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Chloride Catalytic Determination as Potential Tool to Assess Metal Ion Bioavailability in Water

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Abstract: This paper focuses on the development of an environmentally friendly sequential injection (SI) method for the determination of chloride in water samples from dynamic water systems. Chloride quantification is highly relevant, as it may affect metal ion bioavailability and potential toxicity to the environment. The approach was established based on the catalytic reaction of chloride ions in the colorimetric reaction between 3,3′,5,5′-tetramethylbenzidine (TMB) and hydrogen peroxide. Optimisation studies were performed regarding several parameters such as reaction pH, reagent volume and concentration, reaction time, and flow rates. As such, it was possible to obtain a wide dynamic range of 60 to 1000 mM, with a limit of detection and quantification of 17 and 58 mM, respectively, and a relative standard deviation of 7%. Validation was performed by analysing 13 water samples from dynamic water systems, namely seawater, estuarine water, and estuarine harbour water, with the SI method developed and by comparing the results obtained to potentiometric titration as the reference method. The relative error of these comparisons was not significant (<10%). Interference studies were also performed and showed no significant effect on the performance of the system (interference percentage < 10%), proving that a robust and sensitive system was developed.

Keywords: metal ions toxicity; chloride quantification; chloro-complex formation

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1. Introduction

Metal ions play a crucial role in both environmental and human health, as they are essential at trace levels but highly toxic when present at high levels. The contamination of metal ions in water sources is a rising concern, since some of them result from anthropological events, such as industrial discharges, agricultural runoff, and inadequate water and wastewater treatments [1,2]. This is a severe global problem because it is persistent, irreversible, and can degrade the quality of the atmosphere and of life, since it can affect humans via the food chain [3]. Some metal ions are considered micronutrients, namely copper, zinc, and cobalt, but they can still be toxic at high concentration levels. Other metal ions are toxic to humans, even at low levels, namely cadmium, chromium, arsenic, lead, and mercury. These metal ions' toxicity depends on many factors, such as chemical and physical speciation, emphasising the need to gain knowledge regarding each speciation [4].

The most used methods for the quantification of metal ions are atomic techniques, namely inductively coupled plasma optical emission spectroscopy and mass spectrometry (ICP-OES and ICP-MS) and atomic absorption spectrometry. Other techniques, including molecular techniques such as UV/Vis absorption spectrometry, ion chromatography, and electrochemistry are also often employed [5]. The interaction between metal ions and the water matrix influences their bioavailability and the extent to which they become toxic to human life. For example, high chloride concentrations decrease the toxicity of Cu(II), cause no interference in the toxicity of Cd(II), and increase the toxicity of Hg(II) and Pb(II) [4]. Chloride is distributed throughout natural waters and is present in almost all water sources, ranging from 10 to 20 mg/L in river water to 19 g/L in seawater [6]. Additionally, the salt content in water, in the form of dissolved NaCl, can also result in high chloride concentration, contributing to the formation of chloro-complexes. These complexes are coordination compounds formed when metal ions bond with chloride ions. It has become essential to understand how the chloride concentration influences the formation and stability of these complexes. The charge of species involving Ag, Cd, Cu, Ni, and Zn may vary with saline conditions because of the complexation between each metal cation and the chloride ions [7]. Therefore, as the chloride content can influence the ions' electrical charge, it is very important to attain accurate quantification. Waters with high or variable salinity content can affect the chloro-complex formation with the metal ions, conditioning their bioavailability. Some of the previously reported methods for chloride quantification in water samples are listed in Table 1. There are potentiometric methods [8,9], spectrophotometric flow-based methods [10–12], argentometric determination [13], and an atomic absorption spectrophotometry method [14], illustrating a variety of potential detection systems.

Table 1. Previously reported methods for the determination of chloride in water samples.

Water Type	Reagent Used	Dynamic Range (mg/L)	Determination Details	LOD (mg/L)	Ref.
Groundwater	Self-made silver-chloride membrane electrode	0.15–2.9	Potentiometric determination ISE-Cl	0.009	[8]
Aqueous soil extracts and water samples	Ammonium nitrate-nitric acid reagent	10–100	Potentiometric determination (ISE)	0.5	[9]
Natural waters	Mercury (II) thiocyanate	1.98–7.80	Flow injection spectrophotometry	0.5	[10]
Mineral, tap and well waters	Mercury (II) thiocyanate	0.2–8	Multisyringe flow injection spectrophotometry	0.06	[11]
Natural waters	Potassium Persulfate	2.0–20	Spectrophotometric multi pumping flow system	0.7	[12]
Drinking, mineral and river waters	Silver nitrate	3.5–886	Argentometric determination	1.77	[13]
Drinking and groundwater	-	0.03–3	Atomic absorption spectrometry	n.d.	[14]

n.d. not described.

The spectrophotometric methods use mercury (II) salts or persulfate, which can pose a serious limitation from a green chemistry perspective [10–12]. The potentiometric determinations may have some interferences from the matrix, requiring ionic strength adjusting according to the different sources of water [8,9].

Argentometry employs the use of silver nitrate, which is also not environmentally friendly [13]; in the case of atomic absorption, the detection method itself is highly expensive [14].

Therefore, more environmentally friendly, analytical procedures are needed, using chemicals with low toxicity, in small amounts, and with less waste production, alongside more automated techniques [15]. Furthermore, a common denominator in all the methods described (Table 1) is that they were not applied in high or variable salinity content waters (as is the cases of estuarine water and seawater). This work aimed to overcome this drawback while adopting a green chemistry approach, developing a method to quantify chloride in different salinity content waters, namely estuarine waters. The idea was to attain a simple, automated, and sensitive method that uses a greener approach with less toxic reagents and lower waste production. As such, a sequential injection lab-on-valve method was developed, using 3,3′,5,5′-tetramethylbenzidine (TMB) to oxidise hydrogen peroxide, which is a less harmful reagent [16], for the determination of chloride levels in different water bodies. The reaction was between TMB and peroxide, with copper (II) acting as a trigger and chloride acting as a catalyst.

2. Materials and Methods

2.1. Reagents and Solutions

All the solutions used in this work were prepared with analytical grade chemicals and Milli-Q water, MQW, (resistivity > 18.2 MΩ/cm, Millipore, Burlington, MA, USA).

A 2.00 M chloride stock solution was prepared from solid sodium chloride (Merck, Darmstadt, Germany) and used to prepare chloride standard solutions from 0.060 to 1.0 M.

A 0.80 M acetic acid solution was prepared by diluting 4.6 mL of acetic acid (d = 1.05, 100% glacial, Merck, Darmstadt, Germany) in 100 mL of water, with a resulting pH of 2.3. A 5.0 M hydrogen peroxide (H_2O_2) solution was prepared by the dilution of the commercial solution (d = 1.11, 30% Merck, Darmstadt, Germany). The oxidant solution prepared containing peroxide and acetic acid was prepared daily by mixing 100 μL of 5.0 M hydrogen peroxide in 4 mL of 0.80 M acetic acid to obtain a final peroxide concentration of 0.12 M.

The 3,3′,5,5′-tetramethylbenzidine (TMB) solution was prepared by dissolving 24 mg of TMB (Sigma-Aldrich, Saint Louis, MO, USA) in 50 mL of ethanol (96%, Labchem, Greenford, UK) and then adding 50 mL of water, resulting in a 1 mM concentration, as described by Palladino et al. [17]. This solution was stored in a dark bottle and refrigerated.

A 50 mM copper solution was prepared by dissolving 100 mg of copper acetate (Merck, Darmstadt, Germany) in 10 mL of the 0.80 M acetic acid solution.

2.2. Apparatus

A sequential injection lab-on-valve (SI-LOV) method was developed for chloride quantification in natural waters, as shown in Figure 1.

The SI-LOV system consisted of the FIALab 3500 (FIALab, Seatle, WA, USA), connected to an HP computer running the FIALab for Windows 5.0 software, equipped with a syringe pump (2.5 mL), a lab-on-valve six-port selection vale and a USB2000 detector (Ocean Optics, Orlando, FL, USA) connected to a UV-VIS-NR Mikropack Lightsource with FIA-P400-SR fibres (Ocean Optics, Orlando, FL, USA). The absorbance value was registered at 450 nm.

As an additional propulsion system, the FIALab 3500 peristaltic pump was also used, equipped with two PVC propulsion tubes—one with a 1.02 mm inner diameter for a sample or standard and one with a 0.76 mm inner diameter for the acetic acid solution—connected to a Y-shaped confluence. All the connections between the ports and the solutions were established with Gilson polytetrafluoroethylene (PTFE) tubes (inner diameter 0.8 mm, 008T16-080-20).

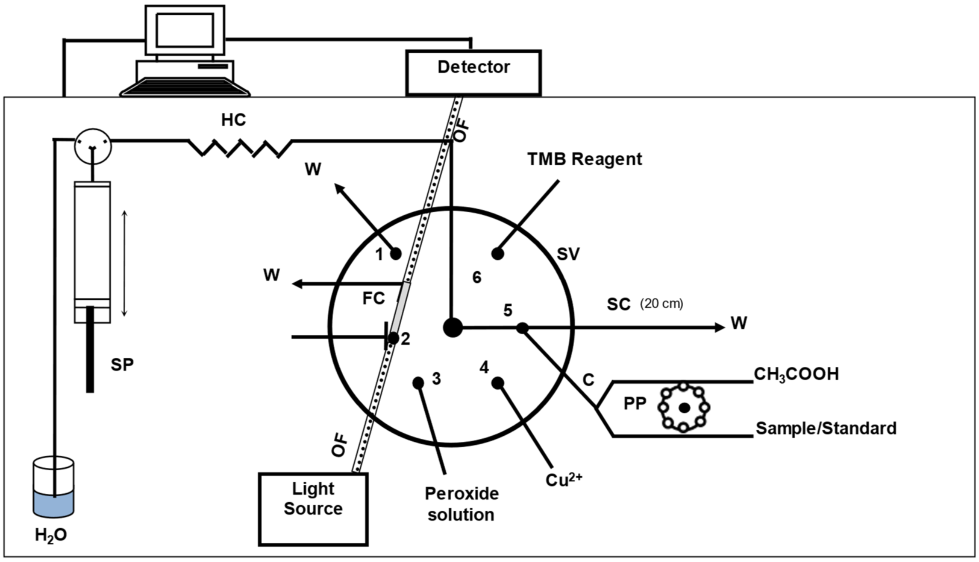

Figure 1. Manifold of SI-LOV system developed for quantification of chloride; SP, syringe pump; HC, holding coil; OF, optical fibre cables; FC, 1 cm flow cell; SV, six-port selection valve; W, waste; SC, 20 cm sample/standard coil; Sample or standard (S/St); CH_3COOH, acetic acid 1.7 M; C, Y shaped confluence; PP, peristaltic pump at 60% counter clockwise; TMB reagent, 3,3′,5,5′-tetramethylbenzidine solution 1 mM; peroxide solution, oxidant solution of 0.12 M peroxide in 0.8 M acetic acid solution; Cu^{2+}, 50 mM copper solution in 0.8 M acetic acid.

2.3. Sequential Injection Procedure

The protocol sequence of the developed SI method is depicted in Table 2.

The cycle was initiated by filling the syringe pump with water and simultaneously loading the sample/standard coil (SC) with acidified sample/standards by the activation of the peristaltic pump (20 s at 60% counterclockwise), propelling a mixture of sample/standard and acetic acid through confluence C (step A). The volume of mixture propelled was enough to fill the SC and to wash out the excess.

Then, the oxidant solution (peroxide in acetic acid), the TMB reagent, and the copper (II) solution were sequentially aspirated to the holding coil (steps B–D), followed by the acidified standard/sample from the SC (step E). The aspirated solutions were stacked in the holding coil (HC), initiating the colour reaction. To promote the reaction extension, a waiting period of 60 s was set (step E).

Finally, the reaction product was propelled to the detector, and the absorbance signal was acquired (step F). For this final step, a three-fold increase in the total volume aspirated was promoted to ensure the washing of the flow cell and holding coil before the next cycle.

The washing of the sample/standard coil, SC, was carried out at the beginning of each cycle as the volume of the sample/standard and acetic acid mixture was over ten-fold that of the SC volume (volume of mixture propelled was 1468 µL and volume of SC was 100 µL).

Table 2. Sequential injection protocol for determination of chloride in water.

Step	Peristaltic Pump (PP)	Syringe Pump (SP)		Selection Valve Position	Volume (µL)	Description
		Valve Position	Flow Rate (µL/s)			
A	On	In	150	-	1000 (SP) /1468 (SC)	Fill the SP with water /Loading the sample/standard coil (SC) with acidified sample/standards

Table 2. *Cont.*

Step	Peristaltic Pump (PP)	Syringe Pump (SP)		Selection Valve Position	Volume (µL)	Description
		Valve Position	Flow Rate (µL/s)			
Steps running in a 3-repetition loop						
B	Off	Out	25	3	100	Aspirate the hydrogen peroxide with acetic acid solution to the HC
C	Off	Out	25	6	5	Aspirate TMB solution to the HC
D	Off	Out	25	4	5	Aspirate copper (II) solution to the HC
E	Off	Out	25	5	30	Aspirate acidified sample/standard from SC
F	Off	Out	-	-	-	Stop period for 60 s to promote reaction extension
G	Off	Out	8	2	540	Propel to detector for absorbance signal acquisition

2.4. Reference Procedure

To evaluate the accuracy of the method developed, the results obtained with the SI-LOV method were compared with those obtained with a reference procedure; potentiometric titration with standard silver nitrate was used. For the titration, a 0.1 M $AgNO_3$ standard solution was used as the titrant against 5.00 or 2.00 mL of water sample, estuarian harbour water or seawater, respectively, with the addition of 2 mL of H_2SO_4 1 M solution.

The signal was acquired using a potentiometer (micropH 2002, Crison, Barcelona, Spain) equipped with a silver ion selective electrode (AgCl-ISE) and a double junction reference electrode (Cat.no 5241, Crison, Barcelona, Spain).

3. Results and Discussion

The sequential injection lab-on-valve method developed based on the oxidation of TMB reagent by hydrogen peroxide, with chloride as the catalyst, was optimised for the determination of chloride in different water samples to enable quantification in a wide range of concentrations. Following the triggering effect of copper (II) on the oxidation reaction described by Zhang et al. (2021) [18], a copper solution was also included. The solutions aspiration order was set as follows: peroxide in acetic acid, TMB reagent and copper (II), followed by the chloride standard or sample. Taking into consideration the number of reagents, four plugs in total, the volume of the copper solution was set to 5 µL as a minimal reproducible value. After setting these conditions, the optimisation studies were performed based on the "one variable" approach, establishing calibration curves for each condition studied.

All the experiments were performed with the aim of increasing the method's sensitivity by evaluating the calibration curve slope, as a higher slope would indicate higher sensitivity.

3.1. Preliminary Studies—Batch-Wise Procedure

The reaction pH was studied, initially also in a batch-wise procedure, by recording the spectra of the formed product obtained for pH values of 1.1, 2.3, and 3.6, with and without chloride (Figure S1). To set up the spectra, a mixture of 1 mL of acetic acid with 30 µL peroxide, 50 µL TMB, and 50 µL copper (II) solution was prepared and used to obtain the blank spectrum (without chloride); then 300 µL 0.2 M chloride standard was added, and the spectrum was recorded as the standard spectrum (with chloride).

At pH 1.1, the absorbance values were very low throughout the entire spectrum for both the blank and the standard (with and without chloride, respectively), making it difficult to distinguish both spectra as they overlapped (Figure S1A). At pH 2.3, an absorbance peak was observed at 450 nm, and there was a clear difference between the blank spectra (without chloride) and the standard spectra (Figure S1B). At pH 3.6, there was also an absorbance peak at 450 nm, but a higher peak was observed at 655 nm and, once again, there was also a clear difference between the blank spectra and the chloride standard spectra (Figure S1C). The highest difference between the blank spectra and the chloride standard spectra, which corresponded to the reaction sensitivity, was obtained with pH 2.3 at 455 nm; for that reason, it was the reaction pH chosen.

3.2. Reaction pH—SI-LOV Method

To maintain the appropriate pH, the peroxide solution and the copper(II) solution were prepared in acetic acid (0.80 M). Additionally, the in-line acidification of the sample and standards was tested, to a final acetic acid concentration of 0.80 M, using the equipment-integrated peristaltic pump. This study required different arrangements in the manifold (Figure 2A) to incorporate the in-line acidification. To attain this acidification, the equipment peristaltic pump was equipped with two tubes, one for the sample/standard introduction and one for the acetic acid introduction (Figure 2B), and a confluence (C in Figure 2B) to promote the efficient mixing of these two solutions. To minimise the consequent sample/standard dilution, a lower flow rate was employed for the acetic acid solution (26.6 µL/s) than for the sample/standard (46.7 µL/s). Two calibration curves were established, one with chloride standards acidified off-line (previously prepared in acetic acid) and another acidified in-line (prepared in Milli-Q water). The acidified standards could be directly aspirated into the system (Figure 2A), but the in-line acidification needed an additional confluence with acetic acid (Figure 2B).

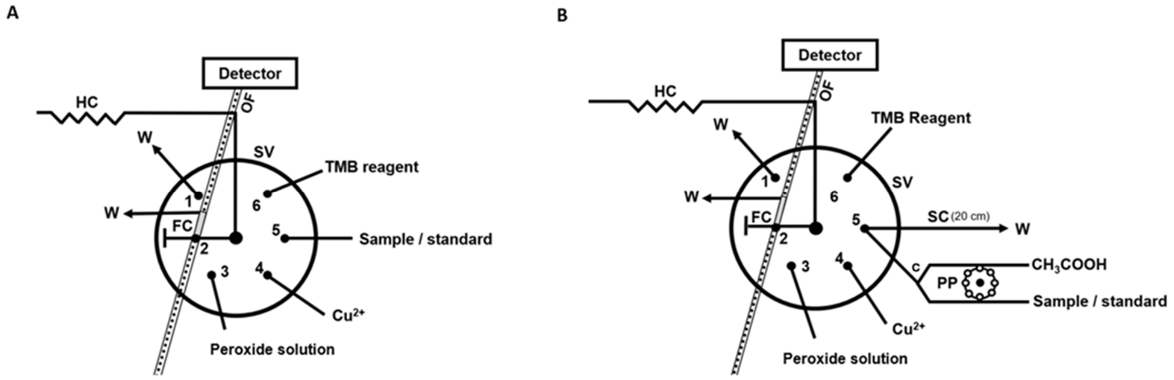

Figure 2. (**A**) Manifold design for introducing the sample and/or standard with off-line acidification; (**B**) Manifold design for the in-line sample and/or standard acidification.

The concentration of acetic acid used in this step was 1.7 M, resulting in 0.80 M after the confluence. The effectiveness of the inline acidification was evaluated by comparing a calibration curve performed with each manifold design.

There were no significant differences between both calibration curves (Figure S2), as the relative deviation between the calibration curves slopes was below 1%, attesting to the efficiency of the in-line acidification. This was a crucial advantage, as it meant there was no need to perform the procedure at the sample collection site.

3.3. Optimisation of the SI-LOV System Parameters

The solutions' aspiration order had been previously set to peroxide, TMB, copper, and chloride standard, all in an acidic medium. Since chloride's role in the chosen oxidation reaction was that of a catalytic effect, a stop period of 30 s was established before propelling it to the detector to promote reaction extension.

The first parameter to be studied was the acidified sample volume, and the volumes of 10, 20, 30 and 40 µL were tested (Figure 3A). The volume that corresponded to the highest slope, meaning the highest sensitivity, was 30 µL, so this was the chosen volume. A higher volume did not improve the sensitivity and resulted in more sample consumption.

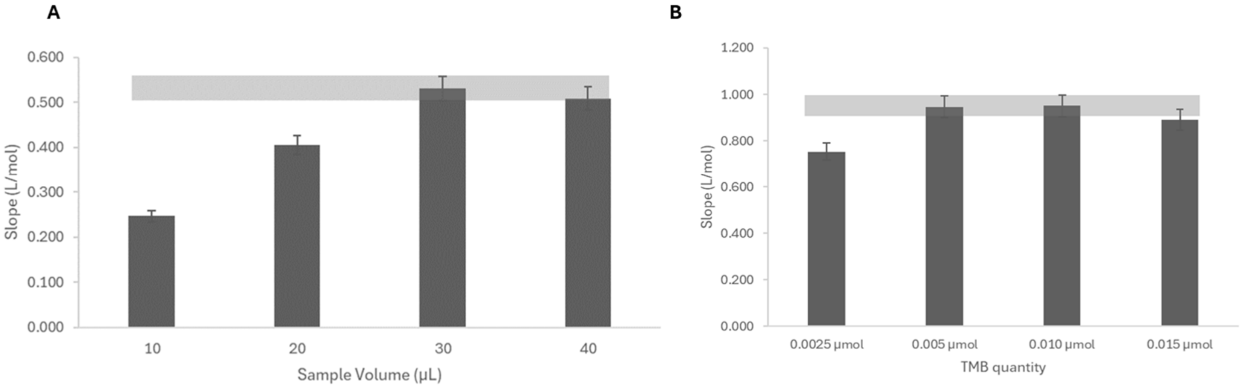

Figure 3. Study of influence using calibration curve slope (method sensitivity) of: (**A**) different sample volumes and (**B**) TMB quantity; the error bars represent a 5% deviation; the light grey shadows over the dark grey bars represents the 5% deviation of the chosen values.

The influence of TMB quantity on the calibration curve slope was studied, where different combinations of the reagent concentration and volume were tested accordingly (Figure 3B). Once the sensitivity increased up to 0.010 µmol of the TMB reagent, this was the chosen quantity, corresponding to 5 µL of the 2.0 mM concentration.

The flow rate of the aspiration of the reagent and the acidified sample (from SC) was tested (Figure S3). In this study, only three flow rates were tested—25, 50, and 100 µL/s—as it was not expected to affect the calibration curve slope. In fact, the different flow rates showed no significant differences in the calibration curve slope (overlapping of the 5% deviation intervals), but as the highest slope was obtained with 25 µL/s, this was the flow rate chosen. The approach of not testing lower flow rates was decided upon to prevent an increase in the cycle time, which would result in a decrease in the determination rate.

As previously mentioned, copper (II) was included as a trigger for the reaction. Therefore, different concentrations were tested, namely 12.5, 25, and 50 mM (Figure S4). This study showed that there were no significant differences between the calibration curve slope obtained using the 25 mM and 50 mM copper (II) solutions (relative deviation of 6%), so 50 mM was chosen for the remaining optimisation to ensure reagent excess.

The hydrogen peroxide volume was also studied (Figure S5) by establishing calibration curves with 50, 75, and 100 µL of peroxide solution; no significant differences were observed in the calibration curve slopes (overlapping of the 5% deviation intervals). Nevertheless, the absorbance signal was better defined with 100 µL, so this was the volume chosen.

Following this, different stop periods were tested, ranging from 0 to 240 s (Figure 4A). The calibration curve slope increased with the increasing stop period; however, for the stop periods of 120, 150, and 240 s, the more concentrated chloride standards resulted in absorbance signals that were too high (A > 1.8) and could not be included in the linear correlation. As such, an alternative solution was set to 60 s to attain a wide application range from 0.060 to 1.0 M, covering all the target water samples (estuarine waters and seawater).

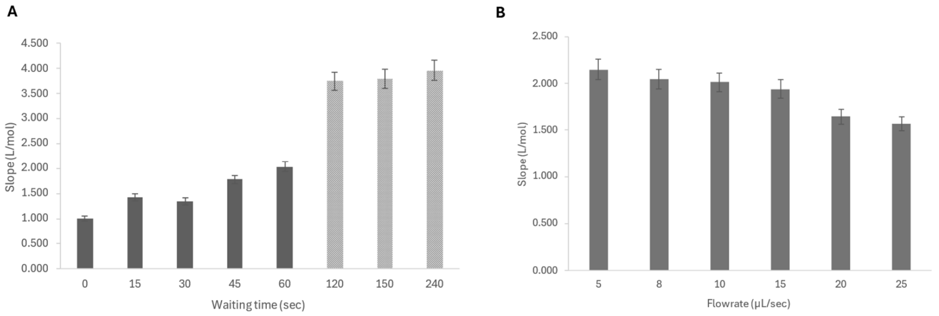

Figure 4. Study of influence using the calibration curve slope (method sensitivity), by (**A**) comparing different flow rates to the detector (light grey bars represent calibration curves with fewer standards); and (**B**) comparing stop periods before propelling to the detector; each error bar represents a 5% deviation.

Then, with all the parameters established and optimised, the flow rate to the detector was also studied, varying between 5 and 25 µL/s. As Figure 4B demonstrates, the calibration curve slope decreased with the increase in flow rate. However, with 5 µL/s, a full cycle took 2.23 min per determination (6.7 min per standard, #3 replicates), resulting in 40 min for a calibration curve (#6 standards), so an alternative solution was to use 8 µL/s, since this allowed us to obtain a calibration curve in 30 min, which was almost 10 min faster.

3.4. Potential Matrix Interferences

The next step was to investigate which ions could potentially interfere with the reaction. Therefore, given the guidelines provided by the World Health Organisation (WHO) [19] and the Environmental Protection Agency (EPA) [20], different levels of possible interferences were tested (Table 3). Two sets of chloride standards of 0.060 M (2 g/L) were prepared, with and without the interfering agent. The absorbance value obtained for the chloride standard without the interfering agent (A_{Cl}) was compared to the chloride standard with the interfering ions solutions (A_{Cl+Int}), calculating the interference percentage as follows: $\%IP = (A_{Cl + Int} - A_{Cl})/A_{Cl} \times 100$.

It is possible to conclude that most of the ions did not present significant interference (interference percentage, IP < 10%), with the exception of sulphate and calcium at the reported concentrations from WHO and EPA, respectively (IP > 30%). However, these concentrations (2.0 g/L of sulphate and 0.50 g/L of calcium) are quite high and do not reflect the content commonly found in water. So, half of those reported values were tested (1.0 g/L of sulphate and 0.25 g/L of calcium), and no significant interferences were observed (IP < 10%). These results indicate that the interference was probably not due to the interfering ion itself but to a much higher ionic content.

Table 3. Study of possible interferences for chloride determination on the SI-LOV method and their respective interference percentage (IP); WHO, World Health Organisation; EPA, Environmental Protection Agency.

Source	Interfering Ion	Interfering Ion Reported Concentration	%IP
WHO	I^-	5.0 µg/L	4%
	Br^-	15 µg/L	−4%
	$SO_4{}^{2-}$	2.0 g/L	−31%
		1.0 g/L *	−3%
	$NO_3{}^-$	50 mg/L	−8%
	$PO_4{}^{2-}$	0.25 g/L	5%
	$NH_4{}^+$	12 mg/L	−3%
	Fe^{3+}	1.0 mg/L	−2%

Table 3. *Cont.*

Source	Interfering Ion	Interfering Ion Reported Concentration	%IP
EPA	NO_2^-	3.0 mg/L	1%
	Mg^{2+}	0.12 g/L	−4%
	ClO^-	1.0 mg/L	6%
	Ca^{2+}	0.50 g/L	−75%
		0.25 g/L *	−2%

* half of the reported concentration.

3.5. Features of the Developed SI-LOV Method

The characteristics of the method developed, including the average calibration curve, the limit of detection (LOD), the limit of quantification (LOQ), and repeatability, are presented in Table 4.

Table 4. Features of SI-LOV method developed: LOD, limit of detection; LOQ, limit of quantification; SD, standard deviation; RSD, relative standard deviation.

Dynamic Range	0.060–1.0 M (2.1–35 g/L)
Calibration Curve (A = slope ± SD × [Chloride] M + intercept ± SD)	A = 1.99 ± 0.28 × [Chloride] M—0.037 ± 0.012
LOD [a]	0.017 M (0.60 g/L)
LOQ [a]	0.058 M (2.0 g/L)
Repeatability, slope RSD [a]	7%
Reagent consumption/per cycle [b]	69 mg CH_3COOH 1.2 mg H_2O_2 3.1 µg TMB 48 µg Cu^{2+}
Sample consumption/per cycle [b]	934 µL

[a] $n = 4$. [b] one cycle corresponds to three replicates.

The LOD and LOQ were determined based on four calibration curves, calculated as three and ten times, respectively, the standard deviation of the intercept divided by the calibration curve slope average, following IUPAC guidelines [21].

The repeatability was assessed by calculating the relative standard deviation (RSD) of four calibration curves on consecutive days. The reagent consumption was calculated based on the total volume of all reagent solutions used in a full cycle. A full cycle corresponded to three replicates of the standard/sample.

3.6. Application to Water Samples

Several water samples were analysed in the system developed, and the results obtained were compared to those obtained by the reference method, which, in this case, was potentiometric titration (Table 5). The relative error percentage (RE%) was calculated as follows: RE% = $(([Cl^-]_{SI\text{-}LOV} - [Cl^-]_{Titration})/[Cl^-]_{Titration}) \times 100$.

Table 5. Results obtained for the determination of chloride in water samples and the respective relative error percentages (RE%).

Type of Water	Sample ID	pH	Conductance (mS/cm)	$[Cl^-]_{Titration}$ (M) ± SD	$[Cl^-]_{SI\text{-}LOV}$ (M) ± SD	RE%
Estuarian Water	#1	7.98	19.3	0.236 ± 0.001	0.219 ± 0.009	−7.1%
	#2	7.61	36.6	0.502 ± 0.002	0.549 ± 0.009	9.3%

Table 5. *Cont.*

Type of Water	Sample ID	pH	Conductance (mS/cm)	$[Cl^-]_{Titration}$ (M) ± SD	$[Cl^-]_{SI\text{-}LOV}$ (M) ± SD	RE%
Seawater	#1	7.86	43.4	0.584 ± 0.002	0.639 ± 0.006	9.4%
	#2	7.00	30.3	0.395 ± 0.001	0.427 ± 0.005	8.1%
	#3 *	7.41	75.1	1.06 ± 0.01	1.02 ± 0.10	−3.6%
	#4	7.25	39.8	0.549 ± 0.001	0.578 ± 0.006	5.3%
	#5 *	7.59	79.1	1.08 ± 0.01	1.08 ± 0.01	−0.3%
Estuarian Harbour Waters	#1	7.26	14.0	0.076 ± 0.001	0.079 ± 0.005	4.1%
	#2	6.85	31.1	0.208 ± 0.002	0.225 ± 0.002	8.1%
	#3	7.10	22.2	0.238 ± 0.001	0.222 ± 0.002	−6.9%
	#4	7.00	15.4	0.169 ± 0.003	0.186 ± 0.005	9.8%
	#5	7.33	10.36	0.092 ± 0.001	0.097 ± 0.003	5.3%
	#6	7.44	18.62	0.136 ± 0.001	0.133 ± 0.005	−2.2%

* samples with chloride levels slightly above the highest standard of the calibration curve.

The titration of seawater samples #3 and #5 resulted in a chloride concentration slightly above 1.0 M. Nevertheless, even with an expected concentration above the dynamic range, the samples were still analysed using the SI-LOV system developed, resulting in a relative error below 5%.

A linear relationship between the two sets of values was established (Figure S6), and the following equation was obtained: $[Cl^-]_{SI\text{-}LOV} = 0.991\ (\pm 0.0516) \times [Cl^-]_{Titration} - 0.0136\ (\pm 0.0270)$. The values in parentheses represent the 95% confidence interval, proving that there were no significant differences between the two sets of results, as the slope was not statistically different from one, and the intercept was close to zero [22].

4. Conclusions

In this work, a sequential injection analysis system was developed and optimised for the determination of chloride content in different water types, with an automated and greener approach. This method was based on the reaction between 3,3′,5,5′-tetramethylbenzidine (TMB) and hydrogen peroxide, with copper (II) as the trigger and chloride as the catalyst, proving to be an efficient method for quantifying chloride, with reduced waste production and reagent consumption, as well as less toxic reagents when compared to the more commonly used methodologies.

With this system, it was possible to obtain a linear dynamic range of 0.060 to 1.0 M, with 0.017 M as the detection limit and 0.058 M as the quantification limit. The method developed was precise and repeatable, with an RSD of 7%. By using in-line acidification, this system further improved in situ sampling by eliminating the manual pre-treatment of the samples. Compared to the reference method, which was potentiometric titration, 13 samples proved to have no significant differences in chloride concentration in the seawater, estuarine, and harbour water samples. This was consistent with the interference assessment where several ions were tested and no significant interferences were observed. High levels of sulphate and calcium resulted in some signal interference, but such levels are hardly encountered in most of the waters.

In conclusion, the SI-LOV method established offers a sensitive and reproducible approach to chloride quantification in various water samples. With a broad dynamic range and reduced reagent consumption, the method aligns with green chemistry principles while maintaining high analytical performance. This methodology is proven to be a potentially valuable tool for water monitoring, as it may affect several other parameters, such as metal ion bioavailability, which can be a critical component in many other water-related studies.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/chemosensors13040124/s1, Figure S1: Study of pH influence on the colorimetric reaction, with and without chloride; (A) pH 1.1; (B) pH 2.3; (C) pH 3.6; Figure S2: Comparison of the calibration curves, with offline acidification (in orange) and with inline acidification (in blue).; Figure S3: Influence on the calibration curve slope (sensitivity) of the aspiration flowrate (of reagent and sample); the error bars represent a 5% deviation; Figure S4: Study the influence of copper (II) concentration on the calibration curve slope (sensitivity); the error bars represent a 5% deviation; Figure S5: Study the influence of the volume of hydrogen peroxide on the calibration curve slope (sensitivity); the error bars represent 5% deviation; Figure S6: Comparison of the results obtained for chloride determination in water samples using the developed SI-LOV method ([Cl-]LOV) and the reference method, the potentiometric titration ([Cl-]Titration). The error bars represent the standard deviation of the measurements, while the black line represents the established linear relationship between the two data sets.

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