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Iron speciation by microsequential injection solid phase spectrometry using 3-hydroxy-1(H)-2-methyl-4-pyridinone as chromogenic reagent

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ABSTRACT

The speciation of iron using the newly synthesized 3-hydroxy-1(H)-2-methyl-4-pyridinone by solid phase spectrophotometry in a microsequential injection lab-on-valve (μSI-LOV-SPS) methodology is described. Iron was retained in a reusable column, Nitrilotriacetic Acid Superflow (NTA) resin, and the ligand was used as both chromogenic and eluting reagent. This approach, analyte retention and matrix removal, enabled the assessment of iron (III) and total iron content in fresh waters and high salinity coastal waters with direct sample introduction, in the range of 20.0–100 μg/L. with a LOD of 9 μg/L. The overall effluent production was 2 mL, corresponding to the consumption of 0.48 μg of 2-metil-3-hydroxy-4-pyridinone, 0.34 mg of $NaHCO_3$, 16 mg of HNO_3, 4.4 μg H_2O_2 and 400 μL of sample. Four reference samples were analyzed and a relative deviation < 10% was obtained; furthermore, several bathing waters (#13) were analyzed using the developed method and the results were comparable to those obtained by atomic absorption spectrophotometry (relative deviations < 6%).

1. Introduction

To monitor the concentration of iron in natural waters is crucial to the knowledge of its distribution of the element in the environment. Moreover, it is important to determine not only the total iron content but also the fraction of both oxidation states: ferrous ion, Fe(II), and ferric ion, Fe(III) due to their dissimilar biological activity and toxicity. Therefore, for iron speciation in natural waters, direct measurements using atomic absorption or ICP techniques are not useful. Instead, spectrophotometric detection can be used for the speciation of iron in a more straightforward and economic way, if suitable chromogenic agents are employed; in fact, similar detection limits can be achieved, in the order of micrograms per liter [1]. However, most of the commonly used reagents for the spectrophotometric determination of iron, namely 1,10-phenanthroline, bathophenanthroline and eriochrome cyanine R [2], are highly toxic, and so alternatives using benign reagents are needed. In our previous work [3], the analytical application of 3-hydroxy-4-pyridinone chelators as chromogenic reagents for iron quantification was studied. A sequential injection method was developed and the 3-hydroxy-1(H)-2-methyl-4-pyridinone ligand proved to be the best choice for a flow analysis application. Although the detection limit was adequate for the iron assessment of natural waters, the application was limited to freshwaters. Additionally, no iron speciation was achieved.

In this work, the use of a 3-hydroxy-4-pyridinone (3,4-HPO) ligand in solid phase spectrophotometry (SPS) approach, combined with a microsequential injection analysis configuration is proposed to achieve iron speciation in both fresh and coastal waters. The chosen approach of SPS aimed to efficiently tackle the complexity of the target matrices, namely coastal waters. The analyte is retained by solid phase extraction and the matrix discarded to waste. After perfusion of the solid material with the ligand, the complex was measured and subsequently directed to waste. In fact, the matrix elimination resulting from the SPS approach [4] enhanced the sensitivity of the spectrophotometric method [5].

To accomplish SPS, a NTA resin was used as solid phase due to its favourable characteristics for this purpose: being relatively transparent to radiation and its affinity for iron (III). Actually,

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NTA resin has been effectively used for retaining iron in a pre-concentration procedure for water analysis [6–8] and in solid phase spectrophotometry detection [9,10]. The proposed method for iron speciation comprises two cycles: (i) direct perfusion of the resin with the ligand for the determination of iron (III); (ii) performing a preliminary in-line mixing of the sample with peroxide and subsequent retention of total iron. To the best of our knowledge, it is the first time that a 3-hydroxy-4-pyridinone ligand, the synthesized 3-hydroxy-1(H)-2-methyl-4-pyridinone, is used to perform SPS detection.

2. Experimental

2.1. Reagents and solutions

All solutions were prepared with analytical grade chemicals and boiled Milli-Q water (resistivity >18 MΩ cm, Millipore, Bedford, MA, USA).

A stock solution of 10 mg/L iron(III) standard was prepared by dilution of the 1000 mg/L atomic absorption standard (Spectrosol, England). Working standards, 0.02–0.1 mg/L in 0.03 mol/L HNO_3, were prepared by dilution of the stock solution.

The 3-hydroxy-1(H)-2-methyl-4-pyridinone ligand (Hmpp) was synthesized as previously described [11]. The ligand solution was prepared by dissolution of Hmpp to a final concentration of 15 mg/L, corresponding to a saturated solution [3]. A carbonate buffer solution, 0.5 mol/L, was prepared by dissolving 4.2 g of $NaHCO_3$ (Panreac, Spain) in 100 mL of water and adjusting the pH adjusted to 10.5 with NaOH. The Hmpp reagent was prepared every other day by mixing the Hmpp solution with the carbonate buffer, in a ratio 4:1.

Nitrilotriacetic Acid Superflow resin (Qiagen, Netherlands), highly cross-linked 6% agarose and bead diameter 60–160 μm, was used as bead suspension for packing the column in the flow cell for iron(III) retention.

Nitric acid 1 mol/L was prepared from the concentrated solution ($d=1.39$; 65%, Merck) and used as washing/conditioning solution to ensure an acidic pH for the pre-concentration.

A hydrogen peroxide solution, 12.8 mmol/L, was prepared from the concentrated solution (perhydrol, 30% H_2O_2, Merk) and used for iron(II) oxidation.

For interference assessment studies, the solutions of the tested ions were obtained from: Al^{3+}, dissolution of the solid $AlK(SO_4)_2 \cdot 12H_2O$ (Steinheim, Germany); Ca^{2+}, Mg^{2+}, Zn^{2+}, Cu^{2+}, dilution from the respective atomic absorption standards 1000 mg/L (Spectrosol, England).

2.2. Sample collection and preparation

Water samples from inland and coastal bathing areas (ESI Table. S1) were collected in polyethylene plastic bottles of 0.5 L capacity at about 30 cm depth. The samples, acidified at collection to pH ≈ 2 (with HCl) according to the collection procedure [1], were introduced directly in the developed system without filtration.

2.3. Microsequential injection manifold and procedure

The microsequential injection lab-on-valve solid phase spectrometry (μSI-LOV-SPS) manifold developed is depicted in Fig. 1.

The μSI-LOV system was a FIAlab-3500 (FIAlab Instruments) consisting of a bi-directional syringe pump (2500 μL of volume), a holding coil and a lab-on-valve manifold mounted on the top of a six-port selection valve.

The detection system comprised a USB 2000 Ocean Optics CCD spectrophotometer, fiber optics cables (FIA-P200-SR, 400 μm), and a Mikropack DH-2000-BAL deuterium halogen light source. FIAlab for Windows 5.0 software on a personal computer (HP Compact) was used for flow programming and data acquisition. The bead column was obtained by packing the NTA superflow resin between the two optical fibers, 10 mm optical path (Fig. 1B). To prevent any resin loss, a PTFE stopper (aligned with the central channel) and a PEEK tube, with inner diameter of 127 μm (#1535 Upchurch scientific), were used (Fig. 1B). All tubing connecting the different components of the flow system was of polytetrafluoroethylene (PTFE) with 0.8 mm inner diameter, including a 1.5 m of holding coil.

The protocol sequence with the respective volumes used for both iron(III) and total iron determinations is shown in Table 1.

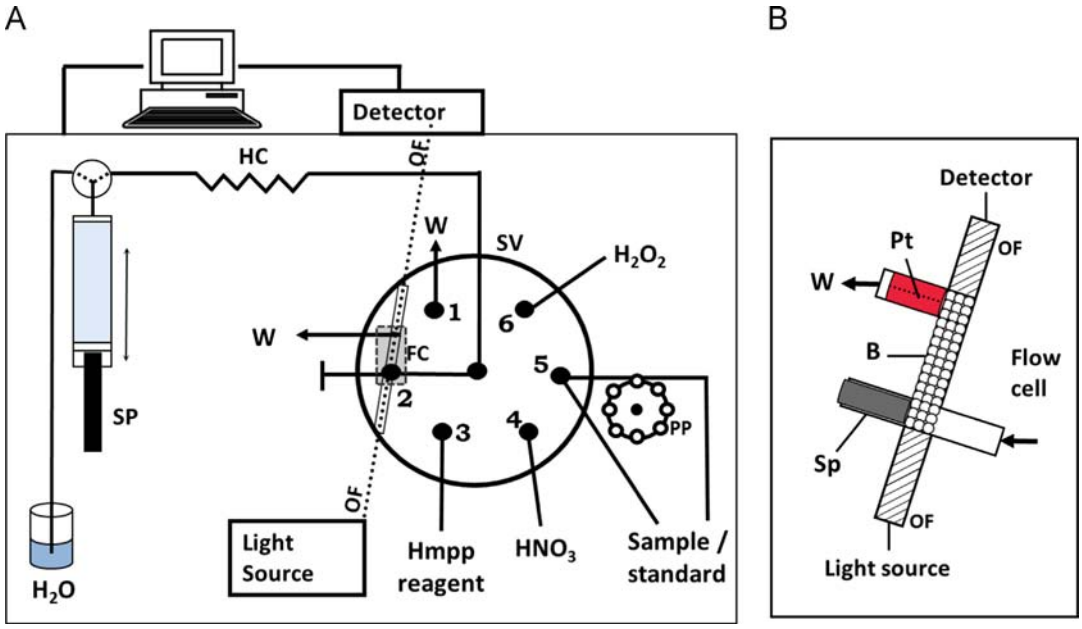

Fig. 1. Microsequential injection manifold developed for the solid phase spectrometry determination of iron with Hmpp: (A) Schematic representation: SP, syringe pump; SV, six port selection valve; HC, holding coil; PP, peristaltic pump; FC, flow cell; W, waste; OF, optical fiber; (B) Detailed scheme of the flow cell where the black arrows represent the flow direction: Sp, PTFE stopper, Pt, PEEK tube with 127 μm inner diameter; B, packed beads column of NTA resin; OF, optical fiber.

Table 1
Protocol sequence for the developed μSI-LOV-SPS for the determination of iron with Hmpp ligand.

Step	Operation	SV position	Volume (μL)	Flow rate (μL/s)
A	Fill the syringe with carrier	–	1000	200
B[a]	Aspirate H_2O_2	6	5	10
C	Aspirate sample/standard	5	400	25
D[a]	Aspirate H_2O_2	6	5	10
E	Propel sample or sample mixture through the NTA column in the flow cell	2	600	10
F	Aspirate of the Hmpp reagent	3	40	25
G	Propel the Hmpp reagent through the NTA column in flow cell with the retained iron and absorbance measurement	2	350	10
H	Aspirate HNO_3	4	250	60
I	Propel HNO_3 through the NTA column in the flow cell for washing/conditioning the column	2	1000	10

[a] Steps present only for the determination of total iron.

After filling the syringe pump with carrier (step A), the sample/standard was aspirated and propelled through the flow cell packed with NTA resin (steps C and E) for retaining iron(III). Then, the Hmpp reagent was aspirated to the holding coil and sent through the column, removing iron from the resin beads by forming the colored, 3,4-HPO iron(III) complex to be measured (steps F and G).

For the determination of total iron, two extra steps were included in the protocol sequence. After filling the syringe pump (Step A), the sample/standard was aspirated between two plugs of peroxide (Steps B–D), promoting the oxidation of iron(II) to iron (III). Then, the oxidized sample/standard was sent to the flow cell packed with NTA resin and iron(III) was retained (Step E).

Afterwards, the determination was carried out as previously described (Steps F and G).

At the end of each cycle, the NTA column was washed and conditioned with nitric acid in order to achieve similar initial experimental conditions for each cycle (steps H and I).

2.4. Reference procedure

The collected bathing waters, both inland and coastal, were analyzed using the atomic absorption method (APHA 3113B) [1] and the results were compared to those obtained with the developed μSI-LOV-SPS method.

For further accuracy assessment, results obtained with the proposed μSI-LOV-SPS system were compared to the certified values of four certified water samples. A river water certified reference material (NRC-SLRS-4), a surface water reference material (NIST-SPS-SW2) and two drinking waters (CA-021a and CA-010a) were analyzed for the evaluation of the accuracy of the developed method.

3. Results and discussion

The reaction between Hmpp and iron(III) has been previously studied and effectively applied in a sequential injection and a microsequential injection procedure [3]. However, in this work, the aim was to achieve a lower dynamic range, to enable iron speciation and also to extend the application to saline samples. In this context, a SPE step was explored using the NTA resin (beads) packed in the flow cell of a microsequential injection analysis lab-on-valve unit. The pre-concentration was attained by propelling the sample/standard solution through the packed column of beads in the flow cell followed by perfusion of the beads with the Hmpp reagent (Hmpp in carbonate buffer).

3.1. Preliminary studies

In our previous work [3], the Hmpp solution and the carbonate buffer solution were mixed in-line to improve reagent stability. In this work, due to the number of available ports, the Hmpp solution and carbonate buffer were previously mixed to produce the Hmpp reagent (described in Section 2.1).

The Hmpp concentration, corresponding to a saturated solution (15 g/L), and the aspiration volume (40 μL) were set from the previous work [3]. Different carbonate concentrations were tested, ranging from 0.03 to 0.1 mol/L; the sensitivity increased up to 0.1 mol/L. Higher concentrations were not tested as they produced a degradation of the Hmpp reagent, easily observed by a color (yellowish) increase.

The stability of this solution was studied by comparing calibration curves of four consecutive days. The results showed no significant impact on the sensitivity (slope variation $< 1\%$ for the first three days and 5% by the fourth day) but a major increase in the detection limit (intercept tripled by the third day). This feature could be explained by the degradation of the ligand with a consequent blank increase. So, an option was made to prepare Hmpp reagent every other day.

3.2. Iron retention in the NTA resin

The NTA resin (beads) was used in a reusable approach: at the beginning of the working day, the flow cell was packed by propelling the beads suspension to the optical path. Whenever a visual decrease in the column size was observed, resulting from the loss of some smaller beads to the waste due to continuous propelling, the column was refilled and/or repacked. The packed column could be used for 2 days, about 180 determinations, with no need for refilling or repacking.

There are two possible approaches when working with solid phase spectrometry: resin beads can be discharged after each measurement in a bead injection approach (BI) or reused in a pre-packed column approach. The latter was chosen as a less expensive (resin saving) option. Having set the column packing procedure, the preparation of the Hmpp reagent and the respective volume to be used, parameters for the SPE step were assessed. The flow rate of propelling through de beads was set as previously reported [9]: 10 μL/s.

3.2.1. Regeneration of the column

Due to the option of a reusable column approach, it was necessary to ensure complete elution of the metal after the measurement and guarantee identical conditions for each cycle. Nitric acid 1 mol/L was used as eluent/conditioner and different volumes were tested: 75, 100, 125, 250 μL, followed by a 4 fold volume of water for rinsing. The efficiency of the washing/regeneration process was assessed by estimating the repeatability (RSD) of a 0.800 mg Fe^{3+}/L standard. The volume of 250 μL resulted in a RSD $< 2\%$ so that was the chosen volume.

3.2.2. Column breakthrough

The column breakthrough corresponds to the maximum amount of iron(III) retained in the beads. Using an iron(III) standard of 0.4 mg/L, increasing amounts of iron(III) were loaded in the packed beads column by executing consecutive cycles with increasing volumes. The corresponding absorbance values were plotted against the mass of iron(III) loaded in the beads (ESI Fig. S1). The signal increased up to 56 ng of iron(III) as the absorbance for both 56 ng and 64 ng of iron(III) was statistically the same ($A=0.042\pm0.003$ and $A=0.043\pm0.002$, respectively). Therefore, 56 ng was considered as the maximum amount of iron(III) retained in the beads column.

3.2.3. Sample/standard volume

Having established the maximum amount of iron(III) to be retained in the NTA resin, the influence of sample volume on the sensitivity was studied. First, a wide range of volumes: 40, 60, 100, 200, 400 and 600 μL were assessed by comparing the signal of an 80 Fe^{3+}μg /L standard. The volumes of 400 and 600 μL produced the higher signals obtained (increase of > 35% compared to the other tested volumes), so, calibration curves with those volumes were compared. The sample volume of 400 μL was chosen as it resulted in a calibration curve with a higher slope value (7% increase) and a lower intercept value (5% decrease) than the calibration curve obtained with a sample volume of 600 μL, thus meaning more sensitivity and lower detection limit.

3.3. Iron speciation

The ligand Hmpp can complex both iron(II) and iron(III), due to its mild oxidizing capacity [3]. So, it was necessary to ensure that only iron(III) was retained in the NTA resin in order to achieve speciation.

3.3.1. Determination of iron(III)

The dispensed volume, of sample/standard plus carrier, propelled through the NTA resin was studied to guarantee that non-retained ions were washed out. Although iron(II) was not expected to be retained, if the dispensed volume was not sufficient, it could remain in the column dead volume and complex with the Hmpp ligand. Two iron(III) standards of 60 μg/L were prepared, one containing iron(II) 110 μg/L. The absorbance values for both standards, with different dispensed volumes, 440, 600, 800 μL (corresponding to 10%, 50%, 100% over the sample/standard volume) were compared. The results obtained showed that 440 μL of dispensed volume was not enough to wash out the non-retained iron(II) from the NTA resin. In fact, the signal for the standard containing both iron(II) and iron(III) was higher (relative deviation = 30%) than the signal for the iron(III) standard (ESI Fig. S2). However, for the dispensed volumes of 600 and 800 μL, the registered signals were the same for both standards (relative deviations < 3%), showing that iron(II) was not retained in the NTA resin (ESI Fig. S2). In order to minimize the waste production, a dispensed volume of 600 μL was chosen.

3.3.2. Determination of total iron - concentration of H_2O_2

In order to determine the total iron content, it was necessary to oxidize the Fe(II) to Fe(III), as Fe(II) was not retained in the beads. Hydrogen peroxide was chosen as the oxidizing agent for the determination of total iron and two steps were added to the analytical cycle, in order to sandwich the sample between two oxidant plugs [9]. The volume of oxidant was 5 μL per plug, the reported minimum volume to attain an effective overlapping [12].

The concentration of hydrogen peroxide was studied within the range 2–64 mmol/L in order to attain a complete oxidation of iron (II) to iron(III). The signal of an iron(II) standard of 110 μg/L was registered for the different peroxide concentrations and compared to the signal obtained for an iron(III) standard with the same concentration (Fig. 2). The results showed that 12.8 mmol/L was the minimal hydrogen peroxide concentration to obtain the same signal with equimolar standards of iron(II) and iron(III).

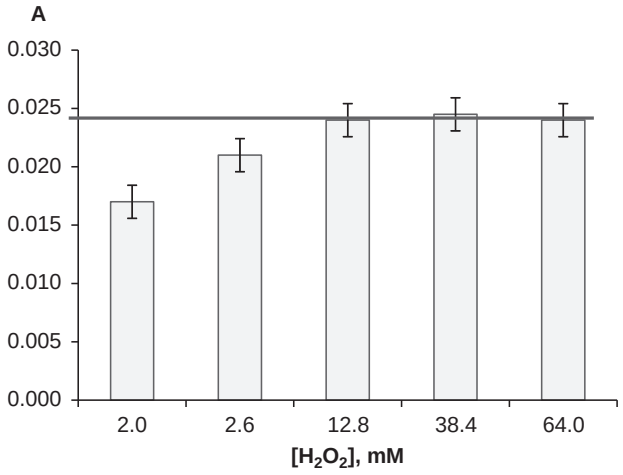

Fig. 2. Study of the effect of the hydrogen peroxide concentration on the signal obtained for 110 μg Fe^{2+} /L standard solution, grey bars; the black line represents the signal obtained for an iron(III) standard with the same concentration.

3.4. Interferences study

3.4.1. Salinity interference assessment

The effect of salinity was studied for the salinity values, 0, 5, 15 and 35, comparing calibration curves using standard solutions with those salinity values. These standard solutions were prepared by adding sodium chloride to the previously used standards to achieve a final concentrations of 0, 22, 45 and 112 g/L of NaCl. The calibration curve resulting from pure iron standards was compared to the calibration curves obtained from the iron standards with added NaCl. The estimated slopes of the curves were assessed at the confidence intervals at 95%. The quality of the regression was tested by residual analysis (i.e. randomness and normality) and by the coefficient of correlation, R^2, which was above 0.987 in all cases. No statistical difference, at 95% confidence level, was observed between the calibration curves with complete overlapping of the slope values (ESI Fig. S3), thus indicating no salinity interference. Therefore, calibration with pure iron standard solutions can be used to analyze samples with higher salinity. So, the developed methodology of iron speciation is applicable to sea water samples as well as to river and estuarine waters.

3.4.2. Possible interference of other bivalent cations

A detailed study of the possible interfering species for the colorimetric determination of iron(III) with the Hmpp ligand was accomplished in the previous work [3]. So, only potential interferences on the SPE step were evaluated, namely for other metal ions that could also be retained in the NTA resin. The metal ions that are likely to be present in natural waters such as aluminium (III), calcium(II), magnesium(II), copper(II) and zinc(II) were those whose interference was tested. Several standard solutions were prepared with the same concentration of iron(III), 60 μg/L, and different concentrations of the foreign metal ions. The tested concentrations were based on the values from the Environmental Protection Agency (EPA) for natural waters and from United Nations Food and Agriculture Organization (UNFAO) for irrigation waters [1]. The signal obtained from the standards with an inter-

fering ion were compared to those obtained with a pure iron(III) standard (Table 2).

No significant interference (< 5%) of the tested metal ions for the tested concentrations (Table 2) was found. Exception made for 1.00 mg/L of copper(II) with an interference percentage of over 10%. However, that concentration is not expected in natural waters.

3.5. Figures of merit

The features of the developed method, namely dynamic range, limit of detection, determination rate and reagent consumption, are summarized in Table 3.

The typical calibration curve corresponds to a mean of four calibration curves with the standard errors between brackets. The LOD was calculated as three times the standard deviation of the intercept ($n=5$), according to IUAPAC recommendation [13]. The quantification rate was calculated in the time spend per cycle plus the time needed for equipment operation. The repeatability was assessed by calculating the relative standard deviation (RSD) of two bathing water samples, one inland and one coastal. The reagent consumption values presented were calculated per determination. A sample consumption of 400 μL and an effluent production of about 2 mL per cycle was obtained.

3.6. Application to natural waters

3.6.1. Accuracy assessment for total iron determination

For the accuracy assessment studies, the protocol sequence was the one for the determination of total iron described in Table 1. In order to evaluate the developed methodology accurateness, four certified water sample were analyzed: two drinking waters, CA-021a and CA-010a, a surface water, NIST-SPS-SW2, and a river water certified material, NRC-SLRS-4 (Table 4). Since the certified value was above the dynamic concentration range, the certified samples were diluted prior to analysis. The repeatability was also evaluated by the calculation of the relative standard deviation (RSD).

The results obtained, RD < 10%, validate the determination of total iron attained with the developed microsequential injection lab-on-valve methodology.

For further accuracy assessment, several river and sea water samples (#13) were assessed with the developed μSI-LOV methodology (μSI-LOV) and the results compared with those obtained by the reference procedure, atomic absorption spectrometry (AAS) (Fig. 3).

A linear relationship between the results obtained with the developed μSI-LOV ($[Fe^{3+}]_{\mu SI\text{-}LOV}$) and the reference procedure ($[Fe^{3+}]_{AAS}$) was established and the equation found was: $[Fe^{3+}]_{\mu SI\text{-}LOV} = 1.004\ (\pm 0.100)\ [Fe^{3+}]_{AAS} + 0.000\ (\pm 0.007)$, where the values in parenthesis are the 95% confidence limits. These figures show that the estimated slope and intercept do not differ from the values 1 and 0, respectively. Thus, there is no evidence for systematic differences between the two set of results [14]. Furthermore, the relative deviations (RD) between the results obtained with the developed methodology and the reference procedure were calculated and the values obtained, RD ≤ 10%, proved that there were no significant differences between the two sets of results (ESI Table. S2).

3.6.2. Iron speciation in bathing waters

The proposed method was applied to several bathing waters, both inland and coastal, for iron speciation (Table 5). The determination of iron(III) was carried out without the oxidation of the analyte. For the determination of total iron, the inclusion of hydrogen peroxide, steps B and D of the protocol sequence detailed in Table 1, ensured the oxidation of iron(II) to iron(III) so the total iron content of the sample was retained in the NTA beads. The calculation of the iron(II) content was calculated by subtracting the iron(III) content from the total iron content (Table 5). The total iron content of the samples previously determined with the reference procedure (AAS) was also included in the table.

The iron content of some samples, Pi2 and P2, were slightly above the dynamic range of the developed method but the

Table 2
Study of possible interference from metal cations in the registered signal of a 60 μg /L iron(III) standard.

Tested cation	Average values in streams [1]	Legislation limits EPA[a]/UNFAO[b] [1]	Tested concentration (mg/L)	Signal interference (%)
Ca^{2+}	15 mg/L	–	25	−2.3
Mg^{2+}	4 mg/L	–	10	4.0
Al^{3+}	400 μg/L	50 μg/L (EPA) 200 μg/L[c] (UNFAO)	2.50	−3.3
Cu^{2+}	4–12 μg/L	1.3 mg/L (EPA) 200 μg/L (UNFAO)	0.50 1.00	−3.8 −14.0
Zn^{2+}	20 μg/L	2 mg/L (EPA) 5 mg/L (UNFAO)	10	2.3

[a] Environmental protection agency.
[b] United Nations Food and Agriculture Organization, irrigation waters.
[c] Value for minimal risk.

Table 4
Certified water samples assessed by the developed μSI-LOV-SPS method; RSD, relative standard deviation; RD, relative deviation.

Certified sample ID	μSI-LOV (mg Fe/L ± SD)	RSD (%)	Certified value (mg Fe/L ± SD)	RD (%)
CA-021a	0.199 ± 0.006	3.0	0.196 ± 0.002	−1.6
CA-010a	0.254 ± 0.011	4.2	0.236 ± 0.003	−7.8
SLRS-4	0.096 ± 0.001	0.8	0.103 ± 0.005	6.4
SPS-SW2	0.103 ± 0.002	2.1	0.100 ± 0.001	−3.2

Table 3
Features of developed microsequential injection methodology.

Dynamic range (μg/L)	Typical calibration curve A = slope × mg Fe^{3+}/L + b	LOD (μg/L)	Quantification rate (h^{-1})	RSD (%), (μg/L ± SD)	Reagent consumption
20.0–100	$A = 0.229\ (\pm 0.007)\ [Fe^{3+}] + 0.007\ (\pm 0.001)$ $R^2 = 0.997\ (\pm 0.003)$	8.5	14 13[a]	2.1% (75.6 ± 1.4)[b] 3.7% (88.6 ± 3.3)[b]	0.48 μg Hmpp 0.34 mg $NaHCO_3$ 15.8 mg HNO_3 4.4 μg H_2O_2[a]

[a] For the determination of total iron.
[b] Sample concentration values in brackets.

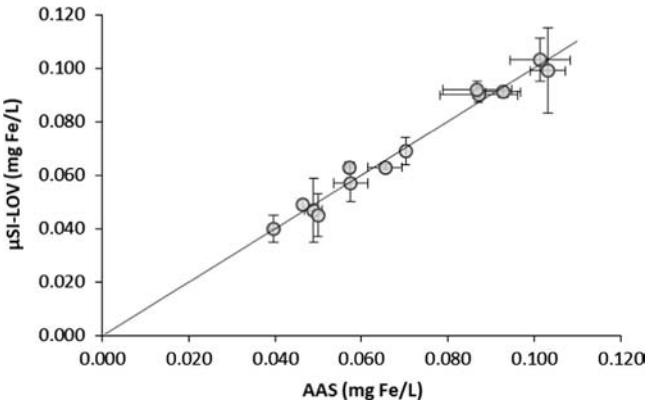

Fig. 3. Accuracy assessment comparing the total iron concentration in river and sea water samples calculated using the developed methodology (µSI-LOV) and using atomic absorption spectrometry (AAS); the line represents the optimal correlation (slope = 1 and intercept = 0).

Table 5
The developed µSI-LOV-SPS methodology was applied to iron speciation in bathing waters; SD, standard deviation; AAS, atomic absorption spectrometry; RD, relative deviation.

Sample		µSI-LOV			AAS	RD (%)
Type	ID	µg Fe^{3+}/L ± SD	µg Fe^{2+}/L	µg Fe/L ± SD	µg Fe/L ± SD	
Inland beach	Pi1	46.0 ± 1.4	16.9	62.9 ± 1.2	65.5 ± 3.6	−4
	Pi2	78.6 ± 2.4	24.7	103 ± 8	101 ± 7	2
	Pi3	46.9 ± 1.0s	18.6	48.7 ± 1.5	46.5 ± 0.0	5
	Pi4	84.0 ± 8.5	6.30	90.3 ± 2.7	87.1 ± 8.6	4
	Pi5	39.0 ± 0.7	29.8	68.8 ± 4.0	70.2 ± 0.0	−2
	Pi6	30.7 ± 7.3	9.8	40.4 ± 4.5	39.7 ± 1.0	2
Coastal beach	P1	45.9 ± 5.0	48.3	94.2 ± 2.3	–	–
	P2	86.3 ± 4.2	23.7	110 ± 5	–	–
	P3	80.4 ± 6.4	14.2	91.7 ± 3.3	86.8 ± 8.1	6
	P4	75.8 ± 3.1	15.3	91.1 ± 1.5	92.8 ± 4.5	−2

results were still in agreement with the reference procedure (when performed).

4. Conclusions

The developed microsequential injection lab-on-valve methodology for iron speciation in bathing waters proved to be an effective, real time, reliable tool for the environmental monitoring of iron. To the best of our knowledge, this is the first application of the 3-hydroxy-4-pyridinone, the 3-hydroxy-1(H)-2-methyl-4-pyridinone (Hmpp) ligand, in a solid phase spectrophotometric (SPS) determination. Although this ligand has been previously successfully used as a selective chromogenic reagent in a greener approach to the spectrophotometric determination of iron [3], its use with the solid phase extraction of the metal ion was a highly advantageous improvement. A more than 4.5 fold sensitivity increase was achieved. The choice of NTA resin for retaining iron (III) attested to be appropriate as it enabled to establish a suitable dynamic range (20.0–100 µg/L) and a direct introduction of the natural water samples. Furthermore, the choice of SPS approach widens the application range to high salinity samples. The combination of the SPE step with the SPS determination, attained by the packing of the beads (NTA resin) in the flow cell, was possible due to the high affinity of the Hmpp reagent for iron which ensured the complete removal of the metal ion following the detection. This feature allowed employing a reusable approach, minimizing reagent consumption and overall analysis cost.

The most remarkable output is to have a single methodology for iron speciation in both low salinity and high salinity water samples, with direct introduction of the sample. Aiming for the environmental monitoring of iron distribution it becomes feasible to compare results from different water sources by assessing all target samples with the same experimental procedure.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2014.03.059.

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