Gas diffusion sequential injection system for the spectrophotometric determination of free chlorine with *o*-dianisidine

Raquel B.R. Mesquita, António O.S.S. Rangel*

Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal

Abstract

A gas diffusion sequential injection system for spectrophotometric determination of free chlorine is described. The detection is based in the colorimetric reaction between free chlorine and a low toxicity reagent *o*-dianisidine. A gas diffusion unit is used to isolate free chlorine from the sample in order to avoid possible interferences. This feature results from the conversion of free chlorine to molecular chlorine (gaseous) with sample acidification. With minor changes in the operating conditions, two different dynamic ranges were obtained enhancing the application both to water samples and bleaches. The results obtained with the developed system were compared to the reference method, iodometric titration and proved not to be statistically different. A detection limit of $0.6\,\mathrm{mg\ ClO^-/L}$ was achieved. Repeatability was evaluated from 10 consecutive determinations being the results better than 2%. The two dynamic ranges presented different determination rates: $15\,\mathrm{h^{-1}}$ for 0.6–$4.8\,\mathrm{mg\ ClO^-/L}$ (water samples) and $30\,\mathrm{h^{-1}}$ for 0.047–$0.188\,\mathrm{g\ ClO^-/L}$ (bleaches).

Introduction

Water disinfection is an essential procedure to ensure public health. To prevent water contamination from bacteria, disinfectants are added, being chlorine among the most commonly used ones. Despite the important role of keeping the water safe, chlorine may also be harmful to human health, causing eye/nose irritation as well as stomach discomfort. The chlorine concentration determines which role it plays, therefore, implying a close monitoring of its value. For drinking water, the goal is having a maximum value of $4\,\mathrm{mg\ Cl_2/L}$ [1].

Chlorine occurs in several oxidation states presenting oxidising properties in all forms except as chloride [2]. As a disinfectant, chlorine is in oxidation state I—hypochlorite, which is a rather unstable form tending to be partially as molecular chlorine. When added to water the chlorine disin-fectant undergoes hydrolysis to form free chlorine consisting of aqueous molecular chlorine (dissolved gas), hypochlorous acid and hypochlorite ion [3].

Determination of free chlorine in flow systems has been based in colorimetric redox reactions, with specific or non-specific reagents, due to its oxidising capacity. Being an oxidant, it also enables electrochemical detection.

All works developed with electrochemical detection, either potentiometric [4–8] or amperometric [9], were based on the flow injection methodology. Whereas for free chlorine spectrophotometric determination, although flow injection was also the methodology adopted in most cases [10–18], there were two exceptions: one including tandem flow with solenoid valves [19] and another with sequential injection [20] methodology.

Most of the colorimetric reactions used in free chlorine spectrophotometric determination implied highly toxic, polluting reagents [11,13–15,17,20]. Tolidine, the most frequently used, is rather specific and sensitive for free chlorine determination, as well as highly toxic, pollutant and carcinogenic.

* Corresponding author. Tel.: +351 225580064; fax: +351 225090351.
E-mail address: aorangel@esb.ucp.pt (A.O.S.S. Rangel).

There is an increasing trend to minimise the use of toxic reagents in flow systems which implies a search for alternative reagents but without loss of determination sensitivity. Following such a tendency there were a couple of works mentioning an alternative reagent for free chlorine colorimetric determination [10,19]. The reagent used, *o*-dianisidine, is not carcinogenic and only slightly pollutant due to its low toxicity, still maintaining good sensitivity. The major drawback presented by *o*-dianisidine was the lack of selectivity. Despite the significant improvement concerning the reagent, the mentioned works [10,19] maintain the disadvantage of a relatively high effluent volume typical of flow injection. There was also unnecessary sample consumption as it is introduced in the system through the peristaltic pump. Additionally, lack of robustness and low commercial availability of the solenoid valves still limits the widespread use of multicommuted flow injection approach.

A more environmental friendly approach in flow analysis results from both the tendency for the use of less toxic and polluting reagents as well as minimisation of effluent volume. For that purpose, a system for free chlorine determination based in sequential injection methodology is described, using the colorimetric reaction with *o*-dianisidine. The proposed system aims for improvement not only in terms of reagent toxicity but also reagent consumption and effluent volume as SIA enables a tighter control of volumes than in continuous flow analysis. Improved robustness is also attained with the use of commercially available and easily computer-controlled selection valves. The reagent chosen was the one presenting lower toxicity for this determination and the lack of selectivity was overcome by separating free chlorine from the sample to avoid interference. This separation was based on the possibility of having free chlorine in the form of molecular chlorine, a gas at room temperature, isolated from the sample through a diffusion membrane. Acidification of the sample with hydrochloric acid was required to assure that all free chlorine was at molecular chlorine form. The dissolved gas would then diffuse from the sample through a hydrophobic membrane at a gas diffusion unit. The diffused gaseous chlorine is converted to hypochlorite by hydroxide and afterwards reacts with *o*-dianisidine resulting in the coloured product being measured.

Experimental

Reagents and solutions

All solutions were prepared with analytical reagent grade chemicals and boiled Milli-Q water (resistivity >18 MΩ cm). Two hypochlorite stock solutions were weekly prepared, one of about 200 mg/L for chlorine determination in waters and another one of 4.7 g/L for chlorine determination in bleaches. Working standards were prepared daily from these stock solution in the dynamic ranges of 0.6–4.8 mg ClO$^-$/L and 0.047–0.188 g ClO$^-$/L for waters and bleaches determination, respectively.

Dianisidine solution 0.2 g/L, was prepared daily by weighing 4 mg of *o*-dianisidine and adding concentrated acetic acid ($d = 1.05$; 100%) to a final concentration of 0.8 M in 20 mL of freshly boiled water.

Hydrochloric acid solution 0.05 M was prepared weekly from proper dilution of the concentrated acid ($d = 1.19$; 37%).

Stock solution of sodium hydroxide 0.4 g/L was prepared monthly by dissolving in 100 mL of water 40 mg of the solid. The working solution was obtained daily by dilution of this stock solution with freshly boiled water to a concentration of 0.1 g/L.

Sample preparation

The water samples were introduced directly in the system without any previous treatment.

The bleaches were previously diluted between 100 and 250 times in order to fit the linear dynamic range.

Apparatus

Solutions were propelled by a Gilson Minipuls 3 peristaltic pump with PVC pumping tubes. The pump was connected to the central channel of an eight port electrically actuated selection valve (Valco VICI 51652-E8). All tubing connecting the different components of the flow system were made of Teflon from Omnifit with 0.8 mm i.d.

A Perspex gas difusion unit (GDU) shown in Fig. 1, with a zig-zag shaped flow channel, Fig. 1I was used as sepa-
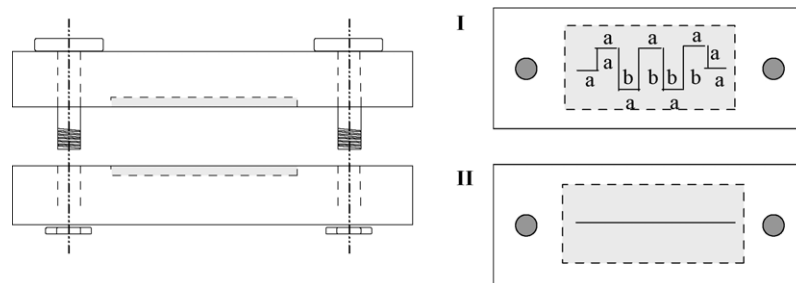

Fig. 1. Gas diffusion unit (GDU), lateral view, channels with inner diameter = 2 mm and depth = 1 mm: (I) top view of the configuration with a zig-zag channel, length: $a = 0.5$ cm, $b = 1$ cm; (II) top view of the configuration with a straight channel, length = 7.5 cm.

Table 1
Protocol sequence for determination of chlorine in waters

Step	Selection valve position	Operation time (s)	Flow rate (μL/s)/direction	Volume (μL)	Description
A	4	4.4	56/a	250	Aspirate hydrochloric acid
B	1	14.1	56/a	800	Aspirate sample or standard
C	4	4.4	56/a	250	Aspirate hydrochloric acid
D	1	14.1	56/a	800	Aspirate sample or standard
E	4	4.4	56/a	250	Aspirate hydrochloric acid
F	5	89	28/p	2500	Propel through donor channel of GDU
G	6	1.5	56/a	85	Aspirate sample from acceptor channel of GDU
H	7	3.5	56/a	200	Aspirate dianisidine reagent
I	8	50	56/p	2800	Propel to detector and signal registration
J	6	4	56/a	230	Washing acceptor channel of GDU
L	3	7.2	56/p	400	Washing of holding coil

Table 2
Protocol sequence for determination of chlorine in bleaches

Step	Selection valve position	Operation time (s)	Flow rate (μL/s)/direction	Volume (μL)	Description
A	4	1.5	17/a	25	Aspirate hydrochloric acid
B	2	1.5	17/a	25	Aspirate sample or standard
C	4	1.5	17/a	25	Aspirate hydrochloric acid
D	5	5.3	28/p	150	Propel through donor channel of GDU
E	6	1.5	56/a	85	Aspirate sample from acceptor channel of GDU
F	7	3.5	56/a	200	Aspirate dianisidine reagent
G	8	50	56/p	2800	Propel to detector and signal registration
H	6	4	56/a	230	Washing acceptor channel of GDU
I	3	7.2	56/p	400	Washing of holding coil

ration device. A Millipore Durapore® membrane filter (ref. HVHP09050) was used between the two channels.

A Hitachi 100-40 UV–vis spectrophotometer with a Starna Brand 75.3 Q flow-cell (20 mm light path, 40 μL inner volume) was used as detection system. The wavelength was set at 453 nm. Analytical signals were recorded in a Metrohm E 586 Labograph strip chart recorder.

A personal computer (Samsung SD700) equipped with a PCL818L interface card, running with homemade software written in QuickBasic 4.5, controlled the selection valve (SV) position and the pump sense and speed.
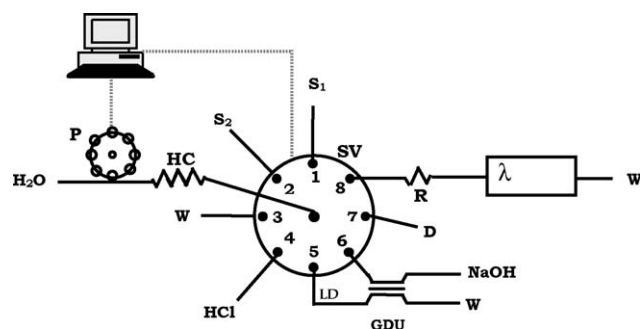

Fig. 2. Sequential injection manifold for the colorimetric determination of chlorine: S_1, water sample; S_2, bleach sample; HCl, hydrocloric acid (0.5 M); D, dianisidine solution (0.2 g/L dianisidine and 0.8 M acetic acid); NaOH, hydroxide solution (0.1 g/L); W, waste; HC, holding coil (6 m); GDU, gas diffusion unit; LD, connection to donor channel (8 cm); R, connection to detector (70 cm); SV, eight port selection valve; P, peristaltic pump; λ, UV–vis spectrophotometer.

Flow manifold and procedure

The manifold for the colorimetric determination of chlorine in waters and bleaches is depicted in Fig. 2. The sequence of the steps and respective time is shown in Table 1 for chlorine determination in waters and in Table 2 for chlorine determination in bleaches.

Results and discussion

Manifold configuration

As previously mentioned the developed system proposes the use of a less toxic reagent, o-dianisidine, which is not specific for chlorine. To overcome this drawback chlorine had to be isolated in order to prevent possible interference.

The manifold was then designed to comply a gas diffusion unit for chlorine separation from the sample. According to previous works [10,19] better results for chlorine isolation should be obtained if the acceptor channel was in a close loop enabling a chlorine pre concentration. Therefore, a comparative study between having the acceptor channel in a close loop and in an open channel was performed. While the open channel approach was easily performed with a single selection valve, the close loop approach required also an injection valve, therefore, two configurations were needed to carry on the study as shown in Fig. 3.

Both configurations were tested under the same operating conditions. Regarding sample acidification and transport to
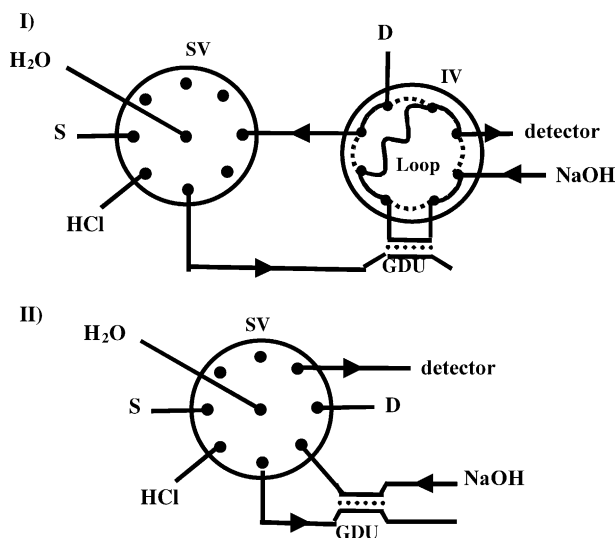
Fig. 3. Configurations for complying a gas diffusion unit (GDU). (I) Configuration with a close acceptor channel; (II) configuration with an open acceptor channel. S, standard; HCl, hydrocloric acid; D, dianisidine solution; NaOH, hydroxide solution; GDU, gas diffusion unit; Loop, dianiside loop; SV, eight port selection valve; IV, eight port injection valve.

the GDU the same sequence (sampling sequence) was used. The sampling sequence was: aspiration of acid, aspiration of sample, and again aspiration of acid, followed by propelling through the donor channel of the GDU. After this sequence, there was a slight difference for each configuration. In the configuration with the closed loop shown in Fig. 3I, the injection valve was kept in the loading position, dashed lines in Fig. 3I, during the sampling sequence. After that and before switching the valve position, the loop of dianisidine, "loop" in Fig. 3I, was filled, the injection valve was then switched to injection position, full lines in Fig. 3I. At this position, both the chlorine in the acceptor channel and dianisidine in the loop were propelled to the detector. In the configuration with the open channel, Fig. 3II, after the sampling sequence chlorine was aspirated from the acceptor channel directly, followed by aspiration of dianiside. Then both plugs were sent to the detector. From the results obtained with both configurations it was concluded that there was no advantage in having the acceptor channel in a close loop. This configuration not only presented a worse sensitivity (decrease of 62% in the slope of the calibration curve) but also required a far more complex design.

Optimisation of the gas diffusion unit

Having set the manifold design for complying the gas diffusion unit, depicted in Fig. 2, the optimisation of the unit was carried out. The GDU was optimised regarding design; two possible alternatives were tested and illustrated in Fig. 1: a GDU with a zig-zag channel in Fig. 1I and a GDU with a straight channel in Fig. 1II. The first one was chosen as it showed an increase in sensitivity of about 17%.

The membrane used for chlorine separation from the sample was a hydrophobic membrane. Two types of hydrophobic membranes were compared: a Millipore membrane and a commercial Teflon membrane. The commercial Teflon membrane presented a decrease of about 23% in sensitivity and lack of mechanical stability in the GDU. This membrane would get all wrinkled inside the GDU with the pressure caused by the aspiration of the chlorine from the acceptor channel, leading to non-repeatable measurements. Therefore, due to both better sensitivity and stability, the Millipore membrane was chosen. Afterwards a study of the pore size was carried out between two Millipore membranes: 0.45 and 0.22 μm. The pore size of 0.45 μm yielded a sensitivity increase of 15% so this was the one chosen.

Optimisation of the SIA system parameters

The volume of dianisidine was set at 200 μL. The volumes of hydrochloric acid and sample were adopted from a similar study described in a previous work [21]; those volumes correspond to the maximum sample volume to obtain a good mixture with the reagent. In the developed system those volumes correspond to an efficient acidification of the sample. Due to the low efficiency of mass transference procedures such as gas diffusion, even that maximum volume of sample was not enough to achieve good sensitivity. Therefore, a study for increasing the volume of acidified sample passing through the donor channel was carried out. To achieve this without increasing the sample volume aspirated, aspiration of a second plug of sample followed by another plug of acid was tested. This procedure resulted in a 2.5 times higher sensitivity.

The flow rate for propelling the acidified sample to the donor channel of the GDU could have a significant influence in the diffusion of molecular chlorine through the membrane. Flow rates of 0.85, 1.70, 2.57 mL/min were tested. The highest flow rate presented the same signal for all standards, probably just the signal for the colour reagent due to very poor diffusion. As for the flow rate of 0.85 mL/min, the sensitivity was just slightly higher (1.2×) than the one for the flow rate of 1.70 mL/min, this was the chosen value as a compromise between diffusion efficiency and determination rate.

The length of the connection between the acceptor channel of the GDU and the selection valve was set to a minimum of 6 cm in order to minimise sample dispersion. The optimisation of the volume aspirated from the acceptor channel of the GDU was a critical parameter in the optimisation process. Different aspiration volumes represent different chlorine concentration due to a gradient established along the acceptor channel. Before proceeding to the optimisation of aspiration volume, a preliminary study with dyes was carried out; it was concluded that the volume to be aspirated should be at least 85 μL. Then, the volumes aspirated varied from the previously set 85–230 μL. The chosen volume was 85 μL as larger volumes did not improve the sensitivity as illustrated
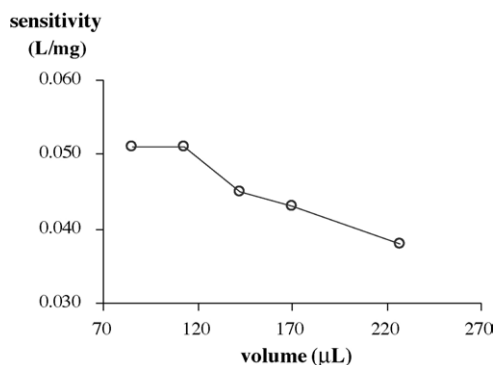
Fig. 4. Influence of the volume aspirated from the acceptor channel of the GDU on the sensitivity.

in Fig. 4. The highest sensitivity implied that the more concentrated part was aspirated.

The concentration of the reagents was also studied as shown in Fig. 5. From the results obtained, a concentration of 0.5 M for the hydrochloric acid, 0.20 g/L for o-dianisidine and 0.2 g/L for sodium hydroxide were chosen.

Interferences

Even though the chosen reagent was not specific for chlorine determination, the use of the GDU ensures that there is no significant interference from other species possibly present in water samples. These potential interferents include metal cations and species with oxidising properties. However, as these species do not diffuse to the acceptor channel of the GDU, no significant interference is produced, as previously described [10].

Application to water samples

Different water samples, both tap and waste water were analysed by the iodometric titration (IT) as reference method [3] and by the developed sequential injection system (SIA) results presented in Table 3.

To evaluate accuracy, a linear relationship between C_{SIA} (mg ClO$^-$/L) and C_{IT} (mg ClO$^-$/L) was established; the
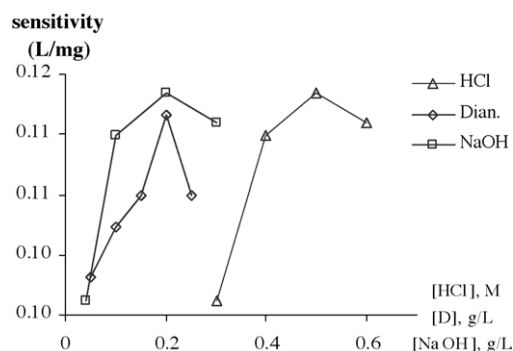

Fig. 5. Optimisation of reagents concentrations.

Table 3
Application of the developed system (SIA) to water samples, and comparison with the reference method, iodometric titration (IT)

Sample	IT (mg ClO$^-$/L)[a]	SIA (mg ClO$^-$/L)[a]	RD (%)
Tap water	0.416 ± 0.015	0.435 ± 0.004	4.6
Waste water water	1.422 ± 0.148	1.481 ± 0.056	4.1
Tap water	1.271 ± 0.031	1.356 ± 0.053	6.7
Waste water	0.837 ± 0.026	0.722 ± 0.013	−13.7
Tap water	0.620 ± 0.093	0.630 ± 0.043	1.6
Tap water	0.804 ± 0.168	0.912 ± 0.088	13.4
Waste water	0.549 ± 0.053	0.554 ± 0.013	0.8
Waste water	0.948 ± 0.041	0.946 ± 0.059	−0.2
Tap water	0.842 ± 0.031	0.961 ± 0.036	14.2
Waste water	0.518 ± 0.080	0.477 ± 0.010	−7.9
Waste water	1.435 ± 0.019	1.253 ± 0.036	−12.7
Waste water	0.717 ± 0.031	0.777 ± 0.009	8.4

[a] Mean ± standard deviation ($n = 3$).

equation found was:

$$C_{SIA} = 0.0433(\pm 0.1664) + 0.962(\pm 0.1797) \times C_{IT}$$

where the values in parenthesis are 95% confidence limits. These figures show that the estimated slope and intercept do not differ statistically from the values 1 and 0, respectively. Therefore, there is no evidence for systematic differences between the two sets of results [22].

Application to bleaches

All bleach samples were previously diluted, as mentioned above, before they were analysed in the SIA system. The reference method, iodometric titration (IT), was also performed with the diluted sample and results are presented in Table 4.

A linear relationship between C_{SIA} (g ClO$^-$/L) and C_{IT} (g ClO$^-$/L) was established to evaluate accuracy, the equation found was:

$$C_{SIA} = 2.48(\pm 9.22) + 0.940(\pm 0.296) \times C_{IT}$$

where the values in parenthesis are 95% confidence limits. As it also happened for water samples the figures show that the estimated slope and intercept do not differ statistically from the values 1 and 0, respectively.

Table 4
Application of the developed system (SIA) to bleach samples, and comparison with the reference method, iodometric titration (IT)

Sample	IT (g ClO$^-$/L)[a]	SIA (g ClO$^-$/L)[a]	RD (%)
1	41.3 ± 1.5	37.8 ± 0.5	−8.5
2	36.5 ± 2.7	34.2 ± 0.4	−6.3
3	17.2 ± 0.2	17.4 ± 0.3	1.2
4	35.3 ± 0.1	38.0 ± 0.2	7.6
5	29.1 ± 0.1	31.8 ± 0.3	9.3
6	17.8 ± 0.1	17.4 ± 0.3	−2.2
7	31.6 ± 0.3	34.9 ± 0.4	10.4
8	31.6 ± 0.9	34.4 ± 0.1	8.9

[a] Mean ± standard deviation ($n = 3$).

Features of the flow system

For each concentration range, typical calibration curves were as follows: (i) water samples, range 0.6–4.8 mg ClO^-/L, $A = 0.1704[ClO^-] + 1.08 \times 10^{-2}$ $(R^2 = 0.998)$; (ii) bleaches samples, range 0.047–0.188 g ClO^-/L, $A = 5.121[ClO^-] + 2.20 \times 10^{-3}$ $(R^2 = 0.995)$. The detection limits for both concentration ranges were calculated according to IUPAC [23] recommendations. For the range of 0.6–4.8 mg ClO^-/L it was 0.5 mg ClO^-/L, and for the range of 0.047–0.188 g ClO^-/L was 5 mg ClO^-/L. The limits of determination were, respectively, 0.6 and 6 mg ClO^-/L.

Precision was evaluated by the determination of the relative standard deviation obtained from 10 consecutive determinations of the same sample, and the results were: 1.5% (0.739 mg ClO^-/L); 1.8% (1.159 mg ClO^-/L).

A complete analytical cycle took about 4 min for water samples (0.6–4.8 mg ClO^-/L) and 2 min for bleaches (0.047–0.188 g ClO^-/L). An analytical cycle is the sum of the time needed for each step plus the time necessary for the port selection in the selection valve. Thus, based on the time spent per cycle, the sampling frequency was 15 and 30 determinations per hour for determination in water samples and bleaches, respectively. This corresponds to a sample consumption per determination, of 1.6 mL for waters and 25 μL for diluted bleaches.

The overall reagent consumption per determination was: 40 μg dianisidine; 9.6 mg acetic acid; 8.5 μg of sodium hydroxide. Regarding the hydrochloric acid consumption per determination, it was 1.4 mg for waters, and 91 μg for bleaches. The total volume of effluent produced per determination is only around 6 mL for chlorine determination in waters and around 4 mL for determination in bleaches.

Conclusions

The use of sequential injection system for chlorine determination with a less toxic non-specific reagent proved to be an effective alternative. The lack of selectivity of the reagent was efficiently overcome resorting to a gas diffusion unit for the chlorine separation from the sample.

The developed flow system allowed chlorine determination in different types of water as well as in bleaches. This was accomplished with a single manifold and minor changes of operational parameters in the controlling software. Thus, by removing two steps of the protocol sequence and changing the times for aspiration sample and acid as well as the propelling through the GDU a different concentration range was obtained.

The major improvements of the presented system towards the previous works are: using a less toxic reagent, maintaining low detection limits, and reducing both reagent consumption and volume of effluent. As the developed system deals with very small volumes of every solution involved, it can be used for unattended in situ monitoring of water treatment plants and tap water networks with no need for frequent refilling of reagent solutions.

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