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Iodine speciation in coastal and inland bathing waters and seaweeds extracts using a sequential injection standard addition flow-batch method

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ABSTRACT

The present work describes the development of a sequential injection standard addition method for iodine speciation in bathing waters and seaweeds extracts without prior sample treatment. Iodine speciation was obtained by assessing the iodide and iodate content, the two inorganic forms of iodine in waters. For the determination of iodide, an iodide ion selective electrode (ISE) was used. The indirect determination of iodate was based on the spectrophotometric determination of nitrite (Griess reaction). For the iodate measurement, a mixing chamber was employed (flow batch approach) to explore the inherent efficient mixing, essential for the indirect determination of iodate. The application of the standard addition method enabled detection limits of 0.14 μM for iodide and 0.02 μM for iodate, together with the direct introduction of the target water samples, coastal and inland bathing waters. The results obtained were in agreement with those obtained by ICP-MS and a colorimetric reference procedure. Recovery tests also confirmed the accuracy of the developed method which was effectively applied to bathing waters and seaweed extracts.

1. Introduction

Iodine is one of the most abundant micronutrient in sea water and it is essential to all animals, including humans. In fact, the human diseases associated with iodine deficiency (IDD's) have been extensively reported by the World Health Organization [1,2]. However, the excessive intake may also result in a negative impact in human health, evidencing iodine importance as micronutrient [3,4].

Iodine may be found in water, air, soil, plants and animals but the most important source is seawater where it is mainly present as iodide (up to 50% in surface seawater) and iodate, with a minor fraction of dissolved organic iodine. So, the determination of the two inorganic forms enables to assess the iodine content and is very important to understand the geochemistry of the oceans [5,6]. Iodate is the most thermodynamically stable form of iodine in seawaters, yet due to phytoplankton and bacteria degradation, iodate is depleted from surface waters originating iodide [6]. Still, iodine levels in bathing waters are expected to be low: about 40 μg I (as I^-) L^{-1} in surface coastal water, 60 μg I L^{-1} in deep ocean water and 3 μg I L^{-1} in fresh and estuarine waters [7]. In the case of marine algae, micro and macro, iodine is mainly present as iodide since both iodate and organoiodine are in the form of monoiodotyrosine and diiodotyrosine [5,6].

Furthermore, in brown seaweed, most of iodine is inorganic, namely iodide, while in green seaweed, iodine is mostly bound to organic molecules [8].

The most common methods employed for iodine determination are capillary electrophoresis and chromatography [5]. However, these methods present a relatively high acquisition cost and, when complex matrices are involved, laborious sample pretreatments are required. In this scenario, it would be quite advantageous to develop alternative methods resorting to common laboratory equipment, namely involving potentiometry and molecular absorption spectrophotometry. Nevertheless, these methods generally do not permit to reach the required detection limits. One way to overcome this drawback, without involving complex wet chemical procedures such as separation methods, could be the application of the standard addition method (SAM). With this calibration method, based on the addition of different and known amounts of analyte to a constant amount of sample, the analyte concentration in the sample is determined by extrapolation [9]. In the end, the minimization of matrix interferences

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Table 1
Bathing waters samples characterization and sampling locations; Temp., temperature; *G*, conductivity; DO, dissolved oxygen.

Sample type	Sample ID	Multiparameter probe					Geographical coordinates	
		Temp. (°C)	pH	*G* (μs cm^{-1})	Salinity	DO, O_2 (mg L^{-1})	Longitude	Latitude
Inland bathing waters	Pi2	9.26	7.00	27	–	11.93	41.810169	−8.416739
	Pi10	16.03	6.26	36	–	8.33	41.810169	−8.416739
	Pi11	17.53	6.94	36	–	9.17	41.847478	−8.41915
	Pi12	18.03	6.88	41	–	9.16	41.597742	−8.458111
	Pi13	17.43	6.59	29	–	8.95	41.609328	−8.40830
Coastal bathing waters	P2	12.67	7.90	49441	32.28	11.05	41.156169	−8.681656
	P3	12.62	7.91	49940	32.74	11.74	41.160739	−8.686025
	P9	13.27	8.02	48067	32.34	14.33	41.695406	−8.849944
	P12	16.91	7.77	53295	35.21	8.04	41.014356	−8.644575
	P13	16.15	7.81	47182	30.81	8.43	41.202553	−9.116675
	P14	16.50	8.07	47338	30.85	8.36	41.161086	−9.103453

of complex sample matrices is accomplished. In a conventional batch mode, SAM is a very laborious and time consuming process, because it requires a calibration curve per sample. Therefore, the implementation of SAM in flow analysis could be an efficient combination. In fact, the advantages of performing the standard addition method using flow injection analysis have already been described [9,10].

In this context, the objective of the described work was to develop a sustainable method for iodine speciation in complex matrices such as bathing waters and seaweed extracts by combining sequential injection (SI) technique with SAM. The bi-parametric determination of iodide and iodate using the same SI manifold with no need for sample pre-treatment was aimed.

The choice of sequential injection approach, among other possible flow analysis modes, was due to its relative advantages, such as the possibility to perform multi-parametric determinations and the easiness to couple different devices, namely gas diffusion/dialysis units, mixing chambers and packed columns, around the selection valve [11].

The iodide determination was attained using a combined iodide ion selective electrode (ISE) due to its selectivity and simple incorporation in the flow system. Furthermore, the use of potentiometric detection is not influenced by the sample colour or turbidity. The SAM was implemented in-line by the sequential aspiration of the different standard solutions and sample.

Iodate was measured indirectly through the determination of nitrite in a sequence of two reactions [12]: (i) oxidation of hydroxylamine by iodate with production of nitrite and iodide; and (ii) Griess reaction for the determination of the formed nitrite: diazotisation of sulphanilamide by nitrite in acidic medium and the coupling of the intermediate with N-(1-naphthyl)-ethylenediamine hydrochloride (N1NED) to produce a coloured azo dye possible of being determined by spectrophotometry. To improve the oxidation of hydroxylamine by iodate, which is a relatively slow reaction, the mixture of the reagent and sample was enhanced by using a mixing chamber (MC), in a flow batch approach. A flow-batch system corresponds to the incorporation of a MC into the flow system, this way combining the advantages of batch and flow analysis, namely improved mixing between reagents and sample in a reproducible way [13]. By choosing this approach, not only a more efficient mixing is achieved, but the possibility of implementing stop periods to increase the reaction time was enabled. As the iodate concentration was calculated through the nitrite determination, based on the Griess reaction, the nitrite content of the samples was also assessed using a previously described method [14]. In the end, the nitrite content of the sample was subtracted from the assessed value that corresponded to the iodate plus nitrite in order to calculate the iodate concentration in the sample. As previously mentioned, to achieve the expected low levels of iodate and to minimize matrix interference, SAM was used and performed in-line. But in this case, fully exploring the flow-batch approach, SAM was accomplished using a single standard solution. With the MC placed in a side port of the selection valve, additions of different volumes of the standard solution combined with different volumes of deionized water (in line preparation of the standard solutions) were sent to the MC and added to the same sample volume for the in line standard addition procedure.

2. Experimental

2.1. Reagents and solutions

All solutions were prepared with analytical grade chemicals and boiled deionized water (specific conductance of less than 0.1 μS cm^{-1}).

Iodide stock solution of 100 mM was prepared by weighing 1.7 g of the previously dried KI (Merck, Darmstadt, Germany) in 100 mL of water. Standard solutions were prepared by dilution of the stock solution in the range 5.00–500 μM.

The ionic strength adjuster solution (ISA) was daily prepared by dissolving 5.1 g of potassium nitrate (Merck, Darmstadt, Germany) in water with addition of iodide to a final volume of 500 mL providing a final concentration of 0.1 μM I^- and 0.1 M KNO_3.

Iodate stock solution of 100 mM was prepared by weighing 2.15 g of the KIO_3 solid (Riedel-de Haën, Seelze, Germany) in 100 mL of water. The standard solution of 20 μM was prepared by dilution.

Hydroxylamine hydrochloride solution was prepared by dissolving 3 g of the reagent (Sigma-Aldrich, Germany) in 18 mL of 4 M hydrochloric acid (Merck, Darmstadt, Germany) and diluting to 200 mL with water. A concentration of 15 g L^{-1} hydroxylamine and 0.36 M HCl were obtained.

A stock solution of *ortho*-phosphoric acid (Merck, Darmstadt, Germany) 5 M was obtained from the concentrated acid (d=1.71, 85%). The colour reagent was prepared by dissolving 5 g of sulfanilamide (Merck, Darmstadt, Germany) in 25 mL of 5 M *ortho*-phosphoric acid and by mixing with 0.5 g of N-(1-naphthyl)-ethylenediamine dihydrochloride, N1NED, (Merck, Darmstadt, Germany) dissolved in water. After homogenizing the mixture the volume was completed to 250 mL and final concentrations of 20 g L^{-1} sulfanilamide and 2 g L^{-1} N1NED in 0.5 M of *ortho*-phosphoric acid were obtained under these conditions.

Artificial seawater was obtained by dissolving 41.5 g NaCl (Merck, Darmstadt, Germany) and 15 g $MgSO_4 \cdot 7H_2O$ (Merck, Darmstadt, Germany) in water to a final volume of 1.5 L, according to Liang et al. [15].

2.2. Sample collection and preparation

River and sea water samples were collected in 500 mL polyethylene plastic bottles at about 30 cm depth and were kept refrigerated until analysed. In situ measurements (Table 1) were performed using a YSI 6920 multiparameter probe. For iodine speciation, the bathing water samples were directly introduced in the system after reaching room temperature.

Samples of seaweeds, *Ulva lactuca* (green algae) and *Porphyra sp*. (brown algae) were collected and kept at −20 °C until analysed. To 1 g or 10 g of wet seaweeds, 50 mL of water was added and kept stirring for 3 h, followed by filtration and centrifugation for 40 min (4000 rpm at 4 °C) according to the protocol described by Hou et al. [16]. The supernatant was directly injected in the developed SI system.

2.3. Sequential injection manifold and procedure

The sequential injection manifold for iodine speciation in bathing waters using the standard addition method is depicted in Fig. 1.

Solutions were propelled by a Gilson Minipuls 3 (Villiers-le-Bel, France) peristaltic pump, P_1, with a Tygon pumping tube connected to the central channel of a ten port selection valve (Valco VICI Cheminert C25-3180EUHB, Houston, USA). All tubing connecting the different components was made of Teflon from Omnifit (Cambridge, UK), with 0.8 mm i.d.

A Crison pH meter GLP 21 potentiometer equipped with a combined iodide electrode (Hanna, Rhode Island, USA) was used and the analytical signals were recorded using a Kipp & Zonen BD 111 (Delft, The Netherlands) chart recorder. To accommodate the combined iodide electrode in the flow system, a wall-jet arrangement was set. A Perspex device was used for a robust arrangement and the flow inlet was at the bottom of the Perspex device.

A Thermo Spectronic Helios γ UV–vis spectrophotometer (Thermo Fisher Scientific, Massachusetts, USA) set at the wavelength of 543 nm and equipped with a Hellma (Müllheim/Baden, Germany) 178.711-QS flow-cell (10 mm light path, 40 μL inner volume) was used as detection system for iodate determination. The analytical signals were recorded using a Kipp & Zonen BD 111 (Delft, The Netherlands) chart recorder.

A personal computer (HP Pavillion zt3000) equipped with a National Instruments DAQcard – DI0 (Austin, TX, USA) interface card, running homemade software, was used to control the selection valve position and the peristaltic pump direction and speed.

An additional peristaltic pump, P_2, (Ismatec mini S-640) pumped ISA solution, with a continuous flow rate of 0.6 mL min^{-1}, to the ISE through an acrylic lab-made T-shaped confluence, *c*, connected to the wall-jet assemblage.

An acrylic cone shaped mixing chamber, MC, with an internal volume of ca. 0.9 mL, containing a magnetic bar, was placed over a magnetic stirrer.

Iodine speciation was obtained by the determination of iodide and iodate and the sequence of steps with the respective time and volumes is shown in Tables 2 and 3, respectively.

The first step of iodide determination (Table 2) corresponds to the aspiration of the standard solution followed by the aspiration of the sample (steps A and B). The plugs are subsequently propelled to the detector (step C) where the mixing with the ISA solution takes place, at confluence *c*, previously to the ion selective electrode where the analyte detection occurs.

For the iodate determination (Table 3), hydroxylamine solution was aspirated to the holding coil, HC, (step A). Then, the 20 μM iodate standard solution and deionised water were sequentially aspirated (steps B and C). Different standard additions were obtained in-line by the combined variation of the aspirated volumes of iodate standard solution (step B) and deionised water (step C), to the final volume of 81 μL. Afterwards the sample was aspirated (step D) and the staked plugs sent to the MC (step E). The reduction of iodate to iodide by hydroxylamine, with consequent production of nitrite, was promoted by a stopping period of 35 s with continuous stirring (step F). After the stop period, the HC is washed (step G) followed by the aspiration of the colour reagent and the mixture from the MC (steps H and I). Then, the flow reversal ensured mixing of the Griess reaction reagents for nitrite determination while propelling to the detector (step J), where the coloured product was measured. After the indirect determination of iodate, the MC is washed by discarding the residual content (steps K and L), propelling of water (step M) and discarding of the cleaning water followed by washing of the HC (steps N and O) leaving the MC ready for next cycle.

Considering the indirect determination of iodate, the nitrite assessed by the developed SI method corresponded to nitrite content in the sample plus the nitrite formed by the hydroxylamine oxidation

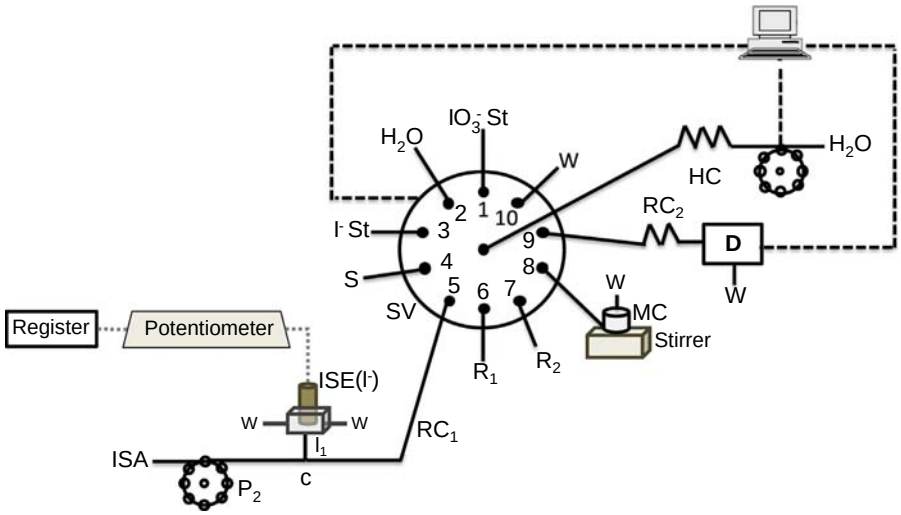

Fig. 1. Manifold for the determination of iodide and iodate in waters using the standard addition method. IO_3^- St, iodate standard solution (20 μM); I^- St, iodide standard solutions; S, sample; R_1, hydroxylamine solution (15 g L^{-1} hydroxylamine and 0.36 M HCl); R_2, sulfanilamide solution (20 g L^{-1} sulfanilamide and 2 g L^{-1} N1NED in 0.5 M of H_3PO_4); ISA, ionic strength adjuster (0.1 M KNO_3 and 10^{-7} M I^-); W, waste; MC, mixing chamber; P_i, peristaltic pumps; RC_1, reaction coil of 12.3 cm, RC_2, reaction coil of 50 cm; HC, holding coil; SV, ten port selection valve; *D*, spectrophotometer (λ=543 nm); *c*, T-shaped confluence; ISE (I^-), iodide ion selective electrode; l_1, tube of 2.5 cm length.

Table 2
Protocol sequence for the determination of iodide using the standard addition method.

Step	SV position	Flow rate (μL s^{-1})	Direction	Volume (μL)	Description
A	3	27.3	a	82	Aspiration of iodide standard solution
B	4	27.3	a	82	Aspiration of sample
C	5	54.2	b	3794	Propelling to the detector (ISE) and signal registration for the iodide determination

Table 3
Protocol sequence for the determination of iodate using the standard addition method.

Step	SV position	Flow rate (μL s^{-1})	Direction	Volume (μL)	Description
A	6	54.2	a	108	Aspiration of hydroxylamine solution
B	1	13.5	a	0–81[a]	Aspiration of standard solution (20 μM)
C	2	13.5	a	81–0[b]	Aspiration of deionised water
D	4	54.2	a	650	Aspiration of sample
E	8	54.2	b	867	Propelling to the MC
F	8	–	–	–	Stop period of 35 s
G	10	54.2	b	108	Washing the HC
H	7	54.2	a	271	Aspiration of sulfanilamide solution
I	8	54.2	a	867	Aspiration of MC content
J	9	54.2	b	4065	Propelling to the detector
K	8	54.2	a	108	Aspiration of residual content of the MC
L	10	54.2	b	163	Propelling to waste
M	8	54.2	b	921	Propelling water to the MC for washing
N	8	54.2	a	976	Aspiration of MC content
O	10	54.2	b	976	Propelling to waste

[a] Different volumes of standard solution increasing from 0 to 81 μL.
[b] Different volumes of water decreasing from 81 to 0 μL.

by iodate. So, nitrite determination in the samples was carried out according to Mesquita et al. [14]. Then, in order to calculate the iodate concentration in samples, the nitrite content of the sample was subtracted from the nitrite concentration obtained by the developed SI method.

2.4. Reference procedures

The reference method used for the iodide determination was the leuco crystal violet (LCV) method [5]. The determination is attained by the oxidation of iodide to iodine by the addition of potassium peroxymonosulfate, followed by the oxidation of the LCV by iodine to produce a coloured product, crystal violet. The absorbance was measured at 592 nm and the iodide concentration was calculated using a calibration curve method with linear dynamic range from 0.10 to 10 μM. For the iodide oxidation step, potassium peroxydisulfate was used instead of potassium peroxymonosulfate.

Since this reference method can only be applied to waters with low salinity values, it was applied to inland bathing waters. For high salinity samples, such as seawater and seaweeds extracts, the ICP-MS method was used as reference procedure. However, with this method (ICP-MS), only the total content of iodine was assessed, so the results were compared to the sum of the iodate plus iodide content.

3. Results and discussion

The main objective of this work was to determine inorganic iodine in bathing waters, both coastal and inland, using the same

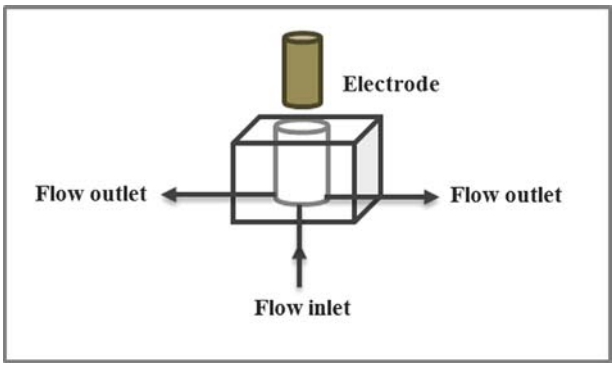

Fig. 2. Schematic representation of the wall-jet configuration.

SI manifold. So, in order to overcome the possible matrix interferences and to achieve low limits of detection, previously discussed, the developed SI method was implemented with the standard addition approach performed in-line. For iodide determination, a potentiometric detection using an ion selective electrode was used due to its selectivity towards the analyte while for iodate determination a colorimetric reaction and a spectrophotometric detection were used [12].

3.1. Determination of iodide

Due to its recognized efficiency, a wall-jet configuration was chosen to incorporate the electrode in the flow manifold (Fig. 2). In this configuration, the electrode is placed in an acrylic device and the inlet flow is at the bottom of the device, reaching the electrode at a perpendicular arrangement (Fig. 2).

Having set the electrode assembly, the carrier composition was studied. According to Ferreira et al. [17], potassium nitrate was used as carrier solution to adjust the ionic strength; different concentrations of potassium nitrate were tested. Additionally, to enable higher stability of the baseline, a low concentration of iodide should also be present in the ionic strength adjuster (ISA) solution. The addition of iodide to the carrier solution also induces a faster signal return to the baseline, so the influence of iodide concentration was also studied (Fig. 3). Concentrations of 0.01, 0.1, 0.5 and 1 M for potassium nitrate and 10^{-8}, 5×10^{-8}, 10^{-7}, 5×10^{-7}, 10^{-6} M for iodide were tested; values of 0.1 M and 10^{-7} M, respectively, were chosen since these values effectively induced a faster signal return to the baseline as shown in Fig. 3 for the addition of different iodide concentrations to the carrier solution.

Regarding the sample volume, 164 μL was chosen, from the tested volumes of 18, 42, 61, 82, 164, 347 and 520 μL as it assured a good repeatability, combined with a faster determination rate.

In order to implement the SAM, the previously chosen sample volume was split in two to accommodate both sample and added standard solution, 82 μL of each. The iodide concentration (C_A) in samples was calculated according to Santos et al. [18]. A linear calibration graph of 10^(ΔE/slope) as a function of the standard iodide concentration added to the sample was established and the iodide concentration value obtained from the equation:

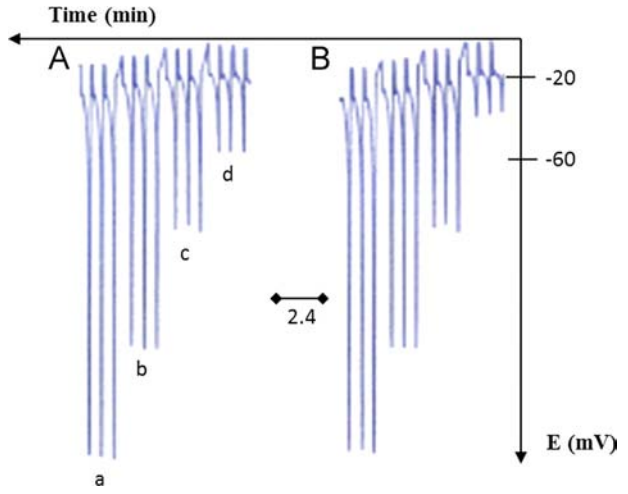

Fig. 3. Signals obtained for tracing an iodide calibration curve, using two concentrations of iodide in the carrier solution: A, 10^{-7} M; B, 10^{-8} M; a, 1.00×10^{-2} M; b, 1.00×10^{-3} M; c, 1.00×10^{-4} M; d, 1.00×10^{-5} M.

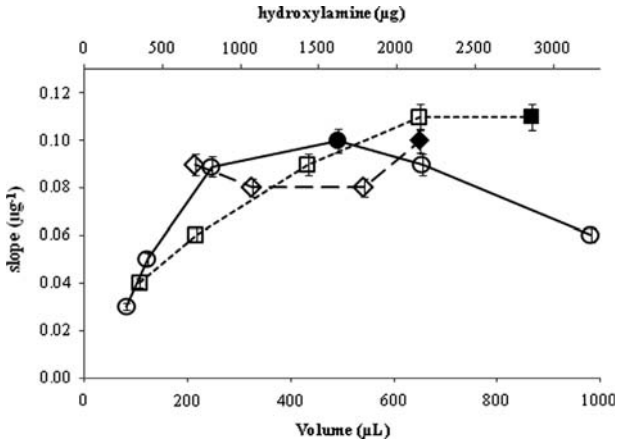

Fig. 4. Study of the influence of sample volume, hydroxylamine mass and the volume aspirated from the MC in the iodate determination: ◇, sample volume; ○, mass of hydroxylamine; □ , volume aspirated from the MC; the chosen values are represented in full black; error bars, represent 5% deviation.

C_A=(intercept × D_S)/(slope × D_{ST}), where the dilution factors of sample (D_S) and standard (D_{ST}) were previously calculated each day.

3.2. Determination of iodate

As previously stated, the aim is to apply the developed SI method to highly complex matrices, namely bathing waters and seaweed extracts, which required the use of the standard addition approach. The iodate concentration (C_B) in the samples was calculated according to Morais et al. [19]: the linear relationship between the recorded absorbance and the added iodate standard mass was established and the iodate concentration in the sample was calculated from the equation: C_B=|(−intercept/slope)/V|, where V is the sample volume.

To assure a good mixing between the solutions involved (added standards, sample and reagents), a mixing chamber (MC) was coupled to the selection valve, in a kind of flow-batch approach. Additionally, it was important to guarantee an efficient oxidation of hydroxylamine by iodate, which is a relatively slow process, so this process was promoted using magnetic stirring to improve the mixing in the MC.

The use of the MC also enabled the in-line standard addition to be performed with a single standard solution and deionised water. Consecutive cycles combined addition of different volumes of the standard solution with different volumes of deionized water to be sent to the MC and added to the same sample volume, guaranteeing, this way, a reproducible standard addition protocol. To select the volumes to be used in the standard addition protocol, the following volumes of iodate standard/water were planned: 0/75, 25/50, 50/25, 75/0 µL. These values took in consideration the minimum reproducible volume to be used in a SI mode, using a peristaltic pump [20]: about 25 µL. The experimental flow rate produced a slightly higher volume, 27 µL and 81 µL as final volume (standard solution and water). In this way, the standard additions were made in-line by aspirating a single iodate standard solution of 20 µM IO_3^- and maintaining the final volume constant.

The use of the MC limited the total volume to ca. 900 µL. The sample volume was studied within the range of 108–650 µL (Fig. 4). Although there was no major improvement in the obtained slope, the volume of 650 µL was chosen due to a slightly better sensitivity and linearity.

The volume of the hydroxylamine solution was set to 108 µL and different concentrations were tested. The corresponding hydroxylamine mass of 1620 µg was chosen from 270, 405, 813, 1620, 2160 and 3240 µg since a higher sensitivity was obtained (Fig. 4). The ratio between hydroxylamine and HCl was kept (2:3) according to Shabani et al. [12].

Having established the volumes (for added standard, sample and reagent) the remaining 61 µL were not used since we did not want to use the mixing chamber maximum capacity (900 µL) in order to avoid any loss of solution and co-contamination.

The flow-batch approach enabled the possibility to have a stop period, with continuous stirring, improving the mixture and the reaction extension. So, aiming to increase the efficiency of the hydroxylamine oxidation by iodate, different stop periods in the mixing chamber were tested. A stop period of 35 s was chosen, from tested values between 0 and 100 s, since higher values did not improve the sensitivity.

Having set the volumes sent to the MC and the stop period, the volume to be aspirated from the mixing chamber was also assessed. Volumes in the range of 108–867 µL were tested (Fig. 4) and the sensitivity increased up to the volume of 867 µL, so this was the chosen volume.

The sulfanilamide reagent was prepared according to Mesquita et al. [14]. A volume of 271 µL was chosen from the tested volumes between 55 and 650 µL since higher values did not significantly improve sensitivity and lower values result in a poor linearity.

Finally, the influence of the tube length from the valve to the detector was studied with reaction coils of 25, 50, 100, 200, and 300 cm. A 50 cm length reaction coil was chosen since there was no significant increase in sensitivity for higher values.

3.3. Interference studies

The possible interference of some ions in the developed biparametric determination of iodide and iodate was assessed. For the iodide determination, the ions tested, namely chloride, nitrite, nitrate and sulphate, were those present in bathing waters that could potentially interfere with the ISE membrane.

As the iodate determination was based on an indirect spectrophotometric determination of nitrite, the possible interference of other ions such as copper, calcium and magnesium [6] was also tested. Nitrite could not be considered interference as it was involved in the calculation itself. The interfering species and corresponding concentrations were based on maximum values mentioned in both Portuguese [21] and international legislation [22]. An exception was made for chloride, as the tested value corresponds to the expected concentration in seawaters [15]. The

solutions of the tested foreign ions were prepared from dilution of atomic absorption standards, for Cu^{2+} and Ca^{2+} (Spectrosol BDH, England), and of stock solutions prepared from the respective solids: $Mg(NO_3)_2 \cdot 6H_2O$, $NaNO_2$, $NaNO_3$ and Na_2SO_4 (all from Merck, Darmstadt, Germany).

Several standards, with 10 μM iodide or 2 μM iodate, and the tested concentration of interfering ions, were prepared to simulate the sample and analysed with the developed SI method. The concentration value obtained from the standard addition curves performed using the standard with and without interfering ion was used to calculate the interference (Table 4).

The calculated percentages of interference were lower than 10%, for expected concentrations of the tested ions, thus indicating no significant interference from natural water samples. For high concentrations of some ions (NO_3^-, Mg^{2+}, Ca^{2+} and Cu^{2+}), the interference values exceeded 10%. However, those values (maximum legislation limits) were not expected in the targeted waters.

3.4. Features of the system

The main features of the developed SI method are summarized in Table 5.

The limit of detection (LOD) and the limit of quantification (LOQ) were calculated according to IUPAC recommendation [23,24], as the concentration corresponding to three and ten times, respectively, the standard deviation of the intercept of five consecutive calibration curves with deionized water.

The standard addition plot corresponds to the mean slope and intercept of ten calibration curves, with deionised water as sample, performed in the same day with the respective standard deviations.

The repeatability was assessed by calculating the relative standard deviations for inland and costal bathing waters with seven calibrations per sample ($n=7$).

The sample frequency was calculated based on the time spent per cycle. A complete analytical cycle took about 1.3 min for iodide determination and 3.6 min for iodate determination. A complete analytical cycle takes into account not only the time in the protocol sequence but also the time required to change valve position and pump direction. Due to the analytical approach of using SAM, each sample represents an entire calibration curve, so for the iodide determination, with 7 iodide standards, two samples are analysed per hour. As for the iodate determination, with the in-line standard preparation and with four standard additions, one sample is analysed per hour. Although the sampling rate is low for a flow analysis method, the possible determination of both parameters with a single manifold along with successful dealing of sample complexity, allowing direct introduction of samples, clearly points out the advantage of the proposed methodology.

The overall consumption values per determination of iodide were: 189.3 mg of KNO_3 and 0.31 μg of KI. The overall consumption values per determination of iodate were: 19.4 mg of hydroxylamine, 17.0 mg of HCl, 65.0 mg of sulfanilamide, 6.48 mg of N1NED and 160 mg of H_3PO_4. The effluent production of the developed SI method was 30.4 mL per determination of iodide and 62.5 mL per determination of iodate.

3.5. Application to bathing waters and seaweed extracts

The developed SI method for iodine speciation was applied to bathing waters and seaweed extracts. The accuracy validation was attained by comparing the results obtained with the developed SI method (SIA) with the results obtained by two reference procedures [7]: a colorimetric method for iodide determination, the leuco crystal violet method (LCV) and inductively coupled plasma with mass detection (ICP-MS) for total iodine determination.

For the colorimetric determination of iodide with the LCV method [7], nine inland bathing waters were spiked with iodide and the results compared to the ones obtained with the developed bi-parametric SI method (SIA). The coastal bathing waters and seaweeds could not be assessed with the LCV method which is not suitable for high salinity samples. A linear relationship between C_{SIA} (μM) and C_{LCV} (μM) was established (ESI Fig. 1) and the equation found was $C_{SIA}=1.03\ (\pm 0.34) \times C_{LCV}+0.248\ (\pm 2.142)$, where the values in parenthesis are 95% confidence limits. These figures show that the estimated slope and intercept do not differ statistically from values 1 and 0, respectively [25].

Table 4
Interference percentages of the tested possible interfering ions for the iodide and iodate determination; UNFAO, United Nations Food and Agriculture Organization values; Portugal, legislated values.

Tested ion	Legislation maximum values (irrigation or streams waters)		Tested concentration (mg L^{-1})	% Interference	
	UNFAO (mg L^{-1})	Portugal (mg L^{-1})		Iodide	Iodate
Cl^-	–	–	28000[c]	−0.4	−9
NO_2^-	–	0.1[b]	0.1	4	–
NO_3^-	–	50[a]	50	−1	77
			25	–	−2
SO_4^{2-}	–	–	250	−1	4
Mg^{2+}	5[b]	50[b]	50	–	52
			0.5		4
Ca^{2+}	15[b]	50[b]	50		77
			25		−5
Cu^{2+}	1.3[a]	5[a]	5		151
			0.25		1

[a] Irrigation.
[b] Streams.
[c] Value according to Liang et al. [15].

Table 5
Features of the developed SI method for iodine speciation by iodide and iodate determination; RSD, relative standard deviation.

Parameter	Standard addition plot[a] (μM)	LOD[b] (μM)	LOQ[c] (μM)	RSD (%)
Iodide	E (mV) = $-59.8 \pm 2.5 \log [I^-] - 52.8 \pm 6.9$	0.14	0.47	4.2 (4.83 ± 0.20 μM)[d]
				5.2 (6.79 ± 0.35 μM)[d]
Iodate	$A = 1.57 \times 10^{-3} \pm 1.4 \times 10^{-4}\ [IO_3^-] - 7 \times 10^{-3} \pm 1 \times 10^{-3}$	0.02	0.06	4.1 (1.93 ± 0.08 μM)[d]
				2.5 (3.77 ± 0.09 μM)[d]

[a] $n=10$.
[b] Limit of detection ($n=5$).
[c] Limit of quantification ($n=5$).
[d] $n=7$.

For determination of total iodine with ICP-MS, 7 samples, 3 seaweed extracts and 4 spiked inland bathing waters, were analysed and the results compared to those obtained with the developed SI method for the bi-parametric determination of iodide and iodate (Table 6).

For the total inorganic iodine determination, a linear relationship between C_{SIA} (μM) and C_{ICP-MS} (μM) was established and the equation found was: $C_{SIA} = 1.00\ (\pm 0.16) \times C_{ICP\text{-}MS} + 0.02\ (\pm 0.26)$, where the values in parenthesis are 95% confidence limits. These figures show that the estimated slope and intercept do not differ statistically from values 1 to 0, respectively [25].

Concerning the values presented in Table 6, the following clarification should be made. Trace values of iodide and iodate were observed for inland bathing waters and seaweeds extracts respectively. So the calculation of the relative deviation was made based on the iodate values for inland bathing waters and iodide for seaweed extracts. As the determination of iodate implied the measurement of the nitrite content, the nitrite concentration was also assessed; in seaweeds extracts, it was below the limit of detection [14]. For the inland bathing waters, the values were: Pi10, $[NO_2^-] = 1.60 \pm 0.05$ μM; Pi11, $[NO_2^-] = 1.55 \pm 0.08$ μM; Pi12, $[NO_2^-] = 1.55 \pm 0.03$ μM; Pi13, $[NO_2^-] = 1.76 \pm 0.08$ μM; and taken into account for the calculations.

To further assess the accuracy, recovery tests were performed using bathing water samples, both coastal and inland. A concentration of 4.00 μM of iodide was added to the samples, with 20 μL of 5 mM iodide in 25 mL of sample. For iodate recoveries volumes of 25, 50, 75 and 100 μL of 1 mM iodate in 25 mL of sample yielding final iodate concentrations of 1.00, 2.01, 3.01 and 4.02 μM. The calculation of the recovery percentage was made according to IUPAC recommendation [26] and the results obtained are presented in Table 7. The iodate values corresponding to the initial concentration were below LOD so they were not included in the table.

The developed SI methodology provided recovery ratios with an average (± standard deviation) of 101 ± 7% for iodide determination and of 100 ± 4% for iodate determination and a statistical test (*t*-test) was used to evaluate if it did not significantly differ from 100% [25]. For a 95% significance level, the calculated *t*-value was 0.300 for iodide and 0.092 for iodate with a correspondent critical value 3.163 and 2.841, respectively, thus indicating the absence of multiplicative matrix interference.

4. Conclusions

The developed SI method using the standard addition approach for the bi-parametric determination of iodide and iodate is an effective process for inorganic iodine speciation, in bathing waters and seaweed extracts. As far as we know, there is no previous work combining flow analysis with the standard addition method for iodine speciation in waters. In fact, only seven articles (ESI Table 1) [27–33] describe the simultaneous determination of iodide and iodate using flow injection analysis (FIA) and none involving sequential injection analysis with the standard addition method (SAM). The FIA approach is usually associated with higher consumption values, due to continuous flow of reagents, and less automation than SI. For iodide determination, several works using

Table 6
Comparison of the results obtained with the developed SI method for iodine speciation by iodide and iodate determination (SIA) to those obtained with ICP-MS for accuracy validation; SD, standard deviation; RD, relative deviation.

Sample source	Sample ID	ICP-MS (μM)	SIA (μM)		RD (%)
		$[I_2] \pm$ SD	$[I^-] \pm$ SD	$[IO_3^-] \pm$ SD	
Seaweed extracts	PA	2.80 ± 0.11	2.89 ± 0.72	< LOD	3.2
	PA2	0.634 ± 0.031	0.702 ± 0.012	< LOD	10.7
	PA4	0.491 ± 0.071	0.484 ± 0.043	< LOD	−1.4
Inland bathing waters	Pi10	1.04 ± 0.11	< LOD	0.940 ± 0.200	−9.6
	Pi11	2.18 ± 0.04	< LOD	2.03 ± 0.01	−7.1
	Pi12	1.13 ± 0.01	< LOD	1.29 ± 0.00	14.2
	Pi13	1.88 ± 0.08	< LOD	1.99 ± 0.21	6.1

Table 7
Recovery percentages obtained with the developed SI method using SAM for iodine speciation in bathing waters; SD, standard deviation; and RSD, relative standard deviation.

Analyte	Sample ID	Initial			Added	Found			Recovery (%)
		$[I^-]$ (μM)	SD	RSD %	$[I^-]$ (μM)	$[I^-]$ (μM)	SD	RSD %	
Iodide	P2	3.61	0.95	26	4.00	7.63	0.50	7	101
	P3	3.27	0.91	28	4.00	7.38	0.58	8	103
	P9	3.72	0.52	14	4.00	7.59	0.51	7	97
	P12	6.11	0.9	15	4.00	10.3	1.7	17	105
	P13	3.02	0.14	5	4.00	7.43	0.74	10	110
	P14	1.82	0.37	20	4.00	5.42	0.37	7	90
		$[NO_2^-]$ (μM)	SD	RSD %	$[IO_3^-]$ (μM)	$[IO_3^-]$ (μM)	SD	RSD %	
Iodate	Pi4	0.37	0.04	10	4.02	3.87	0.36	9	96
	P2	3.61	0.06	2	1.00	0.99	0.07	7	99
	P3	0.700	0.000	0	1.00	0.95	0.12	13	95
				0	3.01	2.96	0.08	3	98
	P9	9.67	0.06	1	1.00	1.07	0.22	20	106
				1	3.01	3.07	0.57	19	102
	P12	3.37	0.13	4	4.02	3.99	0.55	14	99
				4	2.01	2.13	0.05	2	106

potentiometric detection in FIA were described, none of them using SAM [29,32,34,35], and the one [30] applied to marine samples presented one manifold configuration for each parameter and required prior sample treatment. So, as far as we know, this is also the first application of SAM in sequential injection (SI) for the potentiometric determination of iodide in waters.

The herein proposed sequential injection system has the following advantages: programmable flow with higher extent of automation, a single manifold to accommodate the determination of both parameters, no prior sample treatment is required and employs less toxic reagents. Additionally, the developed method can be effectively applied for iodine speciation in both seawater and freshwaters. By combining the SI technique with the standard addition method (SAM), it was possible to minimize the expected matrix interference, especially for seawater and seaweed samples, both for potentiometric and spectrophotometric detection.

Furthermore, preliminary studies shown that the application of SAM enabled to achieve lower limits of detection when compared to the calibration curve method.

Regarding the levels of these parameters found in the analysed samples, the results showed that iodate was depleted in surface seawater (coastal bathing waters) while iodide was present at the micromolar level. This was expected since iodate in surface seawater, in spite of being more stable than iodide, is degraded by bacteria and phytoplankton to iodide. In inland bathing waters, neither iodide nor iodate was detected, indicating the trace presence of those ions. Iodide was present at higher concentrations in seaweeds which confirms that this is the predominant form in macroalgae since iodate is majorly present as monoiodotyrosine and diiodotyrosine [5,6].

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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.2014.01.025.

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