Available online at www.sciencedirect.com
SCIENCE DIRECT®

Talanta 68 (2005) 245–252

Talanta

www.elsevier.com/locate/talanta

Autoadaptative sequential injection system for nitrite determination in wastewaters

M. Baeza [a,b,*], J. Bartrolí [a], J. Alonso [a]

[a] *Sensors and Biosensors Group, Department of Chemistry, Facultat de Ciències, Edifici Cn, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain*
[b] *Escola Universitària Politècnica del Medi Ambient, Rbla. Pompeu Fabra 1, 08100 Mollet del Vallès, Spain*

Abstract

A novel autoadaptative sequential injection system for the analysis of nitrite is described. The automatic determination uses a direct spectrophotometric method, based on the Griess–Ilosvay reaction. In this method the absorbance of the purple azo dye formed is measured at 555 nm. In the sequential injection operation, the sample and the reagent are aspirated and mixed by reverse flow. The sequencing and overlapping of stacked (reagent) zones as well as selection of volumes have been studied in detail. The proposed analytical system is intelligent, simple and robust, allowing for nitrite determination in a double concentration range, by a simple and automatic programmable operation change. These two ranges are 0.0–3.0 and 0.0–20.0 ppm with detection limits being 0.048 and 0.4 ppm, respectively. Next surroundings have been developed allowing autocalibration and independent monitoring of nitrite concentration. The experimental set-up has been evaluated applying it to real samples analysis of very diverse concentration samples coming from a WWTP. The throughput of the method was 12 samples per hour.

Keywords: Autoadaptative analytical system; SIA; Intelligent system; Ion nitrite; Spectrophotometry; Schlieren effect

---

1. Introduction

The increasing demand on process monitoring has led to new analytical systems, which can work during long periods of time with little maintenance and low reagent consumption. Flow injection analysis (FIA) fulfils those requirements and its simplicity and robustness helped in its introduction into the design of monitoring systems [1]. Additional improvements can be obtained exploiting the autoadaptative potentialities of the continuous flow techniques that can automatically modify its operational parameters according with the sample requirements. However, conventional FIA analysers have been designed as closed and dedicated systems useful to work with very well defined sample compositions. When modifications in the system features are needed, they can only be introduced after considering physical changes of the system parameters. This limitation prevents its operation in unattended automatic conditions when high variability in analyte concentrations is expected. This disadvantage can be circumvented easily by the use of an alternative flow technique called sequential injection analysis (SIA), which confers versatility, robustness and high degree of autoadaptative abilities [2].

SIA techniques are based on sequential aspiration of small volumes of sample and reagents into a single channel followed by mixing during their transport to the detector. A simple manifold including a selection valve, a pump and a suitable detector can be adapted for many different chemical determinations. All the operations related to the liquid handling can be automated using an automatic burette as liquid driver and a selection valve. Forward and backward movement of the syringe piston produces the aspiration and impulsion processes while the length of the piston stroke regulates the volumes of the liquids. The sequence of sample/reagent zones used as well as additional unitary operations (e.g., injection, washing) was controlled with the aid of the selection valve [3].

---

[*] Corresponding author. Tel.: +34 93 5796791; fax: +34 93 5796785.
E-mail address: mbaeza@eupma.uab.es (M. Baeza).

0039-9140/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2005.07.038

The introduction of certain intelligence capabilities into analytical systems seems to be the next logical step for future developments. The implementation of this new concept requires the use of automatic self-configuring instrumentation, which is basically incompatible with the usual instrumentation employed in flow injection equipment design. SIA has permitted advantages in this way, but the need of specific control software is considered the main drawback for the use of this flow system technique. In 2002, Lenehan et al. only found, in a SIA review, three references worldwide where SIA control software coupled with data acquisition were developed [4].

In this paper, a sequential injection system is described showing the possibilities of the SIA technique in the design of intelligent autoadaptative monitors for analytical process control. The proposed system for determining nitrite is especially suitable for analysing real samples over the usual detection ranges; it can also operate reliably over long periods of time. A system for nitrite determination at a two concentration ranges are proposed as a demonstrator. In order to obtain the chemical signal corresponding to the sample and depending on its concentration found in a preliminary step, the system automatically adopts a suitable reagent to sample molar ratio. This is selected from two alternative systems designed and previously optimised for two working concentration ranges, without any change on the physical operational parameters previously optimised and only defined by the control software of the system. This system can be useful in a Waste Water Treatment Plant (WWTP) with nitrogen removal. Urban WWTPs that use nitrogen removal processes usually maintain nitrate levels below 30 ppm. Nitrite concentration rarely reaches 1 ppm when plants work properly. However, an excessive load of ammonium inhibits the biological oxidation of nitrite to nitrate so the former may build in the reactor at concentrations well above the usual levels [5]. The developed system can be used to monitoring this parameter in order to make the necessary control actions in the plant for its optimal operation.

The need of control of this parameter in urban wastewater plants [6] industrial flows [7] and natural waters [8–10] shows the importance of nitrite determination. The proposed method was applied to nitrite determinations of real samples proceeding from a pilot WWTP. Two different studies were done: kinetic inhibition of nitrification process with nitrite at very high concentrations (0–1500 ppm) in plant process, and monitoring of respirometric determinations, where degradation of ammonium into nitrite was achieved. In this second study concentration range was much lower (0–20 ppm).

2. Experimental

2.1. Apparatus

The manifold for sequential injection analysis used in nitrite determinations is shown in Fig. 1. It consists in a modular digital burette CAVRO XP 300 (Cavro, USA), equipped with a 2.5 ml syringe, 12.5 μl minimum piston stroke and a three-way valve head. By changing the direction of the piston stroke, liquids are either aspirated or expelled at a constant flow rate between 0.25 and 10 ml min^{-1}. The three-way valve allows load and discharge of the syringe without disturbing the content of the flow lines. The liquids are directed using a six-port valve CAVRO XL Series Smart Valve (SV) (Cavro, USA). The burette and the selection valve were connected to a personal computer via an RS-485 bus. The flow rate, flow direction, volume and selection valve are controlled by home-made software. All flow lines are made of PTFE tube (Tecator, Hoganes, Sweden). The dimensions of the finally selected holding and reaction coils (Fig. 1) are 310 mm × 0.5 mm i.d. and 36 mm × 0.5 mm i.d., respectively.

Spectrophotometric measurements were performed at 555 nm using a single-beam Pharmacia LKB Biochrom colorimeter (Cambridge, USA) equipped with a flow cell of 1 cm optical path length and 8 μl internal volume purchased form Hellma GmbH (Müllheim, Germany). PC communicates

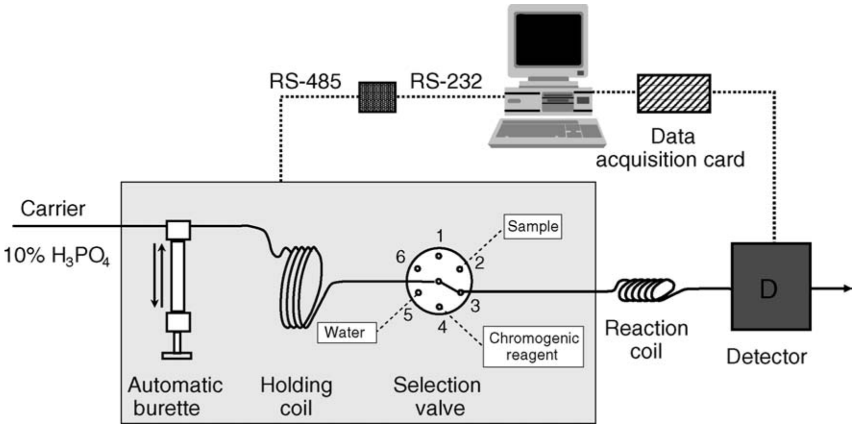


Fig. 1. Autoadaptative sequential injection analysis of nitrite manifold. Holding coil: 310 cm × 0.8 mm i.d. and reaction coil 36 cm × 0.5 mm i.d., syringe volume 2.5 ml (minimum stroke 12.5 μl) and flow rate 0.5 ml min^{-1}.

with the colorimeter through an ADDA-14 analogy to digital conversion card. The developed program was written in C language and was used in all manifold elements control.

2.2. Reagents and solutions

All solutions were prepared using deionised water from a Milli-Q system (Millipore, Billerica, MA, USA).

In preliminary studies, the dye solution was prepared by dilution of stock solution of phenol red (Panreac) 1.13×10^{-2} M in sodium hydroxide 0.1 M (Panreac, 98%). All dilutions were done in sodium hydroxide 0.1 M [11]. The standard Tris (hidroximetyl)aminomethane (Merck) and chlorhidric acid (Panreac, 37%) were prepared and standardized with the appropriate primary standard solution.

The standard nitrite solutions were prepared from a concentrated stock solution of 1 g/l of the sodium salt (Panreac, 98%). The chromogenic reagent was prepared by dissolving 10 g of sulfanilamide (Panreac, 99%) and 1 g of *N*-(1-naphtyl)ethylenediamine dihydrochloride (NED) (Panreac, 98%) in 100 ml of phosphoric acid (Panreac, 85%) in a final volume of 1000 ml in Milli-Q water [12].

2.3. Procedures

2.3.1. SIA software control procedure

The specific software, developed in C language, allows the total real time control of the process. The developed application takes into account: the automatic calibration of system in two ranges of concentration, data acquisition and automatic selection of the best sample/reagent ratio for each nitrite concentration using feedback control.

The homemade control software uses a bus RS-485 for data communication and a ADDA-14 card for data acquisition. Automatic burette management, selection valve and data acquisition are entirely controlled by different subroutines integrated in the main programme module. Other routines with specific actions included in the software are: set-up analysis conditioning, graphic monitoring, data acquisition, data treatment and cleaning cycles. In order to minimize electric noise a digital filter BOXCAR [13] has been implemented in the data treatment routine.

All possible actions of automatic burette and valve were defined in the program. Two files are used to configure the system. The first one with the specific analysis conditions, and the second one where the calibration sequence, analysis and cleaning cycles are defined. High versatility of the system is obtained, while possible operational sequences changes are easily achieved with only few modifications on the specific files.

The software is divided in three sections: system initialization, calibration and finally WWTP monitoring. The first sequence consists in automatic burette and selection valve initialisation and detector stabilization. In the second part the system performs automatic calibration in two different experimental conditions (low and high concentration ranges). This part ends with the adjustment and storage of the two corresponding calibration regression equations. Nitrite standards used in low range calibrations are: 0.0, 0.05, 1.0 and 2.0 ppm while standards used in high concentration range are: 0.0, 2.0, 10.0 and 20.0 ppm. Real sample monitoring stage starts with determination in low range conditions. If the response is higher than 1.5 AU (control valour) the analysis is repeated in high range conditions (0.0–20.0 ppm). Finally, using the corresponding calibration equation obtained previously, the program determines automatically nitrite concentration in the sample. Results are shown in the screen as well as recorder in a file. Sample analysis is repeated three times at the same conditions as the last analysis. If high range conditions are now used and absorbance value is smaller than 0.3 AU (control valour), the sample analysis is repeated at low range conditions (0.0–3.0 ppm).

2.3.2. Nitrite determination

Nitrite ion analysis was based on Griess–Ilosvay reaction [14]. Appropriate sample volume was mixed with sulphanilamide and NED to obtain, through diazotization and coupling reactions, a highly coloured compound that was detected spectrophotometrically as explained before.

Sample and reagent volumes, dispersion and mixing of the aspirated zones and sequence in aspiration are key parameters that have been optimised and that will be discussed later. A “sandwich” optimal sequential aspiration in two complementary concentration ranges was adopted. The total time for each analysis cycle is 5 min. A complete measuring cycle is composed by the following sequence:

1. aspiration of 25 or 12.5 μl of chromogenic reagent (sulphanilamide + NED + H_3PO_4) into holding coil (port 4);
2. aspiration of 150 or 25 μl of sample (port 2) into holding coil;
3. aspiration of 25 or 12.5 μl of chromogenic reagent (sulphanilamide + NED + H_3PO_4) into holding coil (port 4);
4. aspiration of 12.5 μl of MilliQ water (port 5) into holding coil;
5. injection of the content of holding coil into the reaction coil through port 3;
6. impulsion to into the detector using 10% H_3PO_4 solution;
7. absorbance detection at 555 nm;
8. new determination or system cleaning.

3. Results and discussion

3.1. Precision and accuracy of experimental set-up

Initially, precision and accuracy of experimental set-up was evaluated. Experimental precision was considered as the reproducibility of the aspiration/injection processes. This was studied by successive aspirations and injections of a dye (phenol red, 1.13×10^{-2} M) representing the sample and reagent

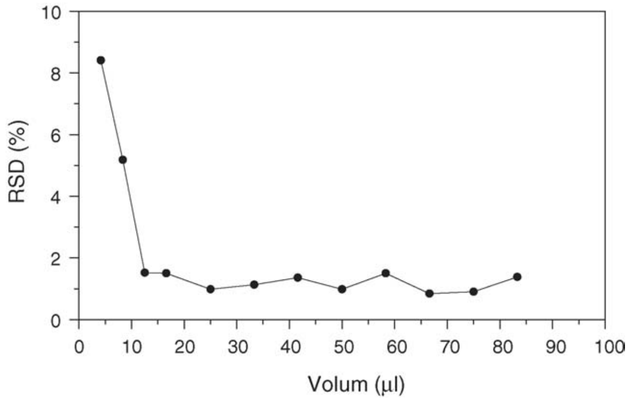


Fig. 2. Repeatability in aspiration/impulsion process. Flow rate: 4.40 ml min^{-1}.

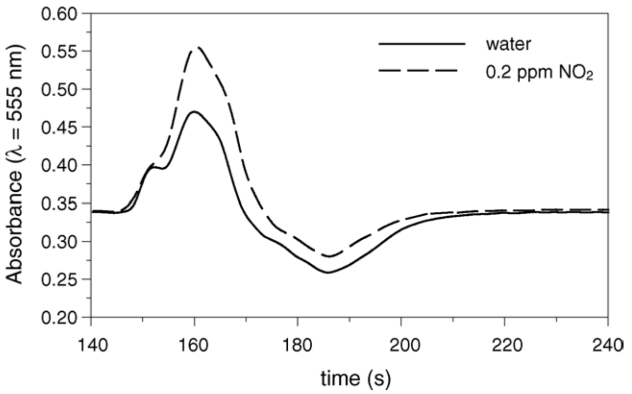


Fig. 3. Schlieren effect on system response for a sequence reagent/sample/reagent (25/125/25 µl) using 10% H_3PO_4 as a carrier solution.

zones. Consecutive aspiration and injections of variable volumes between 4.16 and 83.3 µl were performed. Flow rate was 4.40 ml min^{-1}. Repeatability was calculated as relative standard deviation (R.S.D. (%)) for 20 replicates of each studied volume. Lowest repetitive volume found was 12.5 µl (R.S.D. (%) = 1.52) as shown in Fig. 2. Relative standard deviation for higher volumes was found to be lower than 1.5%, confirming the suitability and precision of the automatic burette for sequential injection operations.

Volume aspiration accuracy was tested for the two first volumes with lower R.S.D.: 12.5 and 16.6 µl. Volumes of a 0.1 M Tris solution previously aspirated and dispensed with the developed system was titrated using a standardized 0.0125 M HCl solution. Obtained results were 12.11 ± 0.03 and 16.22 ± 0.04 µl, with 0.34 and 0.31% R.S.D. for $n = 10$, respectively. Piston speed was also considered as an important parameter in volume aspiration precision. Titrations of different volumes (12.5 and 16.6 µl) were performed as done before at different aspiration flow rates (F_1: 1.78 µl/s and F_2: 4.40 µl/s). For a theoretical volume of 12.5 µl obtained results were: F_1: 12.20 ± 0.05 µl and F_2: 12.207 ± 0.014 µl, and for the second volume (16.6 µl) results were: F_1: 16.23 ± 0.06 µl and F_2: 16.33 ± 0.13 µl. Two factors variance analysis was performed and no significance differences were observed as $F_{\text{cal}} = 1.324 < F_{\text{tab}} = 161.45$.

3.2. *System optimization (optimal reagent/sample volume ratio)*

Optimisation of reagent/sample volume ratio in the reagent/sample/reagent sequence was performed in order to obtaining a robust, versatile and intelligent system, suitable for nitrite ion determination in two concentration ranges. System autoadaptation or intelligence concept can be considered as the possibility of automatic modification of the reagent/sample volume ratio in the mentioned sequence.

Preliminary tests were carried out with different flow assemblies in order to establish the influence of the system variables on the final sample dispersion. To optimise the system, volumes in direct configuration sequence (water/reagent/sample/reagent/water) were studied. Simultaneous increase in sample (25, 50 and 75 µl) and reagent (100, 200 and 300 µl) aspirated volumes and final carrier (water, 12.5 µl) volumes were evaluated. In all cases, anomalous signals due to Schlieren effects [15] were obtained. Differences in refraction index of reagent, water and sample solutions were responsible for these phenomena. This effect is the main cause of parasite absorbencies, higher than those associated to reaction product formation (azo dye).

In order to minimize this effect, water was substituted for 10% H_3PO_4 as carrier solution and sample and reagent volumes were decreased. As shown in Fig. 3, complete elimination of the Schlieren effect is not possible. To avoid this problem, blank subtraction from sample signal was always performed. Additional studies were done in order to establish the optimal aspiration sequence characteristics which allow defining two operational working ranges.

The relations volumes (reagent/sample/reagent in microliters) evaluated were: 25/150/25, 50/150/50, 25/50/25 and 12.5/25/12.5. Nitrite standards compressed between 0 and 10 ppm were used. The first two relations were studied in order to evaluate the effect of increasing the volume of reagent when analyte concentration is low. The last two combinations tried to evaluate the response at higher concentrations (0–10 ppm). In all cases three replicates were performed automatically. An associated error was estimated as $I_{n-1}^{95\%}$. Fig. 4(a) shows the obtained response for the first relation (25/150/25) while in Fig. 4(b) calibration curve correlation is shown at the same scale.

Table 1 shows correlation adjustments in all performed calibrations. It can be observed that when reagent volume was increased while sample volume remained constant (second studied relation 50/150/50) response was not better ($r = 0.995$) and no significant sensibility changes were obtained (0.52 ± 0.22 AU ppm^{-1}).

Reduction in sample volume (relation 25/50/25) resulted in lineal range amplification up to 5 ppm and refraction index effect disappeared when concentration was over 1 ppm, with good correlation ($r = 0.995$) but with less sensibility (0.18 ± 0.02 AU ppm^{-1}) compared to higher volumes.

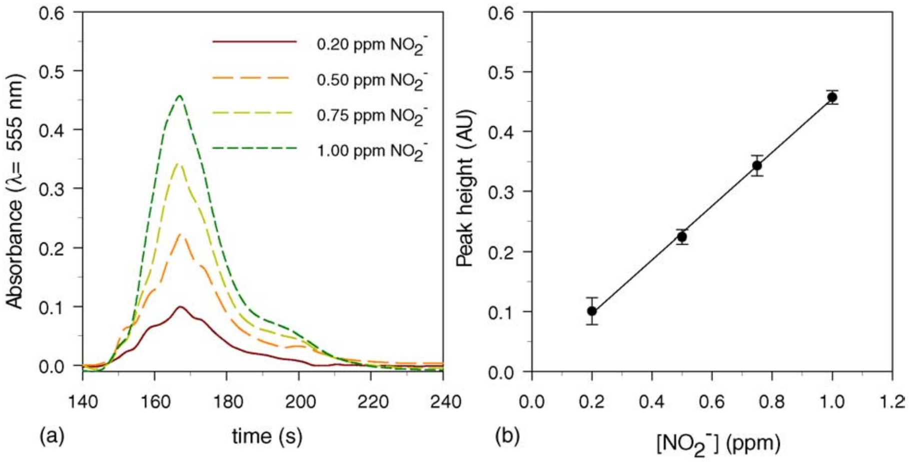


Fig. 4. Obtained responses for a 25/150/25 μl relation: (a) obtained peaks with subtraction of blank solution and (b) calibration regression.

With the aim of getting wider lineal concentration ranges sample and reagent ratios were maintained at 1/2/1. Linear range was upgraded to 10 ppm with lower sensibility (0.079 ± 0.010 AU ppm^{-1}). No refraction index effect was observed when sample concentration was over 0.5 ppm. When sample and reagent volumes were reduced, dispersion increased and thus difference in refraction index at the interfaces was decreased. Under these conditions obtained response is only due to the formed azo dye.

Finally, the selected aspiration sequence was reagent/sample/reagent using a 10% H_3PO_4 as carrier. The optimal ratios were 25/150/25 and 12.5/25/12.5 μl for low range (0.0–3.0 ppm) and high range (0.0–20.0 ppm), respectively. In both conditions it was possible to increase the upper response limit. Fig. 5 shows the obtained responses in the two concentration ranges. Corresponding found calibration curves were: $H\,(\mathrm{AU}) = 0.03 \pm 0.01 + 0.474 \pm 0.009\ [\mathrm{NO_2}^-]$ (ppm) with $n = 8$, and $H\,(\mathrm{AU}) = 0.021 \pm 0.017 + 0.102 \pm 0.006\ [\mathrm{NO_2}^-]\ (\mathrm{ppm}) - 0.0014 \pm 0.0003\ [\mathrm{NO_2}^-]^2\ (\mathrm{ppm}^2)$ with $n = 7$. Associated errors have been calculated as $t_{(n-2)}s$ and $s_{n-1}/n^{1/2}$, respectively.

3.3. System characterisation

Once aspiration sequence and sample/reagent volumes were defined, the quality of the developed method was evaluated in terms of the detection limit, repeatability and reproducibility. The most important features of both system configurations analysis are shown in Table 2.

3.3.1. Detection limits and repeatability

Due to the existence of Schlieren effect, detection limit has been calculated following the strictest criteria proposed by Calcutt and Boddy [16]. Response obtained for a blank solution (MilliQ water) in the different experimental conditions (ratio: sample/reagent) was used for the determination of detection limit. The detection limit was estimated from the standard deviation of 10 pairs of targets

Table 2
Principal parameters of quality of the autoadaptative SIA for the determination of nitrite

Parameter	Low range	High range
Sensitivity (AU ppm^{-1})	0.474 ± 0.009	0.102 ± 0.006
Zone operation (ppm)	0.0–3.0	0.0–20.0
Detection limit (ppm)	0.048	0.4
Optimal ratio reagent/sample/reagent (μl)	25/150/25	12.5/25/12.5
Sample consumption (μl)	150	25
Reagent consumption (μl)	50	25
Repeatability (R.S.D. (%), $n = 10$)	0.88	1.54
Reproducibility (one-way ANOVA)	$F_{\mathrm{cal}} = 0.32 < F_{\mathrm{tab}} = 9.55$ ($n = 6$)	$F_{\mathrm{cal}} = 0.08 < F_{\mathrm{tab}} = 10.13$ ($n = 5$)
Sample throughput (h^{-1})	12	12

Repeatability has been estimated for a standard of 0.75 and 2.5 ppm, respectively.

Table 1
Obtained results in reagent/sample/reagent sequence optimization

Relation volumes (μl) reagent/sample/reagent	Carrier	$H\,(\mathrm{AU}) = a\ (\pm t_{(n-2)}s_a) + b(\pm t_{(n-2)}s_b)\ [\mathrm{NO_2}^-]$ (ppm)	r
25/150/25	10% H_3PO_4	$H = 0.01(\pm 0.03) + 0.45(\pm 0.04)\ [\mathrm{NO_2}^-]$ ($n = 4$)	0.9997
25/150/25	Water	$H = 0.02(\pm 0.08) + 0.48(\pm 0.12)\ [\mathrm{NO_2}^-]$ ($n = 4$)	0.9983
50/150/50	10% H_3PO_4	$H = -0.04(\pm 0.04) + 0.52(\pm 0.22)\ [\mathrm{NO_2}^-]$ ($n = 4$)	0.9950
25/50/25	10% H_3PO_4	$H = 0.03\ (\pm 0.07) + 0.18(\pm 0.02)\ [\mathrm{NO_2}^-]$ ($n = 5$)	0.9950
12.5/25/12.5	10% H_3PO_4	$H = 0.01\ (\pm 0.05) + 0.079\ (\pm 0.010)\ [\mathrm{NO_2}^-]$ ($n = 5$)	0.9993

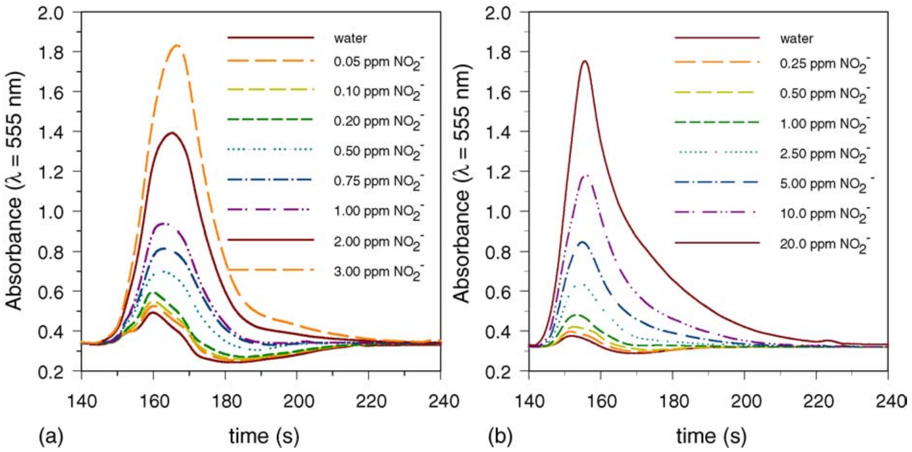


Fig. 5. Obtained response for low and high ranges: (a) 0.0–3.0 ppm and (b) 0.0–20.0 ppm.

made in different days:

$$s_b = \sqrt{\frac{\sum (\text{dif})^2}{n}}$$

The detection criterion (DC) is defined as the minimum detectable signal, because the standard deviation of a nitrite solution can be higher that blanks.

$$\text{DC} = s_b \times 2 + 0.2 \times s_b$$

Replace this value into the correlated equation permits obtain the minimum detectable concentration. The minimum detectable concentration was confirmed with 10 analyses of this standard solution concentration and afterwards the calculation of the minimum detectable signal was obtained by $S_m = \text{DC} + t_s \times s_s$ using the s_s obtained from the 10 analysis of the standard nitrite solution. In all cases the absorbance value of the ion solution was calculated subtracting targets values. S_m value indicates detection limit using the calibration curve. The minimum detectable nitrite ion concentration was 0.048 ppm for low range and 0.4 ppm for high range.

Repeatability was considered as the relative standard deviation for 10 replicates of nitrite solution with an intermediate concentration used in the calibration curves in each of the defined ranges. These concentrations were 0.75 and 2.5 ppm of nitrite for low and higher ranges respectively. For each concentration R.S.D. (%) was calculated by averaging the absorbance of 10 measurements. Obtained values were 0.88 and 1.54%, respectively (Table 2). Results were similar than those obtained in the determination of the repeatability of the aspiration/impulsion procedure without chemical reaction. The obtained repeatability was considered adequate since the R.S.D. value was not higher than 1.5% in the two concentrations used.

3.3.2. Reproducibility

Reproducibility of this method was determined by comparison of slopes of the calibration curves performed on different days for $n = 6$ (low range) and $n = 5$ (high range). Variance analysis of an ANOVA factor was used. It was found that there was no significant differences between calibration curves with a 95% confidence since $F_{\text{calculated}} < F_{\text{tabulated}}$ (Table 2).

3.4. System validation

Developed system was validated with synthetic and real samples determination. After proper calibration efficiency of the system was evaluated with the feedback control program. Two synthetic samples of 2.5 and 5.0 ppm nitrite were analysed. Fig. 6 shows the obtained response for these two samples.

First analysis was performed at low concentration range (0.0–0.3 ppm) conditions. When absorbance showed higher values than 1.5 AU, nitrite concentration in sample was over 3.0 ppm, and thus, analysis is repeated automatically in high range conditions (0.0–20.0 ppm). If absorbance showed lower values than 0.3 AU analysis is repeated at low concentration range conditions.

Finally, autoadaptative system has been validated with real samples proceeding from a pilot plant WWTP where bio-

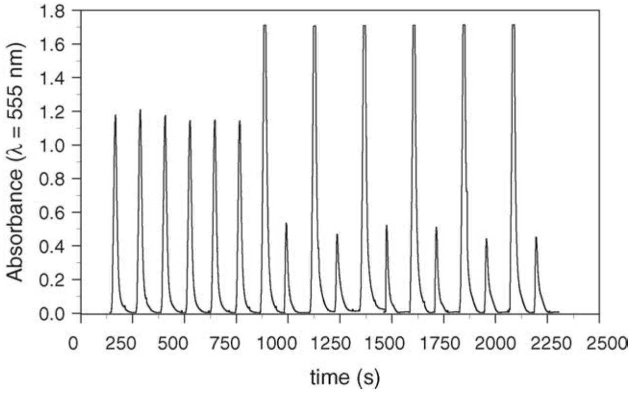


Fig. 6. Obtained response for standard concentration of 2.5 and 5.0 ppm. When the concentration is greater than 3.0 ppm, the analysis is repeated in high range conditions (small peaks).

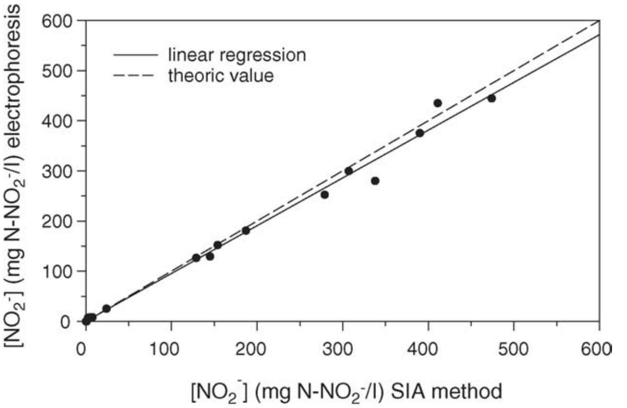
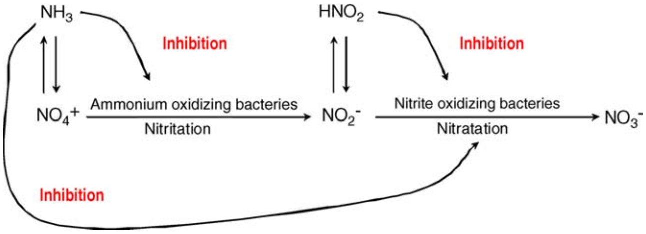


Fig. 7. Comparison of the obtained results by SIA vs. capillary electrophoresis (1 ppm NO_2^- = 0.3043 ppm $N–NO_2^-$).

logic nitrification process was under study [17,18]. Nitrite ion is an intermediate product of this process and thus it is common to observe inhibition in wastewater substrates with high ammonium concentration. Both the ammonium oxidizing and Nitrite oxidizing bacteria are inhibited for their own substrates: ammonium and nitrite. As shown in the following reaction:



Inhibition concentrations are dependent to multiple factors: nitrifying biomass conditioning to NH_3 and HNO_2, temperature, and nitrifying microorganisms concentration.

Analysed samples proceeded from a WWTP pilot plant with high charge, where kinetic studies were under development. All samples were taken directly from the pilot plant and after appropriate filtering (45 μm Millipore) two sample series were analysed.

The first set of samples corresponds to an inhibition nitrite study in biological nitrogen removal of high-strength ammonium industrial waste water [17]. A total of 17 samples with high nitrite concentration (0–1500 ppm) were analysed. Sample dilution was performed when necessary. Obtained results with the developed method were compared with those obtained by capillary electrophoresis. Statistical paired *t*-test and direct regression analysis were used throughout. No significant differences were obtained at 95% confidence level. In paired *t*-test: $t_{cal} = 0.17 < t_{tab} = 2.12$, while in lineal regression a slope of 0.95 ± 0.05 and 0.24 ± 13.7 intercept was obtained (Fig. 7). Correlation coefficient was $r = 0.995$.

Fig. 8 shows the obtained results in lower nitrite concentration samples proceeding from another kinetic study. This experiment use respirometric techniques coupled to the system model to monitor the nitrite concentration of the nitrification process in urban wastewater biological treatment plants [18].

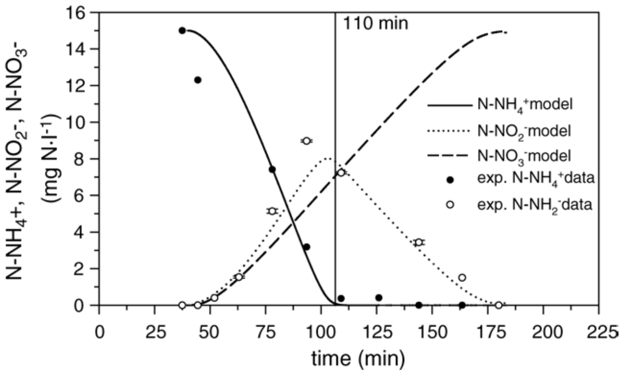


Fig. 8. Real samples of respirometric estimation in WWTP.

In this case no dilution was necessary. Validation of the obtained values was compared with those obtained with the model from the oxygen uptake rate (OUR) [19]. Good adjust is observed as long as predicted values are equal to those obtained by the SIA system. $N–NH_4^+$ analysis were performed through the standard method [12]. $N–NO_2^-$ analyses were performed through the developed method, knowing that 1.0 NO_2 ppm corresponds to 0.3043 $N–NO_2^-$ ppm. No interference effect from sample matrix was observed during both real applications.

4. Conclusions

With all the presented results in the present work, a SIA procedure for the determination of nitrite at two concentration ranges has been developed and validated. The implemented system shows the autoadaptative potent abilities of this kind of systems. Compared with FIA technique, the power of SIA technique is basically instrumental, so that easy automatic modification of its physical operations parameters in order to fit to the sample composition at every moment. This advantage allows the design of intelligent systems, which means that the system allows the sample analysis to be performed in the optimum experimental conditions, depending on the sample composition and on the management software working-out.

Additionally, this technique reduces sample and reagents volumes, which is an important benefit in continuous analysis as it means less maintenance and less waste disposal. This fact favours its operation in unattended conditions during extended periods of time. Using specific instrumentation, this kind of systems can be easily miniaturised.

As can be derived, the intelligent systems design for online monitoring is one of the most promising future applications of SIA techniques. Obtained results in real samples have permitted validation and efficiency demonstration of the developed system.

Acknowledgements

This work was carried out with the financial support given by Ministerio de Ciencia y Tecnología (CICYT; Madrid, SPAIN), through MAT94-0668-C03-03 and MAT97-0720-C03-02 projects. M. Baeza also thanks for the PhD grant of CICYT.

References

[1] P.J. Baxter, G.D. Christian, Acc. Chem. Res. 29 (1996) 515–521.

[2] J. Ruzicka, J.D. Marshall, Anal. Chim. Acta 237 (1990) 329–343.

[3] M.T. Oms, A. Cerda, V. Cerda, Anal. Chim. Acta 315 (1995) 321–330.

[4] C.H. Lenehan, N.W. Barnett, S.W. Lewis, Analyst 127 (2002) 997–1020.

[5] D. Gabriel, J.A. Baeza, F. Valero, J. Lafuente, Anal. Chim. Acta 359 (1998) 173–183.

[6] A. Cerdà, M.T. Oms, R. Forteza, V.V. Cerdà, Anal. Chim. Acta 351 (1997) 273–279.

[7] J.F. van Staden, T.A. van der Merwe, Mikrochim. Acta 129 (1998) 33–39.

[8] S. Motomizu, M. Oshima, L. Ma, Anal. Sci. 13 (1997) 401–404.

[9] A. Cerdà, M.T. Oms, R. Forteza, V. Cerdà, Anal. Chim. Acta 371 (1998) 63–71.

[10] M. Miró, A. Cladera, J.M. Estela, V. Cerdà, Analyst 125 (2000) 943–948.

[11] F. Welcher, Chemical Solutions, D.Van Nostrand Company, Inc., New Jersey, 1966.

[12] APHA-AWWA-WEF, Standard Methods for the Examination of Water and Wastewater, 20th ed., American Public Health Association, American Water Works Association, Water Environment Federation, Washington DC, 1999.

[13] D. Binkley, R. Dessy, J. Chem. Educ. 56 (1979) 148.

[14] L.N. Sawyer, P.L. McCarty, G.F. Parfin, Chemistry for Environmental Engineering, Mc Graw Hill, Maidenhead, UK, 1994.

[15] E.A.G. Zagatto, M.A.Z. Arruda, A.O. Jacintho, I.L. Mattos, Anal. Chim. Acta 234 (1990) 153–160.

[16] R. Caulcutt, R. Boddy, Statistics for Analytical Chemists, Chapman and Hall, London, 1983.

[17] J. Carrera, I. Jubany, N. Carvallo, R. Chamy, J. Lafuente, Process Biochem. 39 (9) (2004) 1159–1165.

[18] A. Guisasola, J.A. Baeza, J. Carrera, C. Casas, J. Lafuente, Water Sci. Technol. 48 (11/12) (2003) 267–275.

[19] A. Guisasola, I. Jubany, J.A. Baeza, J. Carrera, J. Lafuente, J. Chem. Technol. Biotechnol. 80 (4) (2005) 388–396.