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Abstract

Inorganic chloramines are disinfection by-products resulting from the unwanted reaction between chlorine used as disinfectant in swimming pools and nitrogenous compounds brought by bathers. This parameter (total chloramines or combined chlorine) is currently measured on site by a colorimetric method that does not allow to measure only inorganic chloramines. In this paper, a multi-syringe chromatography system combined with a post column derivatization is applied for the first time for the specific detection of the three individual inorganic chloramines (monochloramine, dichloramine and trichloramine). These latter ones are separated using a low-pressure monolithic C18 column, and separately detected after a post-column reaction with the chromogenic reagent ABTS (2,2'-azino-bis-(3-ethyl-benzothiazoline)-6-sulfonic acid-diammonium salt). Development of two ABTS reagents provides discrimination of chlorine and monochloramine that are not separated on the column. Optimization of the experimental conditions enables determination of inorganic chloramines with very good detection limits (around 10 µg eq.Cl_2 L^{-1}) without interferences from other chlorinated compounds such as organic chloramines or free available chlorine. The validation of the whole procedure has been successfully applied to real swimming pools samples.

Keywords: Multi-syringe chromatography; inorganic chloramines; free chlorine; post-column reaction; swimming pools.

1. Introduction

Among disinfection by-products commonly found in swimming pools, inorganic chloramines are the most often encountered at the highest concentrations, with monochloramine (MCA, NH_2Cl) mainly found in water (up to 1180 µg L^{-1}), followed by trichloramine (TCA, NCl_3) (up to 800 µg L^{-1}) and dichloramine (DCA, $NHCl_2$) (up to 650 µg L^{-1}) [1]. Inorganic chloramines are a group of three chemical substances in aqueous solution formed by reactions between chlorine at oxidation state (+I), ammonia and amino acids coming from urea and sweat brought by bathers (such as uric acid, urea, creatinine, L-arginine, L-histidine, glycine) [2].

These chloramines are present in various types of waters where chlorine is used as disinfectant or for biofouling control (drinking waters, swimming pools waters, wastewaters, industrial chlorinated effluents) [1, 3-6]. In particular for swimming pools, respiratory issues, such as asthma, wheeze, cough and lower respiratory tract infections, have been correlated with swimming pool attendance, which is likely due to chlorinated volatile DBPs, such as chloramines and especially TCA [7-9]. TCA is also assumed to cause skin and eye irritations [10]. MCA and DCA have not been yet assumed to be toxic but their roles as precursors of the potent carcinogen N-nitrosodimethylamine (NDMA) have been emphasized by several authors [11-13]. Consequently, reliable, rapid and sensitive methods are required to monitor pool water quality and to assess their mitigation strategies.

Many methods have been proposed for the determination and quantification of chloramines and have been reviewed by Kinani et al. [14]. The most commonly used are based on the redox reaction using N, N-diethyl-p-phenylenediamine (DPD) as the reducing agent with colorimetric

or amperometric detection. The colorimetric method uses the red coloration produced by the reaction between DPD, free chlorine and inorganic chloramines (after addition of iodide) with a spectrophotometric measurement at 510 nm, whereas the titrimetric method employs ammonium sulfate with ferrous ion as the final reagent of the colored solution produced by DPD reaction. These methods are simple and sensitive (quantification limits (LOQ) are around 30 µg eq. Cl_2 L^{-1}), but they suffer from a lack of selectivity. Indeed, organic chloramines may interfere with inorganic chloramines as they react in the same way in the presence of the iodide ions, leading to an overestimation of the real amount of inorganic chloramines in samples with large amounts of organic nitrogen [15]. Another colorimetric method using a derivatization reaction has been developed to differentiate inorganic monochloramine from organic chloramines using phenol type reagent as color reagent [16]. The derivatized product can be measured spectrophotometrically at a wavelength of 655 nm. However, this method suffers from a low sensitivity (LOQ > 270 µg eq. Cl_2 L^{-1}) and from the ability to detect only monochloramine.

To overcome these issues and to allow individual determination of the three chloramines, chromatographic separation using high performance liquid chromatography (HPLC) has been proposed. Separations of chloramines are generally performed using reversed-phase HPLC on a C_{18}-phase column. Direct spectrophotometric detection is possible at specific wavelength for each chloramine (MCA at 240 nm, DCA at 210 or 280 nm and TCA at 220 nm) [17,18]. However, quantification limits of this detection method are generally very high (LOQ >200 µg eq. Cl_2 L^{-1}), and selectivity is also very low as many other co-eluting compounds can interfere in the wavelength range below 250-300 nm. Direct amperometric detection with a working glassy carbon electrode is also proposed with a better sensitivity (quantification limits 2-13 times lower than those obtained by spectrophotometric detection), but interference from oxygen seriously hampers application of this method [19]. Chemical derivatization generally provides

low sensitivity, except when LC-MS/MS is used with a pre-column derivatization of MCA. This method is very sensitive and specific for MCA, but drawbacks are the high cost and complexity of the detection method, and the inability to measure DCA and TCA [20]. The most efficient derivatization method for inorganic chloramines to date is the post-column derivatization with potassium iodide and photometric or electrochemical detection of triiodide ions formed [21]. Although the sensitivity of this method is quite high (LOQ around 30 µg eq. Cl_2 L^{-1}), the post-column reaction is non-specific to inorganic chloramines (potassium iodide can react with many other compounds).

The aim of this study was to develop a low-cost automated analyzer for the individual quantification of MCA, DCA and TCA. So far, the only on-line automated analytical tool for determination of these three inorganic chloramines is membrane-introduction mass spectrometry (MIMS), which uses a semi permeable membrane coupled to a mass spectrometer [15]. This analytical tool is very specific for inorganic chloramines, but lacks of sensitivity (LOQ =60-300 µg eq. Cl_2 L^{-1}), remains costly and requires qualified personnel to handle with MS interpretations. Our aim was thus to propose an alternative analytical method based on the simplicity and versatility of flow injection techniques coupled to liquid chromatography separation for the determination of individual inorganic chloramines.

A multi-syringe chromatography (MSC) system using monolithic column is thus developed to obtain a low-pressure automated portable system with relatively low-cost and simple components, able to be used as an on-line system. In order to achieve better sensitivity and specificity than direct spectrophotometric detection, a new post-column reaction for all inorganic chloramines is proposed using 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), an indicator already used by Solterman et al. [22] for the detection of trichloramine. Optimizations of this post-column reaction and of the

chromatographic separation of chloramines are presented, and the developed method applied on real swimming pool samples.

2. Material and methods

2.1. Reagents and solutions

All chemicals were of analytical reagent grade and used without further purification. Ultrapure water purified with a Milli-Q system (Millipore, USA, resistivity >18 MΩ cm) was used for the preparations of the whole solutions. Sodium hypochlorite solutions were prepared by dilution of a commercially available NaOCl solution (4.00-4.99% active chlorine, Sigma-Aldrich, France) and standardized by iodometric titration. Ammonium stock standard solution (2 g L^{-1}) was prepared from ammonium chloride (Alfa Aesar, Germany). Solutions of ABTS (2,2'-azino-bis-(3-ethyl-benzothiazoline)-6-sulfonic acid-diammonium salt, Sigma-Aldrich) were prepared by dissolving appropriate amount in water. Acetonitrile (MeCN) (HPLC-Gradient phase, Sigma-Aldrich) was used as the organic mobile phase. Formic acid (Fisher chemical, France), sulfuric acid (Fisher chemical), hydrochloric acid (Sigma-Aldrich) and sodium hydroxide (Sigma-Aldrich) were used for pH adjustments.

2.2. Inorganic chloramines standard solutions

Inorganic chloramines are unstable and sensitive to light, so they had to be prepared daily and stored in amber glass or polytetrafluoroethylene bottles at +4 °C and kept out of direct light. Inorganic chloramines solutions prepared were standardized by using DPD/FAS titrimetric method.

MCA standard solution was prepared by slowly adding 5 mL of sodium hypochlorite solution (6 mmol Cl_2 L^{-1}) to 5 mL of ammonium chloride solution (12 mmol L^{-1}) whose pH was prior adjusted at 9 with 0.1 M NaOH (ammonia to chlorine molar ratio was 2:1). Final pH was between 10.5 and 11. The reaction vial was then covered with aluminum foil and stored at +4 °C for 60 min before use.

DCA standard solution was prepared by acidification of the MCA solution with 0.1 M formic acid to reach a pH value between 3.5 and 4. This solution was then stirred at room temperature for 2 h while pH was monitored and readjusted as necessary, and then left for 4 h at +4 °C before use.

TCA standard solution was prepared by addition of 5 mL of a 6 mmol Cl_2 L^{-1} sodium hypochlorite solution (whose pH was previously adjusted below 3 with 0.5 M H_2SO_4) to 5 mL of a 1.9 mmol L^{-1} ammonium chloride solution (ammonia to chlorine molar ratio was 1:3.15). Final pH was between 2.3 and 2.5. The reaction vial was then covered with aluminum foil and stored at +4 °C for 45 min before use.

A solution of MCA and DCA was prepared by additions of 5 mL of a 6 mmol Cl_2 L^{-1} sodium hypochlorite solution (whose pH waspreviously adjusted to 6 with 0.1 M formic acid) to 5 mL of a 6 mmol L^{-1} ammonium chloride solution (ammonia to chlorine molar ratio was 1:1). Final pH was between 5.2 and 5.8. The vial was then covered with aluminum foil and stored at +4 °C for 45 min before use.

For the study on inorganic bromamines, monobromamine (NH_2Br), dibromamine ($NHBr_2$) and tribromamine (NBr_3) were prepared as described by Galal-Gorchev and Morris [23] and by Lei et al. [24] , while bromochloramine (NHBrCl) was prepared following Allard et al.'s method [25].

142 2.3 HPLC separation of inorganic chloramines with direct UV detection

143 Inorganic chloramines separation has been first optimized by using a HPLC system consisting
144 of an L-2130 pump, an L-2400 UV detector, and an L-2200 autosampler (Hitachi Elite
145 LaChrom system, Tokyo, Japan). HPLC separations were performed on a RP-18 Onyx™
146 monolithic column (25 mm x 4.6 mm, Merck, Germany). Detection was performed at 245 nm
147 for MCA, 295 nm for DCA (210 nm for mixtures of MCA + DCA) and 220 nm for TCA. The
148 column temperature was fixed at 25 °C, the flow rate was set at 0.5 mL min^{-1}, and the injected
149 sample volume was 20 µL.

150

151 2.4. Reaction of inorganic chloramines with ABTS

152 The reaction between free chlorine or inorganic chloramines with ABTS was studied using a
153 Jasco V-670 spectrophotometer operating with the Spectra Manager software. The solutions
154 were analyzed in a 10 mm light path quartz cell.

155 Solutions of ABTS were stored at 4 °C and were stable for at least 2 months. Solution A was
156 prepared by dissolving 13.6 mg of ABTS in 100 mL 0.4 M HCl ([ABTS] = 0.25 mM). Solution
157 B was prepared by dissolving 13.6 mg of ABTS in 100 mL of 5 mM phosphate buffer at pH 3.
158 For the kinetic measurements in quartz cells, 1 mL of sample and 2 mL of the appropriate ABTS
159 solution were added and mixed in the cell, and absorbance was measured every 5 s at 405 nm.

160

161 2.5. MSC instrumentation and software

162

163 The device used is described in Fig.1. It included a multisyringe burette (BU-4-S, Crison
164 Instruments, Spain) equipped with four syringes (Hamilton, Switzerland). Three syringes were
165 used: S1 (5 mL; mobile phase: MeCN/H_2O: 20/80), S2 (2.5 mL; ABTS solution A) and S3 (2.5

mL; ABTS solution B). Standard solenoid valves on the head of S1 were replaced by a two-way connector made of Delrin® (Sciware System SL, Spain) in order to resist organic solvents. This connector linked S1 with a new solenoid valve (V1, Takasago, Japan) which was situated outside the syringe module and allowed a maximum operating pressure of 6 bars. V2 and V3 were standard solenoid valves. The manifold was built up with a 0.8 mm internal diameter poly(tetrafluoroethylene) (PTFE) tubing. S1 was connected to one port of a 6-port injection valve module (Crison Instruments) equipped with a 20 µL loop. The three other ports of the injection valve were connected to the sample vial, the monolithic column and waste. The injection loop was filled with the sample thanks to a solenoid micro-pump (MP1) (Bio-Chem, USA). The indicated stroke volume for the micro-pumps was 20 µL. MP1 and V1 were computer-controlled by means of a MCFIA/MPFS system (Sciware System SL) having eight digital 12V output channels.

The separation of chloramines was performed on an Onyx™ monolithic C18 column (25 mm x 4.6 mm, Merck, Germany) at room temperature, using a mixture of acetonitrile and water as mobile phase ($MeCN/H_2O$: 20/80).

A PEEK (polyether ether ketone) T-mixer equipped with a PAT frit (PEEK Alloyed with Teflon, Interchim, France) was used to mix the eluent from the column with the ABTS solution during the post-column derivatization. A 100 µL PTFE tubing (0.5 mm internal diameter) was used to connect the T-mixer to the flow cell to ensure enough time to complete reaction between chloramines and ABTS.

The detection system was composed of an ultra violet-visible light source (DH-2000 Deuterium Tungsten Halogen Light sources; wavelength range 215-2000 nm; Ocean Optics, USA), a FIA-ZSMA PEEK flow cell (50 mm pathlength, 130 µL internal volume, Ocean Optics) and of a USB 2000 miniature fiber-optic spectrometer detector (detector range: 200 - 1100 nm, Ocean

190 Optics) connected to a computer via an USB interface. The analytical wavelength was set at
191 405 nm. Two FC-UV600-2 optical fibers (Ocean Optics) were used to guide the light from the
192 source through the flow cell and further to the spectrophotometric detector. System control,
193 data acquisition and processing were performed using the software package Autoanalysis 5.0
194 (Sciware System SL).

195

196 2.6. MSC procedure

197 The optimized protocol used for the MSC system is presented in Table 1 and summarized as
198 follows:

199 A first cycle (separation/derivatization) was run with ABTS (solution B) to measure only free
200 chlorine. After injection of the sample in the loop (Load position, step 1), chromatographic
201 separation was performed by switching the valve to "Inject" position (step 4) and by dispensing
202 the mobile phase (step 6), while absorbance measurement was carried out at 405 nm. After
203 reloading of the syringes (step 8), a second cycle was run with ABTS (solution A) to measure
204 inorganic chloramines (step 9-13).

205 **Table 1** Analytical procedure for Multi-syringe chromatography analysis of free chlorine with
206 ABTS solution B and inorganic chloramines with ABTS solution A.

207

Step	V1	V2	V3	Injection Valve	Mode	Volume (ml)	Flow rate (mL min^{-1})[a]	MP1 (µL)
1	OFF	OFF	OFF	LOAD	-	-	-	500
2	Get dark spectrum continuous							
3	Get blank spectrum continuous							

4	OFF	OFF	OFF	INJECT	-	-	-	-
5	Start MEASUREMENT				-	-	-	-
6	ON	OFF	ON	INJECT	Dispense	5	0.30	-
7	STOP MEASUREMENT				-	-	-	-
8	OFF	OFF	OFF	INJECT	Pick up	5	5	-
9	OFF	OFF	OFF	LOAD	-	-	-	500
10	OFF	OFF	OFF	INJECT	-	-	-	-
11	Start MEASUREMENT							
12	ON	ON	OFF	INJECT	Dispense	5	0.30	-
13	STOP MEASUREMENT							
14	OFF	OFF	OFF	INJECT	Pick up	5	5	-

a. Flow rate of S1; Flow rates of S2 and S3 are half that of S1 because of half volumes of S2 and S3 compared to S1.

2.7. Swimming pools samples

Samples were collected in five indoor swimming pools located in the Aix-Marseille Provence Metropolis. These five pools were supplied with public water works and were continuously disinfected with bleach at a set point concentration of 1 mg L^{-1} Cl_2 (expressed as free chlorine). Water samples were taken at a depth of 30 cm, stored at 4 °C and analyzed at the laboratory as quick as possible (delay < 1 hour). The sampling took place at peak times with the objective of having a maximum bather load (free swims or classes).

3. **Results and discussion**.

3.1. Inorganic chloramines standard solutions

DPD/FAS titrimetric standardization of chloramines standard solutions was used to provide conversion percentages of chlorine and ammonia into chloramines. In the conditions described

221 in section 2.2, 98 % conversion of free chlorine to monochloramine was achieved within 60
222 minutes, and 95 % of monochloramine was also converted to dichloramine after acidification
223 of the monochloramine solution and completion of the reaction for 6 h.

224 Regarding the trichloramine solutions, different preparation methods were tested as described
225 in the literature [15,22,26]. Chlorine to ammonia molar ratio is usually 3:1 or 3.15:1 with acidic
226 pH between 2.3 and 2.8 but proposed ageing times vary considerably, from 1 h to 1 week. The
227 DPD/FAS determination of the solutions prepared in these various conditions in our laboratory
228 showed that there was always free chlorine and dichloramine in the trichloramine standard
229 solutions, even at very high chlorine to ammonium molar ratio (10:1) as was described by some
230 authors [22].

231 Preparation of trichloramine solutions (according to the protocol described in section 2) and
232 kinetic measurements of chloramines using DPD/FAS titration over 28 h ageing showed that
233 both dichloramine and trichloramine were present immediately after the beginning of the
234 experiment. After 45 min, concentration of trichloramine became stable for 6 h, followed by a
235 slow decrease in trichloramine concentration as well as an increase in dichloramine
236 concentration. Conversion yields of trichloramine were usually around 10 % (based on initial
237 ammonia concentration) after 45 min. This preparation method and ageing time has been chosen
238 for this study in order to reduce the daily preparation time of trichloramine standards.

239 3.2. Inorganic chloramines separation with direct detection

240

241 Optimization of the separation of inorganic chloramines was first studied using HPLC with
242 direct UV detection (on standard solutions with high concentrations of chloramines) as
243 preliminary experiments. Various mobile phases were tested in order to study the separation of
244 inorganic chloramines by HPLC using a monolithic column, which has never been documented

before in the literature (only with traditional particle-packed columns). Results in Table 2 show retention times of the three chloramines and resolution between MCA and DCA whose peaks were the lower-separated ones. The best compromise between TCA retention time (which corresponds to the total analysis time for three chloramines) and MCA-DCA resolution was a mobile phase made of 20 % acetonitrile, leading to short overall retention times and good separation of the whole chloramines. Representative chromatograms for the separation of the inorganic chloramines with this mobile phase are depicted in Figure S1 in the supplementary materials section. Addition of buffers (acidic or neutral) did not improve separation and was not selected for the rest of the study as it could displace equilibrium between chloramines during the column separation. Methanol was also tested as the organic solvent of the mobile phase, but results were not as good as with acetonitrile and it generated higher pressure in the system, which is not recommended for low-pressure MSC systems. Irrespective of the conditions tested, chlorine and MCA have not been able to be separated.

The same experimental conditions (monolithic column, mobile phase made of 20% MeCN, eluent flow rate of 0.5 mL min^{-1}) have then been applied to the MSC system coupled to a UV detector (without post-column reaction).Retention times and resolution of chromatographic peaks were close to those obtained with the HPLC system, giving proper pressures around 3 bars. MCA and chlorine also co-eluted with this MSC system.

Table 2 Retention times of inorganic chloramines for different mobile phase using HPLC with a C18 monolithic column (flow rate was 0.5 $mL.min^{-1}$).

Mobile phase	Retention time (min)			MCA-DCA resolution
	MCA	DCA	TCA	
H_2O	1.11	1.89	>30	2.61
Acetonitrile 10% - H_2O 90%	1.10	1.86	8.84	2.52

Acetonitrile 20% - H_2O 80%	1.09	1.68	6.17	2.29
Acetonitrile 25% - H_2O 75%	1.07	1.60	5.02	2.15
Acetonitrile 30% - H_2O 70%	1.04	1.53	4.30	1.81
Acetonitrile 40% - H_2O 60%	1.01	1.30	2.90	0.91
Acetonitrile 25% - Phosphate buffer pH 7 75%	1.08	1.60	5.02	2.17
Acetonitrile 25% - Acetate buffer pH 4.5 75%	1.07	1.58	5.01	2.11
Methanol 25%- H_2O 75%	1.02	1.50	6.34	1.97

3.3 Optimization of the post-column reaction with ABTS

ABTS was used as a post-column reagent being known to be able to react with halogenated compounds to form the colored $ABTS^{\circ+}$ radical, detectable and quantifiable at 405 nm (ε= 31600 M^{-1} cm^{-1}). ABTS has indeed already been used as a colorimetric indicator for trichloramine [22] and for detection of monochloramine after addition of nitrite [26] but has never been studied for the simultaneous/sequential detection of all inorganic chloramines and free chlorine.

3.3.1. Kinetic measurements by batch experiments

The objective of the optimization of the reaction between ABTS and chloramines was first to find experimental conditions that ensured rapid and total reaction of the three inorganic chloramines, in order to be used later as post-column reaction on our MSC system. Chlorine and inorganic chloramines react with ABTS as follows:

$HOCl + 2\ ABTS + H^+ \rightarrow Cl^- + 2\ ABTS^{\circ+} + H_2O$

$NH_2Cl + 2\ ABTS + 2H^+ \rightarrow Cl^- + 2\ ABTS^{\circ+} + NH_4^+$

$NHCl_2 + 4\ ABST + 3\ H^+ \rightarrow 2\ Cl^- + 4\ ABTS^{\circ+} + NH_4^+$

$NCl_3 + 6\ ABTS + 4\ H^+ \rightarrow 3\ Cl^- + 6ABTS^{\circ+} + NH_4^+$

One must therefore bear in mind that stoichiometry of TCA and DCA reactions are three times and twice that of MCA or hypochlorous acid, respectively. Various experimental conditions were tested (pH, addition of iodide catalyst…) in batch experiments (quartz cell) and all inorganic chloramines (and hypochlorous acid) were found to react quickly and quantitatively with ABTS at pH below 2, without catalyst. Figure 2 displays the kinetics of the $ABTS^{\circ+}$ formation for all studied compounds with ABTS dissolved in 0.4 M HCl. All reactions were complete after 30 s, which was fast enough to be used for post-column reaction. Measured absorbance after reaction of DCA (10^{-5} mol L^{-1}) with ABTS was twice that of MCA and chlorine (10^{-5} mol L^{-1}), as expected. Regarding TCA, only mixtures of TCA, DCA and HOCl have been able to be formed, and the absorbance depicted for the reaction with TCA (concentration of TCA at 10^{-5} mol L^{-1} in the mixture) was calculated by subtracting the absorbance of the expected $ABTS^{\circ+}$ produced by reaction with DCA and HOCl from the total absorbance (originating from the reaction with the three species).

The reaction between ABTS and inorganic chloramine and free chlorine is highly dependent on the reaction pH value, and up to now, discrimination between free chlorine and MCA was not achievable, these two compounds being not separated after the chromatographic step. In our previous experiments, ABTS reacted with all chlorinated compounds at acidic pH and with any of them at neutral or basic pH. However, a fine-tuning of the pH around 3 allowed us to separate MCA and HOCl (Fig. 3). MCA did not react with ABTS at pH 3, whereas HOCl still exhibited quantitative and fast reaction. Differentiation between MCA and HOCl was thus possible by

303 running two experiments: one with ABTS in HCl (ABTS solution A) for free chlorine and
304 inorganic chloramines, and one with ABTS at pH 3 (ABTS solution B) for determination of
305 free chlorine.

306

307 3.3.2. Optimization on the MSC system.

308 The MSC system has then been optimized in terms of the post-column reaction by varying
309 several operational parameters: the ratio between flow rates of mobile phase and ABTS
310 solution, the mobile phase flow rate and the tubing volume /length between the mixer and the
311 detector. As the mobile phase and the ABTS solution were delivered by the same piston pump
312 (by use of a multi-syringe pump in order to simplify the experimental set-up), the only way to
313 change the flow-rates ratio was to change the volume of the syringe of ABTS while keeping
314 that of mobile phase constant (5 mL). Therefore, flow rate ratios of 1, 2 or 5 have been able to
315 be obtained by using 5 mL, 2.5 mL or 1 mL syringes for ABTS. For these experiments, flow
316 rate of mobile phase was set at 0.3 mL min^{-1}, pressure was 2.5 bars, and the volume of the
317 tubing between the mixer and the detector was 100 µL. Table 3 shows that peak areas and
318 resolution between MCA and DCA peaks were better when ABTS flow rate was half that of
319 the mobile phase (which corresponded to the volume ratios used in quartz cells experiments).
320 This ratio was selected for the rest of the study.

321 **Table 3** Influence of the ratio between mobile phase and ABTS solution flow rates on MCA
322 and DCA peaks.

323

	Monochloramine	Dichloramine	Resolution

Ratio of flow rate	Retention time (min)	Area of peak	Retention time (min)	Area of peak	
1	2.05	10.1	3.5	7.4	1.5
2	2.8	11.5	4.03	9.6	2.01
5	2.71	10.1	3.7	4.1	0.7

The mobile phase flow rate and the tubing volume between the mixer and the detector had a direct influence on the post-column reaction time. Volumes from 100 to 300 µL and two flow rates (0.3 mL min^{-1} and 0.5 mL min^{-1}) were tested, and best results (peak areas and resolution) were obtained with a flow rate of 0.3 mL min^{-1} and a tubing volume of 100 µL (Table 4). Considering the mobile phase and ABTS solution flow rates (global flow rate of 0.45 mL min^{-1}), this volume corresponded to a reaction time of 13.3 sec, which seemed sufficient to obtain the best compromise between peak areas and resolution.

Table 4 Influence of the mobile phase flow rate and of the volume of the tubing between the mixer and the detector. Flow rate of ABTS was half that of the mobile phase.

Flow rate (mL min^{-1})	Volume of the tubing (µL)	Monochloramine		Dichloramine		Resolution
		Retention time (min)	Area of peak	Retention time (min)	Area of peak	
0.3	100	2.88	11.5	4.03	9.6	2.01
0.5	100	1.8	4.05	2.6	1.57	2.15
0.3	200	3.11	12.4	4.35	10.9	1.57
0.5	200	2.01	7.6	2.66	3.7	1.78

0.3	300	3.36	10.9	4.61	8	1.69
0.5	300	2.08	7.3	2.82	6.7	1.51

335

336

337 3.4. Interferences from organic chloramines and other halogenated compounds

338 DPD-based methods for inorganic chloramines measurements are known to suffer from strong
339 interferences from other chlorinated compounds, especially from organic chloramines which
340 are formed during chlorination of organic amines and amino acids [15]. These interferences
341 result in "false positives" for inorganic chloramines determination (appearing as MCA or DCA)
342 with DPD standard methods, which strongly hinders application of this method to real samples
343 with complex matrix content. To assess interferences from organic chloramines on our MSC
344 system, glycine was selected as a model organic amino compound. This latter one was indeed
345 already demonstrated to be chlorine-reactive forming organic chloramines (N-chloro or N-
346 dichloroglycine) [27].

347 The ability of the MSC system to differentiate inorganic and organic chloramines species was
348 demonstrated as follows : 1 mL of a glycine solution (100 mg L^{-1} as N) was added to 220 mL
349 of 0.01 M phosphate buffer (pH 7) in a flask, then aliquots of free chlorine stock solution were
350 added so that chlorine/nitrogen mass ratio were from 2 to 16 (chlorine/nitrogen molar ratio from
351 0.4 to 3.2). The concentration of residual chlorine was then determined by the DPD/FAS
352 titration method (Fig. 4a.) and by the MSC system after 30 min (Fig. 4b). As expected, the lack
353 of selectivity of DPD/FAS titration was highlighted, as MCA and DCA were quantified in high
354 quantities while it has already been proven by MIMS measurements (during an experiment
355 performed under the same conditions) that none of them were able to be actually present at low
356 Cl/N mass ratios (<14) [15]. On the opposite, our MSC system only detected free chlorine at

high Cl/N mass ratio, which proved that organic chloramines did not interfere with our system. Indeed, organic chloramines generated by the chlorination of glycine did not react with the ABTS solution A (or B), as was demonstrated by a direct test in quartz cell. Therefore, coelution of an organic chloramine with an inorganic chloramine would not cause interference on the determination of MCA, DCA or TCA. The same results were obtained during chlorination of asparagine (data not shown).

Interferences from other chlorinated disinfection by-products or chlorine-related compounds were also assessed. N-chlorourea, chloroform, bromoform (trihalomethanes), mono-, di- and tri-chloroacetic acids (haloacetic acids) and cyanuric acid (chlorine stabilizer) showed no analytical response with our system, due to the non-reactivity of these compounds with ABTS.

Reactivities of inorganic bromamines were also studied. The results showed that NH_2Br coelutes with NH_2Cl, while $NHBr_2$ and NHBrCl had the same retention time as $NHCl_2$. Nevertheless, NBr_3 has been able to be separated from NCl_3 as their retention times were different (13.9 min for trichloramine and 19.6 min for tribromamine). Further studies are in progress in our laboratory to differentiate inorganic bromamines and chloramines by optimization of the ABTS reagent.

3.5. Analytical features

The analytical procedure of the MSC system was evaluated under the optimized experimental conditions, with ABTS solution A for HOCl and inorganic chloramines determination, and with ABTS solution B for HOCl only (Table 5). Excellent linear regression coefficients were obtained for all chloramines up to at least 7100 µg eq.Cl_2 L^{-1} (100 µmol eq.Cl_2 L^{-1}). The limits of detection (LD) and of quantification (LQ) were estimated using the classical 3s and 10s approaches respectively, *i.e.* calculation of LD and LQ through analysis of the standard deviation of blank measurements (n=8). Limits of detection for the inorganic chloramines were

all around 10 µg eq.Cl_2 L^{-1}, which was comparable to the DPD titrimetric method and 2 to 10 times better than usual MIMS performances [14]. Limits of detection on our MSC system without post-column reaction (direct UV detection) were 100 to 500 times lower, proving efficiency of the ABTS detection. Slope of the linear regression equations of HOCl or inorganic chloramines were all similar after reaction with ABTS (when concentrations were expressed as equivalent Cl_2 L^{-1}, and considering stoichiometry of equations presented in section 3.1), proving that chloramines concentrations were not affected by the chromatographic separation using our experimental conditions (monolithic column for fast separation, mobile phase with water), contrary to what has been previously described when using traditional HPLC method [17]. Regarding sample throughput, the overall procedure took about 30 min, including the two cycles with ABTS solution A and B, allowing the analysis of 2 samples/h.

Table 5 Analytical features of the MSC system developed

Analytical parameter	MCA (or HOCl)[a]	DCA[a]	TCA[a]	HOCl[b]
Limit of detection (µg eq.Cl_2 L^{-1})	9.3	9.9	10.0	29.2
Limit of quantification (µg eq.Cl_2 L^{-1})	31.2	33	33.5	97.4
Linear working range(µg eq.Cl_2 L^{-1})	31-7100	33-7100	34-7100	97-7100
Linear regression equation[c]	A= 0.00116xC+0.23	A=0.0113xC+0.25	A=0.0124xC+0.06	A=0.00926xC+0.061

R^2	0.997	0.998	0.999	0.986
RSD (%)[d]	4.9	3.8	4.4	3.7
Retention time (min)	3.0	4.05	13.9	3.0
RSD of retention time (%)	1.2	1.8	0.4	1.6

a. with ABTS solution A; b. with ABTS solution B; c. A was the area of the peak, C was the analyte concentration (µg eq.Cl_2 L^{-1}); d. RSD was calculated on a 200 µg Cl_2 L^{-1} standard, n=6 replicates.

3.6. Application to real samples

Our multisyringe analytical system was applied to real samples collected in swimming pools of Aix-Marseille Provence Metropolis. We first intended to compare our results with MIMS measurements, which was not available in our laboratory. Samples were sent to another French laboratory equipped with a MIMS for comparison purposes, but quantification for MCA and DCA was unfortunately not possible on real samples, due to interferences from other compounds and between inorganic chloramines. Standard addition method was also not possible for validation, as addition of inorganic chloramines to swimming pool samples resulted in modification of chlorine-inorganic chloramine equilibrium and very large differences between added and measured concentrations in these samples.

Therefore, we chose to assess the accuracy of the multisyringe system by comparison of the results obtained with our MSC system and those obtained by the DPD/FAS titrimetric assay (Table 6). While measurements for free chlorine were in good agreement (no difference at the 0.05 level using Student's t test), significant differences appeared for inorganic chloramines. MCA was detected by DPD/FAS assay in two samples in low concentrations (2 and 3) but not detected by the MSC procedure. DCA measurements resulted in the largest differences between

both methods, with DPD/FAS results always much higher than MSC results. This large discrepancy has been able to be explained by the inability of the DPD/FAS assay to differentiate between inorganic and organic chloramines [28], which was further demonstrated in this study on swimming pool samples. Samples were collected during highly crowded hours (except sample 1 with no inorganic chloramines detectable by the MSC method), and significant amounts of organic chloramines or other DPD/FAS interferents could have been expected due to the reaction of chlorine with organic nitrogenous compounds brought by swimmers. TCA was not quantified by either methods.

Our method could be also applied to other types of waters such as drinking waters, especially when MCA is used as a secondary disinfectant such as in the USA (where significant concentrations of MCA and DCA are expected).

Table 6 Comparative results between our multisyringe system (MSC) and titrimetric assay (DPD/FAS) on various swimming pool samples (mg eq.Cl_2 L^{-1}, mean ± standard deviation, n=2 replicates).

	HOCl		MCA		DCA		TCA	
	DPD/FAS	MSC	DPD/FAS	MSC	DPD/FAS	MSC	DPD/FAS	MSC
1	1.52±0.03	1.60±0.09	<LQ	<LQ	0.58±0.00	<LQ	<LQ	<LQ
2	1.50±0.01	1.52±0.04	0.20±0.00	<LQ	1.02±0.02	0.37±0.05	<LQ	<LQ
3	1.7±0.04	1.60±0.11	0.1±0.01	<LQ	0.7±0.03	0.68±0.04	<LQ	<LQ
4	1.2±0.00	1.15±0.06	<LQ	<LQ	0.98±0.02	0.06±0.01	<LQ	<LQ

5	1.1±0.03	1.06±0.02	<LQ	<LQ	0.5±0.01	0.22±0.03	<LQ	<LQ

4. Conclusion

In this study, a MSC system based on a chromatographic separation with a monolithic column and post-column derivatization with ABTS was developed for the analysis of inorganic chloramines in water. Use of a mobile phase containing acetonitrile and water enabled successful separation of the chloramines with good resolution and limited pressure in the flow system, leading only to the coelution of chlorine and monochloramine. Optimization of the ABTS reaction resulted in the preparation of two ABTS reagent: one for the quantification of free chlorine only, and one for the quantification of chlorine and the three inorganic chloramines. Mono-, di- and trichloramine were quantified by the MSC system with detection limits around 10 µg eq.Cl_2 L^{-1}, a large linear working range and a very good repeatability. Rather as DPD-based methods, our method was not affected by the presence of organic chloramines. This method applied and validated on real samples represents a promising and cheaper alternative to MIMS, both in laboratory (use of the same method on a widely available LC-UV equipment is also possible) and on-site analysis of inorganic chloramines.

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Figure captions

Figure 1- Descriptive scheme of the multi-syringe chromatography manifold for the analysis of inorganic chloramines. MP1=Solenoid micro-pump, V1, V2 and V3= solenoid valves.

Figure 2- Kinetic of the reactions between ABTS solution A (prepared in HCl 0.4M) and free chlorine/inorganic chloramines. Protocol: 1 mL sample + 2 mL ABTS solution A. The concentration of free chlorine, MCA, DCA and TCA were 10^{-5} mol L^{-1}.

Figure 3- Kinetic of the reactions between ABTS solution B (prepared in pH 3 buffer) and free chlorine or MCA. Protocol: 1 mL sample + 2 mL ABTS solution B. The concentration of free chlorine and MCA were 10^{-5} mol L^{-1}.

Figure 4- Residual chlorine concentration as function of mass ratio Cl/N after 30 min of chlorination of an aqueous solution containing glycine a) measured by DPD/FAS titration, b) measured by the MSC system.

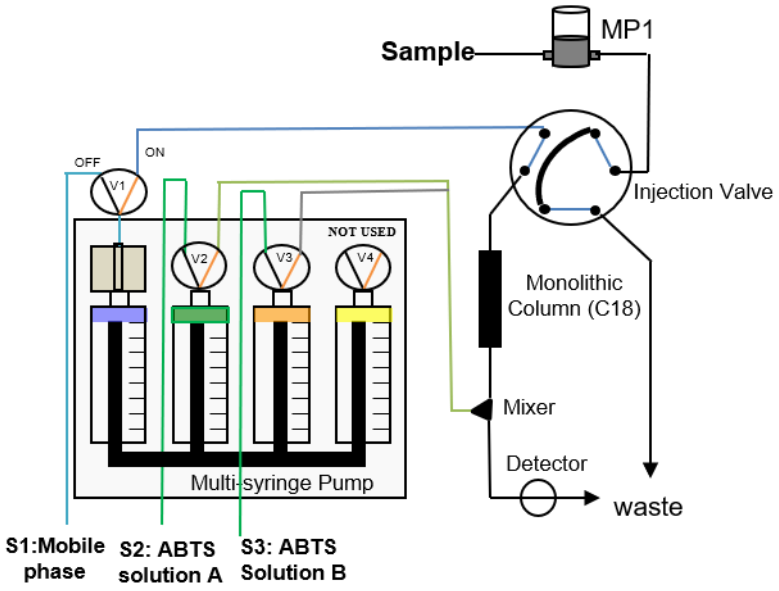
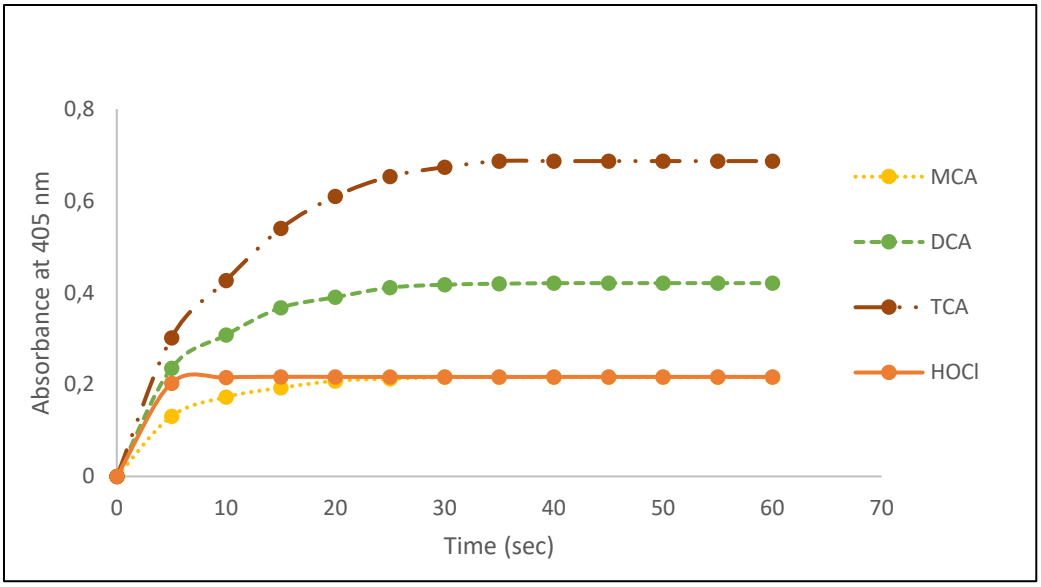


Figure 1.



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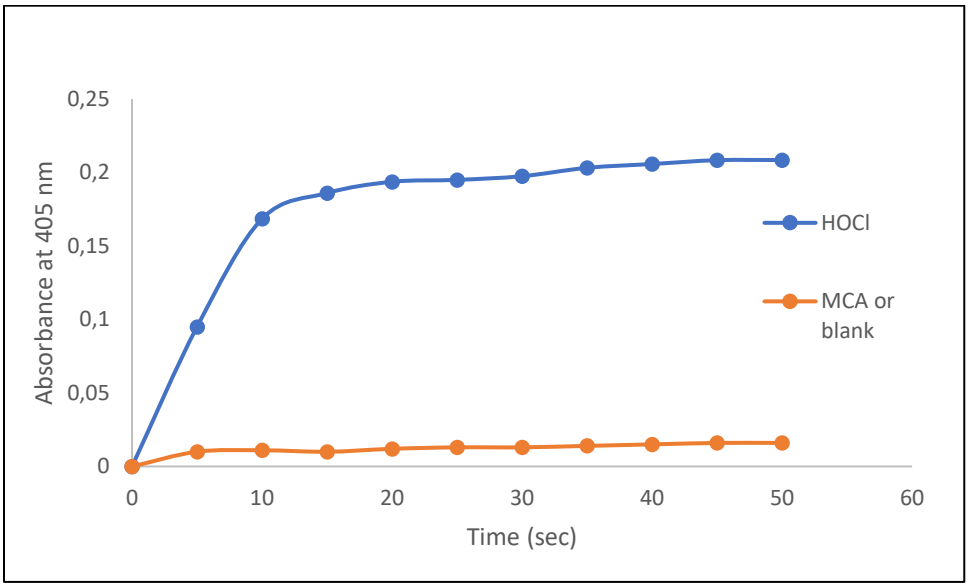
559 Figure 2



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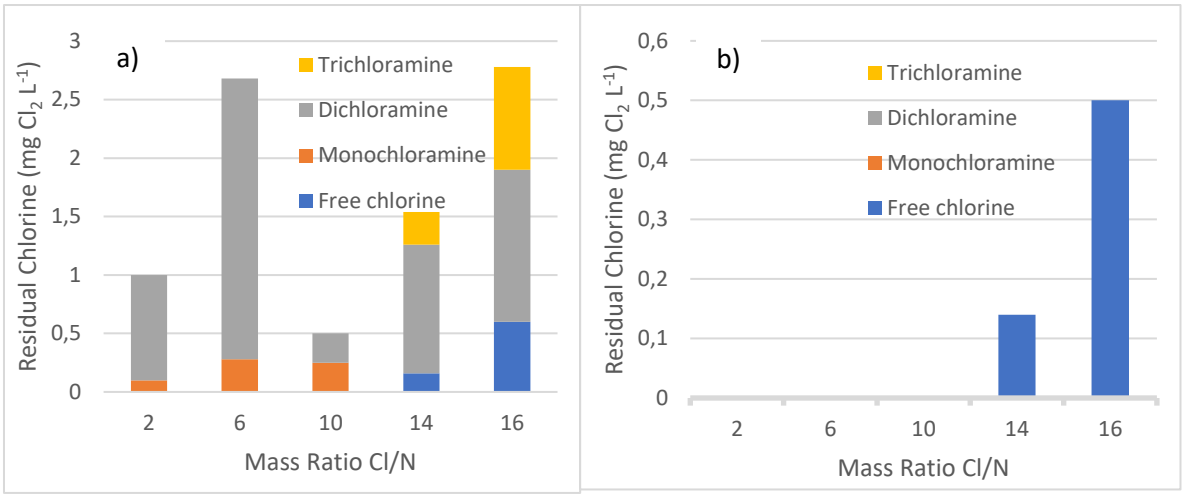
561 Figure 3



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563 Figure 4

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