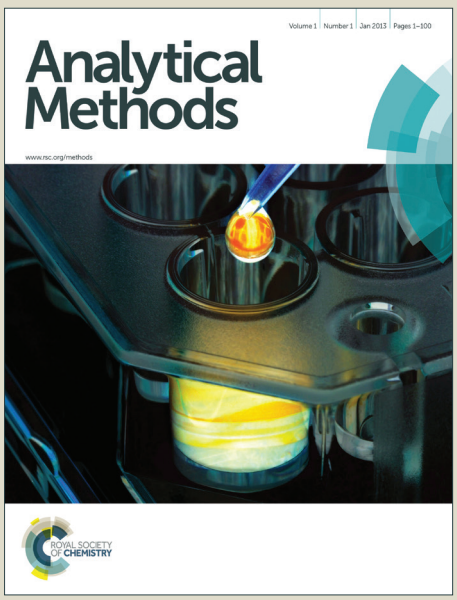
Analytical Methods

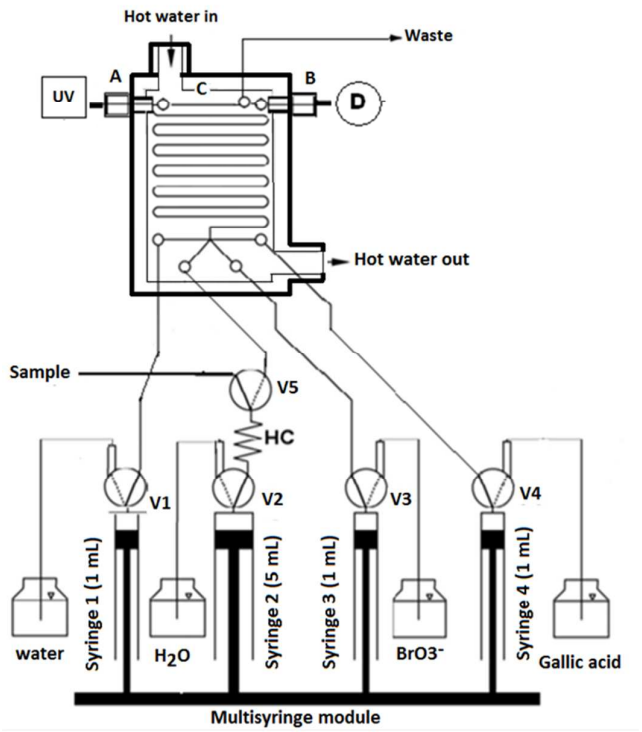
Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/methods

Automatic microfluidic system for catalytic spectrophotometric determination of Vanadium



Automatic integrated system for catalytic spectrophotometric determination of Vanadium in water samples.

Fatima Zohra Abouhiat[1,2], Camelia Henríquez[2], Edwin Palacio[2], Farida El Yousfi[1], Víctor Cerdà[2].

[1] *Department of Chemistry, Faculty of Sciences, University Abdelmalek Essaadi, B.P. 2121 Mhannech II, 93002 Tétouan, Morocco*

[2] *Department of Chemistry, University of the Balearic Islands, Carretera Valldemossa km 7.5, 07122 Palma de Mallorca, Spain tel +34 971173260*

Abstract

An automatic integrated system implementing a catalytic spectrophotometric method for vanadium determination is presented. Thus, a multisyringe flow injection system (MSFIA) was coupled to a monolithic flow microconduit, called chip (CHIP-MSFIA). All reagents and sample were simultaneously propelled into the chip to achieve complete mixing, heating, and measuring inside it. This catalytic spectrophotometric method is based on the oxidation of gallic acid with bromate catalyzed by V(V). The reaction was followed by measuring absorbance change at 384 nm. The incorporation of the detection cell inside the thermostatic zone of the chip allows two kinetic-catalytic determination methods: initial rate and fixed time methods. A critical comparison between these two methods at controlled temperature is presented. Under optimized conditions, the determination of V(V) was performed in the range 0.24-75 µg L^{-1} achieving limits of detection of 0.33 and 0.24 µg L^{-1} for fixed-time and initial rate methods, respectively. Relative standard deviations were between 1- 4%. Finally the initial rate method was successfully applied to determine V(V) concentration in natural and waste water samples. Good recoveries were obtained varying from 94% to 102%. Results were compared with those obtained by the reference method, (ICP-AES), in order to confirm the accuracy of the CHIP-MSFIA method. Additionally, a certified reference material was satisfactorily analyzed.

Keywords:

MSFIA, integrated flow microconduit*,* catalytic spectrophotometric reaction, fixed time method, initial rate method, vanadium.

1. Introduction

Vanadium is widely distributed in the Earth´s crust. It participates in biological processes, being an essential element for some organisms. The impact of vanadium on human health has been studied. It is necessary to normal cell growth at μg L^{-1} levels but results toxic at mg L^{-1} levels [1]. Vanadium exists in various oxidation states and ionic forms varying from monovalent to pentavalent state. Its toxicity is directly related to its oxidation state, hence being the pentavalent vanadium the most toxic for human health [2]. Vanadium is also widely used in industrial processes including the production of special steels, temperature-resistant alloys, glass industry, in the manufacture of pigments and paints, for lining arc welding electrodes and as catalyst. Its combination with non-ferrous metals is of particular importance in the atomic energy industry, air-craft construction and space technology. Thus, vanadium compounds are released in large quantities, mainly by burning fossil fuels and also from various industrial processes. These vanadium compounds are precipitated on the soil drained by rain and groundwater and may be directly adsorbed by plants.

Different analytical methods have been developed for vanadium quantification and speciation in environmental, biological and industrial samples, exploiting different techniques such as spectrophotometry [3-7], flow injection spectrophotometry [8-10], voltammetry [11, 12], thermometry [13], enthalpimetry [14], inductively coupled plasma-atomic emission spectroscopy (ICP-AES) [15], flow injection chemiluminescence [16], liquid chromatography [17, 18] and inductively coupled plasma mass spectrometry (ICP-MS) [17]. Some of these methods are sensitive and accurate but expensive, time-consuming and require skillful operators. Kinetic-catalytic methods are characterized by high sensitivity and selectivity since vanadium acts as the catalyst of the spectrophometric reaction. Moreover, the hyphenation of kinetic-catalytic methods and flow techniques allows the development of automatic, sensitive, rapid, and economic methods.

Thus, the proposed method has been automated using a multisyringe flow injection analysis (MSFIA) system. This flow technique includes the advantages of flow injection analysis (FIA) in terms of mixing of flowing solutions and sequential injection analysis (SIA) in relation to its robustness and versatility [19]. Therefore, MSFIA can be considered as an ideal choice in kinetic measurements since it offers the possibility of simultaneous propulsion of up to four different solutions, e.g. sample and reagents mixed in constant ratio, in a smooth, homogeneous, and pulse-less flow. Moreover, the MSFIA control with the appropriate software

[20] allows the complete automation of the analytical method including instrument and device control, data acquisition and online data process. This high automation degree facilitates the mathematical data process and *initial rate* determination method implementation.

A special monolithic microfluidic conductor device called chip was designed for this system. Similar devices have been previously presented by Cerdà et al. for other applications [21, 22]. In this case, the designed device integrates various steps of the analytical procedure such as: confluent point, mixing coil, and detection cell, all these on a thermostatic chamber, allowing the application of the initial rate and fixed time methods at controlled temperature.

Kinetic methods can be classified according to the mathematical form used in the kinetics reaction, namely: differential or integral. Both cases can be applied in the initial rate and fixed time methods. The initial rate method greatly reduces the time invested in the analytical procedure since only the first seconds of the reaction have to be measured. However, this method presents difficulties in accurately measuring the initial slope, which is subject to errors inherent to the graphical method used. This deficiency can be circumvented by the use of computers to provide the slope of the curve directly in a more precise manner. On the other hand, the fixed time method is easy to understand and data are easily processed. However, sometimes it requires long reaction times to obtain sufficient signal response.

Therefore, in this work a new automatic kinetic-catalytic method for the spectrophotometric determination of vanadium is presented. This method is based in the vanadium (V) catalytic effect on the oxidation reaction of gallic acid by bromate. The oxidation product of gallic acid is a quinone which is an orange-brown compound with a maximum of absorbance at 384 nm. A critical comparison in terms of performance of the fixed time and the initial rate catalytic kinetic spectrophotometric methods for the automatic quantification of vanadium (V) is presented.

2. Materials and Methods

2.1 Reagents and standards.

All reagents and solutions were prepared with milli-Q water (Milli-Q plus, 18.2 MΩ cm^{-1}). A stock standard solution of 1000 mg L^{-1} Vanadium (ammonium monovanadate in nitric acid 0.5 mol L^{-1}) for atomic absorption analysis (Scharlau, Spain), was used to prepare 10 mg L^{-1} stock solution. Working standard solutions (from 1 to 80 µg L^{-1}), were prepared by dilution of the 10 mg L^{-1} stock solution in milli-Q water.

Gallic acid solution 0.08 mol L^{-1} was prepared dissolving 0.38 g of gallic acid (SIGMA-ALDRICH, USA) in 25 mL of buffer solution. 0.36 mol L^{-1} sodium bromate was prepared dissolving 1.36 g of sodium bromate (SIGMA-ALDRICH, USA) in 25 mL.

Prideaux buffer solution (pH=3.4) was prepared by dissolving 12.37 g of boric acid (SIGMA-ALDRICH,USA), 23.06 g of concentrated phosphoric acid (Scharlau, Spain), 12.01 g of glacial acetic acid (SIGMA-ALDRICH,USA), 8.96 g of sodium hydroxide (Scharlau, Spain), and 19.98 g of potassium chloride (Scharlau, Spain) in milli-Q water up to 1000 mL.

2.2 Flow analyzer

The construction of the microfluidic chip driver used in this work is described elsewhere [21]. Thus, this new chip is built using three poly(methyl methacrylate) (PMMA) pieces, integrating a confluence point, a holding coil, and a thermostat chamber into a single device. Additionally, its design includes a detection cell (3 cm optical path) located at the end of the mixing coil and before the liquid exit. For this two lateral holes of UNF ¼ in. 36 fittings were drilled in order to connect the flow cell located at the end of the coil with two optical fibers (400 µm core diameter) to transmit the light from the light source (DH-2000 Deuterium light source TOP Sensor, Eerbeek, Netherlands) to the detector (USB-2000, Dunedin, FL, USA) passing through the reaction mixture inside the detection flow cell, as shown in Fig. 1. The incorporation of the detection cell in the thermostatic zone allows the application of the initial rate method at a controlled temperature.

The multisyringe piston pump module (model Bu 4S), purchased from Crison Instruments S.A. (Allela, Barcelona, Spain), was equipped with three glass syringes of 1 mL (S1, S3, S4) and one of 5 mL (S2), all of TLL SYR series from Hamilton Bunaduz AG (Bunaduz, Switzerland).

The solenoid valves (V1, V2, V3, V4) located at the head of the syringes allowed the connection of the solutions either with the chip, replacing the usual tubing manifold (position ON, activated), or with the respective solution reservoir (position OFF, deactivated) for refilling. S1 and S2 contained water, S3 the BrO_3^- reagent, and S4 gallic acid. An extra external three-way solenoid valve (V5) from Takasago (Nagoya, Japan, type: STV-3-1/4UKG) was powered and controlled via a rear supply port of the multisyringe module. It was used for sample introduction, in ON position.

2.3 Software

The software AutoAnalysis 5.0 (Sciware Systems, S.L., Bunyola, Spain) was used for operational control of flow instrumentation as well as data acquisition and evaluation. AutoAnalysis allowed saving the absorbance values every 5 s during the first minute of the kinetics reaction (table 1.2 step 8). The initial rate method consists of the use of the slope of the kinetic curve in the first seconds of the catalytic reaction. Thus, the slope was estimated using the absorbance measured every 5 s until the lack of linearity of the initial rate, these data were exported in an organized manner, which facilitated the calculation of the slope for each concentration of V(V). Moreover, using this software, the determination method can be easily selected and changed.

This software is adaptable to each instrument by incorporation of dynamic link libraries, which are amenable to communicate and control the individually assembled instruments through a RS232 interface. The program allows user-friendly software tools for method development and automated optimization experiments such as loops, procedures, variables, user inquiries, waiting steps, and definition of conditional inquiries among others.

2.4 Analytical protocol

The analytical protocols proposed for the CHIP-MSFIA system fixed time and initial rate determination methods are presented in tables 1.1 and 1.2, respectively.

For the fixed time method, as can be seen in table 1.1, a volume of 150 µL of sample was aspirated from V5 (step 2). 10 µL of this volume were dispensed towards the chip (step 4) to avoid the dilution of the first part of the sample. Then 39 µL of sample were dispensed into the reaction coil together with the reagents (step 5). Afterwards, syringes were filled (step 6) and the reaction mixture was maintained for 600 s at 40 °C (step 7). After this reaction time, the mixture was pushed through the detection cell with 300 µL of milliQ water to perform the spectrophotometric detection of V (V), and clean the chip (steps 9-11). The absorbance signal was measured at 384 nm, and 512 nm was used as reference wavelength to correct the Schlieren effect. Additional steps were required to overcome the syringe pump backlash, i.e. steps 1, 3 and 8, perform a small flow operation with head valve position OFF in the same direction (dispense or pickup) as in the subsequent step.

For the initial rate method (see table 1.2) the kinetic curve in the first moments of the catalytic reaction is required. For this reason, the absorbance signal was recorded throughout the process. A sample volume of 400 µL was aspirated (step 3) and then, 100 µL of this volume

were dispensed to the chip (step 5) in order to discard the head of the sample plug. The remaining volume of sample was dispensed followed by water, 700 µL in total, together with the reagents (step 6), merging in the confluence point at a high flow rate (i.e. 5.25 mL min^{-1}) until fill completely the detection cell with the central mixing plug. Then, the flow was stopped and the kinetic reaction was allowed to evolve under stop-flow conditions. Meanwhile the syringes were refilled (step 7). The kinetic-catalytic reaction time was 80 s at 40 °C (step 7 and 8). In step 8 a loop was programmed to save the absorbance values as variables in AutoAnalysis each 5 s. Data were easily exported and processed. Then 1.8 mL of water were dispensed (steps 9-10) to expel the mixture and clean the chip for the next analysis. Steps 2 and 4 are required to overcome the syringe pump backlash and ensure the accuracy and reproducibility of the injected volumes.

2.5 Samples

Four well water samples from different locations of Mallorca Island were collected in polyethylene flasks, refrigerated at 4 ºC and immediately transported to the laboratory for analysis. Tap water sample was collected and measured immediately. Samples were measured using the proposed analyzer system and the reference method [23]. Samples previously acidified at 2% with HNO_3 were directly measured in the ICP-AES (Optima 5300 DV, Perkin Elmer® Inc). The obtained values with the ICP-AES method were compared with those obtained with the kinetic-catalytic methods in other to evaluate the accuracy of the proposed method.

The SPS waste water reference material was diluted 1:50 with milli-Q water and neutralized with 0.6 mL of NaOH 0.1 mol L^{-1}. Then, this sample was directly measured using the CHIP-MSFIA analyzer.

3. Results and discussion

3.1 Chip design and system configuration

In this work, an improvement of a previously designed chip [21] has been done by integrating the detection cell inside the device. With this, the temperature of the reaction mixture can be controlled during measurement. This new configuration has allowed the use of both the initial rate and the fixed time methods. Advantages of implementing the initial rate method with controlled temperature, in terms of increasing sensitivity and frequency of analysis, are discussed below.

A careful study of the reagent and sample volumes and their flow rates was carried out to guaranty the maximal concentration of the mixture in the detection cell. For this, a solution of 50 μg L^{-1} of indigo carmine was used. Thus, the volume required for filling the chip completely was 225 μL.

To maximize method sensitivity, a five-fold larger syringe volume was used for sample handling, leading to a 5:1:1 volumetric ratio between sample, gallic acid, and bromate. Also, in order to minimize reagents consumption, both reagents were injected into a slightly longer sample segment, thus applying the zone mixing flow concept instead of continuous confluent mixing.

3.2 Air bubbles removal

One major problem in unsegmented flow techniques is the presence of air bubbles. They alter the flow and mixing pattern of the solutions, affecting the response signal in several detection techniques, increasing the irreproducibility of the determinations. Air bubbles are generally caused by a partly degassing of the solutions at the pressure drop during the aspiration of solutions for syringe refilling [21]. In order to reduce the air bubbles production during aspiration, an air bubble trapping device was placed between the supply tube and syringe head valve (OFF position). Description of the air bubble trapping device can be found elsewhere [21].

In addition, CO_2 is a product of the reaction that takes place inside the chip. Therefore a great number of small bubbles are formed inside the chip during the reaction remaining static inside the chip channel. To efficiently eliminate these small bubbles, it was necessary to dispense periodically a large air bubble to agglomerate and discharge the others.

3.3 Experimental conditions optimization

To evaluate the influence of the principal factors and their interactions in the gallic acid reaction with BrO_3^- catalyzed by V (V), a fractional factorial design (2^{5-1}) was carried out as screening method. The fixed time method was used to develop this first multivariate study. Three central points were added to this design in order to estimate the error of data fit to the model. Independents variables and their experimental domains were: reaction time, from 5 to 15 min; temperature, from 25 to 40ºC; concentration of gallic acid, from 0.01 to 0.09 mol L^{-1}; concentration of BrO_3^-, from 0.2 to 0.5 mol L^{-1}; and pH, from 3 to 4. Other parameters were kept constant, i.e. 39 μL of sample and a flow rate of 0.75 mL min^{-1}.

The studied dependent variable was the ratio between the analytical signal obtained using a standard solution of V(V) 25 μg L^{-1} and a blank (Std/BL). For the fixed time method, the analytical signal was the absorbance peak height, whereas for the initial rate the slope along the first seconds of the kinetic curve. In studies of kinetic-catalytic reactions, frequently is more correct to use this ratio, since the contribution of the uncatalyzed reaction should be taken into account given it can affect the final signal and therefore the sensitivity of the method, providing erroneous optimal values.

For this experimental design (2^{5-1}), data were successfully adjusted to a second order model (R^2=0.98716, Adj=0.92295), with a no significant lack of fit for the 95% of confidence level and a very low pure error (0.00026). In the studied range, the gallic acid concentration was the factor with a highest significant effect followed by the reaction time and the temperature, all with positive effect on the response signal. These are the factors most strongly related to the gallic acid oxidation. On the same way, interactions of these three factors (gallic acid and temperature, reaction time and temperature, and reaction time and gallic acid) were also significant. These results are graphically summarized in the Pareto chart shown Fig S1 in supplementary material. Thus, higher temperature, gallic acid concentration and reaction time increased the reaction extension. 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. Effects of pH and bromate concentration were significant but in lesser extension. The pH of gallic acid solution was fixed at 3.4 due to its low significant effect in the studied range. The studied ranges for the gallic concentration and the reaction time were expanded following the screening results in a second multivariate study. The new experimental design was a central composite of three factors, namely the gallic acid and bromate concentration and the reaction time. The new experimental domain was: gallic acid from 10 to 90 mmol L^{-1}, BrO_3^- from 0.2 to 0.5 mol L^{-1}, and reaction time from 10 to 20 min. Data responded to a quadratic model, without significant lack of fit. Critical values found were 80 mmol L^{-1} of gallic acid, 0.36 mol L^{-1} of bromate, and 15 min of reaction time, at 40 °C. Fig. S2 in supplementary material shows the desirability graphs obtained for these three factors in the experimental domain studied. This function clearly shows the importance of each factor and the critical values obtained. At a reaction time higher than 15 min, the sensitivity did not increase significantly due to the lack of linearity on the kinetic curve. However, in order to improve the frequency of analysis 10 min was selected as reaction time since it was possible to differentiate the signal of blank and a peak of 2.5 μg L^{-1} of V(V). The important increment of sample

throughput with the use of 10 min of reaction time without a significant cost of sensitivity, guided us to select it for following experiments.

Optimal conditions regarding reagents concentration and pH were the same for both, fixed time and initial rate determination methods. We studied the effect of these factors in the initial rate method using a robustness study, varying a 10% around the critical values the selected factors: concentration of gallic acid, bromate and pH. Fig. 2 graphically represents the results obtained in this study for each factor. The existence of maximum levels in the graphs of changes in the factors *vs* analytical signal (Fig 2 a, b and c) allows to confirm the presence of critical values within the studied range. The maximum values obtained at 40 ºC were the same for both methods (initial rate and fixed time methods).

3.4 Analytical parameters. Comparison between initial rate and fixed time methods

As mentioned above, the chip presented in this work allows the automatic application of the initial rate and fixed time determination methods at controlled temperature. Fig. 3.1 shows the profile of the peaks obtained for a calibration curve using the fixed time method. In this case the analytical reaction takes place before reaching the detection cell during a fixed time. Then, a typical profile of a FIA-gram, with a well defined and highly reproducible peak shape is obtained. On the other hand, in the initial rate method, the reagent mixture is propelled and stopped into the detection cell incorporated inside the chip in the thermostated zone, and the peak profile includes the kinetic curve of the analytical reaction obtained in the first minute (Fig. 3.2). Kinetic curves for different concentrations of catalyst can be obtained from the MSFIA-grams obtained with AutoAnalysis software using the initial rate method (Fig. 3.2 A). These diagrams allow the clear visualization of the catalytic effect of V(V) in the kinetic curve. Overlapped averaged fragments of kinetic curves for each concentration of catalyst are represented in Fig. 3.2 (B). The calibration curve is obtained representing the slopes of these curves vs the concentration of V(V) (Fig. 3.2 (C)). Table 2 summarizes main analytical characteristics obtained for both methods under optimal conditions.

The mathematical processing of the initial rate method is much more cumbersome than the one needed in the fixed time method. However, for the selected reaction, the use of the initial rate method provides some significant advantages. The fixed time method requires high reaction times (10-15 min) to achieve sufficient sensitivity to quantify vanadium. Contrarily, the initial rate method requires only few seconds, reducing considerably the time of analysis, i.e. from 10 min

to less than 2 min. This also significantly affects the frequency of analysis, ranging from 6 h^{-1} (injections per hour) in the fixed time method to 30 h^{-1} in the initial rate method.

Furthermore, the initial rate method presents higher sensitivity than the fixed time, achieving better detection limits (LOD): 0.24 µg L^{-1} for initial rate method, and 0.33 µg L^{-1} for the fixed time method. The LOD was calculated as 3 times the standard deviation of the analytical signal of 10 blanks measured under the same conditions and divided by the slope of the calibration curve.

Good repeatability is obtained for both methods. The initial rate method provides a wider linear range (0.24-75 µg L^{-1}) than the fixed time method (0.33-25 µg L^{-1}). The fixed time method linear working range is limited by the maximum absorbance allowed by the Lambert-Beer law, i.e. 25 µg L^{-1} of V(V) reach 1.1 AU. This can be explained taking into account that using the fixed time method the reaction takes place in a larger extension (10 min or more) than in the initial rate method (1-2 min). Thus, given the higher frequency of analysis, working range and better limit of detection of the initial rate method, it was selected for vanadium quantification in real samples.

There are previous works determining vanadium through its catalytic effect [7-9, 24]. Thus, in order to evaluate the advantages and competitiveness of the proposed methods, their characteristics have been compared with those obtained by other similar analytical methods currently reported and summarized in Table 2. Forteza et al [13] and Fukasawa [7] developed two methods based on the same reaction proposed in this work, with thermometric and colorimetric detection, respectively. Fishman et al [24] also proposed a similar method but using persulphate as oxidant. These methods were not automated and required long time of reaction, what precludes their application in routine analysis. The Chip-MSFIA method proposed in this work is the first work in which this reaction has been automated resulting in a fast, sensitive and precise method for vanadium quantification in natural samples.

This monolithic device is very compact which reduces the dimension of the designed manifold reducing significantly sample and reagent consumption and consequently the cost of analysis in comparison with batch variants [7,13] . For example, Fukasawa et al used for each injection: 2500 µL of sample, 180 µmol of gallic acid and 2304 µmol of bromated and Forteza et al used for each injection: 1000 µL of sample, 500 µmol of gallic acid and 3500 µmol of bromated. In our method, the sample and reagent consumption for each injection is 80 µL of sample, 11.2 µmol of gallic acid and 50.4 µmol bromated for initial rate method; and 30 µL of sample, 3.1 µmol of gallic acid and 14.0 µmol bromated for fixed time method. Moreover, the chip was made of

polimethyl-methachrylate, thus it is chemically and mechanically inert for the proposed reaction at working temperature, allowing an infinite number of injections without visible deterioration of the chip. Furthermore, the chip material allowed ease of cleaning and no cross-contamination was observed.

Other authors have reported automated analytical methods with FIA based on similar reactions [8, 9, 25, 26]. Some of these methods [8, 25] present very low LODs, however they need a very high temperature for the reaction (60ºC and 105 ºC), which involves more sophisticated and expensive instrumentation, and the risk of bubble generation inside the flow tubes. Oguma et al. [26] proposed a FIA system based in xylenol oxidation in acid media, which worked at room temperature, but lower sensitivity (LOD: 10 μg L^{-1}), working range and sample throughput (19 h^{-1}) were achieved in comparison with the proposed CHIP-MSFIA system (30h^{-1} for initial rate). Moreover, benefits of using a chip instead of classical flow systems are the compactness of the whole system what leads to simpler configurations and the reduced size of the channels leads in lower reagent and sample consumption resulting in a lower cost and environmental impact per analysis.

3.5 Interferences

The selectivity of the method was assessed by studying the effect of various ions on the catalytic determination of 2.5 μg L^{-1} of vanadium (V). The ions studied and their tolerance levels are summarized in table 3.

It has been reported that some ions, such as Fe(III), Cu(II), and Mo(VI), can affect the reaction between gallic acid and BrO_3^- catalyzed by V(V) [7, 10, 13]. The effect of these ions on the catalytic reaction was studied. Both methods were evaluated. The tolerance level was defined as the interference that yielded a relative error less than or equal to ±10%. Using the fixed time method the tolerance levels obtained for Cu(II), Al(III) and Mo(VI) (1, 20, and 0.5 mg L^{-1}, respectively) were generally higher than the concentrations reported in natural waters (10, 200, and less than 5 μg L^{-1}, respectively) [27]. However, for Fe (III) the found tolerance level (400 μg L^{-1}) was lower than the usual concentration in natural waters (1000 μg L^{-1}, [27]). This fact can affect the applicability of the fixed time method for water samples containing Fe in a concentration equal or higher than 400 μg L^{-1}. The use of masking agents such as fluoride, EDTA, and cationic membranes were not effective since the analytical signal was also suppressed. Regarding the initial rate method, all interferences had a reduced effect. This

reduced effect may be due to the difference in the kinetic reaction catalyzed by V(V) and kinetics of the interferences reaction. This also reaffirmed choosing the initial rate method for vanadium quantification.

3.6 Method validation and application to natural samples

Frequently, samples are preserved in strong acid media until measurement. In consequence, the influence of sample pH in the reaction was evaluated. Fig. S3, in supplementary material, shows the effect of sample pH in the analytical signal. At sample pH lower than 1 a strong increment of the analytical signal was observed. Consequently, samples conserved with 2% of HNO_3 were neutralized before their measurement.

Validation of the proposed method was performed using the three methodologies known for this objective [28]: the analysis of a reference material, the use of a reference method with different physicochemical grounds, and the add-recovery test. The t-student test was employed to evaluate the significant differences between the means compared at the 95% confidence level for three replicates. Results are shown in table 4 together with other samples characteristics such as the content of Fe.

A wastewater reference material SPS-WW2 was analyzed with the proposed CHIP-MSFIA. The t-test for comparison of means revealed that there were no significant differences at the 95% confidence level between the certified value and the results obtained with the proposed method (t- test: 0.4 < 2.96). Well water samples were analyzed with the proposed method. To estimate the matrix effect on the analytical response, samples were spiked with three different volumes of V(V) standard, i.e. 5, 10 and 25 µg L^{-1} (add-recovery test). Satisfactory recoveries, in the range from 94 % to 102 %, were obtained for all samples. These results demonstrate the applicability of the proposed method to determine V(V) in these kind of matrices.

Furthermore, the method was also validated by making a comparison with a reference method. Samples and spiked samples were measured by ICP-AES [23]. All well water samples showed V(V) concentrations close but below the LOD (4 µg L^{-1}) of the reference method. The LOD for the reference method was calculated as 3 S_b/P, where S_b represents the standard deviation of the signal from the 10 x reagent blanks and P measures the slope of the fitted line of the calibration curve. This highlights the advantage of our method in front of the ICP-AES technique

for V(V) determination in terms of sensitivity. Moreover, no significant differences were found between the results obtained with the proposed and the reference method at 95% confidence level (t- test: 0.4-1.9 < 2.92). However, for three of the spiked samples (well water 1 +25 μg L^{-1}, and well water 2 and 3 + 10 μg L^{-1}) with t-values (3, and 3.3) higher than 2.92 but lower than 6.965 which corresponds to 99% of confidence level.

4. Conclusions

In this paper a CHIP-MSFIA system allowing the automation of a catalytic-kinetic method for the determination of V(V) in water samples is presented. The proposed microfluidic chip driver incorporates a thermostated detection cell. This configuration made possible the automation of the initial rate method at controlled temperature, what lead to significantly increase the sampling throughput and the sensitivity of the method, improving the limit of detection in more than an order of magnitude in comparison with the ICP-AES LOD for vanadium. Finally, the validation of the method demonstrated the effectiveness of the CHIP-MSFIA system for automating kinetic-catalytic methods and the applicability of the proposed method in the determination of V(V) in natural waters. The method was satisfactorily applied to well water samples and to a waste water reference material. Also the high reproducibility obtained when analyzing a complex sample matrix like wastewater confirms the appropriate trueness of the proposed method. Thus, the proposed CHIP-MSFIA initial rate method presented is a fast, low-cost, sensitive and robust tool to monitor V(V) in natural water samples.

Acknowledgements: This work was funded by the Conselleria d'Economia, Hacienda, e Innovació of the Government of the Balearic Islands through the allowance to competitive groups (43/2011), and, by the Spanish Ministry of Economy and Competitiveness (MINECO) by the project CTQ2013-47461-R, both projects cofinanced by Feder Funds. F.Z. Abouhiat was funded by the Averroes grants of the European Union.

References

[1] S.v.J.F. Taylor.M.J.C, Analyst, 119 (1994) 1263-1276.
[2] H.G. Seiler, H. Sigel, A. Sigel, Handbook on toxicity of inorganic compounds, 1988.
[3] Q.-z. Zhai, X.-x. Zhang, C. Huang, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 69 (2008) 911-916.
[4] K.F.F. Ashraf.A.M Analytical sciences 17 (2001) 769-773.
[5] J. Gao, X. Zhang, W. Yang, B. Zhao, J. Hou, J. Kang, Talanta, 51 (2000) 447-453.
[6] M.J. Ahmed, S. Banoo, Talanta, 48 (1999) 1085-1094.
[7] K.S. Fukasawa.T, Yamanouchi.T, Analytica Chimica Acta, 171 (1985) 325-332.
[8] S. Nakano, E. Tanaka, Y. Mizutani, Talanta, 61 (2003) 203-210.
[9] M.E. Palomeque, A.G. Lista, B.S.F. Band, Analytica Chimica Acta, 366 (1998) 287-293.
[10] K.S. Fukasawa.T, Unno.A, Analytica Chimica Acta, 183 (1986) 269-274.
[11] R. Dansby-Sparks, J.Q. Chambers, Z.-L. Xue, Analytica Chimica Acta, 643 (2009) 19-25.
[12] S. Sander, Analytica Chimica Acta, 394 (1999) 81-89.
[13] R. Forteza, V. Cerda, Analytical Chemistry, 58 (1986) 453-455.
[14] R. Forteza, J.M. Estela, V. Cerda, Analyst, 116 (1991) 1171-1173.
[15] R.G. Wuilloud, J.A. Salonia, J.A. Gásquez, R.A. Olsina, L.D. Martinez, Analytica Chimica Acta, 420 (2000) 73-79.
[16] J. Liu, B. Qiao, N. Zhang, L. He, Journal of Geochemical Exploration, 103 (2009) 45-49.
[17] C.-C. Wann, S.-J. Jiang, Analytica Chimica Acta, 357 (1997) 211-218.
[18] M. Sugiyama, T. Tamada, T. Hori, Analytica Chimica Acta, 431 (2001) 141-148.
[19] J. Avivar, L. Ferrer, M. Casas, V. Cerdà, Talanta, 84 (2011) 1221-1227.
[20] V. Cerdà, J.M. Estela, R. Forteza, A. Cladera, E. Becerra, P. Altimira, P. Sitjar, Talanta, 50 (1999) 695-705.
[21] F.Z. Abouhiat, C. Henríquez, B. Horstkotte, F. El Yousfi, V. Cerdà, Talanta, 108 (2013) 92-102.
[22] B. Horstkotte, R. Suárez, P. Solich, V. Cerdà, Analytica Chimica Acta, 788 (2013) 52-60.
[23] M.A.H. Franson, L.S. Clesceri, A.E. Greenberg, R. Rhodes Trussell, in: D.d.S. S.A. (Ed.), APHA, AWWA, WPCF, 1992.
[24] S.M.W. Fishman.M.J, Analytical Chemistry, 36 (1964) 1643.
[25] N. Teshima, M. Kuno, M. Ueda, H. Ueda, S. Ohno, T. Sakai, Talanta, 79 (2009) 517-522.
[26] K. Oguma, O. Yoshioka, J. Noro, H. Sakurai, Talanta, 96 (2012) 44-49.
[27] OMS, in: O.M.d.l. Salud (Ed.) Documento de referencia para la elaboración de las Guías de la OMS para la calidad del agua potable, Suiza, 2003.
[28] S.L.R.E. Michael.T, Roger.W, Pure Appl.chemistry, 74 (2002) 835-855.

Table 1.1 Analytical procedure for the determination of Vanadium with the fixed time method

Steps	Device *	Operation **	Comments
1	MS	Pickup 0.040 mL at 0.750 mL/min [1-Off 2-Off 3-Off 4 Off 5 Off]	Syringes refilling
2	MS	Pickup 0.030 mL at 0.750 mL/min [1- Off 2- On 3-Off 4-Off 5-On]	Sample aspiration
3	MS	Dispense 0.010 mL at 0.750 mL/min [1-Off 2-Off 3-Off 4-Off 5-Off]	Overcoming syringe pump backlash
4	MS	Dispense 0.010 mL at 0.750 mL/min [1- Off 2- On 3-Off 4-Off 5-Off]	Dispense sample to chip
5	MS	Dispense 0.039 mL at 0.500 mL/min [1- Off 2-On 3-On 4- On 5-Off]	Mixing of sample with both reagents
6	MS	Pickup 0.184 mL at 2 mL/min [1-Off 2-Off 3-Off 4-Off 5-Off]	Syringes refilling
7	Wait	Wait 600 s	Reaction time
8	MS	Dispense 0.010 mL at 0.750 mL/min [1-Off 2-Off 3-Off 4-Off 5-Off]	Overcoming of syringe pump backlash
9	D	Start measure with 3.3 Hz: Absorbance difference: 384 nm	Measure absorbance when dispensing the reaction mixture to waste
10	MS	Dispense 0.300 mL at 0.750 mL/min [1- Off 2- On 3-Off 4-Off 5-Off]	
11	D	Stop measure	

Table 1.2 Analytical procedure for the determination of vanadium with the initial rate method

Steps	Device*	Operation**	Comments
1	D	Start measure with 8.5Hz: Absorbance difference at 384 nm – 512 nm	Absorbance measurement during expulsion of reaction product to waste
2	MS	Pickup 0.040 mL at 1.224 mL/min [1-Off 2-Off 3-Off 4-Off 5-Off]	Overcoming of syringe pump backlash
3	MS	Pickup 0.080 mL at 0.750 mL/min [1-Off 2-On 3-Off 4-Off 5-On]	Sample aspiration
4	MS	Dispense 0.010 mL at 3.000 mL/min [1-Off 2-Off 3-Off 4-Off 5-Off]	Overcoming of syringe pump backlash
5	MS	Dispense 0.020 mL at 3.000 mL/min [1-Off 2-On 3-Off 4-Off 5-Off]	Dispense sample to chip
6	MS	Dispense 0.140 mL at 0.750 mL/min [1- Off 2-On 3-On 4- On 5-Off]	Mixing sample with both reagents and filling the detection cell.
7	MS	Pickup 0.410 mL at 2.400 mL/min [1-Off 2-Off 3-Off 4-Off 5-Off]	Syringes refilling
8	D	Loop A Read OceanOptic save in “Abs” Mark “Abs” Wait 5s End Loop Repeat 14 times	Reaction time and recording of the absorbance signal each 5 s saving it as a variable to facilitate the data process of the initial rate method.
9	MS	Dispense 0.360 mL at 2.000 mL/min [1-Off 2-On 3-Off 4-Off 5-Off]	Eject the reaction product to waste and clean the chip.
11	D	Stop measure	

* D = Spectrophotometer USB 2000, MS = Multisyringe pump
** All volumes and flow rates refer to the size of syringe 1 (water, 1 mL). For syringe 2, used for sample, volumes and flow rates are fivefold more.

Table 2 Figures of merit and experimental characteristics of catalytic spectrophotometric vanadium determinations. Comparison with similar reported methods

Ref.	Reaction	System	Method	LOD µg L^{-1}	Working Range µg L^{-1}	RSD (%)	T ºC	injection h^{-1}	samples
[7]	Gallic acid+BrO_3^-	Batch	fixed-time 12min	0.5	10-25	10	30		-
[13]*	Gallic acid+ BrO_3^-	Batch	Initial rate	2.5	2.5-25	2			Steel
[24]	Gallic acid+S2O8	Batch	fixed-time 30-60 min	0.1	0.1-8.0		25		natural water
[9]	3,3´DMN[a] +H^+	FIA	fixed-time 100s	200	200-7200	1.9	60	34 h^{-1}	well and river water
			fixed-time 235s	0.032	40-1500	2.3		12 h^{-1}	
[8]	N,N´BHOST[b]+BrO_3^-	FIA		0.008	0.01-3	1.6	60	30 h^{-1}	natural water
[25]	P-anisidine+ BrO_3^-	FIA	fixed-time 370s	0.038	0.1-2	0.8	105	20 h^{-1}	drinking water
[26]	Xylenol orange+H^+	FIA		10	400-5000	2.4		19 h^{-1}	synthetic solution and mixtures
Present work	**Gallic acid+ BrO_3^-**	**MSFIA**	**fixed-time 10min**	**0.33**	**0.33-25**	**2**	**40**	**6 h^{-1}**	**-**
			initial rate 2min	**0.24**	**0.24-75**	**3**	**40**	**30 h^{-1}**	**well water and waste water**

[a] 3,3´DMN , 3,3´ dimethylnaphtidine.
[b] N,N´BHOST, N,N´-bis(-hydroxyl-3-sulfopropyl)-tolidine.
*This method used thermometric determination

Table 3 Interferences tolerance for kinetic-catalytic determination of V(V) using the automated with the CHIP-MSFIA system

Interferences	*Metal tolerance level* ($\mu g\ L^{-1}$)	*Metal concentration in underground waters* ($\mu g\ L^{-1}$)
Al(III)	20000	200 [b]
Cu(II)	1000	10 [a]
Mo(VI)	500	<5 [a]
Fe(III)	400	1000 [a]
I^-	50	18 [b]

a- Concentrations of metals found in groundwater in Palma de Mallorca.

b- Values set by the World Health Organization [27]

Table 4 Vanadium quantification in water samples

Sample	Added ($\mu g\ L^{-1}$)	Found ($\mu g\ L^{-1}$)	Recovery %	Reference method	t-test	Fe ($\mu g\ L^{-1}$)
well water 1	0	1.48±0.05		<LOD		0.3
	5	6.5±0.5	100%	6.1±0.5	1	
	10	11.4±1.5	99%	10.5±0.6	0.9	
	25	27.0±0.7	102%	25.7±0.3	3	
well water 2	0	1.24±0.01		<LOD		27.9
	10	10.68±0.07	94%	9.0±0.5	3.3	
	25	25.6±0.7	98%	25.3±0.6	0.6	
well water 3	0	1.37±0.04		<LOD		38.9
	5	6.4±1.2	100%	5.9±0.6	0.6	
	10	10.9±0.2	96%	9.7±0.6	3.3	
	25	26.6±1.9	101%	26.2±0.6	0.4	
well water 4	0	1.20±0.01		<LOD		3.4
	5	6.2±0.2	100%	5.20±0.06	0.7	
	10	10.6±0.4	94%	10.2±0.8	0.9	
	25	26.0±0.4	99%	25.2±0.5	1.9	
ref(SPS) waste water		501.1±0.6		*500 ± 3	0.4	5000

*This value corresponds with the value reported for the certified reference material (SPS- waste water sample)

Figure Caption

Figure 1. MSFIA system for the spectrophotometric determination of V(V); V1-V5: solenoid valves, HC: holding coil, D detector, A and B: optical fibers which transport the light from the lamp to the detector, C: flow detection cell.

Figure 2. Result of the variation in 10% of the optimum values of the most significant parameters using the initial rate method. (A): Variation of the concentration of gallic acid, (B) pH variations and (C) Variation of the bromate concentration.

Figure 3.1. (A) Peaks profile obtained for a calibration curve of different concentrations of V(V) using the fixed time method, plotting absorbance vs time. (B) Calibration curve obtained for this method, plotting the average of four replicates of absorbance vs [V], error bars are included, RSD<1% for each point of calibration curve.

Figure 3.2. (A) Peaks profile obtained for a calibration curve using the initial rate method, plotting absorbance vs time. (B) Kinetic curves for different concentrations of V(V), plotting absorbance vs time. (C) Calibration curve obtained with the initial rate method, plotting average of three replicates of *slope* vs [V], error bars are included, RSD<1% for each point of calibration curve. The *slope* is the slope of the kinetic curve in the first 60s of reaction.

Supplementary material Figure caption

Figure S1. Pareto chart graphs obtained with the fractional factorial design (2^{5-1}) screening for studied factors: reaction time, temperature, concentration of gallic acid, concentration of BrO_3^-, and pH. Dependent variable: ratio of response signal of a standard of 25 µg L^{-1} of V(V) and Blank. (25 µg L^{-1} / BL).

Figure S2. Desirability graphs obtained with the central composite design for factors: gallic acid, bromate and reaction time. Dependent variable: ratio of response signal of a standard of 25 µg L^{-1} of V(V) and Blank. (25 µg L^{-1} / BL).

Figure S3. Effect of sample pH in the analytical signal. Optimal conditions, 0.08 mol L^{-1} of gallic acid, 0.36 mol L^{-1} of BrO_3^-, 140 µL of sample, 0.75 mL min^{-1} flow rate and a reaction time of 2 minutes at 40 °C.

Figure 1:

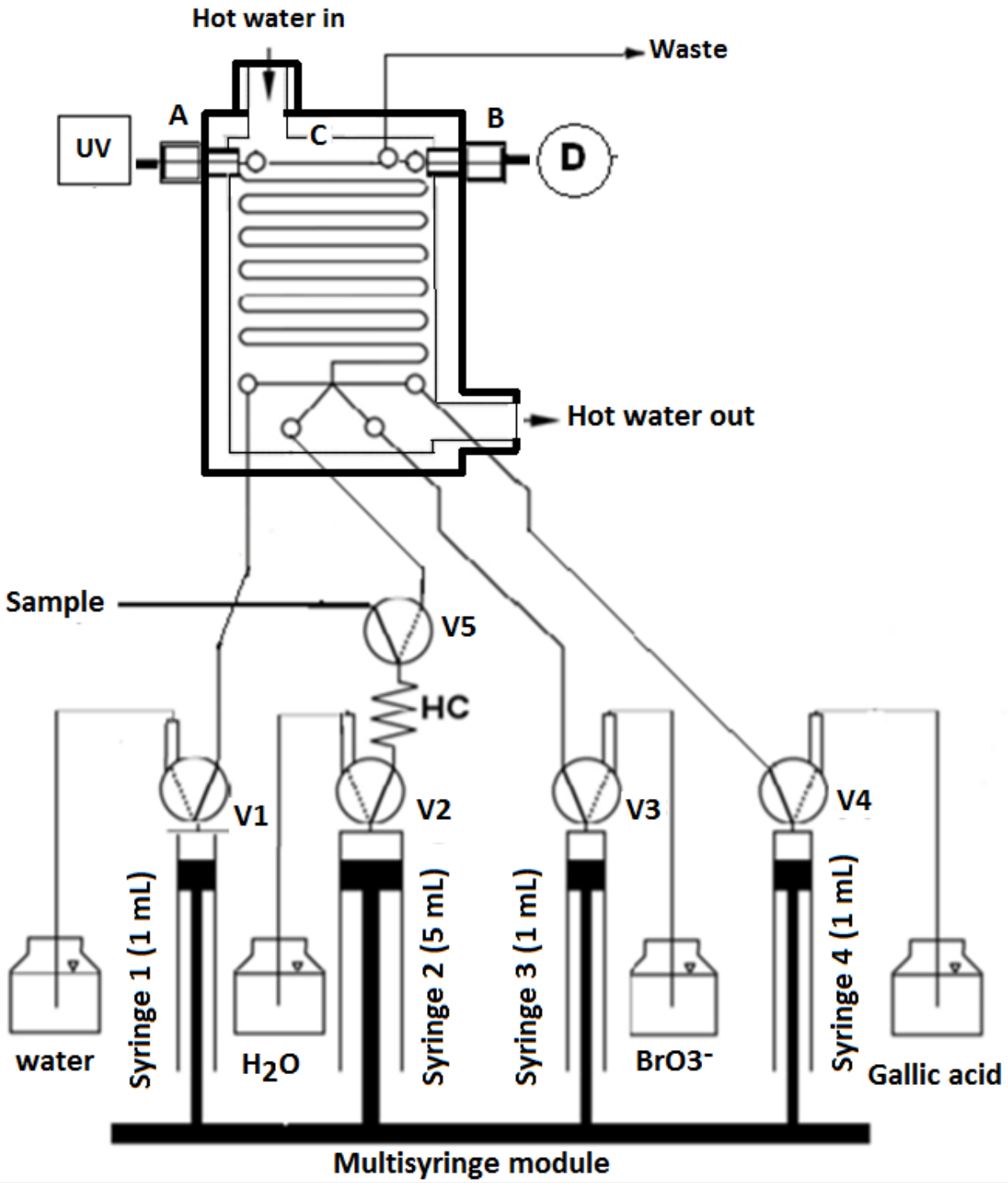


Figure 2:

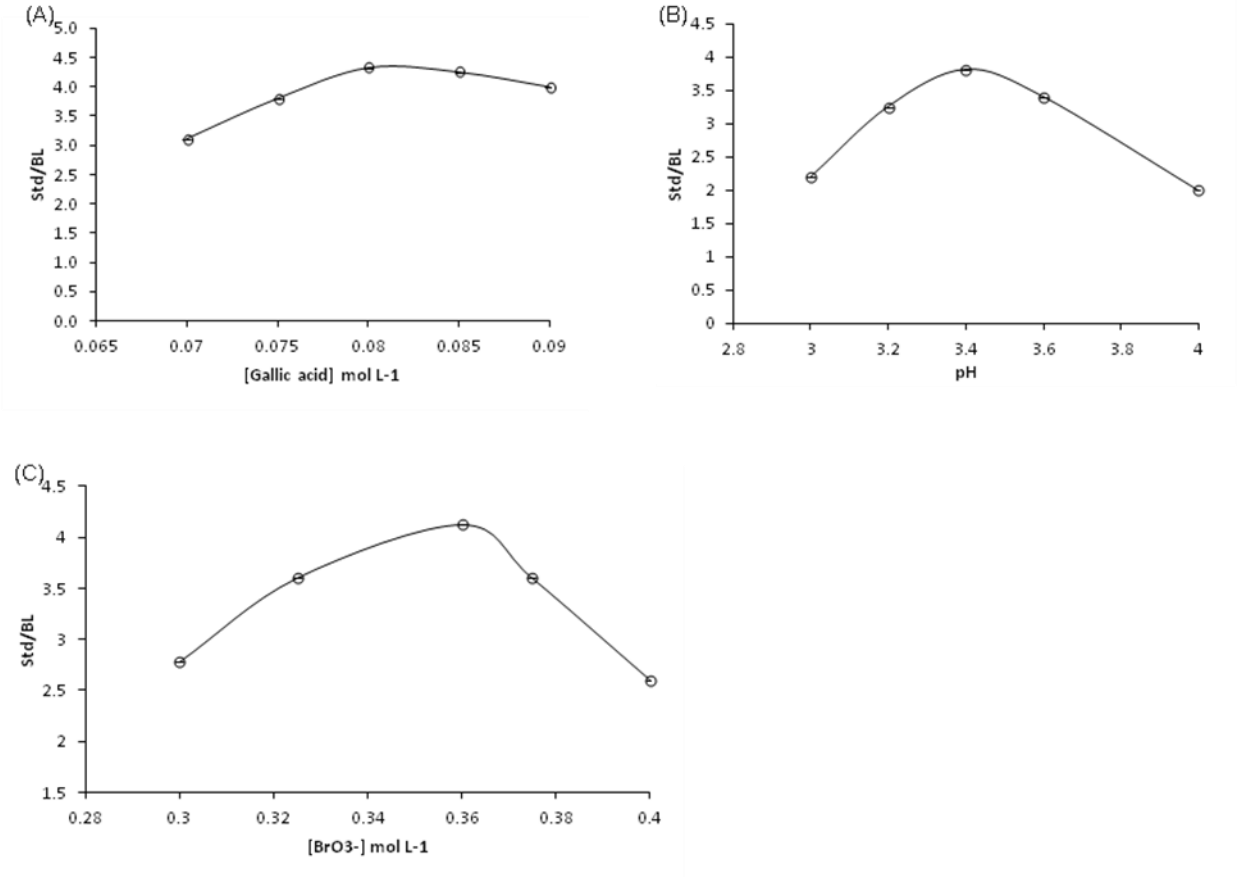


Figure 3.1:

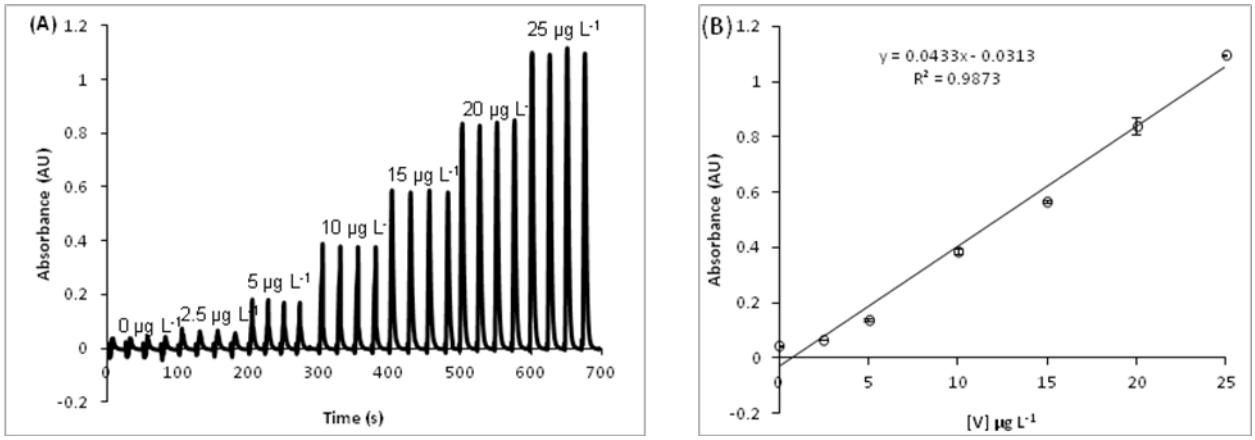
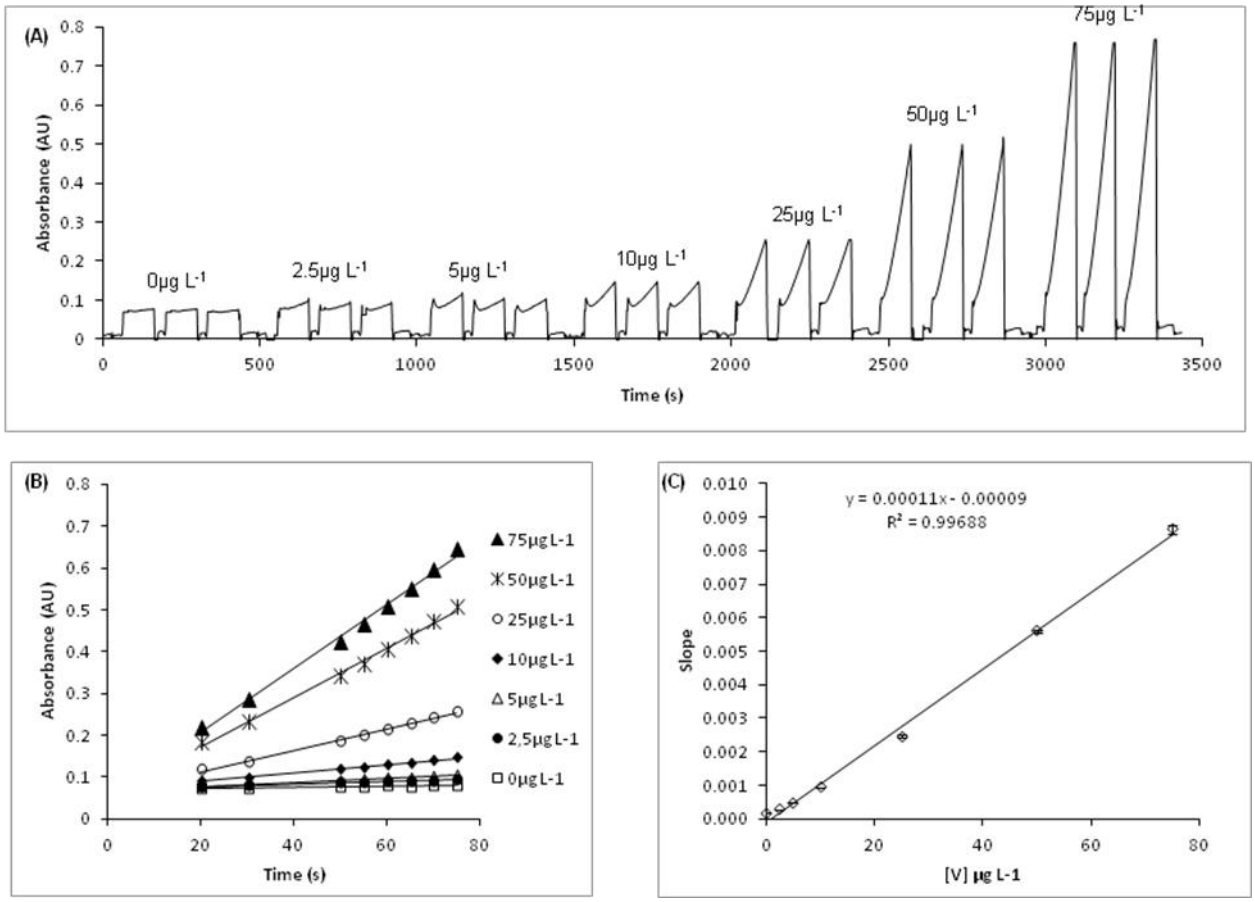


Figure 3.2:



Supplementary material

Figure S1

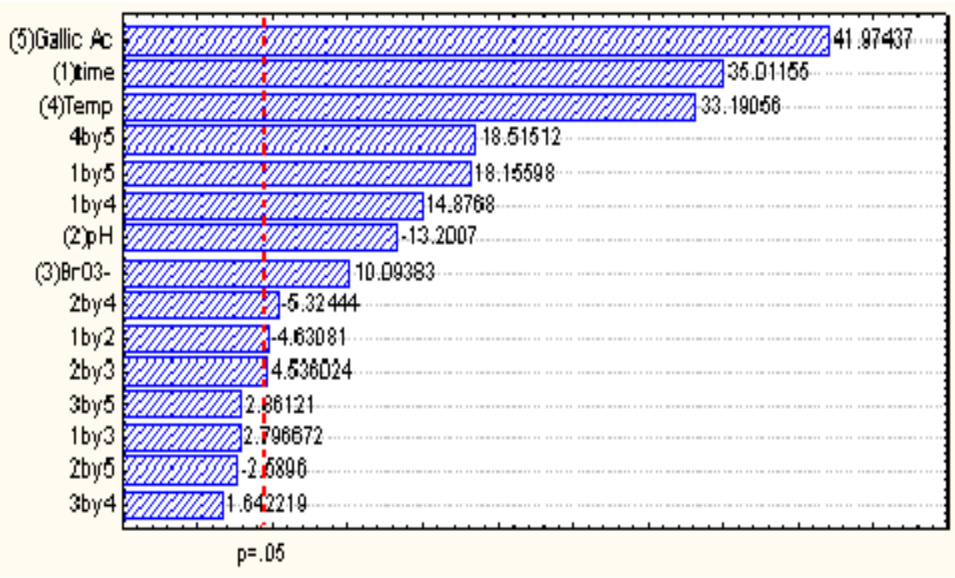


Figure S2.

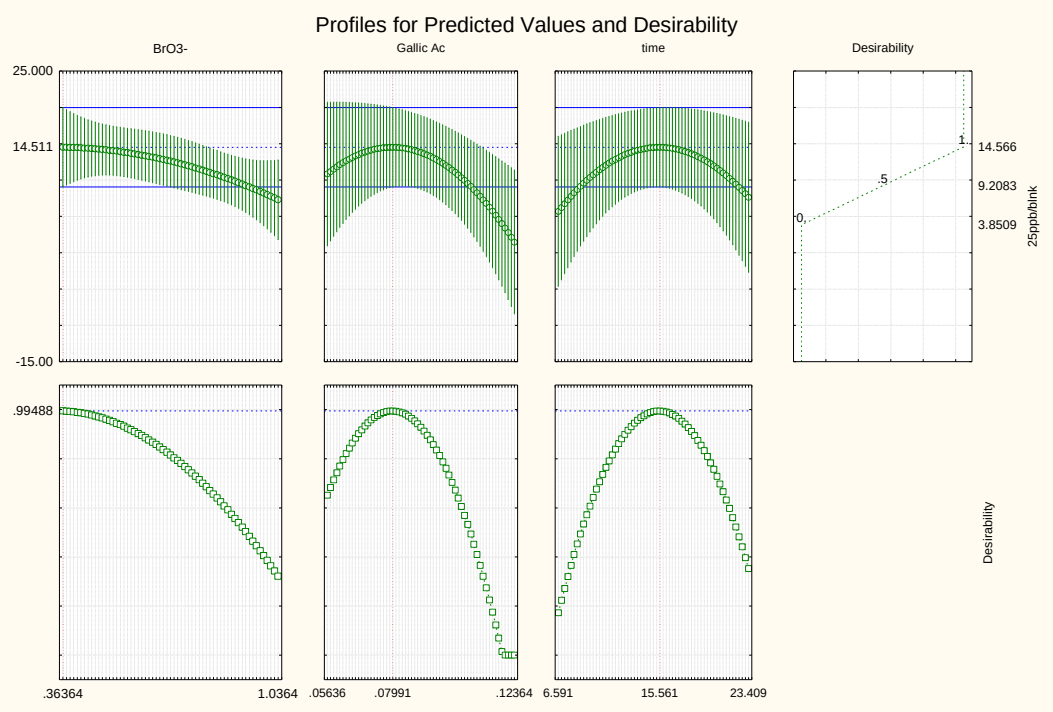


Figure S3.