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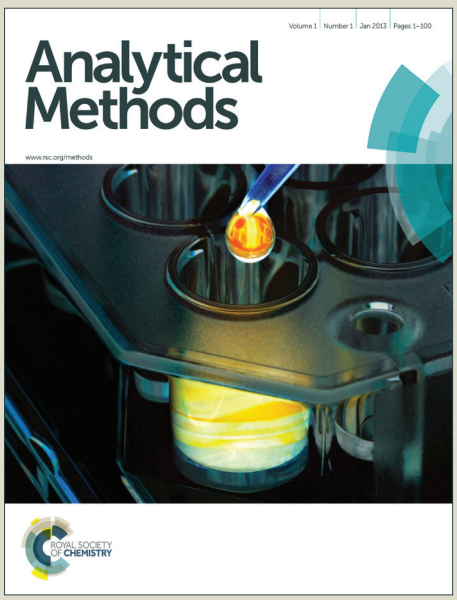

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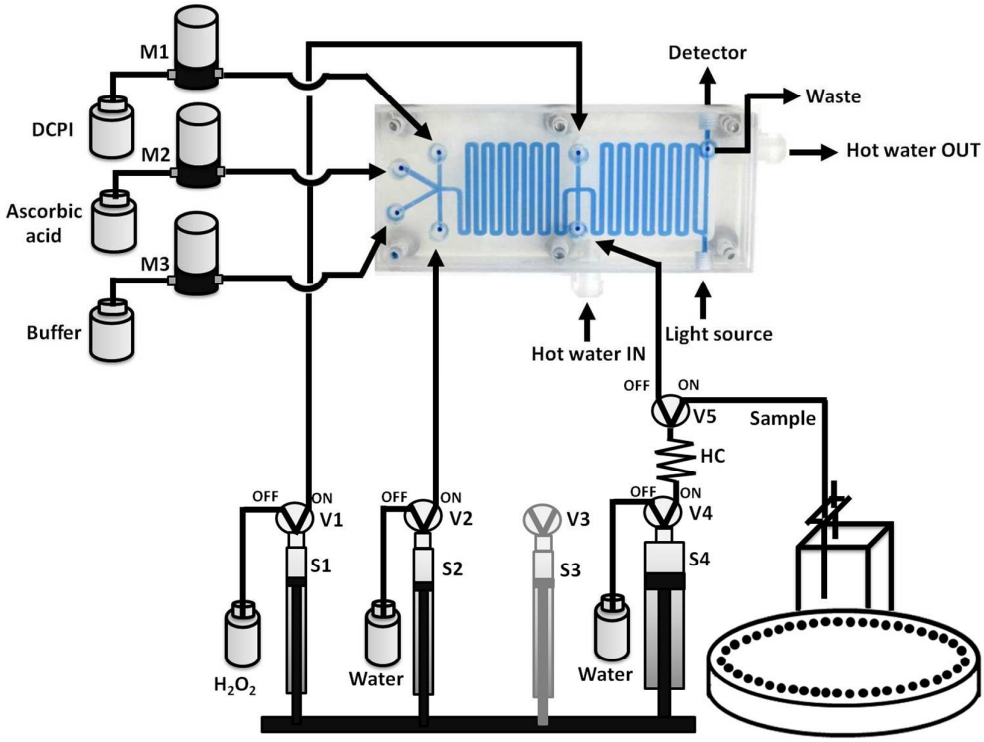

Automated in-chip-catalytic-spectrophotometric method for determination of copper(II) using a multisyringe flow injection analysis-multipumping flow system (Chip-MSFIA-MPFS)

Automated in-chip-catalytic-spectrophotometric method for determination of copper(II) using a multisyringe flow injection analysis-multipumping flow system

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Abstract

In this work a fully automated catalytic-spectrophotometric method for determination of copper at trace level using a multisyringe flow injection system (MSFIA) and a multipumping flow system (MPFS) coupled to a micro-chip (Chip-MSFIA-MPFS) is presented. The reaction is based on the catalytic effect of Cu(II) on the oxidation of the *in situ* reduced form of 2,6-dichlorophenolindophenol $(DCPI)_r$, by hydrogen peroxide. Due to the importance of the mixing order of the reagents, a new design of the chip is proposed. The DCPI, ascorbic acid and buffer are first propelled to mix in the front section of the chip using MPFS. Then this reagent mixture, together with hydrogen peroxide and sample, are simultaneously dispensed to the rear section of the chip by a multisyringe for mixing, heating and absorbance measurement of the product at 600 nm. The optimum conditions are 0.9 mmol L^{-1} DCPI, 3.6 mmol L^{-1} ascorbic acid, 0.8 mol L^{-1} ammonium chloride buffer, pH 10.5, and 0.3 mol L^{-1} H_2O_2. The proposed system is simple, rapid, selective and sensitive. We can determine trace levels of Cu(II) at room temperature (25 °C). The main analytical characteristics of the proposed method are detection limit of 0.12 µg L^{-1} of Cu(II), working range of 0.4-35.0 µg L^{-1} of Cu(II), and relative standard deviation of 0.79 % (10 µg L^{-1} Cu(II), n = 15). The system was successfully applied to water samples, certified reference material (CRM) of river and waste water, acid digested multivitamins and animal tissues with a sample throughput of 31 injections h^{-1}.

Keywords: automated, catalytic spectrophotometric, chip, multisyringe, multipumping

Introduction

Copper is an essential micronutrient for living organisms. It is necessary for growth and is present in various proteins involved in biological processes [1-3]. WHO (1996) has estimated that the average copper requirement is 12.5 µg kg^{-1} body weight per day for adults and 50 µg kg^{-1} body weight per day for infants [4]. The intake of copper in human is *via* water, food, or skin contact [5, 6]. However high copper level has toxic effects [1, 2, 7] and over a long-term may cause vomiting and liver damage [4, 8]. Copper is released to the environment from many sources, such

as fertilizers, wood preservatives, fungicides, algaecides or corrosion of copper water pipes. Increased copper levels in environment can be dangerous for water plants, fish and invertebrates, which can bioaccumulate Cu and incorporate it into the food chain [4, 9]. Generally, copper concentration in natural water is very low (μg L^{-1} level) [4, 8]. Therefore, sensitive methods are required for measurements of trace amounts of copper in environmental samples.

There are several sensitive techniques for trace copper determination, such as voltammetry [10, 11], flame atomic absorption spectrometry (FAAS) [6, 9], inductively coupled plasma-atomic emission spectrometry (ICP-AES) [12, 13], and inductively coupled plasma-mass spectrometry (ICP-MS) [14, 15]. However, these methods require expensive instrumentation, e.g. ICP-MS, ICP-AES. The cost of FAAS is much less than ICP, but needs a pre-concentration step, e.g. liquid-liquid extraction [6], cloud point extraction (CPE) [16], solid phase extraction (SPE) [17], in order to measure at μg L^{-1} levels with appropriate precision. Voltammetry is also capable but often faces interferences. Therefore some researchers tried to find procedures for increasing selectivity [18, 19]. For example, Fu et al. proposed three-dimensional L-cysteine self-assembled monolayers (SAM) functionalized gold nanoparticles/single-walled carbon nanotubes/glassy carbon electrode (L-cys/AuNPs/SWCNTs/GCE). They applied this electrode to improve the performance of detecting trace Cu(II) in stripping voltammetry for determination of Cu(II) in natural water samples [19]. Catalytic spectrophotometry on the other hand is a simple, sensitive, robust and very inexpensive method for trace level determination of Cu(II) [20-23]. Recently, a number of kinetic methods which were developed for determination of trace Cu(II) concentration have been reported [21-28].

Flow techniques are widely used in chemical analysis due to its many advantages, such as low volumes of sample and reagents (μL scale), greater reproducibility, and the possibility of measurement without the need to attain the final equilibrium state. Multisyringe Flow Injection Analysis (MSFIA) is a flow technique that includes the advantages of Flow Injection Analysis (FIA), in terms of efficiency of mixing, and of Sequential Injection Analysis (SIA) with regards to robustness and versatility [29, 30]. MSFIA is an automated system, completely controlled through computer software which includes data acquisition and a very precise control of operation time. Therefore, it is a very appropriate flow technique to automate kinetics measurements. The efficient and highly reproducible mixing of solutions obtained with MSFIA are the critical requirements needed for kinetic-catalytic determinations [31, 32]. Moreover the combination of MSFIA with a multipumping flow systems (MPFS) increases versatility of these flow techniques [33, 34]. The use of solenoid micropumps allows large liquid volumes to be propelled in a short time. These small devices are low-cost, robust, and can be employed for both sample-reagent introduction and manifold commutation. Furthermore, the pulsating flow generated by micro-pumps promotes better mixing of solutions than other flow techniques [34]. For these reasons, MSFIA-MPFS has been proposed as a more efficient and versatile combination of flow methods [34].

Kinetic-catalytic analytical methods require a strict control of experimental conditions, especially the temperature during the reaction progress and measurement. However it is not common to find thermostated detection flow cell. Therefore, we have designed a microfluidic device, called “Chip”, which is combined with flow techniques for the automation of kinetic-catalytic methods.

Abouhiat et al. presented a Chip-MSFIA for the spectrophotometric-catalytic determination of iodide based on the Sandell-Kolthoff reaction [31]. Later, Phansi et al. proposed another chip design which included the detection cell in a thermostated zone [32]. This new configuration allows the use of the initial rate determination method. This Chip-MSFIA method was applied to spectrophotometric-catalytic determination of Mo(VI) in water samples and medicinal products. In general, these chips are made of poly(methylmethacrylate) (PMMA) and integrates different steps in the analytical procedure, such as the confluent point, the mixing coil, and the detection cell. All these are on a thermostated chamber to control the temperature during measurement [32, 35]. The automated Chip-MSFIA system has proved to be very useful for catalytic reactions for obtaining high sensitivity, accuracy, and precision measurements [31, 32, 35].

In this work, a Chip-MSFIA-MPFS is proposed for the spectrophotometric-catalytic determination of Cu(II) based on its catalytic effect on the oxidation of reduced form of 2,6-dichlorophenolindophenol $(DCPI)_r$ by hydrogen peroxide in a NH_3/NH_4Cl buffer [20]. In this process the order of addition and mixing of the reagents is very important towards the products formation. DCPI is first reduced with ascorbic acid, with a colour change from dark blue to colourless. This reduced compound is then oxidized by H_2O_2, with Cu(II) as a catalyst, to give a blue-violet product. Therefore, a new design of the chip, specially adapted for this reaction was constructed and coupled to a MSFIA-MPFS system. This allowed the determination of Cu(II) in water, certified reference material (CRM) of river and waste water, and acid digested multivitamins, and animal tissues.

Experimental section

Reagents and standards

All chemicals were of analytical reagent grade. Milli-Q water (Milli-Q plus, 18.2 MΩ cm^{-1}) was used for preparing the standard and reagent solutions. Working Cu(II) standard solutions were prepared by appropriate dilution of stock AAS grade 1000 mg L^{-1} Cu(II) solution (copper(II) nitrate in 0.50 mol L^{-1} HNO_3, Scharlau, Spain). 1 mmol L^{-1} 2,6-dichlorophenolindophenol (DCPI) stock solution was prepared by dissolving 73 mg of 2,6-dichlorophenolindophenol sodium salt hydrate (Fluka, Switzerland) in 250 mL of a solution containing 210 mg $NaHCO_3$ L^{-1} (Probus, Spain). The DCPI solution was kept in a refrigerator prior to use [20]. The 10 mmol L^{-1} ascorbic acid solution was prepared by dissolving 440 mg of ascorbic acid (Scharlau, Spain) in water and made up to 250 mL. A 0.30 mol L^{-1} H_2O_2 solution was prepared daily by appropriate dilution of a concentrated solution (9.97 mol L^{-1}, Scharlau, Spain). Ammonia-ammonium chloride buffer solution was prepared by mixing appropriate quantities of 2 mol L^{-1} NH_3 solution and 2 mol L^{-1} NH_4Cl, and made up volume with Milli-Q water [36]. A 2 mol L^{-1} NH_3 was prepared from concentrated (14.80 mol L^{-1}) ammonia solution (Scharlau, Spain) solution. A 2 mol L^{-1} NH_4Cl was prepared by dissolving 5.35 g of NH_4Cl (Panreac, Spain) in 50 mL of water.

Reagents for interference study

Appropriate solutions of Fe(III), V(V), and Cd(II) were prepared from AAS grade stock 1000 mg L^{-1} Fe(III), V(V), and Cd(II), respectively (Scharlau, Spain). A stock solution of Fe(II), 1000 mg L^{-1}, was prepared by dissolving 1404 mg of ammonium iron(II) sulphate 6-hydrate (Panreac, Spain) in

water and made up to 20 mL. Stock solution of Mg (II), 1000 mg L^{-1}, was prepared by dissolving 203 mg of $MgSO_4.7H_2O$ (Probus, Spain) in 20 mL Milli-Q water.

Sample collection and preparation

Rain water was obtained from domestic Mallorca cisterns. The water is used for drinking and household use. Samples of demolition leachate wastewater were collected from different areas of Mallorca. All 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 acetate membrane (Sartorious Stedium Biotech, Germany).

A Certified Reference Material of river water (SLRS-4, National Research Council Canada) was adjusted to pH 7.0 before analysis. A stock Quality Control Material of waste water (SPS-WWW2, batch 106, Spectrapure Standards, Norway) was diluted with Milli-Q water, and adjusted to pH 7.0.

Two multivitamin samples, Multicentrum and Pharmaton®, were used for method validation. One tablet of Multicentrum contains 500 µg of copper (Multicentrum 1.39 ± 0.01 g/tablet), whereas Pharmaton has 1000 µg of copper per tablet (Pharmaton® 1.02 ± 0.01 g/tablet). For each product, 10 tablets were powdered in an agate mortar. Then 209 mg of the Multicentrum and 153 mg of Pharmaton® powder were accurately weighed and transferred into 100 mL Teflon digestion vessels. Then 10 mL of ultrapure concentrated HNO_3 (65%) (Scharlau, Spain) were added and the closed vessels 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 multivitamin sample digestion was 1000 W, 200 °C, 30 minutes. The digested solutions were cooled to room temperature and then evaporated to reduce their volume to a small drop. Finally, the volume was adjusted to 100 mL with MilliQ water to obtain a 750 µg L^{-1} and 1500 µg L^{-1} copper solution for Multicentrum and Pharmaton®, respectively. In order to compare results obtained for samples directly diluted with that from digestion, copper medicine solution ("Cobre") were analyzed with only a dilution step or digested using a similar procedure for solid sample, as described previously.

Solutions of pig heart tissues were obtained by microwave digestion. Tissue sections of pig heart were first lyophilized for 36 h. Then 0.500 g of the lyophilized tissues were accurately weighed and transferred into 100 mL Teflon digestion vessel, 8 mL of ultrapure concentrated HNO_3 (65%, Scharlau, Spain) and 2 mL of H_2O_2 (30%, Scharlau, Spain) were added and the closed vessels were placed in the microwave oven. The microwave program was 10 min increasing to 120 °C, 5 min at 120 °C, then 10 min increasing to 200 °C, 15 min at 200 °C, and finally 20 min for ventilation. After digestion, solutions were cooled to room temperature and evaporated to reduce their volume to a small drop. The volume were finally adjusted to 25 mL with HNO_3 2% v/v.

ICP-AES was used as a reference method for the quantification of Cu(II) in samples previously acidified with concentrated HNO_3 (65%, Scharlau, Spain) to 2% v/v. An ICP-AES (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 measurements was 324.752 nm. All measurements were done in triplicate.

Flow conduit chip

The chip was constructed from three rectangular poly(methylmethacrylate) (PMMA) plates, with modification to the design previously reported by Abouhiat et al. [31] and Phansi et al. [32]. One piece, with dimensions 115 x 44 x 10 mm, was used for the flow channels and the other two pieces, 115 x 44 x 10 mm and 115 x 44 x 15 mm, respectively, formed the thermostated unit, as shown in Fig. 1. Two serpentine channels, 1.5 mm wide x 1.0 mm deep x 39 mm long, together with various confluence points, were made using an Alarsis 3-axis PC controlled CNC milling machine (Fig. 1(a)). Threads of ¼" 28 fittings were drilled in this flow plate for connecting to tubings for the reagents, carrier and sample, and to the waste outlet line. At the outlet end of the second serpentine groove a straight channel of 40 mm was made spanning the width of the plate. This formed the detection flow cell. At the two ends of this linear channel two threads of UNF ¼" 36 fittings were drilled for connecting the optical fibers (Fig. 1(a)). As for the other two plates, a rectangular cavity, 45 x 41 x 8 mm for the thinner plate and 45 x 41 x 13 mm for the thicker base plate, was milled out (Fig.1 (b)). These two plates were then glued together so that the two cavities formed a reservoir for the circulation of thermostated water. The grooved plate was then glued, with the grooves facing downwards, to the upper plate of the reservoir so that the outlet section of the chip, with the detection flow channel, was over the reservoir. To glue the various plates together, a thin film of methacrylic acid was first applied, the plates tightly clamped together, followed by UV curing for 1 h.

Flow analyzer

The final automated Chip-MSFIA-MSFS system is shown in Fig 2. A multisyringe piston pump module (model Bu 4S) was purchased from Crison Instruments S.A. (Allela, Barcelona, Spain). The module was equipped with two 1 mL glass syringes (S1, and S2) and one 5 mL glass syringe (S4). Solenoid valves (V1, V2, and V4) allowed connection of each syringe with either the chip (position ON, activated) or with the respective solution reservoir (position OFF, deactivated) for refilling. Solutions in the syringes were H_2O_2 in S1, and Milli-Q water (carrier) in S3 and S4. Furthermore, an external three-way solenoid valve (V5) from Takasago (STV-3 1/4UKG, Nagoya, Japan) was powered and controlled using an auxiliary supply port of the multisyringe module. V5 was used for sample introduction (position ON), and its common position was connected to a holding coil of 255 cm length and 1.00 mm i.d. (2000 µL volume). The MPFS set-up consists of three solenoid micropumps (BIOChem Valve, NJ, USA) with stroke volumes of 8 µL. The MPFS system was used for propelling DCPI (M1), ascorbic acid (M2), and ammonia-ammonium chloride buffer solutions (M3). The flow rate of MPFS is set by the frequency and volume dispensed at each pulse.

All tubings of the flow system were of PTFE with 0.8 mm i.d. The chip has a continuous flow through the cavity of water from a thermostated bath (Selecta, Barcelona) via silicon tubes (ca. 20 cm, 1 cm id). The cavity served as a heated source to accelerate the reaction in the

serpentine channel. For batch analysis, a 45 position autosampler with 10 mL sample vials (Crison Instruments S.A., Barcelona, Spain) was connected to the sampling tube. The multisyringe module and the autosampler were connected in series via a RS232C interface to a PC for remote software control (AutoAnalysis 5.0, Sciware Systems SL, Bunyola, Spain). A DH-2000 deuterium lamp (TOP Sensor Systems, Eerbeek, Netherlands) was used as light source, and an USB-2000 miniature CCD spectrophotometer (Ocean Optics Inc., Dunedin, FL, USA) as the detector.

Analytical protocol and flow method

The procedure for the determination of Cu(II) with chip-MSFIA-MPFS is shown in Table 1. First, the three reagents were mixed (Step 1) by activation of the micropumps M1, M2, and M3 to propel 0.250 mL of DCPI, ascorbic acid, and ammonia-ammonium chloride buffer into the front section of the chip (Fig. 2). During this process the colour of solution in the channel changed from a dark blue colour ($DCPI_{ox}$) to colourless ($DCPI_r$). In Step 2 0.100 mL of carrier (S4), 0.020 mL of carrier (S2), and of H_2O_2 (S1) were rapidly dispensed into the chip to clean out the interconnecting channels between the two serpentine sections of any reagent solution. In Step 3 1.900 mL of the carrier solution in Syringe 4 was pumped through the rear section of the chip to clean out the serpentine channel and the flow cell. Then 0.250 mL of sample was aspirated *via* V5 (Step 4) into the holding coil HC (using the multi-syringe). A 0.05 mL of the sample in the HC was next dispensed (Step 5) into the chip so that this plug at the head of the sample plug, which is diluted by the solution in the tubing between the valve V5 and the chip, would not be mixed with the reagents in the next step. The remaining 0.200 mL of sample and the equivalent volume (0.040 mL) of the mixed reagents (in the first section of the chip) and the hydrogen peroxide (in S1) were next loaded in the detector zone of the chip. The flow was stopped for 5 seconds for the oxidation process to proceed (Step 6) and then 1.900 mL of the reacting solution was pumped through the detector flow channel. The absorbance was then measured at 600 nm, with a reference absorbance measured at 760 nm to eliminate any schlieren effect (Steps 7-9). Finally all 4 syringes were refilled with their reagents with the pistons returned to their initial positions.

Results and discussion

Optimization of the experimental conditions

Multivariate optimization

Fractionated two level (2^{4-1}) factorial design was used to screen the four important parameters for their effects. The concentration of DCPI, ascorbic acid, H_2O_2, and ammonia-ammonium chloride buffer were varied within the experimental range of 0.30-0.50 mmol L^{-1}, 2.00-4.00 mmol L^{-1}, 0.02-0.10 mol L^{-1}, and 0.10-0.50 mol L^{-1}, respectively. These values refer to the concentration in the reagent reservoirs. The temperature was maintained at room temperature (25 °C), buffer pH set at 10.5 and the stopped-time at 5 s. Differences between standard and blank signals could not be observed after 5 seconds using pH less than 10, but standard and blank signals were very different when a pH 10.5 was used. At pH higher than 10.5 the capacity of ammonia-ammonium chloride buffer decreased, therefore pH 10.5 was selected for all experiments.

The measured signals for these experiments were the absorbance peak heights. The *ratio* of the analytical signal of standard 15 μg L^{-1} Cu(II) and blank (standard/blank), and the difference between the absorbance peak height of standard and blank divided by the concentration of the standard *(slope)* ((standard-blank)/15) were selected as response variables in order to obtain the maximal relationship or differences between the catalysed and uncatalysed reactions. Using these two response variables, and taking into account the increasing blank signal, the experimental conditions were obtained with the highest sensitivity.

Fractionated two level (2^{4-1}) factorial design fitted well to a 2-way interaction for both standard/blank and slope observations without any significant lack of fit (r^2=0.99) and a very low absolute error. The statistical results obtained from standard/blank and slope (shown in the supplementary material Table A1) indicated that concentration of DCPI, ascorbic acid, H_2O_2, ammonia-ammonium chloride buffer, interaction between concentration of DCPI and ascorbic acid, and interaction between concentration of DCPI and buffer are significant factors. The analytical response increased at higher H_2O_2 and buffer concentration. For ascorbic acid, the analytical response decreased with the increment of ascorbic acid concentration. This is because an excess of ascorbic acid could reduce the oxidised blue-violet product to the colourless product again. However, a too low ascorbic acid concentration may not be enough to reduce the DCPI to colourless product, so increasing the blank signal. Therefore, the ratio between DCPI and ascorbic acid concentration was selected at 1:4, which is the lowest concentration of ascorbic acid that can completely reduce DCPI to colourless product.

The profiles of predicted values and desirability function were used to define the optimal experimental conditions and the behaviour of both response variables. The analytical response obtained for standard/blank *ratio* was negative, while analytical response obtained for *slope* was positive. This apparent contradiction is due to the increment of blank signal with the DCPI concentration which affects more the *ratio* than the *slope* response variable. The maximum response zone of the profile of predicted values and the desirability function for *ratio* and *slope* correspond to DCPI, and were 0.40 mmol L^{-1} DCPI, 2.0 mmol L^{-1} ascorbic acid, 0.1 mol L^{-1} H_2O_2, and 0.5 mol L^{-1}, ammonia-ammonium chloride buffer. However, the significant interaction between DCPI and ammonia-ammonium chloride buffer concentration require further study of this condition, In this case a Doehlert design was used in a concentration range of 0.40-1.00 mmol L^{-1} for DCPI, and 0.50-1.00 mol L^{-1} for buffer (at 25 °C, 0.1 mol L^{-1} H_2O_2, stopped time 5 s).

Doehlert design fitted well to a 2-way interaction for both *ratio* and *slope* response without any significant lack of fit, and a very low absolute error. The analytical response increased at high concentration of buffer. For DCPI the analytical response were similar to the results in fractionated two level (2^{4-1}) factorial design. The maximum response of the profile of predicted values and the desirability function obtained for *ratio* and *slope* of DCPI were 0.40 and 0.90 mmol L^{-1}, respectively (data shown in the supplementary material Fig. A1 (a) and Fig. A1 (b)). However, the distortion of ratio response with the increasing blank signal with the DCPI concentration led us to select the *slope* as the most appropriate response variable. Therefore, 0.90 mmol L^{-1} of DCPI was selected as optimal. The standard signal increased with buffer concentration but also the blank signal. The maximum response zone of the profile of predicted values and the desirability function obtained for *ratio* and *slope* of buffer are 0.85 and 1.00 mol L^{-1}, respectively. However, buffer concentrations higher than 0.80 mol L^{-1} did not vary

significantly the *slope* (shown in the supplementary material Fig. A1 (b)), and higher buffer concentration increased the blank signal. Therefore, 0.80 mol L^{-1} was selected for this work.

Effect of H_2O_2 concentration

The concentration of H_2O_2 was optimized by an univariate procedure at 0.90 mmol L^{-1} DCPI, 3.6 mmol L^{-1} ascorbic acid, 0.80 mol L^{-1} ammonia-ammonium chloride buffer, pH 10.5, 25 °C, and stopped-time of 5 s. The signal of 15 µg L^{-1} Cu(II) standard and blank (water) were observed. The results indicated that the signal for the standard increased with the concentration of H_2O_2, while the blank signal was not significantly different. Therefore, 0.30 mol L^{-1} H_2O_2 was selected for this work, obtaining a maximum ratio between standard and blank signal which remained constant at higher H_2O_2 concentration, as shown in Fig. 3.

Effect of stopping time

The effect of the stopping time was observed by injecting standard Cu(II) at 0.4-40.0 µg L^{-1} under optimum experimental conditions; 0.90 mmol L^{-1} DCPI, 3.6 mmol L^{-1} ascorbic acid, 0.80 mol L^{-1} ammonia-ammonium chloride buffer, pH 10.5, and 0.30 mol L^{-1} H_2O_2 (at 25 °C). The results indicated that the sensitivity (*slope*) increased when the stopped-time were increased (Fig. 4). On comparing continuous flow with stopped-time of 5 s, the sensitivity of stopped-flow mode was very different from continuous flow. The limit of detection (LOD) obtained for the stopped-flow was much lower than the LOD obtained for continuous flow. The linear range of 0.4-35.0 µg L^{-1} Cu(II) was obtained for both continuous flow and stopping flow for 5 s. A stopped-time greater than 5 s would increase the sensitivity. However, the LOD did not significantly differ between 5 and 20 s stopped-time. The linear range decreased to 1-30 µg L^{-1} Cu(II) for stopped-times 10-12 s. Therefore, a stopped-time of 5 s was selected for this work in order to obtain highest sensitivity, low LOD, high sample throughput, and the widest working range.

Effect of temperature

Temperatures between 25 and 35 °C was studied using standard Cu(II) 0.4-35.0 µg L^{-1} and the optimum reagents conditions: 0.90 mmol L^{-1} DCPI, 3.6 mmol L^{-1} ascorbic acid, 0.80 mol L^{-1} ammonia-ammonium chloride buffer, pH 10.5, 0.30 mol L^{-1} H_2O_2, and stopped-time of 5 s. The thermostated chamber of the chip was used for control of the temperature during the experiments. The calibration equations obtained at 25 °C and 35 °C were $A = (6.4 \pm 0.1)x10^{-3} C + (1.4 \pm 0.3)x10^{-2}$ ($r^2 = 0.997$) and, $A = (7.3 \pm 0.1)x10^{-3} C + (1.8 \pm 0.3)x10^{-2}$ ($r^2 = 0.998$), respectively (A = absorbance, C = concentration of Cu(II) (µg L^{-1})). The limit of detection at 35 °C (LOD=0.09 µg L^{-1}) was only slightly lower than that obtained at 25 °C (LOD=0.12 µg L^{-1}). However at temperature higher than 35 °C, although the sensitivity increased, many bubbles were formed affecting the reproducibility of the measurement. The small difference between the sensitivity and the LOD with temperature and the significant higher reproducibility at the lower temperature indicate that 25°C is the more suitable temperature. Even using room temperature, the thermostated chamber provides a thermal stability to improve the reproducibility.

Analytical characteristics

Analytical features obtained using the proposed Chip-MSFIA-MSFS system are summarized in Table 2. Flow profile and calibration curve (Fig. 5) were obtained under the optimum conditions: 0.90 mmol L^{-1} DCPI, 3.6 mmol L^{-1} ascorbic acid, 0.80 mol L^{-1} ammonia-ammonium chloride buffer, pH 10.5, 0.30 mol L^{-1} H_2O_2, stopped-time 5 s, and 25 °C.

Other analytical methods based on copper kinetic-catalytic redox reaction have been reported [20-22, 24, 25, 37]. Some of these methods are summarized in Table 2. In order to evaluate the advantages and competitiveness of our new proposed method, their characteristics have been compared with those obtained by other similar analytical methods currently reported. Most of these methods are not automated, since they are carried out batch-wise [20, 22, 24-28]. FIA methods has also been used for increasing the degree of automation of analysis [21]. However, reported FIA methods are not fully automated since the samples are manually injected. Prodromidis *et al.* proposed the determination Cu(II) based on the catalytic activity of Cu(II) on the oxidation of leucocompound of the 2,6-dichlorophenol, $(DCPI)_r$, by hydrogen peroxide in ammonia-ammonium chloride buffer solution [20]. The reaction is rapid, sensitive, and has a high tolerance to other metal ions, especially Fe(III) and Mg(II) which are present in water samples. Moreover, the reaction is simple, and could be applied to different types of samples. Therefore, this reaction was selected for this work. The Chip-MSFIA-MPFS improved the sensitivity and precision obtained with batch method due to the full automation and computer control of the system. Besides, the significant effect of temperature is controlled in the chip during both the reaction step and measurement. The reagents and sample are dispensed by the multisyringe module with a very high precision, allowing complete mixing, heating, and measurement inside the chip. The MPFS is a very good system for mixing reagent in a short time due to its turbulent flow and precise flow rate. Therefore, the fully automated Chip-MSFIA-MPFS is a very rapid, sensitive, precision, accuracy, and robustness method for Cu(II) determination in water, multivitamin and tissue samples.

Interference study

The interference effect of some foreign ions in the proposed reaction have been studied in a previous work [20], which showed that only V and Cd interfered significantly. However, water samples in Mallorca have high concentrations of Fe and Mg. Therefore, Fe(III), Fe(II), Mg(II), V(V), and Cd(II) were studied in this work using a Cu(II) standard of 10 μg L^{-1}. The system was found to be tolerant to Fe(III) and Fe(II) up to 1000 μg L^{-1} level, with changes in the absorbance of the catalytic reaction less than 5%. The tolerance ratio of Cu to Fe of 1:100 allows Cu determination in samples such as Mallorca natural water with Fe content lower than 1000 μg L^{-1}. Medicinal samples can also be measured by sample dilution to decrease the Fe interference. The system could tolerate Mg(II) up to 3500 μg L^{-1}. At Mg(II) concentration higher than 3500 μg L^{-1} the analytical signal increased producing a positive error. For V(V), and Cd(II), the tolerance level was 20 μg L^{-1}. However, in general water samples have V(V), and Cd(II) at concentrations lower than this value. The high sensitivity of the present method allows avoiding interference effect by just sample dilution.

The tolerance limit of the Chip-MSFIA-MPFS system is lower than the previous work proposed by Prodomidis et al. [20]. Our new system has higher sensitivity, and the interferences study was carried out with a 10-times lower concentration of Cu(II) standard of 10 μg L^{-1}. This allows the application of the proposed method to a wider diversity of samples, including those with very low concentrations, such as natural waters.

Application and validation

The proposed method has been validated according to the IUPAC directives [38]. For this purpose, two certified reference material of water sample were used, as well as a reference method (ICP-AES), and also spiked samples for the spike-recovery test. The Cu(II) content in CRM (SLRS-4, National Research Council, Canada) obtained using the Chip-MSFIA-MPFS method (1.8 ± 0.1 µg L^{-1}) agrees well with CRM reported value (1.81 ± 0.08 µg L^{-1}). The Cu(II) content in Quality Control Material of waste water (SPS-WWW2, batch 106, Spectrapure Standards, Norway) obtained using our Chip-MSFIA-MPFS method (1999 ± 6 µg L^{-1}) is not significantly different from that QCM reported value (2000 ± 10 µg L^{-1}).

The analysis of rainwater and drinking water samples demonstrates the applicability of the proposed method to different matrix of water samples. The concentration values obtained with the Chip-MSFIA-MPFS agree well with those obtained with ICP-AES, as shown in Table 3. No significant differences were found between these methods at a 95% confidence level by t-test (t-obs<t-crit =2.92). The Cu(II) content in rainwater and drinking water is very low. Therefore three concentration levels 5, 10 and 15 µg L^{-1} Cu(II) were spiked into the samples to evaluate the matrix effect on the reaction. The recoveries obtained were between 94 and 105 %. It should be highlighted that Cu(II) levels are notably high in some collected rain water samples, probably because Cu(II) was released from alloy pipes leading to the reservoir.

Furthermore, the proposed method was applied to multivitamin tablets (Multicentrum and Pharmaton®), multivitamin solution (Cobre), and tissue samples after appropriate sample pre-treatment. The observed Cu(II) value was not significantly different from the reported value for the multivitamin samples. No significant differences were found between these methods at a 95% confidence level by t-test (t-obs < t-crit =2.92) as shown in Table 4. The standard 10 µg L^{-1} Cu(II)) were spiked into the samples to evaluate the matrix effect on the reaction. The recoveries obtained were between 96 and 107 %. Moreover, the Cu(II) value in "Cobre" medicine solutions obtained from direct dilution was similar to Cu(II) level obtained from digestion. This means that the proposed method is not affected by the matrix of this multivitamin product.

Conclusions

This is the first proposed automated method which uses MSFIA-MPFS coupled to a chip for catalytic spectrophotometric determination. The chip is a new design in order to reduce the 2,6-dichlorophenolindophenol (DCPI) prior to its oxidation by hydrogen peroxide reaction catalyzed by Cu(II). The Chip-MSFIA-MPFS system is very suitable method for measurement of trace Cu(II) with high sensitivity (µg L^{-1} level), precision, and accuracy. The reaction is rapid allowing high sample throughput. Moreover, the proposed system is fully automated and controlled by a computer which improves its repeatability, efficiency and robustness. The proposed method was applied to determine Cu(II) in water, multivitamin, and tissue samples. It has been validated using two different certified reference materials of water, a reference method (ICP-AES) and spike-recovery study.

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Appendix, Supplementary material

Supplementary data associated with this article can be found in online version.

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Table 1. Automatic procedure for the determination of Cu(II) with chip-MSFIA-MPFS.

Step	Device	Operation	Flow rate* (mL min^{-1})	MSFIA				MPFS			Comments (time duration, s)
				S1	S2	S4	V5	M1	M2	M3	
1	MP	Pump reagents 0.250 mL	1.200	off	off	off	off	on	on	on	Reduction of DCPI (12.5 s)
2	MS	Dispense 0.100 mL	6.000	on	on	on	off	off	off	off	Cleaning of the interconnecting channels between the two serpentine sections (1.0 s)
3	MS	Dispense 1.900 mL	10.000	off	off	on	off	off	off	off	Cleaning of reaction zone of chip (11.4 s)
4	MS	Pickup 0.250 mL	3.000	off	off	on	on	off	off	off	Loading sample into HC (5 s)
5	MS	Dispense 0.050 mL	1.000	off	off	on	off	off	off	off	Dispensing head zone of sample to chip (3 s)
6	MS	Dispense 0.200 mL	1.000	on	on	on	off	off	off	off	Dispensing of sample, reduced DPCI, and H_2O_2 into detection zone of chip (12 s)
		Wait 5 seconds									Oxidation for 5 s
7	D	Start recording (600, 760 nm)									Start absorbance measurement at 600 nm, with reference absorbance at 760 nm
8	MS	Dispense 1.900 mL	2.000	off	off	on	off	off	off	off	Dispense the reacting solution into detector flow channel and out to waste (57 s)
9	D	Stop recording									
10	MS	Pickup 2.000 mL	12.000	off	off	off	off	off	off	off	Syringes S1-S4 refilled with solutions (10 s)

* Flow rate of S4 (volume 5 mL)

MP: multipumping system, MS: multisyringe system, D: Detector

Table 2. Figures of merit and experimental characteristics of spectrophotometric kinetic-catalytic Cu(II) determination. Comparison with similar reported methods**.**

Reaction	λ_{max} (nm)	System	Method	Type of samples	LOD (µg L^{-1})	Working range (µg L^{-1})	RSD, % (µg L^{-1}, n)	T (°C)	Throughput (h^{-1})	Ref.
2,6-dichlorophenol $(DCPI)_r$ + H_2O_2	562	batch	fixed-time, 5 s	alloys, foodstuffs, pharmaceutical, environmental samples	-	5-50, 50-300	2.5 (30, n=5)	25	-	[20]
Ponceau S + sodiumsulfide	560	batch	fixed-time, 10 min	Tap water, waste water, and Cow's liver tissue	0.4	2-400	2.24 (200, n=7)	25	-	[22]
3-methyl-2-benzolinone hydrazone + H_2O_2	390	batch	fixed-time, 40 min	River water, and ground water	0.27	0-50	3.5 (20, n=12)	35	-	[24]
hexacyanoferrate(III) + cysteine	390	batch	fixed-time	Sea and river water samples	0.15	0-6.35	0.4-3.9	25	-	[23]
hexacyanoferrate(III) + citric acid	420	batch	fixed-time, 30 min	tab water, river water, and seawater	4	6.35-25,400	3.5	60	-	[25]
disodium-6-hydroxy-5-[(4-sulphophenyl)azo]-2-naphtalenesulphonic acid + H_2O_2	478	batch	initail rate, first period 6 min	Fruit, wine, and milk samples	5	16.7-318	8.80 (25, n=5)	25	-	[26]
xylidyl blue + H_2O_2	570	batch	fixed-time, 3 min	Tap water, wastewater and drinking water	0.85	2.5-75	0.5-3.6	30	-	[27]
brilliant cresyl blue + ascorbic acid	624	batch	fixed-time, 7 min	Artificially prepared wastewater and natural drinking water	0.025	0.1-5	0.45 (3, n=5)	40	-	[28]
redox reaction of cysteine with Fe(III) in the presence of 2,4,6-Tris(2-pyridyl)-1,3,5-triazine	593	FIA	fixed-time	natural water, and serum samples	0.005	0.05-8	1.3 (1, n=10)	room tempera-ture	30	[21]
2,6-dichlorophenol $(DCPI)_r$ + H_2O_2	600	Chip-MSFIA-MPFS	fixed-time, 5 s	water samples, pharmaceuticals, and animal tissues	0.12	0.4-35	0.79 (10, n=15)	25	31	Present work

Table 3. Validation data for Cu(II) quantification in water samples.

Samples	Proposed method					ICP-AES		Proposed method vs ICP-AES
	Dilution factor (Final volume/sample volume)	Added ($\mu g\ L^{-1}$)	Found ± S.D. ($\mu g\ L^{-1}$)	Spike-Recovery (%)	Cu(II) in sample ± S.D. ($\mu g\ L^{-1}$)	Added ($\mu g\ L^{-1}$)	Found ± S.D. ($\mu g\ L^{-1}$)	t obs t crit 95%=2.92
Collected rain water 1	-	-	14.6 ± 0.3		14.6 ± 0.3	-	15.3 ± 0.6	0.94
		5	19.8 ± 0.3	103				
		10	24.3 ± 0.5	97				
		15	29.7 ± 0.5	101				
Collected rain water 2	10	-	8.7 ± 0.3		88.1 ± 2.8	-	91.3 ± 3.0	0.78
		5	13.6 ± 0.2	98				
		10	19.2 ± 0.2	105				
		15	22.8 ± 0.5	94				
Collected rain water 3	10	-	14.4 ± 0.3		147.9 ± 1.6	-	146.1 ± 3.0	0.52
		5	19.7 ± 0.5	105				
		10	24.1 ± 0.3	96				
		15	28.9 ± 0.4	97				
Collected rain water 4	-	-	0.56 ± 0.02		0.56 ± 0.02	-	< LOD*	
		5	5.3 ± 0.4	95				
		10	10.8 ± 0.2	102		10	10.6 ± 0.3	0.67
		15	15.4 ± 0.2	99				
Drinking water1	-	-	0.52 ± 0.02		0.52 ± 0.02	-	< LOD	
		5	5.3 ± 0.3	96				
		10	9.9 ± 0.3	94		10	9.8 ± 0.7	0.21
		15	15.0 ± 0.1	96				
Drinking water 2	-	-	0.43 ± 0.64		0.43 ± 0.64	-	< LOD	
		5	5.5 ± 0.3	102				
		10	10.0 ± 0.3	96		10	10.4 ± 0.4	0.79
		15	15.2 ± 0.5	98				

* LOD of ICP-AES for Cu(II) analysis is 0.6 $\mu g\ L^{-1}$ Cu(II).

Table 4. Validation data for Cu(II) in multivitamin and tissue samples.

samples	Proposed method				ICP-AES	Proposed method vs ICP-AES	Reported Cu(II) in multivitamin supplement product
	Added (μg L^{-1})	Found ± S.D. (μg L^{-1})	Recovery (%)	Cu(II) in sample	Cu(II) in sample	t obs t crit 95%=2.92	
Multicentrum	0	12.3 ± 0.2		497.4 ± 7.7	503.6 ± 5.6	0.65	500
	10	22.8 ± 0.4	105	(μg/pellet)	(μg/pellet)		(μg/tablet)
Pharmaton	0	12.9 ± 0.1		1005.0 ± 4.9	996.7 ± 7.6	0.92	1000
	10	23.6± 0.2	107	(μg/pellet)	(μg/pellet)		(μg/tablet)
"Cobre"[a]	0	15.1 ± 0.1		365.6 ± 3.3	364.2 ± 5.5	0.23	362.6
(digestion)	10	24.7 ± 0.1	96	(μg/mL)	(μg/mL)		(μg/mL)
"Cobre"[b]	0	14.4 ± 0.2		359.4 ± 5.6	362.6 ± 6.5	0.37	362.6
(direct dilution)	10	24.4 ± 0.2	101	(μg/mL)	(μg/mL)		(μg/mL)
Tissue sample 1	0	15.2 ± 0.2		151.5 ± 2.5	152.7 ± 2.7	0.33	
(Pig heart solution)	10	25.3 ± 0.9	101	(μg/L)	(μg/L)		
Tissue sample 2	0	8.9 ± 0.3		177.5 ± 5.9	174.20 ± 5.5	0.41	
(Pig heart solution)	10	185 ± 0.8	96	(μg/L)	(μg/L)		

a = The "Cobre" solution samples was prepared by digestion.

b = The "Cobre" solution samples was prepared by direct dilution from package.

Legends to figures:

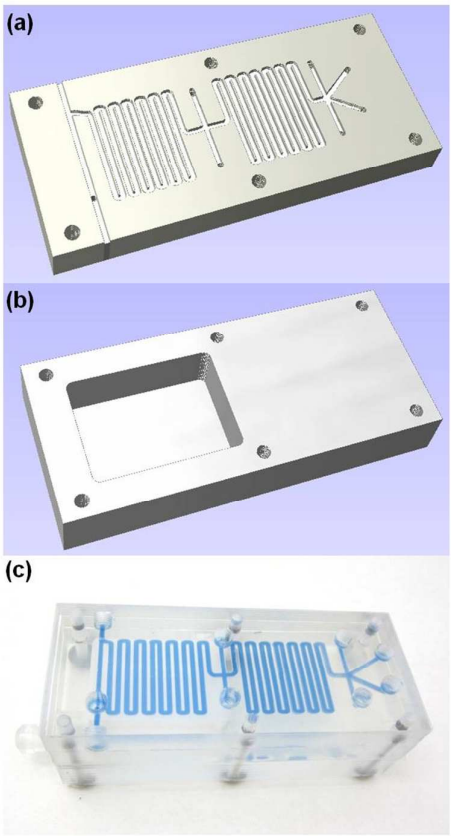
Fig. 1. 3D-schematic diagrams illustrating the chip fabrication: (a) channel plate (b) central and base plates and (c) photo of the complete chip.

Fig. 2. Scheme of the analytical system setup used for the kinetic determination of Cu(II). S: syringe (S1: 1 mL, S2: 1 mL, S4: 5 mL), V: solenoid valve, M: micropump, HC: holding coil of 255 cm and 1.00 mm id, C: PMMA chip, Detector: CCD miniature optical fiber spectrophotometer (measure: 600 nm, schlieren effect correction: 760 nm).

Fig. 3. Effect of H_2O_2 concentration. Experimental conditions: 0.90 mmol L^{-1} DCPI, 3.6 mmol L^{-1} ascorbic acid, 0.80 mol L^{-1} ammonia-ammonium chloride buffer, pH 10.5, 25 ^{o}C, stopped-time 5 s.

Fig. 4. Effect of the stopped-time. Experimental conditions: 0.90 mmol L^{-1} DCPI, 3.6 mmol L^{-1} ascorbic acid, 0.80 mol L^{-1} ammonia-ammonium chloride buffer, 0.30 mol L^{-1} H_2O_2, pH 10.5, and 25 °C.

Fig. 5. Flow profile and calibration curve was obtained from the Chip-MSFIA-MPFS system under the optimum conditions: 0.90 mmol L^{-1} DCPI, 3.6 mmol L^{-1} ascorbic acid, 0.80 mol L^{-1} ammonia-ammonium chloride buffer, pH 10.5, 0.30 mol L^{-1} H_2O_2, stopped-time 5 s, and 25 °C.



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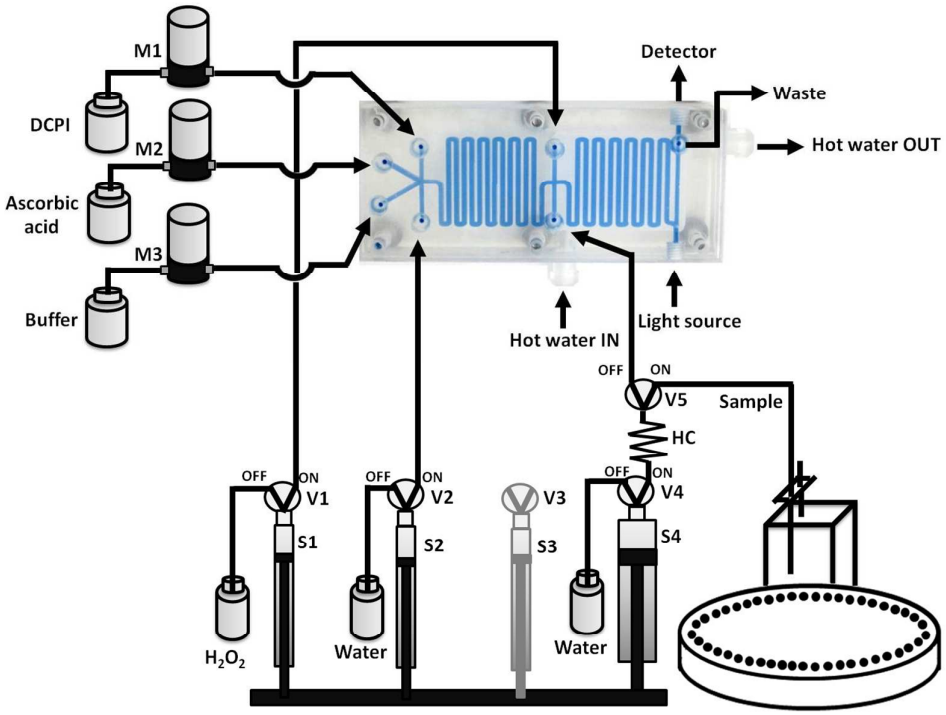

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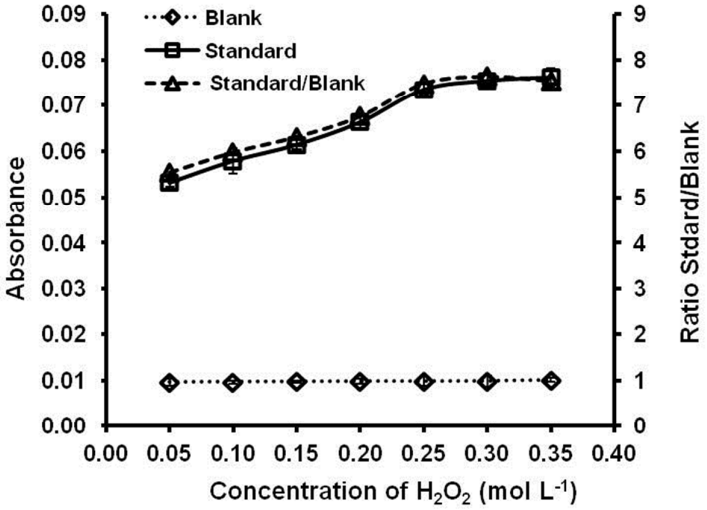

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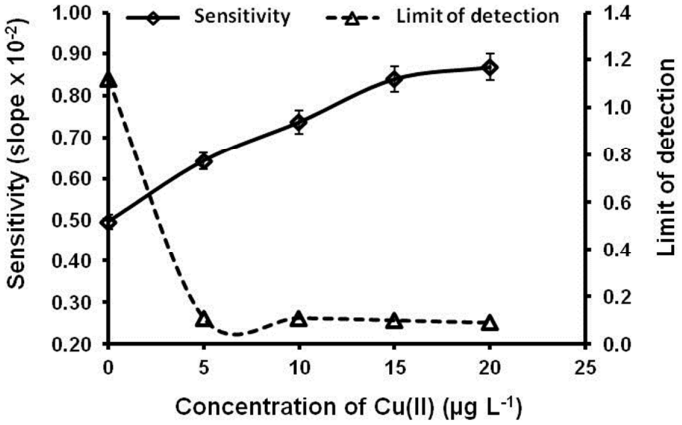
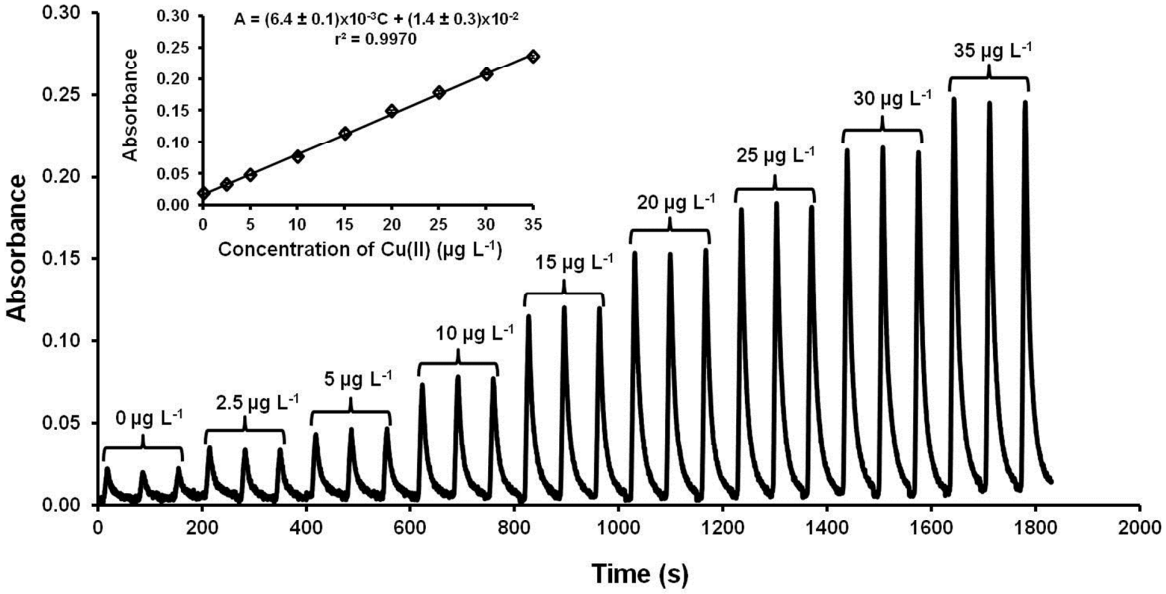


Fig. 4. Effect of the stopped-time. Experimental conditions: 0.90 mmol L^{-1} DCPI, 3.6 mmol L^{-1} ascorbic acid, 0.80 mol L^{-1} ammonia-ammonium chloride buffer, 0.30 mol L^{-1} H_2O_2, pH 10.5, and 25 °C.



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