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SPECTROPHOTOMETRIC DETERMINATION OF AMMONIUM BY AN rFIA ASSEMBLY

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The paper deals with the spectrophotometric determination of ammonia with a reverse Flow Injection Analysis assembly. The procedure is based on the modified reaction of Berthelot in which the sample acting as carrier merges with NaClO in basic medium; then a second reagent (salicylic acid, sodium hydroxide and sodium nitroprusside) is inserted into the resulting mixture. The calibration graph was linear from 0.5 to 20 ppm; L.O.D. 0.3 ppm NH_4^+; the sample throughput was 32 h^{-1} and the repeatability was 0.7% (n = 9, 5 ppm). The day-to day reproducibility resulted in an RSD of the slope of 4.2% (n = 8). The method was applied to fertilizers and pharmaceutical formulations. The separation and pre-concentration of the analyte was also tested by addition of a separation device in which the ammonia passed into an acceptor solution containing a pH indicator and the calibration graph obtained was linear from 0.1 to 5 ppm NH_4^+.

INTRODUCTION

Ammonia is a compound present in environmental samples,[1,2] aquaculture effluents,[3] industrial wastewaters,[4,5] plants[6-8] and soil,[9] in food digest as an indicator for total nitrogen,[10] in pharmaceutical formulations,[11,12] etc.

Many methods have been proposed for its determination including: coulometric titration of NH_3 with hypobromite,[13] fluorimetrically by derivatisation with o-phthaldehyde,[14]conductometric after separation through a membrane,[15] using the hypochlorite luminol chemiluminescence reaction,[16,17] potentiometric with a differential system[10] or indirect by amperometric detection of ammonium ions.[18] Usually, the spectrophotometric detection is favorite for practical applications and it is performed based on the adaptation of classical methods such as Nessler reaction,[19-21] Berthelot reaction[2,3,9,22] or this reaction modified by replacing phenol by salicylate[1,8]. Other possibilities for spectrophotometric detection are based on the indirect determination of ammonia using a mini solid-phase reactor where Ag^+ ions are transformed into a soluble $[Ag(NH_3)_2]^+$ complex that is further determined with bromopyrogallol red (BPR)[11] or a mixture of BPR and *o*-phenanthroline.[23]

A strategy often employed in flow continuous systems, to separate and/or preconcentrate on-line ammonium, is based on the use of microporous membranes that are permeable to the produced ammonia, gas usually obtained by mixing the sample with NaOH. The acceptor stream is different, depending of the detection system, which can be conductimetric,[24] fluorimetric[25] or more often spectrophotometric. In this case, it is usual an acceptor stream containing a pH indicator: namely, cresol red,[26] phenol red,[27] bromothymol blue[5,28,29] (this has also been employed in a portable system to determine ammonium in industrial liquid affluent streams[30]), or an indicator mixture, such as phenol/bromocresol purple,[31] thymol blue/cresol red,[4] or bromocresol violet/bromothymol/cresol red.[32,33] Other separation devices have been used, based on microporous PTFE tube for different detection strategies,[34-36,25] just as a pervaporation unit.[37]

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Translation of a batch analytical method into continuous-flow techniques results in some advantages like the increase rate of analysis, the reduction of the reagent and sample consumption, the increase of the precision, ease of automation, etc. When the sample availability is not a problem, the reagent consumption can be minimized using the reverse Flow Injection Analysis (rFIA) methodology, based on injecting small reagent aliquots into a sample–carrier stream.

This paper is dealing with the ammonium determination in real complex samples. Two different strategies were studied: *(i)* the pH of the sample was made basic which transformed ammonium into ammonia, which was separated from the sample matrix by diffusion through a microporous membrane, and induced a spectrometric measurable color change of the pH indicator in the acceptor stream; and, *(ii)* an adaptation to the flow conditions of the Berthelot reaction, which produced indophenol blue. This analytical process proposes an rFIA assembly and it is based on the transformation of ammonia into chloramine followed by the production in two steps of indophenol blue in the presence of a hypochloride excess in a basic medium. Corresponding chemical reactions are depicted in Fig. 1.

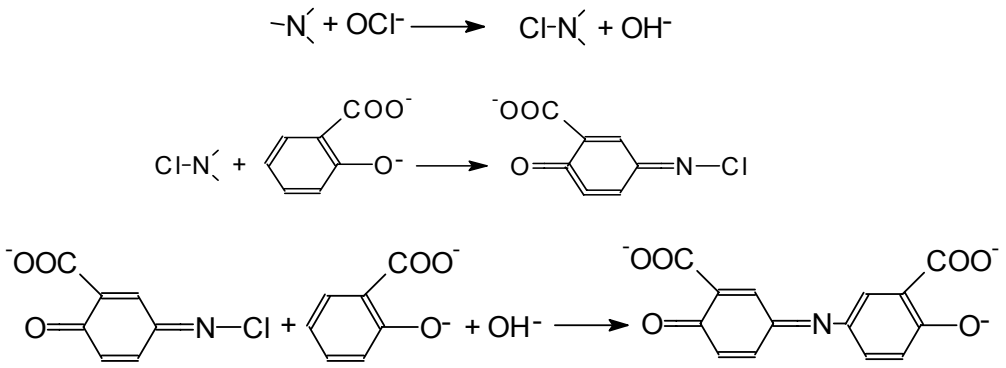

Fig. 1 – The Berthelot reaction used for ammonia determination.

As proposed in formerly published papers[1,8] the salicylate can be used instead of phenol. As catalyst acetone,[38] Mn^{2+}, [31, 39]sodium tetraborate decahydrate[2], hexacyanopherrate (II)[40] and sodium nitroprusside can be used, which proved to be the most suitable;[38,41,42] so they were used in this study.

EXPERIMENTAL

Reagents

A stock solution containing 1000 ppm of NH_4^+ was prepared by dissolving the required quantity of $(NH_4)_2SO_4$ (Probus). The subsequent dilutions were daily prepared.

For the Berthelot reaction, the reagent 1 was prepared by mixing 0.5 g of NaOH (Panreac) with 8 mL of 2.5% NaClO (Panreac) and levelled to 100 mL. The hypochloride concentration was every day determined by the iodometric method. The reagent 2 was obtained by mixing 1 g of salicylic acid (Guinama), 0.5 NaOH (Panreac, 97%), and 0.9 g of sodium nitroprusside (Panreac); then the resulting mixture was levelled to 100 mL. This solution was daily prepared and kept protected from room light.

For the gas diffusion separation and preconcentration study, the pH indicator stock solution was obtained by dissolving 0.1 g of cresol red and 0.3 g of thymol blue in 200 mL of water. The acceptor stream was daily prepared by diluting 10 mL of the indicator stock solution to 500 mL.

All solutions were prepared using purified water with 18 MΩ/cm (Nanopure II, Sybron Barnstead).

rFIA manifold

The flow manifolds used in this work were made by assembling the following components: peristaltic pumps (Gilson Minipuls 2); injection valve (Rheodyne); PTFE tubing with internal diameter 0.8 mm (Gilson); and, PTFE connector devices shaped as “arrow tip”. The spectrophotometric measurements were performed with a Hewlett Packard spectrometer (model 8453) provided with a flow-through cell (Hellma) with an internal volume of 13 μL and optic path of 10 mm.

The home-made gas diffusion device consisted of two PTFE blocks with carved zigzag channels, sandwiching a Fluoropore membrane filter of 0.5 μm pore size and 150 μm thickness (® Millipore, Ireland) and a PTFE connector shaped as “arrow tip” (See Fig. 2).

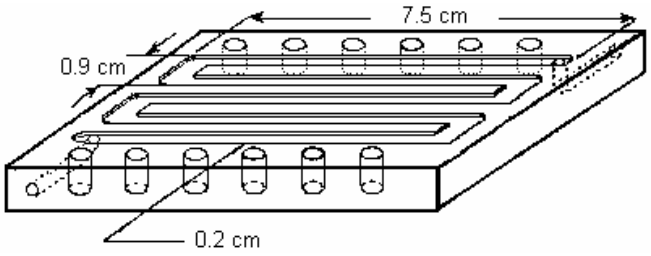

Fig. 2 – Methacrylate piece of the sandwich type gas-diffusion unit. The gas diffuser is made from two methacrylate blocks with a sandwiched porous membrane. The groove carved in the pieces formed a channel that is split by the membrane.

Procedures

The scheme of the rFIA manifold is depicted in Fig. 3. The sample solution flowing at 2.2 mL min^{-1}, merged with reagent 1 (flow-rate 0.6 mL min^{-1}) and the reaction took place in the first coiled reactor (length 141 cm). Then 90 μL of the reagent 2 were injected and the coloured compound formed in a second reactor (length 278 cm) was detected at 640 nm.

A second flow system including a gas diffusion device (see Fig. 4) was also prepared. The sample stream (0.6 mL min^{-1}) merged with NaOH 0.5 M (0.6 mL min^{-1}) and formed gaseous ammonium penetrate through the membrane of the gas diffusion device into the acceptor stream (1.5 mL min^{-1}). The pH diminution, through a coiled reactor 40 cm long, induced a colour change of the indicator solution detected at 590 nm.

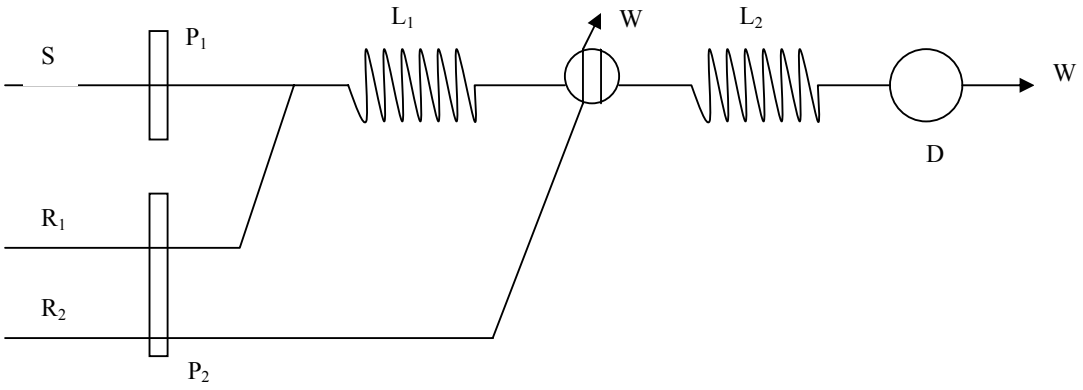

Fig. 3 – Schematic rFIA manifold. P_1 and P_2, peristaltic pumps; I, injection valve; S, carrier-sample solution stream, R_1, reagent 1; R_2, reagent 2; L_1, reactor 1; L_2, reactor 2; D, spectrophotometric detector; and W, waste.

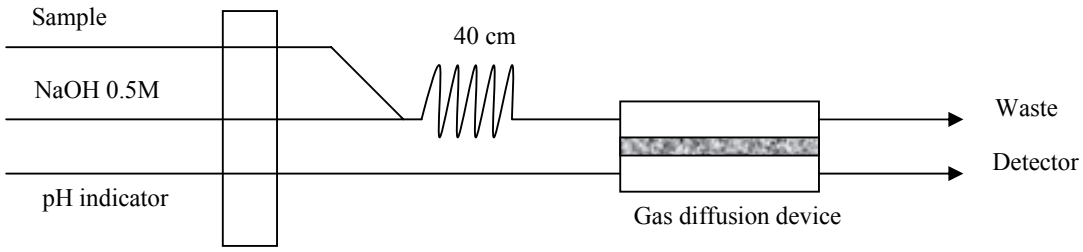

Fig. 4 – Continuous-flow manifold used for ammonia separation and spectrophotometric measurements based on the color change of a pH indicator and gas diffusion device.

RESULTS AND DISCUSSION

Preliminary assays

The performance of the membrane for the separation and preconcentration of the ammonium through a gas diffusion unity was tested, using the flow manifold described in Fig. 4. It resulted in a linear calibration

graph from 0.1 to 5 ppm; A = 0.08 + 0.30·C, R^2 = 0.9964; where A is the absorbance at 590 nm, and C is the ammonium concentration in mg L^{-1}). When the flow rates of the donor and acceptor solutions are not equal, a difference in pressure is created that can forces some liquid to traverse the membrane towards the slower moving flow. So, when the flow rate of the acceptor solution was lowered to 0.8 mL min^{-1}, the blank increased from 0.07 to 1.07 of absorbance avoiding a correct measurement. This inconvenience was probably due to the simultaneous pass of the NaOH from the sample into the acceptor solution. The interference of NaOH was avoided using the Berthelot reaction, which requires a basic medium. Further work to propose a flow assembly was performed without the gas diffusion device.

Previous assays with flow manifolds based in the Berthelot reaction were performed using reagents based on phenol. In addition to the toxicity of phenol (analytical residues aggressive with the environment), its water solution is not stable, being necessary to use a water-organic solvent mixture. This provided a very high blank signal from the phenol-nitroprusside[2] and at the same time increases the toxicity of the solution. Using the rFIA manifold described in Fig. 3, but with reactor 1 length 200 cm and reactor 2 length 250 cm, the blank for a reagent solution of 5 g phenol, 2 g NaOH and 0.09 g sodium nitroprusside presented 0.60 absorbance. These drawbacks were avoided by replacing phenol by salicylate.[1]

Influence of temperature

The Berthelot reaction is slow and therefore difficult to be adapted to a continuous-flow analysis. The influence of the temperature on the height of the analytical outputs was studied with a rFIA manifold similar with the one depicted in Fig. 3, but with the length of reactor 1 of 200 cm, reactor 2 of 250 cm; the carrier-sample flow-rate was 0.8 mL min^{-1} and the flow-rate of reagent 10.4 mL min^{-1}. Reagent 1 was a mixture of 0.75 g salicylic acid, 0.2 g of NaOH and 0.75 g of sodium nitroprusside, levelled to 100 mL. Reagent 2 contained 2 g of NaOH and 4.5 mL of 2.5% NaClO in a 100 mL flask. Reactor 2 can not be heated because this produces gas bubbles which result in irregular flow-rates changes and in spurious spectrophotometric signals. The temperature for reactor 1 was varied over the range 20–60 ºC and for every temperature two samples of 3 and 5 ppm were tested. As shown in Fig. 5 the increase of the temperature resulted in decreasing the signals and in increasing the absorbance of the blank solution. Further work was performed at room temperature.

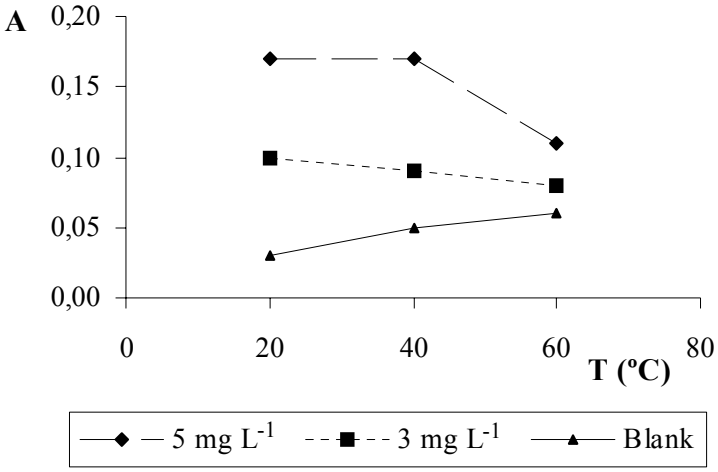

Fig. 5 – Temperature influence applied to reactor 1.

Optimisation of the hydrodynamic parameters

The hydrodynamic parameters of the rFIA were optimised with the aid of a multivariate method known as MSM (Modified Simplex Method). Five FIA parameters were investigated: flow-rate of the carrier-sample stream, Q_1; flow-rate of reagent 1, Q_2; length of the reactor 1, L_1; length of reactor 2, L_2; and, injected volume of reagent 2, V. Every analytical signal (sample minus blank) was calculated as the average

of at least ten reproducible measurements. Two series of experiments were performed, the first with 13 vertices using a sample solution of 5 mg L^{-1} and investigating the above reported parameters over the ranges: Q_1 and Q_2 from 100 to 900 pump display units (arbitrary units), L_1 from 50 to 300 cm, L_2 from 50 to 400 cm and V from 0 to 30 cm of the tube added to the minimum volume of the injection valve. The used reagents were: reagent 1 made with 2 g NaOH, 4.5 mL 2.5% NaClO in 100 mL flask and reagent 2 prepared with 0.75 g salicylate and 0.75 g nitroprusside in 100 mL water. The second simplex consisted of 10 vertices and the parameters were investigated over the ranges: Q_1 from 200 to 800 pump units, Q_2 from 100 to 600 pump units, L_1 from 100 to 300 cm, L_2 from 50 to 300 cm and V from 0 to 30 cm of the tube added as external volume of injection valve (loop). The best hydrodynamic parameter set was found to be: Q_1, 323 pump units (corresponding to a flow-rate of 2.2 mL min^{-1}), Q_2, 202 pump units (0.6 mL min^{-1}), L_1 141 cm, L_2 278 cm and V 6 cm (corresponding to a total injected volume of 90 μL).

Optimisation of the chemical parameters

The concentration of the reagents was re-optimised using the hydrodynamic parameter set chosen in the former empirical work of optimisation. The sample contained 10 mg L^{-1} of NH_4^+ and for the beginning the same reagent concentrations as those from the optimisation of the hydrodynamic parameters. For every concentration tested the blank and the analytical signal were measured. The best concentration was the one giving the highest absorbance at the lowest blank.

The concentration of sodium nitroprusside was tested from 0.2 to 1.3 g in 100 mL. The analytical signal was increasing slowly up to 0.9 g/100 mL while the obtained blank was constant and small in the same range and started to increase thereafter. A 0.9 g sodium nitroprusside /100 mL concentration was considered as optimal.

The concentration of salicylate was optimised in the range 0.4 to 1.2 g/100 mL. The absorbance of the blank was constant in the entire interval studied, while the analytical signal was fast increasing up to 0.6 g/100 mL and then slowly up 1 g/100 mL which was considered as the optimum value.

Using reagent 1 with the formerly optimised concentration, the influence of the concentration of the constituents of reagent 2 was investigated. The sodium hypochloride concentration (stock solution 2.5%) was studied in the range 2 to 10 mL NaClO/100 mL. The signal was increasing with the concentration of sodium hypochloride, until a plateau was reached. The higher signal and minimum blank were obtained with the reagent prepared with 8 mL NaClO in 100 mL solution, equivalent to 0.2 g NaClO / 100 mL.

The medium of the reaction was the last to be optimised. It was investigated from 0 to 3 g NaOH/100mL solution. While the blank was almost constant in all the interval, the analytical signal increased up to 0.2 g NaOH/100mL, remained constant until 1 g NaOH/100 mL, and then decreased. As more safe and robust value was chosen 0.5 g NaOH/ 100 mL (see Fig. 6).

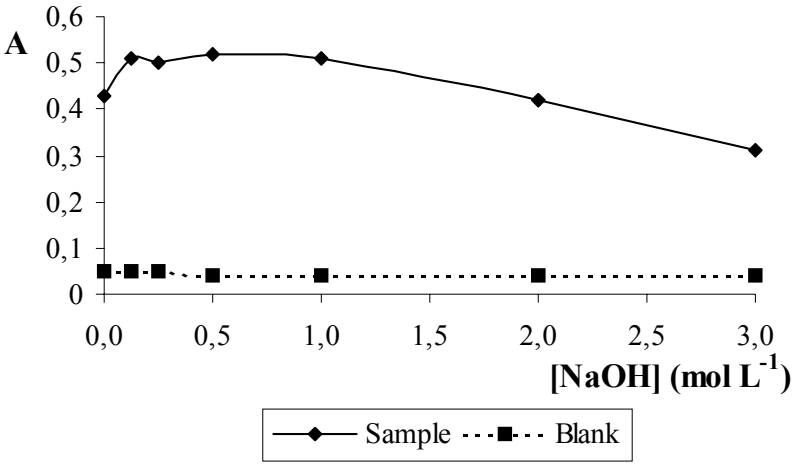

Fig. 6 – Influence of the concentration of NaOH used as medium of reaction.

Analytical Figures of Merit

The calibration graph was linear over the range 0.5-20 mg L^{-1}, which fitted the linear equation A = 0.096 + 0.1158 C, with a correlation coefficient R^2 0.9986 (n 6 replicates; A is the absorbance outputs at 640 nm and C the ammonium concentration in mg L^{-1}). The limit of detection was 0.3 mg L^{-1} NH_4^+, calculated as the concentration of ammonium providing a signal equal to the blank plus three times the standard deviation.

The sample throughput was 32 h^{-1} and the repeatability of the method, calculated as RSD, was 0.7% (n = 9 and sample concentration 5 mg L^{-1}).

The day-to day reproducibility was calculated by preparing 8 independent calibration graphs in different days and with freshly prepared solutions. The RSD of the slope was 4.2%.

The influence of the foreign compounds was studied by preparing solutions containing 5 mg L^{-1} of ammonium and the tested compound. Results, as the average of at least four reproducible determinations, were compared with those obtained from pure ammonium solutions of the same concentration. Relative errors are depicted in Table 1 and, as can be observed, ferric and cupric ions are the most relevant interferences. The use of EDTA to minimize the effect of the inhibitors was not possible, due to the decomposition of the sodium nitroprusside used as catalyst.

Table 1

Study of the interference of different ions found as potential interferents in ammonium samples (the tested concentration of NH_4^+ was 5 mg L^{-1})

Interference	Concentration (mg L^{-1})	Output	Relative error (%)
Cl^-	1000	0.72	4,3
SO_4^{2-}	1000	0.70	1,5
CO_3^{2-}	100	0.73	5,8
HCO_3^-	100	0.71	2,9
SO_3^{2-}	100	0.68	-1,5
Ca^{2+}	500	0.71	2,9
K^+	100	0.69	0
Fe^{3+}	5	0.68	-1,5
Cu^{2+}	5	0.66	-4,3
Zn^{2+}	100	0.71	2,9
Citrate	100	0.695	0,7
Urea	120	0.67	-3

Real sample analysis

The proposed method was used to determine the ammonium concentration in real samples with complex matrices. One fertilizer and one commercially available pharmaceutical formulation were investigated. Both were diluted to a concentration around 5 mg L^{-1} NH_4^+ on the basis of the composition given by the manufacturer. The results (see Table 2), compared with the label claim, showed a good correlation for the pharmaceutical preparation and approximate for the fertilizer, which can be considered normal taking into account the differences in the precision, homogeneity and the quality control required for each types of products. Further on, a study of the recovery was performed. The samples diluted to 5 mg L^{-1} according to the label claim were spiked with two concentrations of NH_4^+, 3 and 5 mg L^{-1}. The recoveries obtained varied between 92 and 107% in both kind of samples (see Table 2).

Table 2

Determination and recovery of ammonium in real samples

Sample	Concentration according to label claim (mg L^{-1})	Concentration found (mg L^{-1})	NH_4 added (mg L^{-1})	NH_4 found (mg L^{-1})	Recovery (%)
Gesal (liquid fertiliser)	5	8.4		4.9	98
		8.7	5	4.5	92
		8.4		5.2	104
		8.6		3.2	106
		8.75	3	3.05	102
		8.75		3.05	102

(continues)

Table 2 (continued)

Benadryl (pharmaceutical preparation)	5	5.2	5	5.3	102
		5.1		5.3	104
		5.1		5.2	102
		5.2		3.15	105
		5.1	3	3.21	107
		5.2		3.18	106

CONCLUSIONS

Two flow assays for ammonium were investigated. The first one is based on the separation of the analyte through a porous membrane to an acceptor flow, containing a pH indicator solution. Despite of the simplicity of the first strategy, the presence of carbon dioxide and other gas permeable substances in the samples results in poor accuracy. Carbon dioxide also has a buffering action, which causes a decrease in sensitivity.[43] On the other hand, with the membrane tested, the results were good only when the acceptor flow rate was bigger than the donor flow rate. This is due to the NaOH passage through the membrane by the difference in the pressure; probably a smaller pore size could improve the results.[28,30]

The spectrometric rFIA method is based on the Berthelot reaction that was adapted and optimized. The optimized method was used with good results on commercially available formulations. With the use of membranes NaOH is not interfering in the Berthelot reaction, which requires a basic medium and thus the separation/preconcentration unit may be coupled to the rFIA system based on the Berthelot reaction. Nevertheless, the linearity range and LOD of the different flow systems based on the Berthelot reaction for the ammonium determination proposed in the literature, fluctuate in a large interval depending of the experimental conditions, the use of catalysts or preconcentration systems, etc. So, it is possible to find methods over the interval 20-250 mg L^{-1} [22] to 0.005-1 mg L^{-1} [26]. The linearity range and LOD obtained by the present rFIA system (0.5-20 mg L^{-1} and 0.3 mg L^{-1}, respectively) are right for the kind of samples analyzed, and they could also be applied to the quality control of the drinking water, since the maximum concentration admissible in this kind of samples is 0.5 ppm.[44] On the other hand, the use of a reverse FIA manifold allows to minimise the waste of reactive.

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