Greener and wide applicability range flow-based spectrophotometric method for iron determination in fresh and marine water

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Abstract

A flow-based method for the spectrophotometric determination of iron in recreational waters, both fresh and marine (variable salinity content), was developed. For that purpose, 3-hydroxy-4-pyrydinone ligand functionalized with an ether function was synthetized and used as chromogenic chelator (1-(3'-methoxypropyl)-2-methyl-3-benzyloxy-4-(1H)pyridinone - MRB13) for iron quantification. This water-soluble reagent was previously reported as a greener alternative to quantify iron, due to its low toxicity and a more environmental friendly synthesis. Furthermore, it also displayed a high affinity and specificity for iron. With the main objective of quantifying iron in a variety of water types (different matrices and iron content), two strategies were developed, one of them including on-line solid-phase extraction (SPE), and the other without resorting to a SPE process. Water matrix clean-up and iron enrichment was achieved using a nitrilotriacetic acid resin column. The potential interference of metal ions usually present in water samples was assessed and no significant interference (<10%) was observed. The limits of detection were 11 and 2.9 µg L^{-1} without and with SPE, respectively. For one determination (three replicates), the corresponding consumption of MRB13 is 90 µg, sodium hydroxide is 1.4 mg, and boric acid is 5.6 mg. The method was applied to certified water samples and the results were in agreement with certified values. The developed method was also applied to fresh and marine water, and recovery ratios of 103 ± 4 and 101 ± 7 without and with SPE, respectively, were achieved.

Keywords: Flow analysis, 3-hydroxy-4-pyridinone, solid-phase extraction, NTA

1. Introduction

Iron is a micronutrient essential for living organisms; however, as it can be introduced in the environment by human activity, it is important to monitor its content, namely in aquatic systems [1–3]. At low concentrations, iron is vital for almost all living organisms, participating in a wide variety of biological processes; nevertheless, in excess, iron becomes dangerous, producing also aesthetic effects, thus affecting the colour, taste and odour of the water [1,2,4]. In this context, iron quantification has been of particularly interest to environmental analytical chemists, aiming for new methods to improve the limit of detection, use low toxicity reagents, and reduce reagents consumption and effluents production. As flow analysis techniques can be quite useful for this purpose, several works were described (Table 1).

Table 1. Analytical characteristics of developed spectrophotometric flow system for iron determination in water samples (presented in descending chronological order).

System	Type of water	Sample volume (µL)	SPE	Reagent	Sample throughput (h^{-1})	LOD (µg L^{-1})	Ref.
SI	Fresh and marine	650 800	- NTA	MRB13	20 8	10.9 2.9	This work
µSI-LOV	River, ground, tap, sea and estuarine	40-700	NTA	CP256		7.3	[5]
pFI	Sea	300	-	Ferrozine	90/40*	3.1/0.57*	[6]
rFIA	Bottled, tap and lake	-	-	Ferrozine	20	2	[7]
µSI-LOV	River	150	-	MRB12	50	15	[4]
SI	Sea	15 mL	-	Sulfosalicylic acid and EDTA	30	90	[8]
SI	River and sea	903	- NTA	CP256	58/42** 28/24**	33/3** 27/3**	[9]
SI	Natural	5000	-	PEG-HPO	24	48	[10]
SI	River	700	-	1.10-phenantroline	-	10	[11]
µSI-LOV	Fresh and marine	400	NTA	Hmpp	14	9	[12]
FA	Fresh	420	-	SCN^-	64	60	[1]
SI	Waste and environmental	100	-	SCN^-	-	200	[13]
SI µSI-LOV	Natural	300 50	-	3,4-HPO	102 90	83 7	[14]
SIA	River, well, ground, potable and sea	250	-	Ferrozine	41	0.15	[15]
MSFIA	Drinking	5000	Modified	Chrome azurol S	6	5.6	[3]

MSFIA	Treatment unit	3200	Amberlite XAD-4 Modified Amberlite XAD-4	Chrome azurol S	-	2.3	[2]
SI-LOV	Industrial waste	45	-	5-Br-PSAA	18	25	[16]
FIA	River	500	-	DPD	20	0.02	[17]
FIA	Tap and bottled	500	-	DPD	25	0.01	[18]
SI	Waste, tap and river	150	-	1.10-phenantroline	-	12	[19]

Ref. – reference; SPE – solid phase extraction; SI – Sequential injection analysis; pFI – programmable flow; µSI-LOV – micro sequential injection lab-on-valve; CP256 – hexadentate 3-hydroxy-4-pyridinone ligand; rFIA – reverse flow injection analysis; FA – Flow analysis, HMPP – 3-hydroxy-1(H)-2methyl-4-pyridinone; MSFIA – multisyringe flow injection analysis; PEG-HPO - 3-hydroxy-4-pyridinone ligand functionalized with an hydrophilic ethylene glycol chain; 5-Br-PSAA – 2-(5-bromo-2-pyridylazo)-5-[N-n-propyl-N-(3-sulfoptopyl)amino]aniline; FIA – flow injection analysis; DPD – *N,N*-dimethyl-*p*-phenylenediamine.
*different values correspond to different flow strategies-stop in holding coil/stop in flow cell.
**different values correspond to the use of different flow cells.

The recommended methods for iron quantification [20–22] are based on atomic absorption spectrometry, inductively coupled plasma spectrometry, molecular absorption (like the phenanthroline colorimetric procedure) and chemiluminescence. However, atomic absorption and emission methods present some limitations like its high cost and non-portability of the corresponding equipment, low tolerance for high salinity samples, together with the need for a skilled operator. Due to their inherent simplicity of operation and lower lost, colorimetric methods have been extensively studied, but the use of toxic reagents (phenantroline, thiocyanate, bathophenantroline, 2,2-bipyridyl, eriochrome R and cetyltrimethylammonium) [23] is nowadays a concern, being the green chemistry principles a priority. The chemiluminescence method, using a luminol-hydrogen peroxide system, is highly sensitive for iron determination; however, this reaction is not specific for iron and other ions such as manganese (II), chromium (III), cobalt (II) and copper need to be separated prior to detection [21].

In the present work, a low toxicity iron chelator was employed for the development of a spectrophotometric flow-based method. The chosen reagent is a 3-hydroxy-4-pyridinone, (3,4-HPO) ligand functionalized with an ether function to increase solubility in water [24]. These iron chelators with chromogenic properties display significant advantages as a low toxicity reagent

with high affinity and specificity for iron(III) [12,14,24], making them an attractive choice for the quantification of iron from a green chemistry perspective.

In the last few years, some 3,4-HPOs with different substituents in different positions have been developed, including the latest 1-(3'-methoxypropyl)-2-methyl-3-benzyloxy-4-(1H)pyridinone (MRB13), the chelator used in the present work. The way the synthesis of MRB13 is achieved is also important to remark, as its route of synthesis is more efficient and sustainable if compared to the one for other ligands [24].

Besides aiming to use a low toxicity colorimetric agent, the objective of this work was to design a flow method that could be applied to recreational water samples with different salinities. In fact, most of previously reported methods do not cope with salinity interference, including those involving atomic absorption or emission spectrometry. To accomplish this objective, in this work a versatile method involving two different strategies, one including a solid phase extraction (SPE) process, is proposed. The SPE was used both to remove the sample matrix interference and/or the enrichment of the analyte. This in-line SPE process has been increasingly used as sample pre-treatment due to some advantages over other extraction techniques, namely liquid-liquid, as little or no organic reagents are employed [25,26]. With this purpose, a column with nitrilotriacetic acid (NTA) was employed, as it was previously reported that in certain conditions (pH = 2), NTA has the capability of specifically retain Fe(III) [5]. This retention occurs because NTA in the fully deprotonated form acts as a sequestering agent and this property is pH dependent [27].

By combining the capabilities of in-line SPE, it was possible to devise a flow method to measure iron in fresh and marine water, with favourable analytical features over previously reported ones.

2. Experimental

2.1. Reagents and solutions

All solutions were prepared with analytical grade chemicals and MilliQ water, MQW (resistivity > 18 MΩ cm, Millipore, USA).

A stock solution of 50.0 mg L^{-1} of Fe(III) was prepared by dilution of the respective 1000 mg L^{-1} atomic absorption standard solution (Fluka, Germany). An intermediate solution of 4.00 and 1.00 mg L^{-1} of Fe(III) solution was prepared by dilution of the 50.0 mg L^{-1} stock solution. Working standards from 5.00 to 80.0 μg L^{-1} with 0.01 mol L^{-1} of nitric acid were weekly prepared by dilution of the 1.00 mg L^{-1} solution. Working standards from 50.0 to 600 μg L^{-1} with 0.01 mol L^{-1} of nitric acid were weekly prepared by dilution of the 4.00 mg L^{-1} solution.

A 0.01 mol L^{-1} nitric acid solution was prepared by dilution of the concentrated solution (d = 1.39; 65%, Merck; Germany).

A 0.50 mol L^{-1} borate buffer solution was prepared by dissolution of the solid (H_3BO_3, Aldrich, Germany) in a solution of 0.2 mol L^{-1} NaOH (Panreac, USA), with the final pH adjusted to 10.0 with sodium hydroxide.

A stock solution of 40.0 mmol L^{-1} of 3-hydroxy-4-pyridinone (MRB13, molar mass = 233.1 g mol^{-1}) was prepared by dissolution of the corresponding quantity of the reagent in water. A 0.6 mmol L^{-1} of MRB13 [24], reagent solution, was daily prepared by dilution of the stock solution in water.

Artificial seawater was prepared according to Kester et al (1967). This seawater solution was composed by: 23.926 g kg^{-1} NaCl (Merck; Germany), 4.008 g kg^{-1} Na_2SO_4 (Merck; Germany), 0.677 g kg^{-1} KCl (Merck; Germany), 0.196 g kg^{-1} $NaHCO_3$ (Merck; Germany), 0.098 g kg^{-1} KBr (Merck; Germany), 0.026 g kg^{-1} H_3BO_3 (Aldrich; Germany), 0.003 g kg^{-1} NaF (Merck; Germany), 0.05327 mol kg^{-1} $MgCl_2{\cdot}6H_2O$ (Merck; Germany), 0.01033 mol kg^{-1} $CaCl_2$ $2H_2O$ (Merck; Germany), and 0.00009 mol kg^{-1} $SrCl_2{\cdot}6H_2O$ (Fluka; Germany). Standards of Fe(III) were prepared with artificial seawater and acidified with nitric acid 0.01 mol L^{-1}.

All solutions used for the interferences assessment (Al, Ca, Co, Cu, Mg, Mn, Ni and Zn) were prepared by diluting commercial atomic absorption standard solution (1000 mg L^{-1}, Spectrosol, England).

A stock solution of 1000 mg L^{-1} of Fe(II) was prepared by dissolution of the corresponding quantity of ammonium iron (II) sulphate hexahidrate (Merck, Germany) in 0.5 mol L^{-1} nitric acid. An intermediate stock solution of 50.0 mg L^{-1} of Fe(II) was prepared by dilution of the 1000 mg L^{-1} standard solution. An intermediate solution of 1.00 mg L^{-1} of Fe(II) solution was prepared by dilution of the 50.0 mg L^{-1} intermediate stock solution. Working standards from 5.0 to 80.0 µg L^{-1} with 0.01 mol L^{-1} of nitric acid were prepared by dilution of the 1.00 mg L^{-1} solution. Work standards prepared similarly as Fe(III) working standard solutions.

2.2 Preparation of the NTA column

NTA resin (60 – 160 µm, NTA Superflow, Qiagen, Netherlands) was used as sorbent for SPE of Fe(III) and packed in a laboratory-made column with 25 mm length of Tygon tube (Gilson, France), 1.85 mm i.d. and 67 µL inner volume. Approximately 100 mg of NTA resin was suspended in water and introduced as a slurry in the column between two pieces of dishwashing foam.

2.3 Apparatus

Solutions were propelled by a syringe pump of 5 mL (Crison, Spain) controlled by computer software. The pump was connected to the central channel of a ten-port electrically actuated selection valve (Valco VICI Cheminert C25-3180D 06B – 0699C, USA) with a PTFE tubing.

An injection valve (Valco VICI Cheminert 60736-E45 230, USA) was connected to the selection valve by the port 7 and 8. All the components of the flow system were connected by PTFE tubing from Omnifit (0.8 mm i.d., UK). The syringe pump, the selection valve and the injection valve were controlled by AutoAnalysis Station 5.0 computer software (Sciware, Spain).

As detection system, an Ocean Optics USB 4000 charged coupled device (CCD) detector spectrophotometer (USA) equipped with a pair of 600 mm optic cable and a Mikropack DH-2000-BAL deuterium halogen light source was used. An Ultem® flow cell (SMA-Z-50 cell, Ocean Optics, USA) with 50 mm optical path and silica windows and 130 µL inner volume was used.

2.4 Flow manifold and procedure

The flow manifold for the developed method for spectrophotometric determination of Fe(III) in waters is depicted in Fig. 1.

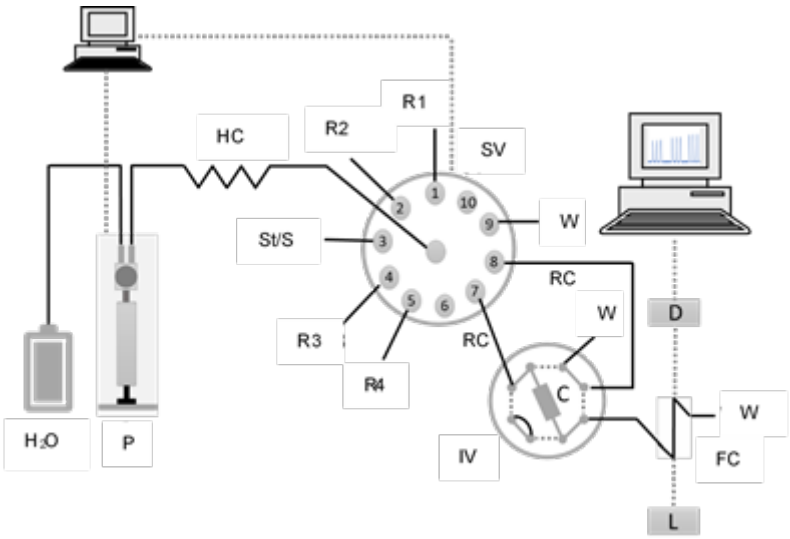

Fig 1. Flow manifold for Fe(III) determination in waters. St/S – standard solution or sample; R1 – MRB13 solution (0.6 mmol L^{-1}); R2 – borate buffer solution (pH 11); R3 – nitric acid solution 0.01 mol L^{-1}; R4 – nitric acid solution 0.5 mol L^{-1}; C – NTA resin column; P – syringe pump; SV – selection valve; IV – injection valve; HC – holding coil (300 cm); RC – reaction coils (10 cm); D – Ocean Optics USB 4000 CCD; L – light source; FC – Z flow cell (50 mm path length); W – waste.

The sequence of steps for iron determination and respective volumes are shown in Table 2. The method is subdivided in two different parts, corresponding to the different strategies for Fe(III) determination.

The sequence of steps described from A to D corresponds to the determination of iron without SPE (FA). This strategy can be chosen for samples that do not need matrix cleaning and/or pre-concentration of the analyte. Reagent, buffer solution, and sample were sequentially aspirated to the holding coil (steps A to C), and the mixture sent to the detector for absorbance measurement (step D).

Table 2. Protocol sequence for the iron determination in waters by (i) Strategy without solid phase extraction, FA: A – D; (ii) Strategy with in-line solid phase extraction SPE-FA: E – M.

Strategy	Step	SV position	IV position	Volume (mL)	Flow-rate (mL/min)	Description
Preliminary steps before starting consecutive cycles				5.000	-	Syringe reset position - syringe fill with carrier
				1.000	5.000	Propel carrier to waste
FA	A	1		0.250	3.529	Aspirate MRB13 solution
	B	2		0.020	3.529	Aspirate borate buffer solution
	C	3		0.650	3.529	Aspirate standard/sample solution
	D	8	ON	2.100	3.529	Propel through the CCD detector for Fe quantification
SPE-FA	E	3		0.800	3.529	Aspirate standard/sample solution
	F	7	ON	1.400	1.500	Propel through the NTA column
	G	1		0.250	3.529	Aspirate MRB13 solution
	H	2		0.020	3.529	Aspirate borate buffer solution
	I	7	OFF	1.500	2.000	Propel through the CCD detector for Fe quantification
SPE-FA - washing & conditioning	J	5		0.200	3.529	Aspirate HNO_3 solution 0.5 mol L^{-1}
	K	7	OFF	0.500	3.529	Propel through the NTA column – cleaning step
	L	4		0.250	3.529	Aspirate HNO_3 solution 0.01 mol L^{-1}
	M	7	ON	0.500	3.529	Propel through the NTA column – reconditioning step

The steps from E to I describe the sequence for the determination of iron with SPE (SPE-FA), providing the sample matrix (high salinity) clean-up and/or preconcentration of iron. In this case, the sample was aspirated to the holding coil and then sent through the column towards the waste (steps E and F).

Subsequently, the plugs of reagent and buffer were aspirated to the holding coil (steps G and H), the injection valve was switched and the plugs sent through the column to the detector (step I). At the end of each sample analysis (one cycle that corresponds to three replicas) the NTA column was washed and reconditioned with nitric acid 0.5 and 0.01 mol L^{-1} respectively (steps J – M).

2.5 Water sample collection and preparation

Water samples from various recreational locations from Porto district (Portugal) were collected 20 cm below the surface. The samples were filtered with Acrodisc 25 mm syringe filters 0.45 µm (Pall, USA) and acidified with nitric acid (0.01 mol L^{-1}) according to the reference sampling procedure [4]. Samples were kept refrigerated until analysis.

2.6 Reference procedure

For comparison purposes, the determination of iron in waters was carried out by the reference procedure with inductively coupled plasma – optical emission spectrometry (ICP-OES) [4], in a Perkin Elmer Optima 7000 dv (USA) equipment. Results were compared with those obtained with the developed flow method.

Additionally, the developed flow method was applied to certified water samples available for the determination of trace elements: ERM-CA615 (ground water), ERM-CA011 (hard drinking water), SLRS4 (river water, CRM, Canada) and TM27.3 (lake water, Canada). The certified water samples were diluted in order to fit the linear range of the calibration of the developed method.

3. Results and discussion

Regarding the flow system configuration (Fig. 1) an option was made to use a selection valve coupled to an injection valve. The selection valve was used to select, in a versatile way, the different solutions and samples with the respective volumes. The injection valve was used to incorporate of the NTA resin column in a loop assembly. This configuration enabled the analyte retention in the NTA to be carried out in one direction, and the elution in the opposite direction, thus minimizing the compaction of the column, without aspirating through the column. Additionally, when the SPE process was not necessary, the injection valve only acts as a flow path to the detector.

The development of the two strategies of the flow system involved several studies to assess the influence of some variables. Those parameters were optimized in order to use low volumes of reagents and standards, increase the determination rate and also increase the sensitivity for the iron determination.

3.1. Development of the FA strategy – iron determination without SPE

The use of this newly reported chelator, MRB13, for the spectrophotometric determination of iron was studied. In a recent work [24], the features of this ligand and some parent ligands were compared in a flow mode for iron measurement. However, no application for iron quantification with the use of this chelator (MRB13) was described.

In the present work, the performance of MRB13 was compared with a parent ligand (MRB12) already referred by Mesquita *et al* as a chromogenic chelator for iron determination [10] in waters. For that purpose, the selected volumes for reagent, buffer and standard were those stated in the same reported work. There were no significant differences in the slope or intercept in the calibration curve (<10%), using the two different ligands. In the reported work [18], a carbonate buffer was used. However, in flow systems, carbonate buffer may generate bubble formation. So, in this work an alternative borate buffer was tested. A 0.6 mmol L^{-1} carbonate buffer and 0.5 mmol L^{-1} borate buffer, both with pH≈10 were compared; no significant differences in the slope (<10%) of the calibration curves were observed. Therefore, borate buffer was the selected solution.

The effect of MRB13 solution concentration (1.2, 0.6 and 0.3 mmol L^{-1}) on the calibration curve (slope and intercept) was also assessed. Using 1.2 and 0.60 mmol L^{-1}, no significant differences were observed in the slope (<10%) of the calibration curve; however, the intercept decreased about 50%. When using the 0.30 mmol L^{-1}, the slope decreased 12%. Then, the concentration of the MRB13 reagent solution was set to 0.60 mmol L^{-1}. The volumes of aspiration of standard, buffer solution and MRB13 solution were also evaluated. First, the volume of 500 µL of sample/standard [10] was set, and then the results compared with those obtained with 550, 650 and 750 µL. No significant differences were observed in the slope of the calibration curve; however, a decrease of 16% in the intercept was observed when using the volumes of 650 and 750 µL. So, the volume of sample/standard was set to 650 µL. The influence of the volume of buffer was also evaluated: volumes of 34 and 20 µL were tested. As the results for the two volumes did not displayed significant differences (<10%), 20 µL was the chosen volume.

3.1.1. Interference studies

The potential interference of some metal ions that can be present in water samples was tested. As evidenced on Table 3, no significant interference in the iron determination was observed.

Table 3. Interference study of some metal ions, commonly present in natural waters, in the iron determination. Values for the concentration of ions that can be present in water streams [20].

Tested ion	Water streams µg L^{-1}	Tested µg L^{-1}	Interference in Fe determination %
Al^{3+}	400	400	-0.9
Ca^{2+}	15000	15000	-5.5
Co^{2+}	0.2	10	-1.7
Cu^{2+}	< 12	100	+0.6
Mg^{2+}	4000	5000	-3.8
Mn^{2+}	7	100	+0.6
Ni^{2+}	1	100	0.0
Zn^{2+}	20	100	-1.2

As the main goal of this work was to propose a method that could cope with different salinities, the influence of this parameter was studied. For that purpose, standards were prepared in MQW, artificial sea water, and seawater diluted 1:2 (to mimic the composition of an estuarine water). Using iron standards prepared in artificial seawater, the signals were erratic, possibly due to the high refraction signal produced during the detection. This phenomenon is usually called schlieren effect, caused by refractive index gradients between the saline solution and the other flowing aqueous solutions. With the standards prepared in ultrapure water and lower salinity content water, no significant differences were observed (< 10%) in the slope and intercept of a typical calibration curve (n=3).

3.2. Development of the SPE-FA method

In order to overcome the above-mentioned problem associated with salinity and also to carry out analyte enrichment, targeting a better detection limit, an on-line solid-phase extraction process was implemented (Fig. 1).

Then, some variables were evaluated. The first one was the influence of the sample loading volume. By increasing the volume, an increasing enrichment factor should be obtained. The use of 650, 800 and 900 µL volumes were compared. An increase of 25% of the slope was observed when the volume was increased from 650 to 800. On the contrary, for a 900 µL loading volume, the slope decreased, and the stability of the repeatability of the absorbance signals also decreased. This could be due to physical processes associated with some alteration of the

positioning of the sorbent inside the column. So, the sample volume of 800 µL was the one chosen.

The flow rate for the sample loading (0.5, 1.0, 1.5, 2.0 mL min^{-1}), and the one for the iron elution (1.0, 1.5, 2.0 mL min^{-1}) from the NTA column was also assessed. The chosen flow rates were 1.5 mL min^{-1} for the loading step and 2.0 mL min^{-1} for the extraction step. These flow rates were chosen as a compromise between sensitivity and determination rate.

3.2.1. Interference studies

The influence of salinity was a parameter of study for the determination of iron with SPE, as this water property can interfere in the quantification. For that purpose, standards were prepared in ultrapure water, artificial sea water [28], and seawater diluted 1:2 (this last one to reproduce approximately the composition of an estuarine water). The calibration curve for these different standards (prepared with different matrix) were compared, and no significant differences for the slope and intercept were observed (< 10%). Therefore, the salinity of the standards did not influence the absorbance signal and so, the developed flow procedure can be applied to different water samples. The NTA resin acts as a matrix clean-up process; this occurs at the loading step, being the iron retained at the NTA resin, while the water sample matrix is transported away from the resin and discarded.

The interference of metal ions was not assessed at this strategy, because the use of MRB13 reagent showed to be specific as iron chelating agent (see section 3.1.1). As the eluting agent is MRB13, no interferences are expected in this strategy for iron detection.

Some previous studies pointed out that iron at ferrous state (Fe^{2+}) is not retained by the NTA resin [9,12] and, because of that, by using this SPE strategy the detected ion would be the iron in ferric state (Fe^{3+}). This issue was revisited: standards of Fe^{2+} were prepared between 5.0 and 80.0 µg L^{-1} and analyzed with SPE system; the absorbance signal of the different standards did not statistically differ from the absorbance of the blank solution (< 5%). This confirms that, by using the NTA, only the determination of Fe(III) is performed. However, this is not a problem for this study, as it is not expected to find significant ferrous iron concentrations in superficial waters, if compared with ferric iron.

3.2.2. NTA column breakthrough

The breakthrough of the NTA packed column was evaluated. This value would correspond to the maximum quantity of Fe(III) that could be retained by the column. This was estimated by increasing the quantity of the analyte that perfuses the column (increasing the standard concentration) and calculating the recovered quantity of iron (in mass). The absorbance was

plotted against the mass of iron; the signal increased until 1.6 µg of iron, that corresponds to a 2.00 mg L^{-1} standard. For higher concentration values, the absorbance signal maintained constant, possibly having reached the breakthrough of the NTA column.

However, using the 2.00 mg L^{-1} iron standard, the stoichiometric ratio (1Fe:3MRB13), between iron and MRB13 reagent is almost reached; so, this value could not be the breakthrough of the NTA column, but merely a lack of reagent. Actually, this is not a problem because the 2.00 mg L^{-1} standard is twenty-five times higher than the highest standard concentration of the calibration curve.

3.3. Application to water samples – accuracy assessment

3.3.1. Certified water samples

For accuracy assessment, the developed flow system was applied to determination of iron in certified water samples. For that objective, the two strategies for iron determination were tested (Table 4). The relative deviation between the certified value and the one obtained with the developed system were below 10%, validating the developed method for iron determination.

Table 4. Comparison of the results obtained with the developed flow system for iron determination in certified water samples with the certified value for iron; direct determination (FA) and with on-line SPE (SPE - FA). RD – Relative deviation.

Sample ID	$[Fe^{3+}]_{certified}$ µg L^{-1}	$[Fe^{3+}]_{FA}$ µg L^{-1}	RD %	$[Fe^{3+}]_{SPE-FA}$ µg L^{-1}	RD %
Ca011	198 ± 5	196 ± 7	-1.0	-	-
SLRS4	103 ± 5	98 ± 3	-5.0	110 ± 5	+6.6
TM27.3	10.9 ± 0.3	11 ± 9	0.0	11 ± 1	+5.0
Ca615	5.1 ± 0.3 mg L^{-1}	5.19 ± 0 mg L^{-1}	+1.6	4.9 ± 0.3 mg L^{-1}	-4.3
				5.0 ± 0.6 mg L^{-1}*	-1.6

*sample diluted 1:100 in artificial sea water [28].

3.3.2. Recovery studies

Since the concentration of a number of the analyzed samples were below the limit of detection of the developed system and/or the reference procedure (ICP-OES), recovery tests were performed for both strategies. The recovery percentages calculations were made according to the IUPAC recommendations [29] and the results are depicted in Table 5. The developed flow

methodology for the determination of iron in fresh and marine water provided recovery ratios of 103 ± 4 without SPE and 101 ± 7 with SPE (average ± standard deviation). The statistical t-test for a 95% significance level was calculated for the two strategies of iron quantification.

For the direct determination, t-value was 0.184 and the correspondent critical value was 3.163. For the determination of iron with SPE, the calculated t-value was 0.410 and the correspondent critical value was 3.163. The statistical t-test for both strategies of iron determination indicates there is no evidence of systematic errors or the presence of some matrix interference [30]. Therefore, the developed system can be applicable for the quantification of iron in a variety of waters samples with different salt content.

Table 5. Recovery percentages obtained with the developed flow-based system in FA mode (samples F1, F2 and F3) and SPE – FA mode (samples M1, M2 and M3).

Type of water	Sample ID	$[Fe^{3+}]_{initial}$ µg L^{-1}	$[Fe^{3+}]_{added}$ µg L^{-1}	$[Fe^{3+}]_{found}$ µg L^{-1}	Recovery (%)
Fresh waters	F1	26.3 ± 5.1	50.0	74.2 ± 3.0	95.8
			200	233 ± 11	103
	F2	34.4 ± 3.6	50.0	85.3 ± 3.6	102
			200	245 ± 5	104
	F3	16.0 ± 0.0	50.0	70.8 ± 3.0	109
			200	219 ± 8	102
Marine waters	M1	< LOD	30.0	28.7 ± 1.1	95.7
			60.0	62.2 ± 8.8	104
	M2	< LOD	30.0	32.5 ± 5.5	108
			60.0	57.4 ± 1.4	95.7
	M3	11.3 ± 1.5	30.0	43.9 ± 1.0	109
			60.0	67.9 ± 3.0	94.3

3.4. Features

The dynamic ranges of both strategies as well as the calibration curves and the limit of detection and quantification (LOD and LOQ, respectively) for the determination of Fe were summarized in Table 6.

The LOD and LOQ values were calculated according to IUPAC recommendations as the concentration corresponding to the sum of three and ten times (for limit of detection and

quantification respectively) the standard deviation to the mean value of ten consecutive blank solution measurements [31,32].

Table 6. Features of the developed flow-based system for iron quantification, FA, flow analysis system without SPE; SPE-FA, flow analysis system with solid phase extraction; LOD, limit of detection; LOQ, limit of quantification; SD - standard deviation.

Strategy	Dynamic range ($\mu g\ L^{-1}$)	Typical calibration curve[a] A = (slope ± SD) $\mu g\ L^{-1}\ Fe^{3+}$ + intercept ± SD	LOD ($\mu g\ L^{-1}$)	LOQ ($\mu g\ L^{-1}$)
FA	25.0 - 800	$A = (2.21 \times 10^{-4} \pm 1 \times 10^{-6})\ Fe^{3+} + 0.045 \pm 0.001$	10.9	32.4
SPE-FA	5.0 – 80.0	$A = (6.62 \times 10^{-4} \pm 2 \times 10^{-5})\ Fe^{3+} + 0.043 \pm 0.002$	2.9	12.1

[a] n=3;

The calibrations curves presented in Table 6 correspond to the mean slope and intercept of three curves with the respective standard deviation. The repeatability was assessed by calculation of the relative standard deviation (RSD) of twelve replicate analysis of a standard (four consecutive cycles); the RSD for Fe determination with FA strategy was 2.5% (200 $\mu g\ L^{-1}$) and with the SPE-FA strategy was 3.8% (40 $\mu g\ L^{-1}$).

A complete analytical cycle (three replicas) for the determination of iron with the FA strategy takes 3 min, and for SPE-FA strategy takes 8 min (including the NTA column washing). The corresponding consumption values for a complete analytical cycle (three replicas) were: 90 µg of MRB13, 1.4 mg of sodium hydroxide and 5.6 mg of boric acid.

4. Conclusions

The developed flow-based methods for iron quantification in surface recreational water proved to be an efficient tool for water monitoring and applicable to the determination of iron in different salinity content waters (fresh and marine). The described method enables iron(III) determination with two possible strategies: a direct approach (FA) and using solid phase extraction (SPE-FA); within the same manifold. Water samples with a relatively high iron content and low salinity concentration, were assessed without resorting to the SPE strategy. If the water samples presented high salinity levels and/or a low concentration of iron, it was possible to resort to the SPE strategy with a NTA resin column incorporated in the system, enabling to clean-up the sample matrix and/or pre-concentration of iron(III).

The choice of a newly reported iron chelator [7] as a colorimetric reagent proved to be successful as both the limit of detection and quantification were better than the previously reported with similar chelators [5,9,12], except when resorting to a long pathlength flow cell.

Additionally, MRB13 has a simpler and cheaper synthesis meeting the requirements of green chemistry guidelines [7]. MRB13 reagent proved to have high affinity and specificity for iron [10,24], similar to other parent ligands, as no significant interferences were observed in the presence of other ions commonly present in natural waters (< 10%).

The incorporation of the NTA column, as a SPE strategy, in the flow-based system proved to be an effective choice to quantify low concentration of iron and to apply to high salinity waters. With SPE-FA strategy, there was the discarding of the matrix, resulting a matrix clean-up, and analyte enrichment, thus improving the detection limit from 10.9 μg L^{-1} to 2.9 μg L^{-1}.

Overall, combining the new chelator MRB13 with an in-line SPE process, an efficient method was devised for iron determination in recreational waters, displaying a low reagent consumption, low effluent production using low toxicity reagents.

The developed method was applied to certified water samples (ground, river, lake and drinking water) and the results were in agreement with the expected results.

The portability of the system makes it appropriate for the in-situ monitoring of iron in water bodies.

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