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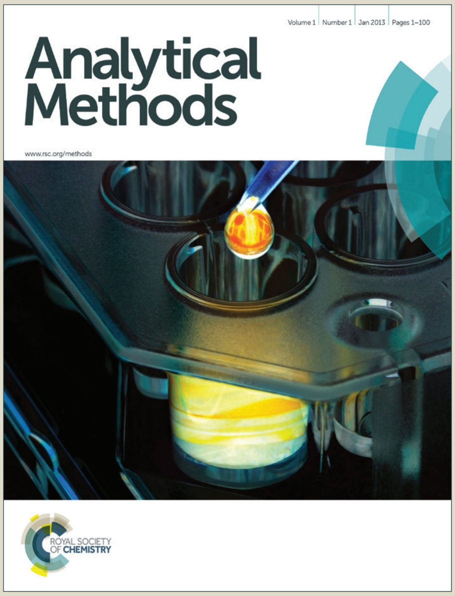

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Kinetic-catalytic method for sequential determination of iron and copper using a chip coupled to a multipumping flow system

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ABSTRACT

A kinetic catalytic spectrophotometric method for sequential determination of iron and copper is presented in this work. For this, a multipumping flow system (MPFS) has been coupled to a monolithic flow conductor, called chip .The reaction is based on the catalytic effect of Cu(II) and Fe(III) on the hydroxylamine oxidation in a basic medium. The addition of EDTA inhibits the Cu(II) effect and become the reaction selective to Fe(III). The nitrite produced is spectrophotometrically measured using the modified Griess reagent. Due to the importance of mixing order of the reagent, a special design of the chip is proposed. Sample, hydroxylamine and buffer (with EDTA for Fe and without EDTA for Cu determination) have been simultaneously propelled into the thermostated first section of the chip for mixing and reaction at 40 ºC. Then, this mixture and the modified Griess reagent (sulphanilamide and N-(1-naphthyl)ethylenediamine) have been injected by confluence into the second section of the chip. Under optimal conditions, the determinable range was 3-75 µg L^{-1} and 70-350 µg L^{-1} for Cu(II) and Fe(III) respectively, with sample throughput of 32 $h^{-1}$for Fe(III) and 39 h^{-1} for Cu(II). Limits of detection were 0.95 µg L^{-1} for Cu(II) and 21 µg L^{-1} for Fe(III). Relative standard deviation (n=6) were 1.6 % for 50 µg L^{-1} the Cu(II) and 1.3 % for 100 µg L^{-1} the Fe(III). The proposed method has been successfully applied to sequential determination of Cu(II) and Fe(III) in waste water and pharmaceutical samples. ICP-OES has been used as reference method for validation.

Keywords: kinetic catalytic method, chip-MPFS, Fe(III), Cu(II), flow analysis

1. INTRODUCTION

Iron and cupper are two essential elements in the environment and they have important roles in different biological systems. Either excess or deficiency of these metals in the living organism can lead to biological disorder [1]. The main way of their incorporation into the food chain by plants and microorganisms, is the absorption of their aqueous species. For that, they are consider as water quality parameters and their concentration in drinking waters are restricted to 0.3 mg L^{-1} for Fe and 0.01 mg L^{-1} for Cu [2, 3]. However, their concentration in surface water can vary from 0.5 to 10 mg L^{-1} [2] and from 0.0005 to 1 mg L^{-1} [3] respectively. Due to the high demand of analysis of these metals in aqueous samples, simples and sensitive analytical methods for their monitoring are require.

Fe and Cu have a high capacity to form colored complexes with organic molecules. The difference in the kinetics of these complexes formation as well as the displacement of the absorption maximum has been used for their simultaneous determination [4-7]. For example the method proposed for Ohno et al.[5] is based on the Cu(II) and Fe(II) complex formation with 2-(5-bromo-2-pyridylazo)-5-[N-n-propyl-N-(3-sulfopropyl)amino]aniline (5-Br-PSAA) at pH 4.6. In this case, the differentiation between these metals was relies on the presence of a reducing agent, ascorbic acid. Consequently, two samples zones and measurement wavelengths were used, one for each analyte. Similar strategy were used for Paipa et al. [4], which measured at two wavelengths to differentiate the absorbance signal of the Fe and Cu complex formed with 5-sulfosalicylic acid.

The catalytic effect of these metals in colorimetric and fluorometric reactions has used also for independent determination of Fe and Cu [8-10]. The main advantage of using catalytic methods relies on its notable high sensitivity because the analyte is the catalyst. However, just one method has been reported for simultaneous determination of Fe and Cu based on a catalytic reaction. In this method, Lunvongsa et al.[6] discriminated the Fe and Cu catalytic effects on the oxidative coupling reaction of N,N-dimethyl-p-phenylenediamine with 1,3-phenylenediamine by changes in the buffer pH and reagent addition order.

Fe and Cu are able to catalyze the hydroxylamine oxidation by dissolved oxygen in a basic medium. Significant differences in the catalytic effect of these two metals could be achieved by changes in experimental conditions such as the kind of buffer or a complexing agent addition. Thus, the catalytic effect of Cu is favoring using borate buffer [11] and the use phosphate buffer with EDTA enhanced the catalytic effect of Fe and inhibit the Cu effect [12]. This differentiation has been previously used to determine one of these metals in the presence of the other such as the methods presented by Gomez et al [12] and Cladera et al [13] which used thermometric and spectrophotometric detection for Cu and Fe determination, respectively. In the spectrophotometric variant [13], the nitrite formed during the catalytic hydroxylamine oxidation were monitoring by the azo dye formation using the Griess reagent, sulphanilamide and N-(1-naphthyl)ethylenediamine.

Flow techniques have be used for automation the analytical procedure in order to guaranty the control of experimental conditions such as mixing and reaction times, and to reduce reagents and sample consumption [14]. Flow injection analysis (FIA) has been the flow technique widely used for catalytic method automation [15]. Other modalities and coupling such as Lab-on-valves coupled to a sequential injection system (LOV-SIA) [5] and cloud point extraction coupled to flow injection system (FI-CPE) [7] has been also reported.

Among the different existents flow techniques, the multi-pumping flow system (MPFS), based on the use of micro-pumps as liquids drivers, has been presented as an excellent alternative for analytical procedures automation. Some of its advantages are the reduced size of the systems, and the economy and portability of its components. Moreover, in terms of flow pattern, the pulsed flow generated by the micro-pumps improves of reagent and samples mixture and the heat transfer, and increases the analytical frequency [16]. However, this flow technique has been scarcely used for catalytic method automations [15, 17]. The MPFS has a high versatility and flexibility of the flow system networking, especial in combination with a monolithic flow conductor [9]. The monolithic flow conductor, called "chip" were firstly used for the authors in the automation of catalytic analytical methods which require temperatures different to environment for reaction and/or measurement [18, 19].

In this work, a full automated kinetic catalytic method for Fe and Cu determination is developed. A MPFS coupled to a chip is proposed for the analytical procedure automation and system size reduction, improving the mixing efficiency of the implied reagents and the temperature control in the integrate reaction coil. In the proposed method for Fe and Cu catalytic determination by hydroxylamine oxidation, the use of chip is especially advantageous since two reactions take place in two different moments of the analytical path: the first one is the catalytic reaction requiring high temperature, and the second one for indicative reaction at room temperature. Main features to be highlighted of Chip-MPFS combination are the simplicity and the low costs of the flow system and controlling circuits, favoring economic and miniaturized flow analyses.

2. Materials and methods

2.1 Reagents and standards

All reagents were of analytical grade and were provided by Scharlau (Barcelona, Spain).and all solutions were prepared in MilliQ water (MilliQ plus, <18.2 MΩ cm^{-1}). Working Iron (III) and Copper (II) standard solutions were prepared by appropriate dilution of stock AAS grade 1000 mg L^{-1} of Fe (III) and Cu(II) solutions respectively

Phosphate buffer of 0.1 mol L^{-1}, was prepared by mixing 4.35 g of dipotassium hydrogen phosphate and 0.73 g of EDTA (0.01 mol L^{-1}) in 250 mL of water. Borate buffer of 0.1 mol L^{-1} was prepared by dissolving 9.53 g of sodium tetraborate in 250 mL of water. Sodium hydroxide solution 3.0 mol L^{-1} was used for pH adjusting to 12.5 both buffer solutions. Hydroxylamine solution 0.075 mol $L^{-1}$was prepared by dissolving 0.52 g of hydroxylamine hydrochloride in 100 mL of water. The Griess reagent was daily prepared by dissolving 0.3 g of sulphanilamide solution (SPA) and 0.3 g of N-(1-naphthyl)ethylenediamine (NED) (ACROS, Geel, Belgium) solution in 0.8 mol $L^{-1}$of HCl and 100 mL of water.

2.2 Sample collection and preparation

Wastewater, buildings demolition leachate as well as pharmaceutical products samples were used to evaluate the proposed methods. Two samples of demolition leachate were collected from different areas of Mallorca (Balearic Islands, Spain). The water samples were collected in plastic bottles (previously cleaned with HNO_3 10% v/v) and transported to the laboratory , where they were filtered through 0.45 μm cellulose nylon membrane to separate any particular matter and measured without further treatment. A certified reference material of wastewater (SPS-WWW2, batch 106, Spectrapure Standards, Norway) was diluted with Milli-Q water, and adjusted to neutral pH.

Three different pharmaceutical samples were used, two in tablet format: Hidropolivit A Mineral (Laboratorios Menarini, S.A. Spain) and Supradyn (Bayer Hispania, S.L. San Joan Despi, Spain), and one in liquid format Cobre (drinking ampoule, Labcatal Ibérica, S.L.Spain). Each tablet of Hidropolivit (1.89 ± 0.01 g/tablet) contains 2 mg of Fe and 1 mg of Cu, whereas each tablet of Supradyn (4.82 ± 0.05 g/tablet) contains 14 mg of Fe and 0.9 mg of Cu. For analysis, 95 mg of Hidropolivit and 106 mg of Supradyn tablets previously powdered in agate mortar were accurately weighed and transferred into100 mL Teflon digestion vessels. 10 mL of ultrapure concentrated HNO_3 (65%) (Scharlau) were added and vessels were closed and placed in a microwave oven (Milestone, START D) to digest the samples. The oven is equipped with a 2450 MHz microwave power supply (0-1200 W), a 6-position turntable and 100 mL Teflon liners with 355° rotatable pressure release valves, resistant up to 350 psi and 210 °C. The step of microwave program for pharmaceutical sample digestion was 1000 W, 200 °C, 30 min. The digested solutions were cooled to room temperature and then evaporated to reduce their volume to a small drop. Finally, the volume was diluted to 100 mL with water to obtain a 0.2 mg L^{-1} of Cu and 2.7 mg L^{-1} of Fe for Supradyn and 0.5 mg L^{-1} of Cu and 1.1 mg L^{-1} of Fe for Hidropolivit extracted solutions.

Aliquots of 20 µL of liquid pharmaceutical sample, “Cobre”, was diluted to 50 mL with water and directly measured. Each ampoule of 2 ml has 725.2 µg of Cu dissolved in 0.1 g glucose and purified water.

ICP-OES was used as a reference method for the quantification of Fe(III) and Cu(II) in water samples previously acidified with concentrated HNO_3 to 2% v/v. An ICP-OES (Optima 5300 DV, Perkin Elmer® Inc.) equipped with a Gem Tip Cross-flow pneumatic nebulizer (Waltham, MA, USA) was operated using the following instrumental parameters: RF generator power 1300 W, RF frequency 40 MHz, plasma argon flow 15 L min^{-1}, nebulizer argon flow 0.8 L min^{-1}, auxiliary argon flow 0.5 L min^{-1}, integration time 5 s and aspiration rate 1.5 mL min^{-1}.

Wavelength for emission measurement was 239.562 nm, 324.752 nm for Fe(III) and Cu(II) respectively. All measurements were in triplicate.

2.3 Flow conduit chip

The chip designed for this work involves two reactions zones, (Fig 1). The first one, for the catalytic reaction, includes a four-way confluence point followed by a serpentine reaction coil of 210 µL (1.0 mm wide x 0.8 mm deep x 27.8 mm long), for sample, NH_2OH and buffer mixture, all over the thermostatic chamber. The second one was used for indicative reaction. It has a three-way confluence point followed by a reaction coil of 105 µL, for mixture the sample zone with the Griess reagent, all this outside of the thermostatic chamber. The general construction of the chip is described elsewhere [20]. It consist of three blocks of PMME, two of 92x49x10 mm, and another of 92x49x15 mm, in the first block the flow channels and confluence points were drilled using a 3-axis PC controlled CNC milling machine (Alarsis, Spain). Threads of ¼” 28 fittings were drilled in the upper part of this block to connect the supply tubes for reagents and sample/carrier as well for the output flow to the detection flow cell. The second block was then glued applying a thin film of acrylic acid prior to tight fixation and UV curing time of 1 hour. For thermostatic chamber two rectangular cavities, 27x28x9 mm for the thinner piece and 27x28x14 mm for the thicker were made in the second and the third block only under the first reaction coil prior to glue both block together. After UV curing, two flow connectors were attached to allow continuous flushing of hot water through the cavity.

2.4 Flow analyzer

A diagram of manifold of automated Chip-MPFS system is shown in Fig 1. Four solenoid micropumps (BIO-Chem Valve, NJ, USA) controlled by a MPFS/MCFIA-module (Sciware Systems, Bunyola, Spain) were used for solutions management. Solenoid micropumps with nominal 25 µL per stroke were used for sample injection (SMP1), hydroxylamine (SMP2), buffer solution (SMP3) and Griess reagent (SMP4), propelled with a flow rate defined by the frequency of pulses. A three way solenoid valve (V) was placed before SMP3 for selective and automatic propulsion of two different buffers: tetraborate for Cu and phosphate with EDTA for Fe determinations.

Tubing of PTFE (0.8 mm id) was used for connections. A 45 position autosampler with 10 mL sample vials (Crison Instruments S.A, Barcelona, Spain) was connected to the sampling tube and was used for the automation of sample and standard introduction. A white, super bright, LED diode (Sciware Systems SL) was used as light source. An USB2000 miniature CCD spectrophotometer (Ocean Optics Inc., Dunedin, FL, USA) was used as detector. Both, LED lamp and spectrophotometer were directly coupled to a PMMA cell support (Sciware Systems SL, Bunyola, Spain) holding a 10 mm path-length flow cell from Starna (type 75.1 SOG, Essex, UK). Spectrophotometric measurement was performed at 542 nm, using 680 nm as correction wavelength to reduce the Schlieren effect. According with Dias et al [21] dual-wavelength measurement is the widest and more efficient option for compensating the Schlieren effect in flow analysis with spectrophotometric detection, especially when the component “mirrors”, regarding light reflection, is dominant like occur in turbulent flow conditions typical of the pulsed flow pattern presented in MPFS. The MPFS/MCFIA module, the autosampler and the spectrophotometer were connected via RS232C or USB interface to a PC for remote software control using AutoAnalysis 5.0 (Sciware Systems SL, Bunyola, Spain). A thermostated bath (Selecta, Barcelona, Spain) were used for propel hot water through the chip thermostatic chamber via silicon tubes (ca.20 cm, 1cm id).

2.5 Analytical protocol and flow method

The analytical procedure for the sequential determination of iron and copper is presented in **Table 1**. Aliquots of 700 µL of sample were dispensed via SMP1 (step 1), followed by 2 mL of buffer (Phosphate buffer for Fe(III) and borate buffer for Cu(II) determination) for cleaning the sample tube and the chip. Then, 75 µL of sample, hydroxylamine and buffer were simultaneously propel and mixed (step 3) into the first section of the chip by SMP1, SMP 2, SMP3, which were activated at 3.0 mLmin^{-1} (Fig 1). This mixture was incubated (60 s for Fe(III) and 30 s for Cu(II)) at 40 ºC (step 4) allowing to progress the catalytic reaction. After that, this reacting mixture was propelled to the second section of the chip and further to the detector, carried by 200 µL of buffer (via SMP3) and mixture with 200 µL of Griess reagent via SMP4.The transient signal of absorbance was register at 542 nm, and correction at 680 nm (step 5-8). Additional volume of buffer at high flow rate (1,5 mL at 5 mL min^{-1} via SMP3) was propelled to clean the system (step 7), avoiding memory effects.

3. Results and Discussion

3.1 Preliminary consideration and univariate optimization

The particular novelty of this work was the determination of two analytes, Fe and Cu, using the same reaction. The analytical system comprises two chemical reactions; hydroxylamine oxidation to NO_2^- catalyzed by Fe and Cu (catalytic reaction) and the Griess reaction to monitoring the NO_2^- generated (indicative reaction). Therefore, the indirect determination of Fe and Cu has hampered the implementation of the initial rate method, so the fixed time method has been used in this work. The reaction time was set by stopping the catalytic reaction mixture in the first section of the chip. Therefore, the reagents and the sample were introduced by confluence within the chip using micropumps with relatively high internal volume (25 µL) which were activated synchronously at a high flow rate (3 mL min^{-1}) to ensure efficient solutions mixture and minimize the time involved in the sample inserting step. Four pulses of samples (75 µL) were dispensed for each injection, considering the minimal pulse number required to guarantee enough sensitive and repeatable analytical signal (relative standard deviation < 5%). The indicative reaction occurs instantaneously, then, the Griess reagent and the catalytic reaction mixture were dispensed though the detection cell at high flow rate minimizing the time require for this step, increasing by this, the analytical frequency. Following these considerations the sample volume and the flow rate were fixed for both analyte determination, and the reaction time were selected by the study of the kinetic curve of each analyte using the two different buffers aforementioned (Fig 2).

Borate buffer (Fig 2A) favor the catalytic action of Cu resulting in a high initial rate during the first 30s followed by the signal stability. However the catalytic effect of Fe was negligible with this buffer. Whereas, phosphate buffer with EDTA, favor the catalytic effect of Fe which causes a high and constant reaction rate during the firsts 2 min. This solution inhibit the catalytic effect of Cu, reducing significantly the reaction rate up to 60 s. Above this time the reaction rate start to increase. Based on these, borate buffer and 30 s of reaction time, and phosphate buffer with EDTA and 60 s of reaction time were selected for Cu and Fe discrimination.

EDTA concentration effects on Fe and Cu catalysis were studied in the range of 0.002 to 0.1 mol L^{-1} comparing the analytical signal obtained for two standard solution, one of 250 μg L^{-1} of Fe and the other one of 250 μg L^{-1} of Fe + 250 μg L^{-1} of Cu. The increment of EDTA concentration enhanced the catalytic reaction, thus the analytical signal of both standard solutions. At 0.002 mol L^{-1} and at 0.1 mol L^{-1} differences between signals obtained for both standard solutions is observed. It is due to the catalytic effect of Cu is presented. However, at 0.01 mol L^{-1} of EDTA the analytical signals obtained using both standard solutions coincide due to the total inhibition of Cu catalytic effect. Therefore 0.01 mol L^{-1}EDTA was chosen for further experiments in order to obtain the maximal discrimination for both catalysts.

3.2 Multivariate optimization of experimental conditions

In order to simplify the number of solutions and the system configuration, the optimization of factors as temperature, concentration of Griess reagent, concentration of NH_2OH and pH were performed by multivariate experimental design. Optimum values of these factors should maximize the sensitivity for each analyte and the discrimination between them.

A full factorial design (2^4) was carried out as screening method to evaluate the influence of these four factors and their interactions in the sequential determination of the Fe(III) and Cu(II). The experimental domains were: reaction temperature, 25- 45ºC; concentration of Griess reagent, 0.1% - 0.5% for both NED and SPA; concentration of NH_2OH, 0.01- 0.19 mol L^{-1}; and pH, 12.2-12.8. Other parameters were kept constant (see table 2). Three central points were added to this design in order to estimate the error of data fit to the model. The analytical signals obtained by the injection of three standards solutions (100µg L^{-1} Cu(II), 250 µg L^{-1} Fe(III) and 100µg L^{-1} Cu(II) + 250 µg L^{-1} Fe(III)) were used as dependent variables.

For both Fe and Cu determination, data were successfully adjusted to a second order model with no significant lack of fit for the 95% of confidence level and a very low pure error . (see table S1). In the studied range, for Fe, the NH_2OH concentration was the factor with the highest significant effect, followed by the temperature and the interaction of them. A significant curvature effect was also observed. Whereas, for Cu temperature and interaction of NH_2OH concentration and pH were the factors with the highest significant effect followed by the pH and the Griess reagent, all with a positive effect on the response signal.

In order to improve the study of NH_2OH concentration, the pH and their interaction a Doelhert matrix were design expanding the experimental domain of this two factors according the previous screening (pH: 11.6-12.8 and NH_2OH: 0.1-0.3mol L^{-1}). The Griess reagent was fixed at 0.3% due its low significant effect observed in the screening and the temperature was fixed in 32 ºC for simplify the design. The profiles for predicted values and desirability clearly shows the importance of each factor and the critical values obtained. A maximum on the desirability function were observed for 12.5 of buffer pH.

A second Doelhert matrix was design to improve the study of NH_2OH concentration, the temperature and their interaction in both determinations (table S1). The ratio of the analytical signal of standard 100 µg L^{-1} Cu(II) + 250 µg L^{-1} Fe(III) and blank (Std/BL) was selected as response variables. Data of these design were fitted to a 2-way interaction model using both ratio and slope response without any significant lack of fit, and a very low absolute error. Analytical responses for both metals were increase with temperature (positive effect) and decrease with NH_2OH concentration (negative effect). However, temperature above 40 ºC promoted bubbles formation causing serious problems in terms of reproducibility. For that reason 40 ºC was selected as working temperature.

The model suggests that the critical value for NH_2OH was outside of the experimental domain selected. For this, the studied ranges for NH_2OH concentration was expanded in a univariate study for both determinations (Fig 3). The maximal difference between peak height of Cu catalyzed reaction and non catalyzed (Cu Std- BL) were obtained using 0.03 mol L^{-1} of NH_2OH and regarding to Fe catalyzed reaction (Fe Std-BL), 0.1mol L^{-1}. However, in order to simplify the number of solutions inserted in the system, an intermediate concentration of NH_2OH trying to minimize the loss of sensitivity for both measurements was attempted. According with this, 0.075 mol L^{-1} of NH_2OH concentration was selected for the following experiments. The optimum conditions used in Chip-MPFS system are summarized in **table 2**.

3.3 Analytical features

The catalytic effect on the reaction is more pronounced for Cu than for Fe which was corroborated for the higher initial rate obtained in presence of Cu. This means a higher sensitivity for Cu than for Fe and it is in agreement with the slopes of calibrations curves obtained for each ion. (See Table 3). Consequently, the limit of detection (LOD), defined as three times the standard deviation of ten measurement of the blank signal intensity, was lower for Cu (0.95 µg L^{-1}) than for Fe (21 µg L^{-1}). Following this idea, the working range for Cu (3.0-75.0 µg L^{-1}) starts in a lower concentration than for Fe (70.0 –350.0 µg L^{-1}), which is also wider. This difference in working ranges is suitable for application in environment samples where Cu usually appear in lower concentration than Fe. The precision of the methods, calculate as the relative standard deviation of six independent measurement carried out for 50.0 µg L^{-1} of Cu(II) and 150.0 µg $L^{-1}$of Fe(III) standard solution, were 1.6% and 1.3% for Cu and Fe, respectively. The calibration curve was obtained by plotting the peak height versus the concentration of Fe and Cu standard solution (Fe(III) +Cu(II)) in both determination. Fig 4 depicted the peak profiles of calibration curves obtained under optimal conditions. The high peak heights of the blank observed in Fig 4A indicate that the solution of phosphate buffer and EDTA favor the non-catalyzed reaction more than borate buffer solution (Fig 4B). This fact could affect the working range for Fe. Regarding the low reagent consumption and waste generation, the proposed method used only 6 µg of SPA and NED, and 0.39 µg of hydroxylamine for each injection. It generated 2.12 mL of waste per injection and 9.07 mL per sample analysis, including 3 injection replicates and sample tube cleaning.

For comparison purpose, table 3 summarized the analytical characteristics of similar catalytic methods for simultaneous determination of Fe and Cu, most of them automated with flow techniques. Only two of these methods are based on the catalytic hydroxylamine oxidation. The first one [12] developed for the thermometric determination of Cu, was not automated and presents higher LOD (50 µg L^{-1}) than the present method (0.95 µg L^{-1}). The second one [13], used spectrophotometric detection was automated with FIA and used. This method presents higher sensitivity and lower limit of detection (2 µg L^{-1}), works at higher temperature (45ºC) and has lower reaction time (60 sample h^{-1}) than the proposed method, however it used only for Fe determinations.

The method proposed by Lunvongsa et al.[6] presents very low limit of detection for both analyte (0.05 and 0.02 µg L^{-1} for Cu and Fe respectively) and relatively high sample throughput. However, it requires two different flow systems for measuring both analytes and work at higher temperature than that proposed in the present work.

Other two methods reported for simultaneous determination of Cu and Fe has been included on table 2 for comparison, however these methods are not catalytic, they are based complex formation. Ohno et al.[5] proposed a Lab-on-valve (LOV-SIA) system, however, besides its advantages of this flow technique modality this method present higher limit of detection and lower analytical frequency than the presented method. Paipa et al. [4] proposed a methods not automated and with very high limit of detection (0.02mg L^{-1} for Fe and 1.14mg L^{-1} for Cu), low sensitivity and requires that samples contents a higher concentration of Cu respect to Fe, limiting its applicability. Durukan et al [7] presented a method with a very low LOD, however, it needs very high temperature for the reaction (70ºC), which involves more sophisticated and expensive instrumentation, and the risk of bubble generation inside the flow tubes. Three of this methods were automated with flow techniques, SIA [5], FIA [6] and FI-CPE [7], nevertheless, the sample throughput obtained for the Chip-MPFS is significantly higher than those obtained for these methods.

3.4 Interference study

In the proposed procedure for Cu(II) determination, concentration of Fe (III) similar or higher to Cu(II) concentration increases the analytical signal in approximately 35 %. This effect was not linear dependent of Fe(III) concentration and it was offset using a mixture of Cu(II) and Fe(III) at the same concentration as standard solutions for calibration. However, this positive interference of Fe disagree with Jardin and Jarbas [11], who observed an inhibit effect of this metal in the hydroxylamine oxidation catalyzed by Cu. Regarding the procedure selective to Fe(III), using buffer containing EDTA, Claderas et al [13] reported that Co(II), Cr(III), Cu(II) at concentration of 40, 40 and 10 time higher than Fe(III) cause an analytical signal increment higher than 10%. These ratios between Fe and these potential interferences are seldom found in natural samples or pharmaceutical formulations. Other potential interferences of this variant are Ca(II) and Mg(II) which are presented in very high concentrations in samples such as very hard waters or demolition leachate. Due to the interest of applying this method on different water sample, interferences of Ca(II) and Mg(II) in the catalytic reaction were studied in details, together with the Mn(II) which are presented in relative high concentration in pharmaceutical formulations. For this, 150 µg L^{-1} standard analytical signals of Fe and Cu were evaluated with various concentrations of Ca , Mg and Mn. Increments in the concentration of Ca between 10 and 1000 µg L^{-1} and from 25 to 5000 µg L^{-1} for Mg caused a significant increase in the analytical signal corresponded to Fe. However, the analytical signal kept stable for higher concentrations of these ions. For that reason, 1 mg L^{-1} of Ca(II) and 5 mg L^{-1} of Mg(II) were added to each standard solution of the calibration curve for the measurement of samples with high concentration. Similar interfering effect was observed for the Mn, when the analytical signal was increased in the presence of 150 µg L^{-1} of this metal. However, due to the low concentration of Mn (II) in samples matrix correction for this analyte was not necessary. To the best of our knowledge this kind of interference were not reported before, however deeper investigation on the theme is out site of the scope of this communication.

3.5 Validation and applications

The proposed Chip-MPFS method was applied to the sequential determination of Fe and Cu in demolition leachate, wastewater and pharmaceutical samples (Tables 4 and 5). Fe(III) and Cu(II) content in the quality control material of wastewater (SPS-WWW2, batch 106, Spectrapure Standards, Norway) obtained with the Chip-MPFS method were in agreement with those reported. Not significant differences between these values were confirmed by the t-Student test for means comparison, being the t-observed (2.5 for Fe and 2.7 for Cu) lower than t-critical (2.92) for 95% of confidence (table 4).

Demolition leachate samples were analyzed with the proposed method (table 4). Two concentration levels (20 and 30 µg L^{-1}) for Cu(II) and (75 and 100 µg L^{-1}) for Fe(III) respectively were spiked into each samples to evaluate the matrix effect on the reaction. The recoveries obtained were between 91 and 103% for all samples and both elements. Furthermore, the method was also validated by comparison with a reference method (ICP-OES) and not significant differences were found at a 95% confidence level (t-obs< t-crit= 2.92). However, in case of Fe(III), one of the spiked sample (Leachate water 1 +100 µg L^{-1}) the t-observed (4.0) was slightly higher than 2.92 but lower than 6.96, which corresponds to the t-critical for 99% of confidence level.

The proposed method was also applied to pharmaceutical samples analysis. Tables 5 summarize the results of Cu and Fe in pharmaceutical samples. In all cases, the t-observed was lower than 2.92. Then, no significant differences between the values obtained by the proposed method and reported were found at the 95% confidence level.

4. Conclusions

In this study, the effectiveness of coupling of an MPFS with a monolithic flow device (Chip) for the automation of kinetic-catalytic analytical methods has been demonstrated. The proposed procedure allowed the very sensitive and accurate determination of µg L^{-1} of Fe and Cu based in their catalytic effects on the same reaction (hydroxylamine oxidation).To the best of our knowledge this is the first proposed method based on this reaction for the simultaneous determination of these two metals. The chip design included two reaction coils with different thermostatic zones allowing different temperature control for each involved reaction (catalytic and indicative reaction).The analytes discriminations were achieved by changing the buffer composition and the addition of a complexing agent using a very simple manifold configuration. The method was satisfactory performed for Fe and Cu determinations in wastewater, demolition leachate and pharmaceutical samples. It was validated using a certified reference material of wastewater, a reference method (ICP-OES) and spike recovery study. The benefit of the use calibration solutions with the same concentration of both analytes for the compensation of interferences effect was confirmed by the accurate results for Cu and Fe in different samples.

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Table 1 Analytical procedure for the determination of Fe(III) and Cu(II).

Step	Function	Operations
1	Dispense sample	SMP1 [0.7mL, 4.0mL/min]
2	Clean the chip	SMP3 [1.5mL, 5.0mL/min,] V[a] [On/Off]
3	Dispense sample, buffer and NH_2OH	SMP1 [0.075mL, 3.0mL/min] – SMP2 [0.075mL, 3.0mL/min] – SMP3 [0.075mL, 3.0mL/min] V[a] [On/Off]
4	Wait [b]	
5	Start measurement	Start absorbance measurement at 542nm, with correction at 680nm
6	Dispense mixture with Griess reagent	SMP3 [0.20mL, 2.5mL/min] V[a] [On/Off]– SMP5 [0.20mL, 2.5mL/min]
7	Clean the chip	SMP3 [1.5mL, 5.0mL/min,] V[a] [On/Off]
8	End measurement	

[a] V [On] Phosphate buffer propeller for Fe determination; V [Off] Borate buffer propeller for Cu determination.

[b] 60s for Fe determination and 30 s for Cu determination

Table 2 Optimum condition of the Chip-MPFS system for determination of Fe(III) and Cu(II)

Parameter	**Optimum values**	
	Fe(III)	**Cu(II)**
Buffer solution (mol L^{-1})	0.1 Phosphate +0.01 EDTA	0.1 Borate
pH	12.5	12.5
Reaction time (s)	60	30
[NH_2OH] mol L^{-1}	0.075	0.075
Griess reagent (%)	0.3	0.3
Reaction temperature (°C)	40	40
Sample volume(µL)	75	75
Flow rate of reaction (mL min^{-1})	3	3

Table 3 Figures of merit for determination of Fe(III) and Cu(II). Comparison with similar reported methods.

Ref	Reaction	λ (nm)	System	Samples	LOD (μg L^{-1})	Working rang (μg L^{-1})	RSD %	T (ºC)	Throughput (h^{-1})
[12]	NH_2OH+O_2		batch		50 (Cu)	50-500	6		
[13]	NH_2OH+O_2	542	FIA	natural waters and white wine	2 (Fe)	3.5-150	2.4	45	60
[6]	N,N-dimethyl-p-phenylenediamine +1,3-phenylenediamine	650	FIA	tap and river water	0.05 (Cu)	0-8	1.4	50	24
					0.02 (Fe)	0-2	1.5	50	27
[5]	2-(5-bromo-2-pyridylazo)-5-[N-n-propyl-N-(3-sulfopropyl)amino]aniline +Cu(II) or Fe(II)	580	LOV-SIA	artificial mixture and industrial wastewater samples	50 (Cu)	100-2000	2		18
		558			25 (Fe)	100-5000	1.8		18
[4]	5-sulfosalicylic acid + ammonia	423.5	batch	pregnant liquid	1140 (Cu)	3.8-250 (mg L^{-1})	1.3		
		488.5			100 (Fe)	0.30-20 (mg L^{-1})	1.2		
[7]	Triton X-114 non-ionic surfactant + Eriochrome Cyanine R		FI-CPE	food and water sample	0.57 (Cu)	1.0-35	2.3	70	
					0.33 (Fe)	1.5-25	1.9		
Present work	NH_2OH+O_2	542	Chip-MPFS	wastewater and pharmaceutical samples	0.95 (Cu)	3-75	3	40	39
					21 (Fe)	70-350	2	40	32

Table 4 Determination of Cu(II) and Fe(III) in demolition leachate samples using the Chip-MPFS method

Sample	Analyte	Added (µg L^{-1})	Found (µg L^{-1})	Recovery	Reference method ICP-AES (µg L^{-1})	Recovery	t-obs <2.92
WastewaterRef (SPS)	Cu(II)	0	1850 ± 93		2000± 10[a]		2.7
	Fe(III)	0	4850 ± 100		5000 ± 25[a]		2.5
Leachate water 1	Cu(II)	0	2.7 ± 0.2		2.8 ± 0.5		0.3
		20	21 ± 1	92%			
		30	30 ± 2	91%	31 ± 2	94%	0.6
	Fe(III)	0	<LOD		10 ± 2		
		75	75 ± 6	100%			
		100	100 ± 3	100%	116 ± 6	106%	4.0
Leachate water 2	Cu(II)	0	1.3 ± 0.9		1.3 ± 0.5		0.0
		20	21.8 ± 0.3	103%			
		30	32 ± 5	102%	29 ± 3	92%	0.9
	Fe(III)	0	69 ± 16		70± 1		0.1
		75	145 ± 6	101%			
		100	164 ± 9	95%	173 ± 7	103%	1.3

[a] This value corresponds to the value reported for the certified reference material (SPS-waste water sample).

Rec: Percentage of recoveries of Add-Recovery test obtained for the Chip-MPFS method

t-obs, values of t-student obtained in the comparison of found values with the proposed method and those found with the reference method for 95% of confidence (t critic 2.92)

Table 5 Determination of Cu(II) and Fe(III) in pharmaceutical samples using the chip-MPFS method

pharmaceutical samples	Ion	Found (mg g^{-1})	Reported (mg g^{-1})	t-obs <2.92
Supradyn	Cu(II)	0.206 ± 0.016	0.187 ± 0.009	1.8
	Fe(III)	2.9 ± 0.01	2.9 ± 0.1	0.4
Hidropolivit	Cu(II)	0.507 ± 0.003	0.528 ± 0.026	1.4
	Fe(III)	1.03 ± 0.01	1.06 ± 0.05	0.7
Labcatal	Cu(II)	52 ±0.002[a]	50.0±0.5[a]	2.4
	Fe(III)	152 ± 6[b]	150 ± 1.5[b]	0.6

[a] These values corresponds to µg L^{-1}

[b] These values corresponds to µg L^{-1}, 150 µg L^{-1} of Fe were spiked into the sample in order to evaluated the procedure in this matrix sample.

Figure Caption

Fig 1. Manifold of the analytical system, Chip-MPFS, used for the sequential determination of Fe(III) and Cu(II). V: solenoid valve, SMP: solenoid micropumps, D: detector.

Fig 2. Buffer composition effect in the kinetic curves obtained using Cu (100 µg L^{-1}) and Fe (250 µg L^{-1}): (A) 0.1 mol L^{-1} of $B_4O_7^{2-}$ buffer, pH=12.5, and (B), 0.1 mol L^{-1} of HPO_4^{2-} buffer, pH=12.5, plus 0.01 mol L^{-1} of EDTA. Working conditions: 0.1 mol L^{-1} of NH_2OH, 0.3 % of SPA, 0.3 % of NED, 75 µL of sample dispensed at 3 mL min^{-1}, and at room temperature.

Fig 3. Effect of NH_2OH concentration in the determination of Fe and Cu with the Chip-MPFS.

Fig 4. Peaks profile and calibration curve obtained with the Chip-MPFS (A) for Fe determination using phosphate buffer + EDTA and (B) for Cu determination using borate buffer.

Supplementary material figure caption

Table S1 Characteristics of different multivariate experimental designs used for the optimization experimental conditions for Fe(III) and Cu(II) determination with the Chip-MPFS.

Fig 1

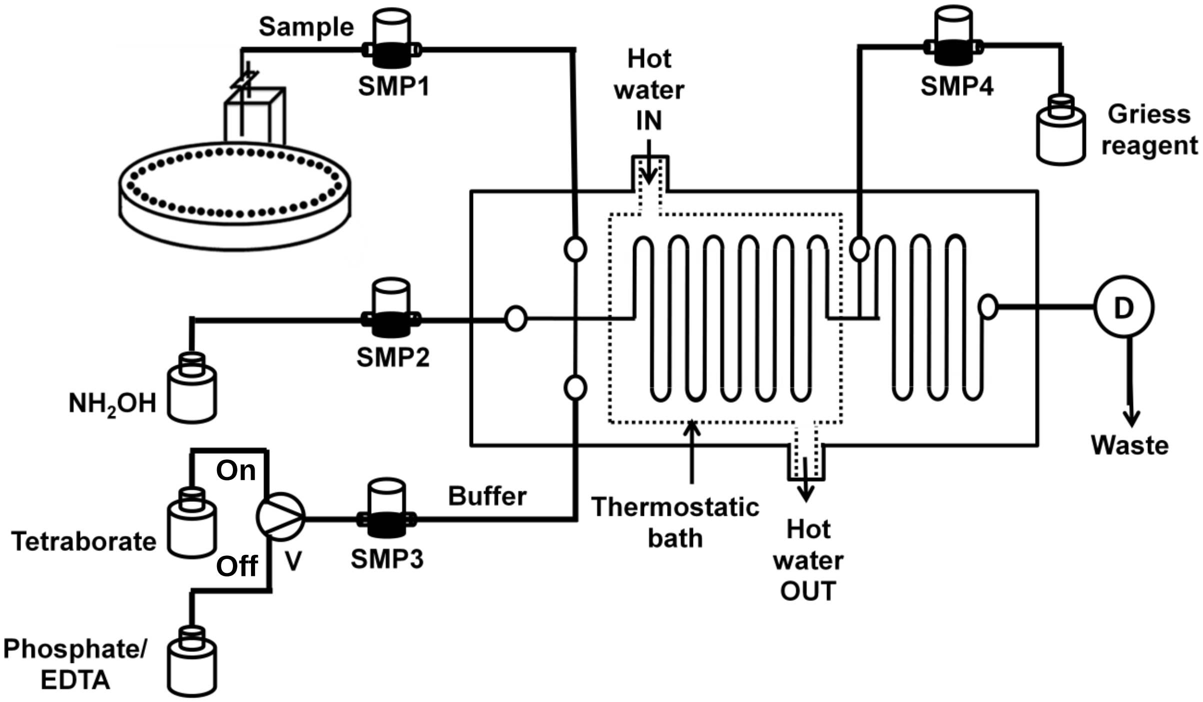

Sample
SMP1
Hot water IN
SMP4
Griess reagent
SMP2
NH$_2$OH
D
Waste
On
Tetraborate
Off
V
SMP3
Buffer
Thermostatic bath
Hot water OUT
Phosphate/ EDTA

Fig 2

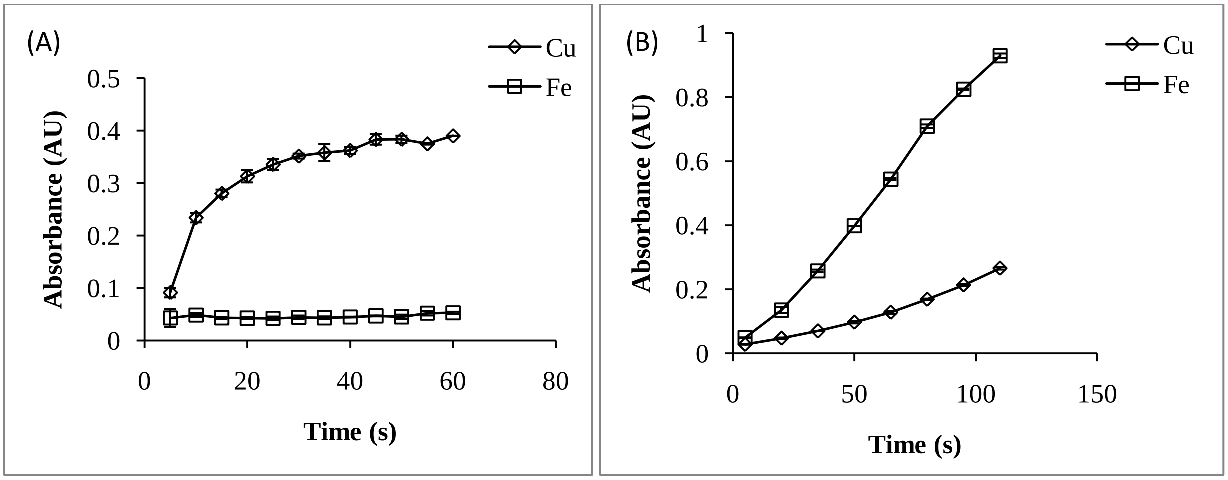

(A)
Cu
Fe
Absorbance (AU)
0.5
0.4
0.3
0.2
0.1
0
0
20
40
60
80
Time (s)
(B)
Cu
Fe
Absorbance (AU)
1
0.8
0.6
0.4
0.2
0
0
50
100
150
Time (s)

Fig 3

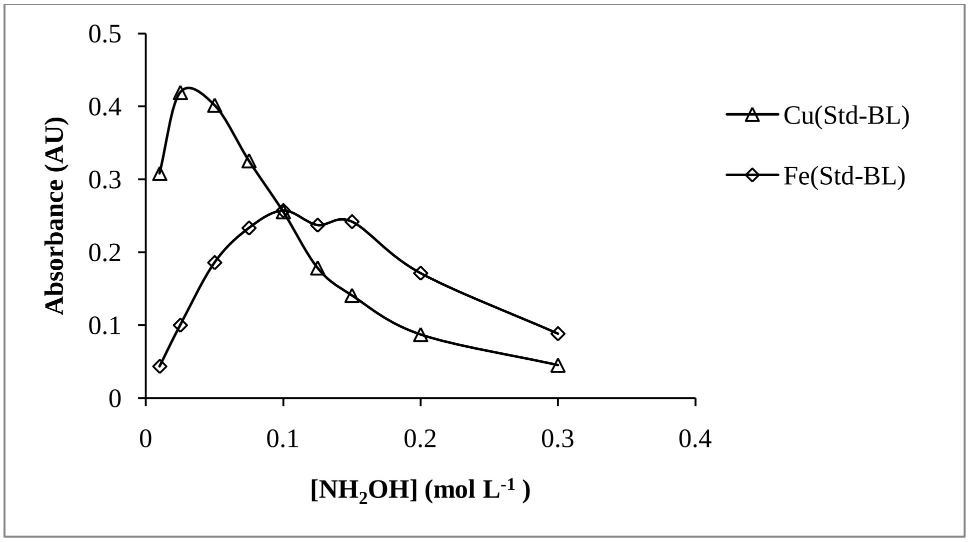

0.5
0.4
0.3
0.2
0.1
0
Absorbance (AU)
0
0.1
0.2
0.3
0.4
[NH_2OH] (mol L^{-1})
Cu(Std-BL)
Fe(Std-BL)

Fig 4

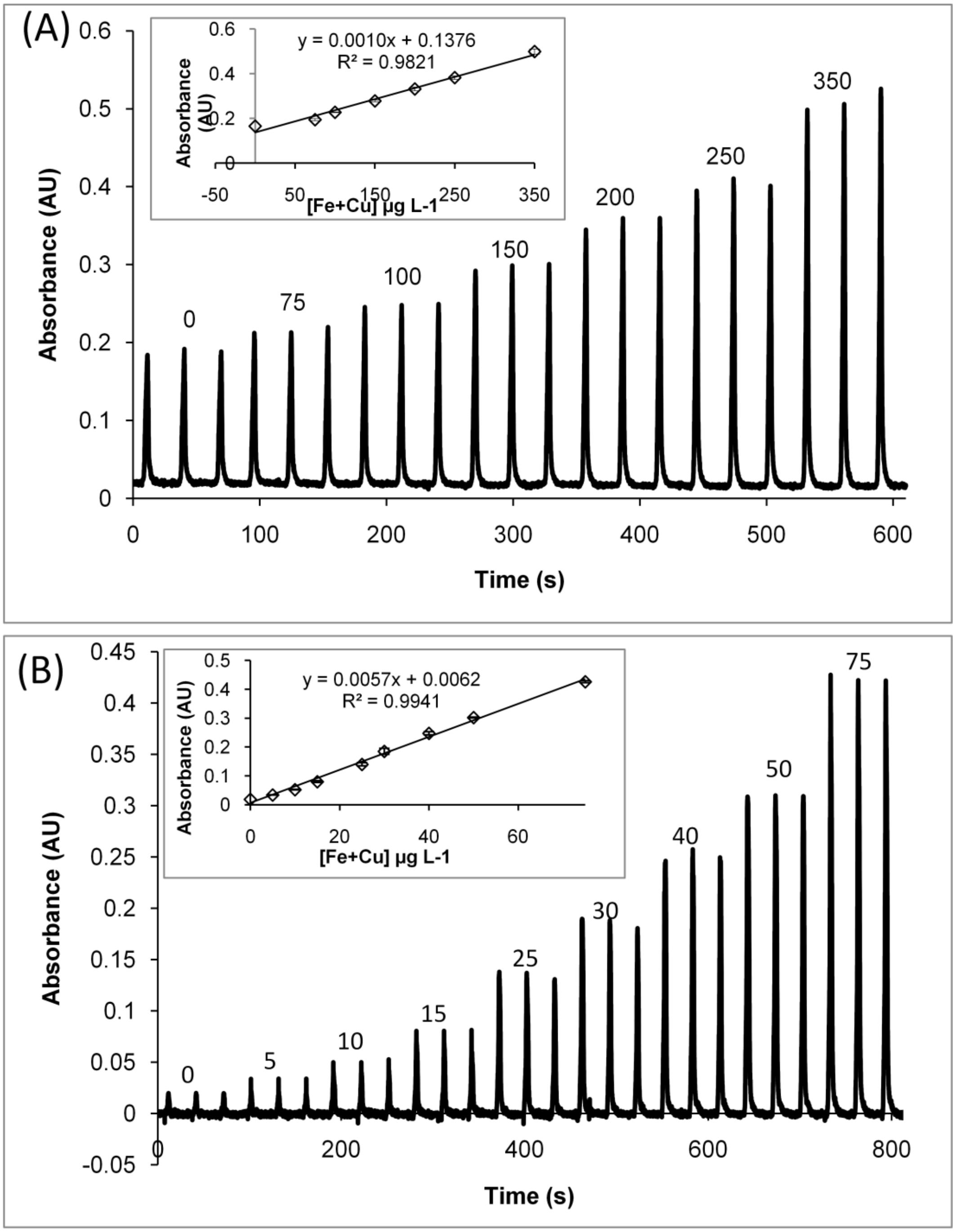

(A)
y = 0.0010x + 0.1376
R² = 0.9821
Absorbance (AU)
[Fe+Cu] µg L-1
0
75
100
150
200
250
350
Absorbance (AU)
Time (s)
(B)
y = 0.0057x + 0.0062
R² = 0.9941
Absorbance (AU)
[Fe+Cu] µg L-1
0
5
10
15
25
30
40
50
75
Absorbance (AU)
Time (s)

Fig 1

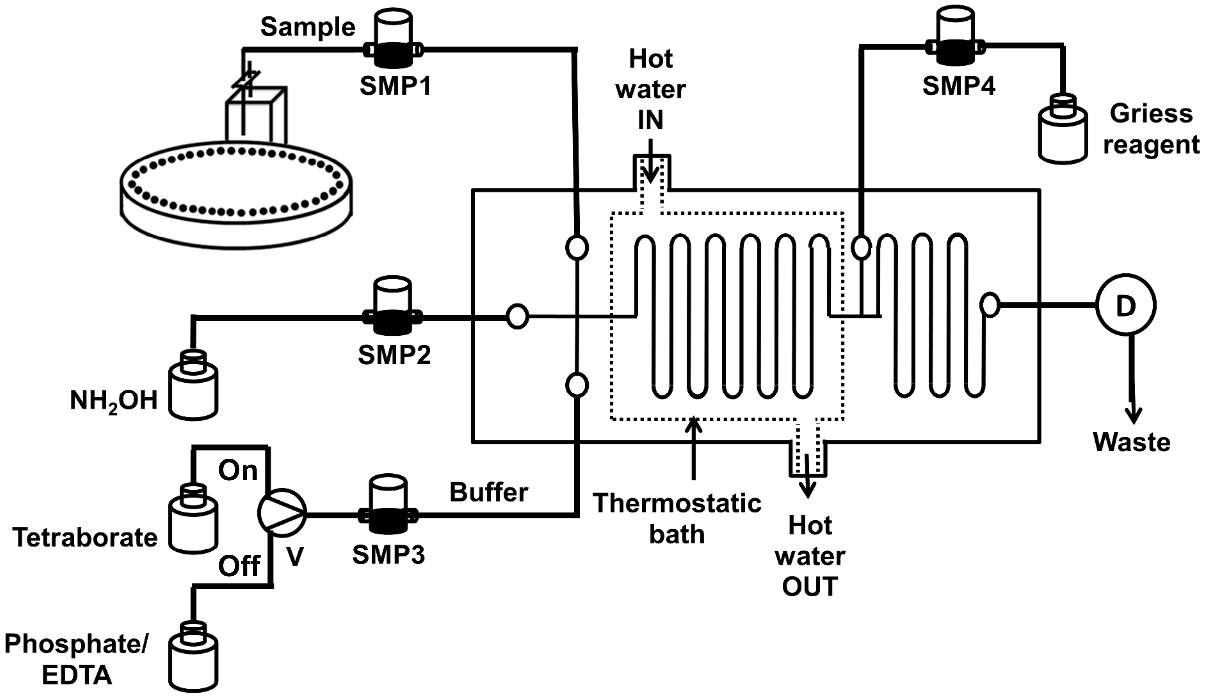

Sample
SMP1
Hot water IN
SMP4
Griess reagent
SMP2
D
NH$_2$OH
Waste
On
Tetraborate
Buffer
Thermostatic bath
Hot water OUT
V
SMP3
Off
Phosphate/ EDTA

Fig 2

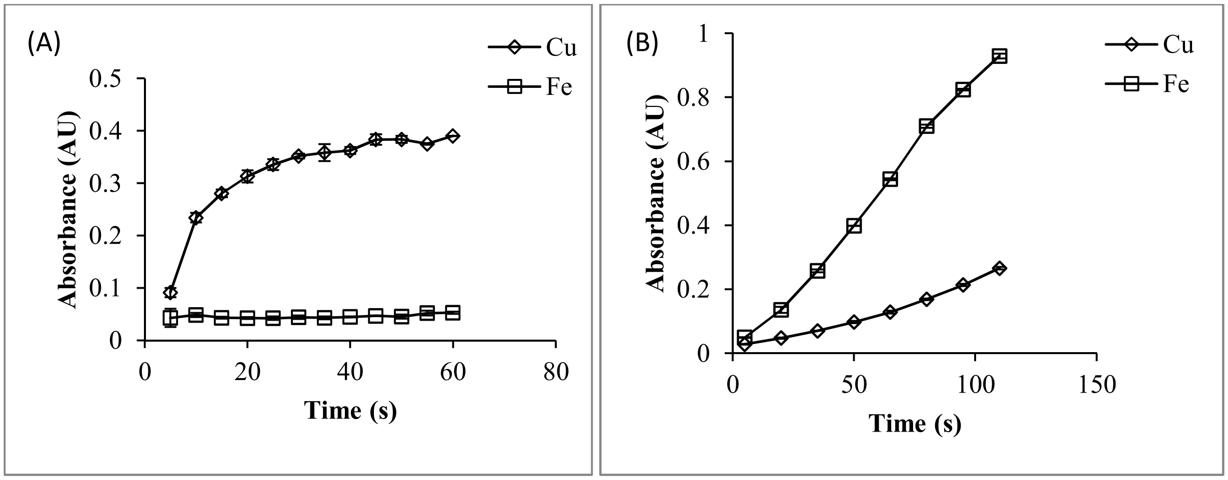

(A)
Cu
Fe
Absorbance (AU)
0
0.1
0.2
0.3
0.4
0.5
0
20
40
60
80
Time (s)
(B)
Cu
Fe
Absorbance (AU)
0
0.2
0.4
0.6
0.8
1
0
50
100
150
Time (s)

Fig 3

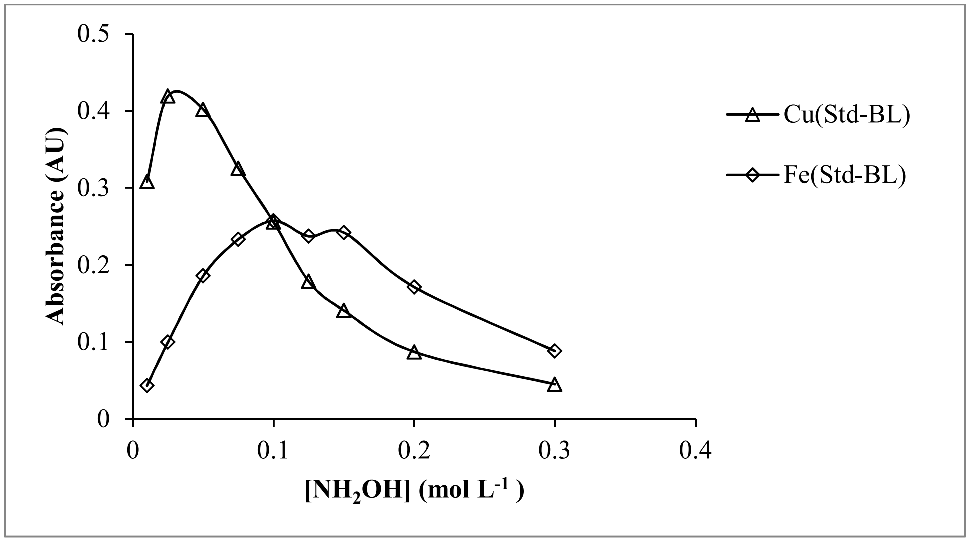
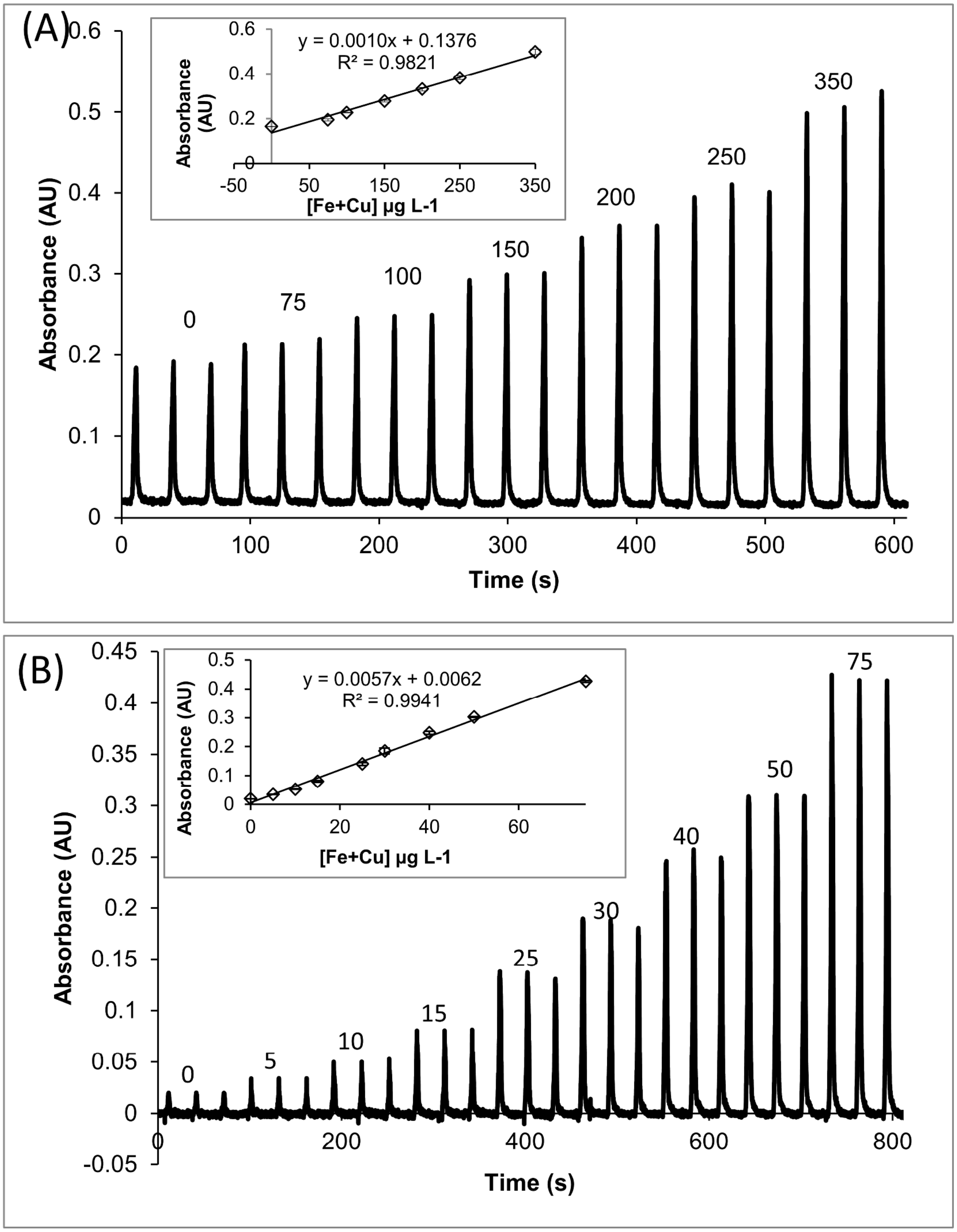

0.5
0.4
0.3
0.2
0.1
0
Absorbance (AU)
0
0.1
0.2
0.3
0.4
[NH$_2$OH] (mol L^{-1})
Cu(Std-BL)
Fe(Std-BL)

Fig 4


(A)
Absorbance (AU)
0.6
0.5
0.4
0.3
0.2
0.1
0
0
100
200
300
400
500
600
Time (s)
0
75
100
150
200
250
350
Absorbance (AU)
0.6
0.4
0.2
0
-50
50
150
250
350
[Fe+Cu] µg L-1
y = 0.0010x + 0.1376
R² = 0.9821
(B)
Absorbance (AU)
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
-0.05
0
200
400
600
800
Time (s)
0
5
10
15
25
30
40
50
75
Absorbance (AU)
0.5
0.4
0.3
0.2
0.1
0
0
20
40
60
[Fe+Cu] µg L-1
y = 0.0057x + 0.0062
R² = 0.9941