination and speciation, given that this metal cannot be measured by methods involving amalgam formation. AdSV is highly sensitive, but dwell in the use of specific reagents (surface-active ligands), which must form complexes, involving adsorption and desorption steps; such sensitivity is not important in bio-minery applications, where high iron concentrations are expected [14].

Hg, Pt and carbon electrodes have been used as the working electrode (WE), some of them have been modified to increase sensitivity measured using different electrochemical techniques, such as anodic and cathodic stripping, cyclic voltammetry, chronoamperometry, potentiometric stripping analysis and differential pulse polarography, among others. These proposed techniques have been recently compressively reviewed, including three tables that classify the available techniques in iron analyses using mercury, platinum or carbon electrodes [13].

Platinum was not widely used as WE in the electrochemical field during the twentieth century; however, in recent years it has become one of the most prevalent choices [14]. The first report that mentioned platinum for electrochemical iron recognition was published in 1972 [15]. Platinum, being a noble metal, has many advantages; it can tolerate extreme pH ranges without being affected and diminishes the possibility of spills when Hg hanging-drop electrodes are used, because of that we chose Pt as our

working electrode. Sensors for detecting ions using voltammetric or amperometric techniques have gained importance in recent years, as they offers a number of advantages over its potentiometric counterpart, principally the ability to detect more than one electroactive species at a time. A relatively new electrode material, boron doped diamond, have been described recently and used to measure iron in acidic conditions [16]. But the set-up used (stirred batch) and the curved amperometric calibration curves obtained would make this sensor not easy to apply in the industrial environment. Techniques based on the flow injection system (FIA) coupled with electrochemical detection have been widely proposed for a high number of analytes and detection modes. FIA systems allow continuous or semi-continuous analytical quantification, are easy to use, have automation potential, offer high sample h^{-1} capabilities, low costs per sample, and a very good analytical performance [17].

In this study we characterize a FIA system, which includes an electrochemical wall-jet type cell, which allows iron speciation and quantification without the use of reagents or pre-treatments other than, if necessary, sample dilution. By choosing the appropriate potential, ferric or ferrous ions react over the Pt WE, allowing their simple and rapid quantification. We applied our method to samples from acid mine drainages and from cultures of iron-oxidizing microorganisms, and compare our results with a well-established method used in the industry (1,10-phenanthroline).

2. Materials and Methods

2.1. Construction of the electrodes

Pt working electrodes (WE), $Ag/AgCl_{sat}$ reference electrodes (RE) and stainless steel counter electrodes (CE) were designed and constructed in the laboratory using electrochemical grade Pt and Ag wires, and grade 301 steel.

A Pt wire (0.7 mm diameter, 1 cm long) was bound to a brass screw using conductive silver epoxy and subsequently enclosed in epoxy resin (Distraltec EP Systems, Buenos Aires, Argentina) in proportions 100:30 resin (Dicast 750) and hardener (Dicure 364) using 5 mL syringe as a mold. Curing was carried out at 80°C in an oven for 48 h. After removal from the syringe, the Pt side was polished using papers of different grain followed by alumina (up to 0.3-micron), until it acquired a mirror-like appearance. No bubbles or cracks were observed in the WE around the Pt wire under a binocular loupe.

The RE used was constructed by placing a previously chlorinated 3 mm diameter Ag wire in a ~~blue~~ micropipette tip (1 mL). A porous plug was used to minimize leakage. The tip was filled with a KCl saturated solution, and the wide end closed with Parafilm to minimize evaporation of the solution. Stability was measured with a tester and a commercial Calomel electrode before use. The CE was a stainless steel coil (from a 1 mm diameter wire).

2.2. Design and construction of the flow cell

A wall-jet cell was designed and built in our laboratory as the main component of our FIA system. The cell was constructed of clear acrylic, allowing the connection of a WE, RE and CE. In this cell, the carrier passes through a glass nozzle of 0.5 mm (internal diameter) and meets the aligned WE. This configuration can increase response sensitivity and prevent the formation of a concentration gradient in the vicinity of the electrode surface, where the analyte is consumed. The cell internal volume was about 1 mL (Figure 1).

The carrier (and supporting electrolyte) solution was 100 mM KCl at pH 2.0; the flow rate was 1 mL min^{-1}. The system also features a low pressure, six-position manual injection valve (Rheodyne model 5012). A 500-µL loop was used in all the experiments presented here.

2.3. Analytical methods

Cyclic voltammetry (CV) was used to study the response of the Pt electrode to Fe^{2+}/Fe^{+3} and Cu^{+2} (as a possible interference to our method, in batch quiet solutions. Chronoamperometry was used to quantify Fe in the FIA system we present here; in our method, Fe^{2+} and Fe^{+3} were measured each separately at a given electrode potential, total Fe was calculated as the sum of both ions. In addition, total iron and ferrous iron were measured using the 1,10-phenanthroline colorimetric method; ferric ion was determined by the difference between the two values, as previously described [18]. The colorimetric and the proposed FIA amperometric methods were compared along this work. The pH and redox potential were measured with commercial electrodes.

2.4. Calibration solutions and samples

Solutions were prepared using double osmosis water. $FeSO_4.7H_2O$ and $FeCl_3.6H_2O$ were used to prepare ferrous and ferric iron calibration solutions, respectively. Salts were dissolved in water previously adjusted to pH 2 with HCl and 100 mM in KCl. The electrochemical techniques used were cyclic voltammetry and chronoamperometry. A potentiostat TEQ-03 (Ing. Sobral, La Plata, Argentina) or a Gamry-300 (Gamry Instruments, PA, USA) was used. Using chronoamperometry, we obtained well-defined peaks as the samples were injected in the carrier flow; the peak high (current) was used and correlated to the analyte (ferric or ferrous ion) concentration (see Figure S1).

Real samples were used in this study. A sample of acid mine drainage (AMD) from the mining area of Andacollo, IV region of Chile was measured. AMD pH was 2.1, with an Eh of ca. 491 mV). The sample was measured after dilution using standard atomic absorption spectrometry; the results obtained indicated that Fe, Cu and Zn were predominant (16.05, 0.400 and 0.322 gL^{-1}, respectively). Co was also found at low concentration (5.7 mgL^{-1}), and As was not detected < 0.02 mg/L^{-1}). Also, a bacterial bio-oxidation process was followed. Both were quantified by our amperometric FIA sensor and the 1,10-phenanthroline colorimetric method, as a reference and established method.

2.5. Microorganisms and culture medium

A culture of *Acidithiobacillus ferrooxidans* DSMZ 11477 was used. Cells were grown in culture medium 9K, which had the following composition (gL^{-1}): $NH_4H_2PO_4$, 3.0; $MgSO_4 \cdot 7H_2O$, 0.15; KCl, 0.15 and $FeSO_4 \cdot 7H_2O$, 44.0. The kinetics of *A. ferrooxidans* iron oxidation was followed during some days. Media was inoculated with 10% of an actively growing culture (ca. 10^7 cells mL^{-1}). pH was not regulated during the experiments, and triplicate assays were done.

3. Results and Discussion

3.1. Cyclic voltammetry studies

Two current peaks were registered when $Fe^{2+/3+}$ were present in the solution, at shown in Figure S1. An anodic peak, at 518 mV and a cathodic peak, at 454 mV are evident. These peaks correspond to the iron oxidation-reduction reaction and each current peak could be used for the detection of the corresponding iron ions, as denoted in Eq. 1 and 2.

$$Fe^{2+} \rightleftharpoons Fe^{3+} + e^- \quad \text{(Anodic reaction – peak 1)} \qquad [1]$$

$$Fe^{3+} + e^- \rightleftharpoons Fe^{2+} \quad \text{(Cathodic reaction – peak 2)} \qquad [2]$$

The voltage differential between the peaks (ΔE_p) was 64 mV (Figure S1, scan rate 25 mVs^{-1}), this is similar to the theoretical value for ferrocyanide/ferricyanide coupling for one electron transfer in a quasi-reversible system (59.2 mV) [19]. We also studied the reversibility of the system by modifying the scan

rate (Figure S2), roughly straight lines were obtained by plotting peak currents *vs.* the square root of scan rates (ip *vs.* $v^{1/2}$, data not shown); the peak measurement was approximated, given that with our electrode size we have probably some non-planar diffusive component. The linearity of ip *vs.* $v^{1/2}$ plots also shows that the heterogeneous electron transfer was quasi-reversible and the overall reaction was basically diffusion controlled.

3.2. Quantification of ferric and ferrous ions

Calibration curves were constructed. Current was measured using chronoamperometry and a FIA system (Figure 1), a technique that measures current while applying a fixed potential. The potential applied to quantify ferric and ferrous ions was 100 mV above their respective anodic and cathodic peaks (Figure S1), to assure that small changes in the RE potential will not change the current signal. Therefore, the applied potential was 618 mV for ferrous ions and 354 mV for ferric ions.

Calibration curves were constructed from 0.01 to 10 gL^{-1} for ferrous and ferric ions with the objective of characterize the analytical system. This exploratory study shows that a concentration/current relationship was obtained between few mgL^{-1} to 10 gL^{-1} for ferrous and ferric ions (chronoamperometry). At high concentrations (above 2 gL^{-1}) the linear relationships deteriorate (see Figure S2). We postulate that this phenomenon could be related to a saturation process, or passivation of the WE. To study the loss of linearity at high concentrations, an experiment was performed, by using a carrier solution containing 3.5 gL^{-1} of either, ferrous or ferric ion, allowing Pt WE constant exposure to each iron species, being the WE polarized continuously. Figure S3 shows that both ions at this relatively high iron concentration affect the electrode (probably by passivation); the ferric ion seems to be more efficient affecting (decreasing) the WE current: it is know that ferric ions are highly corrosive and oxidative [5]. The current diminution is probably related to the formation of insoluble iron complexes over the Pt electrode, which in turn can diminish the surface area available for the reaction. This phenomenon influences the range of iron ion

quantification, suggesting that after the quantification of a number of samples with high ferric ion concentration, subsequent samples are in risk of being under-estimated since the amperometric signal would have decreased (Figures S2 and S3). If well some electrochemical or mechanical ways to maintain the WE operational at high iron concentrations could be eventually envisioned, they were not investigated in this paper.

We decide to use as a practical calibration range for all further experiments values from 10 to 1500 mgL^{-1}, to avoid (or delay) the problems related to high iron concentrations (Figure 3). The statistical analysis shows that the anodic and cathodic currents are linearly associated with ferrous ion (R^2 = 0.996) and ferric ion (R^2 = 0.991) concentration, respectively. The detection limit calculated for the amperometric sensor was at 15±2 mgL^{-1} (2.7 x 10^{-4} M) of iron for both ions. Detection limit was chosen as three times the peak to peak noise of the amperometric trace. Figure 4 shows typical traces for the determination of ferric and ferrous ions at several concentrations; relatively low noise, high repeatability ~~reproducibility~~ and base-line stability are shown, when our FIA system was used.

Repeatability values were estimated using the data obtained with during a 2 h long single session, by using a new assembled FIA system, where the WE was polished at the beginning of the session; we maintain the same operator, location, carrier solution and standards. Reproducibility values were estimated by using data obtained during one month, in 6 different days. After the Pt WE was polished, the FIA cell was assembled every working day; different RE, standards and carrier solutions were used; operator and location were the same. Repeatability shows values of RSD (relative standard deviation) of 2.39 and 1.58% for ferric and ferrous ions, respectively (n = 16, 500 ppm of either ion). Reproducibility shows values of RSD of 2.56 and 1.91% for ferric and ferrous ions, respectively (n = 20, 500 ppm of either ion).

While the majority of analytical methods seek great sensitivity in iron quantification, this is unnecessary for bio-mining applications, since iron values are usually elevated in hydrometallurgical and

biohydrometallurgical processes [20]. Also the iron concentrations are often high in acid mine drainages [21, 22]. This holds true for the production of bioleaching bacteria biomass, where iron concentration can reach 9 gL^{-1} [21], though in tailings and acid mine drainage sites concentrations can reach up to 10 gL^{-1} [22]; still, in some laboratory and industrial applications concentrations can reach higher levels. Amperometric iron sensors based in Pt WE detectors have reported detection limits from 1.2 x 10^{-3} to 3 x 10^{-10} M. These reports each had different objectives, such as water and food analysis. None of them focus on the mining sector, where high acidic, corrosive conditions are expected, as those reported in this study [13].

3.3. Factors that influence the signal and interferences

The flow velocity, sample volume and chemical and physical characteristics of the carrier solution can affect the detector (Pt WE), and thereby the generation of the current signals; preliminary work was done to choose these conditions (data not shown). The chosen parameters were maintained constant throughout the work described in this paper. However, sample composition can also affect the readings, as some ions can interact with the iron (or the Pt working electrode) and cause erroneous measurements.

The present study took into account potential interferences likely found in the mining samples: ferrous and ferric ions as interferences in the quantification of ferric and ferrous ions, respectively, and copper in the quantification of both iron ions. Generally, in pregnant leach solutions (PLS) obtained from leached and bioleached minerals with low copper content, iron concentration is between 1 a 5 gL^{-1}, and copper between 0.5 and 1 gL^{-1},while other metals such as Pb, As, Hg, etc., are found at concentrations below 0.1 gL^{-1}, depending on the geological characteristics of the mine [22].

To examine the potential interference by these ions, ferrous ions were quantified at 100, 500 and 1500 mgL^{-1} in the presence of different ferric ion concentrations (0, 100, 500 and 1500 mgL^{-1}) (Figure 5A), and another similar experiment was performed, but where ferric ions were quantified in presence of

different ferrous ion concentrations (the last acting as possible interference), as shown in Figure 5B. Each point was measured both, by the amperometric sensor and the 1,10-phenanthroline colorimetric method. The quantification of ferric and ferrous ions in the presence of their reduced or oxidized counterparts shows no significant interference effect. The colorimetric method shows better accuracy, quantification at a constant 25 mgL^{-1} of ferrous and ferric ions in the presence of different concentrations of their reduced or oxidized ions produced an error nearing 20%. This error is due to the proximity of the detection limit in the amperometric sensor, which is less sensitive than the colorimetric method.

Copper ions can also interfere with the proposed method. In the treatment of secondary minerals with low copper content, copper is the third most abundant ion after ferric and ferrous ions, as copper is the metal of interest in the mining output [23]. The present study determined that Cu ions could interfere with iron quantification under some particular conditions. When Cu and Fe ions were present in the samples, CV studies shown four current peaks, corresponding to two redox reactions on the surface of the electrode (see Figure S4); for comparison, solutions containing just Cu are presented in the same figure. Previous studies have characterized copper redox reactions on a Pt electrode in the presence of chloride ions [24-28]. The chloride in our study is found in the electrolyte support (KCl 100 mM). The reported reactions are as follows, where C represents a chemical step (in solution) and E an electrochemical step (at the electrode):

Anodic reaction:

E: $Cu - e^- \rightarrow Cu^+$ (Peak 1, Figure S4) [3]

C: $Cu^+ + (n+1)Cl^- \rightarrow CuCl_{(n+1)}^{n-}$ [4]

E: $CuCl_{(n+1)}^{n-} - e^- \rightarrow Cu^{2+} + (n+1)Cl^-$ (Peak 2, Figure S4) [5]

Where, n = 0 when the result is the formation of CuCl, n = 1 when $(CuCl_2)^-$ forms, and n = 3 when $(CuCl_3)^{-2}$ forms [29].

Cathodic reaction:

E: $Cu^{2+} + e^- \rightarrow Cu^+$ (Peak 3, Figure S4) [6]

C: $Cu^+ + 2Cl^- \rightarrow CuCl_2^-$ [7]

E: $CuCl_2^- + e^- \rightarrow Cu + 2Cl^-$ (Peak 4, Figure S4) [8]

Previous studies report that the relative prevalence of these copper species will depend on the concentration of chloride in the medium. The potential of the current peaks of copper and iron can overlap, depending on the concentration of the sample being quantified.

Ferric and ferrous ions were quantified in the presence of different copper concentrations. Ferric ion quantification was not affected at low ferric ion concentrations (100 mgL^{-1}) and copper concentrations lower than 200 mgL^{-1}. But at copper concentrations of 200 mgL^{-1} and above, ferric ion concentration may be overestimated. This is because in this electrochemical technique (chronoamperometry) readings are made at a potential 100 mV higher than the potential of the current peak in the iron redox reaction, which would correspond with an overlap of copper and ferric peaks.

Copper was not a significant interference in ferrous ion measurements as long as it does not exceed 500 mgL^{-1}. The current peak for copper (peak 2, Figure S4) can overlap and lead to overestimation in the quantification of ferrous ions when current is high, but this is not an important factor at lower concentrations.

3.4. Quantification of iron in real samples

Considering the previously mentioned points, real samples can contain all three important ions: Fe^{2+}, Fe^{3+} and Cu^{2+}, to minimize the interference of copper in the current signal during iron quantification an appropriate dilution can be carried out to reduce copper concentration.

To this end, a dilution assay was carried out with a sample from acid mine drainage (AMD). Figure 6 displays voltammograms resulting from different sample dilutions. At sample dilutions 1:101 and 1:11 there were two current peaks corresponding to ferrous and ferric ions. However, at dilutions between 1:4 and 1:2 an additional current peak, corresponding to copper, was present. Dilution was necessary in this type of sample, because the Fe concentration was higher than 2 gL^{-1}. Our results shown values of 0.053 ± 0.004 and 0.076 ± 0.0024 gL^{-1} for the amperometic and the 1,10-phenanthroline methods, respectively (ferrous ion). Also, 15.89 ± 0.12 and 15.48 ± 0.14 gL^{-1} values were obtained, for the amperometic and the 1,10-phenanthroline methods, respectively (ferric ion). Error was relatively high for ferric ions (with sample concentration underestimated by about 20%) when concentrated solutions were used; the source of the error is still unknown, but was solved by extensive dilution or by using the standard addition method, as shown in Figure S5.

Ferric and ferrous ions were also quantified to characterize the bio-oxidation of iron by *Acidithiobacillus ferrooxidans*, a microorganism that obtains energy from the oxidation of iron and is therefore used in bioleaching/biooxidation processes. Figure 7 shows the oxidation of ferrous ions by the bacterial culture and the increase in ferric ions over time. The kinetic begins with a concentration of approximately 1 gL^{-1}, due to the initial bacterial inoculation of 10% (equivalent to an approximate initial bacterial density of 10^6 cells mL^{-1}). Observations of the behavior of the redox potential in the culture media with and without bacterial inoculation shows some iron oxidation without microbial inoculation (control) due to oxygen, but those changes were small. The complete bio-oxidization of all of the available iron occurs when bacteria was present (see Figure 7) [30]. The difference in the quantification of bio-oxidized iron using

the proposed and the reference (colorimetric) methods was minimal, as shown in Figure 7 and Table 1. Moreover, when kinetic parameters were calculated by means of either data, similar volumetric productivity values were obtained (Table 1), showing that the differences among methods are not revelant. The evolution of the redox potential (Eh) during the *A. ferrooxidans* culture is shown in Figure S6. The measurement of Eh is used as an alternative method to stimate the realationship between both Fe species.

4. Conclusions

The amperometric sensor presented here is a convenient method, superior to most of the habitually used to measure iron ions in processes such as bio-leaching, bio-oxidation, ferric leaching, passivation control, where high concentrations of iron and other minerals, as well as extreme acidic conditions are normally found in these industrial applications. The calibration range encountered in the FIA system and electrochemical configuration fits perfectly the needs for the proposed use. Moreover, by tuning carrier flow-rate, sample loop volume, and/or working electrode area, sensitivity and lineal range can be easily changed to cope with different analytical needs. Another interesting feature is that FIA amperometric systems can be easily automated, allowing on-line biomining process monitoring [31]. High Fe concentrations (especially ferric ions) have a deleterious effect over the Pt WE, possibly by the formation of insoluble oxides over the electrode; because of that, very high ferric concentrations were avoided in our calibration curves. Fe^{2+} and Fe^{3+} suffer no interference from the oxidized and reduced iron form, respectively. Our experiments showed that continuous electrode polarization at high Fe concentrations (Fe was included in the carrier solution) lead to WE passivation. We demonstrate with this experiment that limiting the Fe exposure to seconds (by using a FIA system) and/or diluting the sample (by dilution in the carrier flow), passivation is only observed after the measurement of hundreds of samples. This

means that our FIA systems can be used during a full working day, the only required maintenance being a daily polishing step.

Although Cu is not interference if CV is used as analytical technique, Cu peak could interfere with ferric ion measurement at high Cu concentrations, if amperometric measurement at one fixed potential is used. Fortunately, at relevant concentrations and conditions related with cooper mining samples the relationship between ferric ion and Cu ion, and with appropriate dilution, no interference was found. Some anions as sulfate could be found in high concentrations in real samples, the interference of this compound could eventually occur, if well it is not expected (given its relative concentrations among Fe species and their electrochemical characteristics). Real samples, containing a very complex matrix of inorganic and organic molecules (*Acidithiobacillus ferrooxidans* culture and an acid mine drainage sample) were successfully measured. We compare the proposed method with a colorimetric one (1,10-phenanthroline), that is of standard use to speciate iron in the industry; this colorimetric method measure ferric iron as the difference of total and ferrous iron, in two steps. The electrochemical FIA method used here is able measure directly ferric or ferrous ions, depending on the applied potential to the Pt WE. The proposed reagent-free amperometric system is simple and affordable, opening the possibilities of on-site, real-time mining process monitoring that will increase the efficiency and productivity of chemical or biological-based ore solubilization.

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Figure and Table Captions

Figure 1. Schematic representation of the flow injection system used, with details about the electrochemical flow cell design. Parts are not in scale.

Figure 2. Cyclic voltammograms of Fe^{2+}/Fe^{3+} ions (100 mgL^{-1} each), in water pH = 2.0, 100 mM in KCl. Scan rates were 1, 5, 10, 25, 50, 100, 200 and 500 mVs^{-1}.

Figure 3. Calibration curve of ferrous (—●—) and ferric (—○—) ion, using the amperometric FIA sensor presented here. Deviations are ± 2 SD (n = 3).

Figure 4. FIA Chronoamperometry data for injection of ferric (A) and ferrous (B) ions. Concentrations of 10, 20, 40, 60, 80 and 100 mgL^{-1} were measured, noted as 1, 2, 3, 4, 5, and 6, respectively.

Figure 5. Quantification of iron by the amperometric FIA sensor (—) and by the 1,10-phenanthroline colorimetric method (—) in Fe^{2+}/Fe^{3+} solutions. A) Fixed concentrations of ferrous ion of 100 (■, □), 500 (●, ○), and 1500 (★ ,☆) mg L^{-1} in the presence of different concentrations of ferric ion. B) Fixed concentrations of ferric ion of 100 (■, □), 500 (●, ○), and 1500 (★ ,☆) mgL^{-1} in the presence of different concentrations of ferrous ion. Deviations are ± 2 SD (n = 3).

Figure 6. Voltammograms at different dilutions of an acid mine drainage sample. At low dilutions, a Cu peak is evident (corresponding to peak 1 at Fig. S4). In the insert, higher dilutions are show (up to 1:101) where the copper signal disappears. All voltammograms show Fe^{2+}/Fe^{3+} peaks. Scan rate was 25 mVs^{-1}.

Figure 7. Bio-oxidation of iron by an *Acidithiobacillus ferrooxidans* culture. Ferrous (●, ○) and ferric ions (■, □) were quantified using and the amperometric FIA sensor (—) and the 1,10-phenanthroline method (—). Deviations are ± 2 SD (n = 3).

Table 1. Measurement of the bio-oxidation of iron by *Acidithiobacillus ferrooxidans*. Our method (amperometic) was compared with the 1,10-phenantroline one, used as reference; percentages express the relationship among both methods. Volumetric productivities were calculated using data from both methods, for the total time period studied (0 – 71.5 h). ND = not detectable.