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Multi-pumping flow system for the determination of nitrite and nitrate in water samples

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Abstract. A multi-pumping flow system for the spectrophotometric determination of nitrite and nitrate is described. The determination of nitrite is based on the Griess-Ilosvay reaction. Nitrate can be determined after its on-line reduction to nitrite using hydrazine sulphate in alkaline medium. Calibration was linear up to 3 mg NO_2^- L^{-1}, with a limit of detection ($3s_b/S$) of 0.013 mg NO_2^- L^{-1}, an injection throughput of 55 injections h^{-1} and a repeatability (RSD) of 0.5% for the direct determination of nitrite. Two calibration graphs within the ranges 0.039–7 mg NO_3^- L^{-1} and 0.026–5 mg NO_2^- L^{-1} were run for the determination of nitrate and nitrite under reducing conditions, respectively. A limit of detection of 0.039 mg NO_3^- L^{-1} was obtained. An injection throughput of 27 injections h^{-1} and an RSD lower than 1.5% were achieved. The method was successfully applied to the determination of nitrite and nitrate in water samples.

Keywords: Multi-pumping flow system (MPFS); nitrite; nitrate; on-line reduction; hydrazine sulphate

Nitrogen is an essential nutrient for the growth of plants and animals. Nitrate and nitrite are the most common forms of this element to be found in aquatic systems. The determination of nitrite and nitrate is of great interest in order to understand their biogeochemistry in aquatic systems and their role on the process of eutrophication. In this sense, high levels of nitrite and nitrate may be attributed to the effect of human activities such as agriculture [1–3].

On the other hand, flow techniques have proved to be suitable tools for the in-situ determination of nutrient species in natural waters since these techniques are fast, flexible, rugged and portable [2]. Most reported flow procedures for the determination of nitrite and nitrate are based on classical flow techniques (i.e. flow injection analysis (FIA) and sequential injection analysis (SIA)). In these approaches, nitrite determination is usually based on the Griess-Ilosvay reaction [1, 2]. Nitrate determination commonly involves reduction of nitrate to nitrite followed by spectrophotometric detection of nitrite formed. Nitrate reduction is usually carried out using copperized cadmium. However, the shortcomings of this heterogeneous reduction method are noteworthy (i.e. nitrite can be reduced to hydroxylamine and ammonia, loss of reduction efficiency with time, ...) [4]. An alternative to the heterogeneous method is the homogenous reduction using hydrazine and copper ions as reducing agent and catalyst, respectively. One of the main advantages of this approach is that the phosphate inter-

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ference is avoided with regard to the heterogeneous method [5].

In the last ten years, new flow techniques such as multicommutated flow injection analysis (MCFIA) [6], multi-syringe flow injection analysis (MSFIA) [7] and multi-pumping flow systems (MPFS) [8] have been developed. These multicommutated flow techniques can be regarded as a possible alternative to classical flow techniques since they combine the advantages given by FIA such a high injection throughput with those given by SIA such as a minimal reagent consumption and waste generation. Moreover, the possibility to develop fully automated systems using multicommutated techniques has also been demonstrated [9]. Several MCFIA environmentally friendly methods have been proposed for the determination of nitrite and/or nitrate [10–12]. By contrast, no applications are found for the MPFS determination of nitrite and nitrate, as it is still a young technique. MPFS was first described by Lapa et al. and it is based on using solenoid micro-pumps as liquid propelling units. Micro-pumps, that are ideal for dispensing high-purity or aggressive fluids, are responsible for sample-reagent introduction and for manifold commutation. Moreover, the unique features of MPFS (i.e. simplicity, versatility, low cost…) make this technique very appropriate for the on-line monitoring of nutrients [8, 13].

In the present paper, a novel MPFS system for the spectrophotometric determination of nitrite and nitrate in water samples has been developed. Nitrite determination is performed using the Griess-Ilosvay reagent. Nitrate is determined after its on-line reduction using hydrazine. The results obtained have been compared with those found in several FIA and SIA assemblies.

Experimental

Reagents

All chemicals were of analytical reagent grade. For preparation of all solutions Millipore-purified water was used.

Stock standard solutions of nitrite (1000 mg NO_2^- L^{-1}) and nitrate (1000 mg NO_3^- L^{-1}) were prepared from sodium nitrite (Sigma) and sodium nitrate (Sigma), respectively. Working standards were prepared by diluting the stock solutions.

The chromogenic reagent was prepared by dissolving sulfanilamide (20 g L^{-1}) (Sigma) and N-(1-naphthyl)ethylenediamine dihydrochloride (0.5 g L^{-1}) (Merck) in 1.0 mol L^{-1} hydrochloric acid (Panreac).

The reducing agent was prepared daily by dissolving hydrazine sulphate (Sigma) and cupric sulphate pentahydrate (Sigma) in 0.5 mol L^{-1} sodium hydroxide (Panreac). The final concentration of hydrazine sulphate and $CuSO_4$ in the reducing agent was 2 g L^{-1} and 0.02 g L^{-1}, respectively.

Instrumentation and flow manifold

The MPFS system (Fig. 1) consists of four solenoid micro-pumps (M1–M4) (Bio-Chem Valve Inc., Boonton, New Jersey, USA) (www.bio-chemvalve.com/biochem-valve.html) and a solenoid valve (V1) (Takasago Electric Inc, Nagoya, Japan) (www.takasago-elec.co.jp/cgi-bin/en/index03.html).

Solenoid devices are computer controlled through an interface I/O digital card that has eight digital relay input channels and eight digital relay output channels. A maximum of eight solenoid pumps and/or valves can be used. These solenoid devices are set on a mother board connected to the relay outputs. A power source of 12 V is required to activate the solenoid devices. The power source, the I/O card and the input and output channels have been integrated into a module (Sciware, Palma de Mallorca, Spain) (www.sciware-sl.com) [9, 14]. In order to prolong the lifetime of the solenoid valve, a solenoid protection system (Sciware) is required.

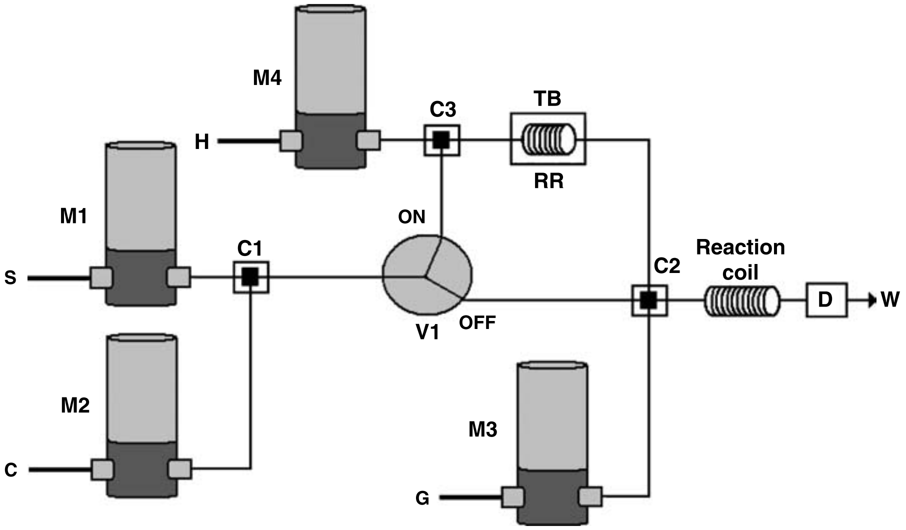

Fig. 1. Experimental MPFS set-up proposed for the determination of nitrite and nitrate. *S* Sample; *C* carrier; *G* chromogenic reagent (modified Griess reagent); *H* reducing agent; *W* waste; *D* detector; *RR* reduction reactor; *M1–M4* micro-pumps *V1* commutation valve; *C1–C3* cross junctions; *TB* thermostatic bath

Table 1. Results of the re-calibrations of the micro-pumps

Micro-pump	Mean stroke volume (μL)	*n*	RSD (%)
M1	20.9	9	2.4
M2	29.8	9	2.2
M3	28.8	7	3.3
M4	28.9	8	2.9

RSD Relative standard deviation; *n* number of re-calibrations.
Re-calibrations were performed once a week as it is described elsewhere [14].

The indicated stroke volume for all the micro-pumps is of 25 μL (Ref. 120SP12-25) except for M1 which is of 20 μL (Ref. 120SP12-20). The stroke volume should be re-calculated as has been reported previously [14, 15]. Table 1 shows the results of the re-calibration of the micro-pumps. As can be observed, the dispensing precision of the micro-pumps used is good (i.e. $\leq$3.3%). In this set-up, micro-pumps of 20 and 25 μL were selected as a compromise between injection throughput, sensitivity and dispensing precision.

For instrumental control, data acquisition and processing, Auto-Analysis software (Sciware) was used [16].

A USB 2000 miniature fiber optic spectrometer (Ocean Optics, Dunedin, FL, USA) (www.oceanoptics.com) equipped with an 18 μL inner volume (1 cm optical path; Hellma, Müllheim, Germany) was used as detector. A halogen lamp with a power source of adjustable intensity (Sciware) was selected as light source. Dual-wavelength detection (540, 700 nm) was used to overcome Schlieren effect.

In order to keep constant the temperature of the reduction reactor, a thermostatic bath was incorporated into the MPFS assembly.

Manifold was constructed with 1.5 mm i.d. (used for connecting the reagents with the inlet of the micro-pumps) and 0.8 mm i.d. (used for the rest of the system) poly(tetrafluoroethylene) (PTFE) tubing.

Procedure

In the system four solenoid micro-pumps (M1–M4) and a solenoid valve (V1) were required. These micro-pumps (M1–M4) were responsible for the insertion of the sample, the carrier, the chromogenic reagent and the reducing agent, respectively.

Two procedures were proposed for the determination of nitrite and nitrate. For the direct determination of nitrite, the sample is inserted into the system by means of micro-pump M1. Afterwards, the sample merges with the chromogenic reagent stream (M3 is switched on) in the reaction coil. The resultant flow is propelled to the detector (M2 is activated).

The procedure used for the determination of nitrate involves a reduction step to convert nitrate to nitrite. Valve V1 should be in the On position in order to direct the flow to the reduction reactor, which is immersed in a thermostatic bath. Thus, the sample is directed to the reduction reactor (M1 is activated) where it is mixed with the reducing agent (M4 is switched on). Then, the flow is stopped. After 40 sec, it is propelled to the reaction coil (M2 is switched on) and it is mixed with the modified Griess reagent (M3 is activated). Finally, the formed product is delivered to the spectrophotometer.

Sample conditioning

Using the proposed MPFS system, a certified sample [MOOS-1 (seawater)] (National Research Council of Canada) has been analysed. Several ground water samples from Mallorca were also analysed. These samples were filtered through a membrane filter of 0.45 μm pore size prior to analysis.

Results and discussion

Optimisation of experimental parameters

Various physical and chemical parameters were optimised following the univariate method.

Different sample volumes within the range 0.1–0.5 mL were tried in order to investigate their effect on analytical signal. The peak height absorbance increased with increasing volume. Nevertheless, it was noticed that the effect of sample volume was not significant above 0.3 mL. Therefore, a sample volume of 0.3 mL was chosen for subsequent experiments.

Chromogenic reagent flow-rates within the range 0.5–5 mL min^{-1} were tested with the aim of decreasing the dilution of the sample. In order to insert the same amount of chromogenic reagent, its concentration was varied proportionally to flow-rate. The analytical signal decreased as the flow rate was increased. A flow-rate of 2.5 mL min^{-1} was selected as lower flow-rates affected negatively the base-line.

The influence of the reaction coil length was examined over the range of 25–300 cm using coiled and knotted reactors. For this experiment, 0.3 mL of a 2 mg L^{-1} nitrite standard were inserted into the system. It was observed that the analytical signal increased by increasing the length of the reaction coil. However, if the reaction coil is longer than 150 cm it has little effect on the sensitivity and, moreover, the injection throughput is decreased. On the other hand, knotted reactors provide better analytical signals than the coiled ones. This fact is in good agreement with literature data [17]. Therefore, a 150 cm length knotted reactor was selected for further experiments.

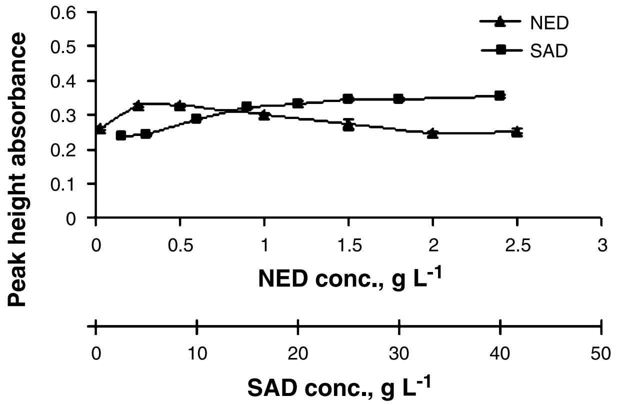

Fig. 2. Effect of the concentration of sulfanilamide (*SAD*) and N-(1-naphthyl)ethylenediamine dihydrochloride (*NED*) in the chromogenic reagent. For this experiment, 0.3 mL of a 2 mg L^{-1} nitrite standard were inserted into the system

The effect of the concentration of sulfanilamide (SAD) and N-(1-naphthyl)ethylenediamine dihydrochloride (NED) in the chromogenic reagent was investigated within the ranges 2.5–40 and 0.025–2.5 g L^{-1}, respectively. As can be seen in Fig. 2, peak height absorbance increased with increasing SAD concentration up to 20 g L^{-1}, above which it remained constant. Thus, a concentration of 20 g L^{-1} was chosen for subsequent experiments since it was found that higher SAD concentration affected the base line. Figure 2 also shows that an optimum analytical signal is achieved at a NED concentration of 0.5 g L^{-1}. Above this value, the sensitivity decreased. The chromogenic agent was prepared in hydrochloric acid since the Griess-Ilosvay reaction takes place in acidic medium. Therefore, hydrochloric concentration was optimized in the range 0.25–1.5 mol L^{-1}. At these conditions, sensitivity and repeatability were not affected by hydrochloric concentration. A concentration of 1 mol L^{-1} was used in order to ensure acidic conditions when the determination of nitrate was required.

The effect of the parameters involved in the reduction reaction was also studied with the aim of obtaining the maximum conversion rate of nitrate to nitrite. For this purpose, 0.3 mL of a 2.5 mg L^{-1} nitrate standard were inserted. The signal obtained was compared to that of a 2.5 mg L^{-1} nitrite standard under the same conditions.

The concentrations of hydrazine sulphate and sodium hydroxide in the reducing agent were optimised in the ranges 0–5 g L^{-1} and 0–1 mol L^{-1}, respectively (Fig. 3). The peak height absorbance of nitrate increased with increasing hydrazine sulphate concentration up to 1 g L^{-1}. Above this value, the sensitivity slightly decreased. It was found that the analytical signal of nitrite decreased with increase of hydrazine sulphate concentration. In this sense, the decrease observed in the formation of the diazo dye may be attributed to the decomposition of nitrite by the reducing agent or to the interference of hydrazine in the Griess-Ilosvay reaction [5]. A hydrazine sulphate concentration of 2 g L^{-1} was selected for further studies as it provided the maximum reduction efficiency. By contrast, the concentration of sodium hydroxide has little effect on the analytical signal of nitrite. The analytical signal of nitrate increased as the sodium hydroxide concentration was increased up to 0.5 mol L^{-1}. Thus, a sodium hydroxide concentration of 0.5 mol L^{-1} was chosen as optimum since it gave the best sensitivity and reduction efficiency.

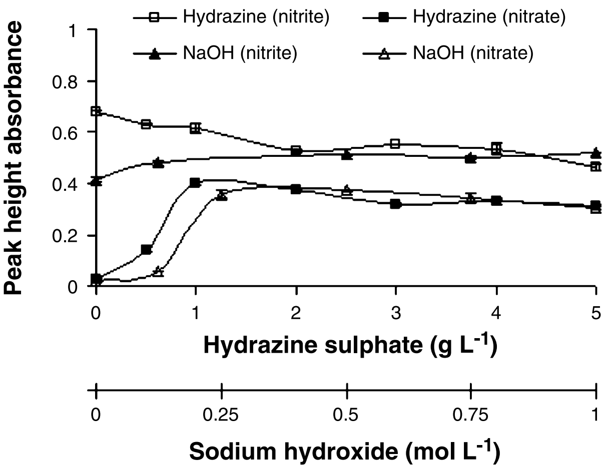

Fig. 3. Effect of the concentration of hydrazine sulphate and sodium hydroxide in the reducing agent

Copper sulphate was used as catalyst. Different copper sulphate concentrations in the reducing agent within the range 0–40 mg L^{-1} were tried. The best sensitivity and reduction efficiency were attained in the range 20–30 mg L^{-1}. As the best results concerning repeatability were achieved with a copper sulphate concentration of 20 mg L^{-1}, this value was kept for further experiments.

Reduction times within the range 0–70 sec were assayed in order to investigate their effect on signal response. Peak height absorbance of nitrate was constant in the range 30–50 sec and decreased at reduction times below and above these values. The optimum reduction time was chosen as 40 sec since it gave the best reduction efficiency together with a good repeatability.

The influence of the temperature of the reduction reactor was evaluated from 25 to 55 °C. The maximum peak height of nitrate was achieved in the range 40–50 °C. A temperature of 40 °C was selected as a compromise between reduction efficiency and repeatability.

Analytical parameters

Direct determination of nitrite

The calibration graph for the direct determination of nitrite was run in the concentration range of 0.013–3 mg L^{-1}, and conformed to the equation peak height absorbance = 0.343[NO_2^- (mg L^{-1})] + 0.0074; $r = 0.9997$; $n = 9$). The limit of detection (LOD), calculated as $3s_b/S$, where s_b is the standard deviation for 10 measurements of the blank and S is the slope of the calibration graph, was 0.013 mg L^{-1}. The repeatability (R.S.D) calculated from 10 repeated injections of a standard containing 1.5 mg L^{-1} nitrite was 0.5%. An injection throughput of 55 injections h^{-1} was achieved.

Determination of nitrate

The conversion of nitrate to nitrite is not complete. For this reason, two calibration graphs were obtained under reducing conditions. One calibration graph was run for nitrite standards and the other one for nitrate standards. The analytical curve for nitrite ranged from 0.026 to 5 mg L^{-1} (peak height absorbance = 0.2053[nitrite (mg L^{-1})] − 0.001 with a correlation coefficient $r = 0.9991$ and $n = 10$). The LOD was 0.026 mg L^{-1} and the repeatability calculated from 10 replicates using 0.3 mL injections of a 2.5 mg L^{-1} nitrite solution was 1.4%. On the other hand, the calibration graph for nitrate was linear in the range 0.039–7 mg L^{-1} (peak height absorbance = 0.1271 [nitrate (mg L^{-1})] + 0.0202; $r = 0.9988$; $n = 11$). A LOD of 0.039 mg L^{-1} was achieved. The repeatability calculated from ten successive injections of a standard containing 4 mg L^{-1} nitrite was 1.3%. An injection throughput of 27 injections h^{-1} was achieved.

The conversion rate of nitrate to nitrite can be calculated from the slopes of the two calibration graphs. Thus, a reduction efficiency of 62% was obtained.

To sum up, three calibration graphs were required for the analysis of nitrate (i.e. one for the direct determination of nitrite and two for the procedure involving on-line reduction) (Fig. 4). Nitrite is determined using the direct procedure. The concentration obtained allows to calculate the contribution of the absorbance due to nitrite (A1) to the total absorbance (A2) in the procedures involving on-line reduction. For this purpose, the equation of the calibration graph for nitrite standards under reducing conditions is used. The absorbance due to nitrate (under reducing conditions) (A3) can be calculated by subtracting A1 from A2. Thus, nitrate concentration can be obtained by substituting A3 in the equation of the calibration graph for nitrate standards.

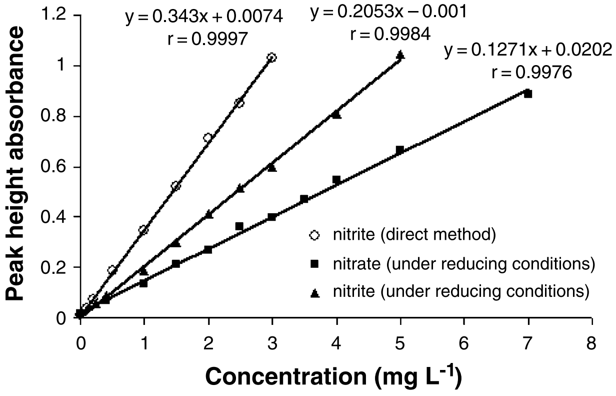

Fig. 4. Calibration graphs obtained for the determination of nitrite and nitrate

Interference studies

The effect of several foreign species on the determination of nitrite (Table 2) and nitrate (Table 3) was investigated at the 5% interference level.

As can be seen in Table 2, the main interference in the direct determination of nitrite is due to Fe^{3+}. It was observed that this cation could be tolerated up to 50 mg L^{-1} by using a solution of sodium fluoride (0.25 mol L^{-1}) as carrier stream.

Regarding the determination of nitrate (Table 3), the strong interference of Mg^{2+} that precipitates as hydroxide due to the alkaline medium needs to be highlighted. According to Cerdà et al. [1], interfering ions such as Mg^{2+}, Fe^{3+} or Fe^{2+} can be removed prior to analysis by using cation-exchange resins or by precipitation in alkaline medium and filtration. Thus, a cation-exchange column was placed before the reduction reactor and it was found that magnesium was tolerated up to 20 mg L^{-1}. However, the injection

Table 2. Tolerance level of several species in the determination of nitrite (1.5 mg L^{-1} NO_2^-)

Ion added	CO_3^{2-}	Urea	Fe(II)	Fe(III)	Sb^{3+}	Ca^{2+}	SO_4^{2-}	NH_4^+	Pb^{2+}	Cl^-	Mg^{2+}
Tolerated concentration (mg L^{-1})	>1000	>1000	50	10	200	>1000	>1000	>1000	250	>1000	>1000

Table 3. Tolerance level of several species in the determination of nitrate (2.5 mg L^{-1} NO_3^-)

Ion added	CO_3^{2-}	Urea	Fe(II)	Fe(III)	Sb^{3+}	Ca^{2+}	SO_4^{2-}	NH_4^+	Pb^{2+}	Cl^-	Mg^{2+}
Tolerated concentration (mg L^{-1})	100	500	5	5	5	200	500	30	10	500	<2.5

throughput was decreased (i.e. 10 injections h^{-1}) as micro-pumps could not overcome the back-pressure from the cation-exchange column. For this reason, the interfering ions were eliminated by precipitation. Moreover, this latter strategy allows higher concentrations of the interfering ions.

Analysis of samples

The accuracy of the proposed methodology was evaluated in the determination of nitrite in a certified reference material [MOOS-1 (seawater)]. Three replicates of this sample were analyzed. The certified and the obtained values were 0.141 ± 0.007 mg NO_2^- L^{-1} and 0.142 ± 0.006 mg NO_2^- L^{-1}, respectively. The Student's *t*-test for mean comparison did not reveal any statistically significant difference at the 0.05 significance level. The *t*-calculated value (0.37) was inferior to the critical one (4.30) for 2 degrees of freedom.

On the other hand, four ground water samples collected near fields treated with fertilizers were analysed. Nitrite was below the LOD in the samples tested. The results obtained for nitrate determination using the proposed MPFS methodology (145 ± 3, 74 ± 3, 72.8 ± 1.2 and 24.5 ± 1.0 mg NO_3^- L^{-1}) were in good agreement with those obtained by means of ionic chromatography (146.5 ± 0.7, 78.5 ± 1.7, 74.3 ± 0.5 and 26.4 ± 0.8 mg NO_3^- L^{-1}). The Student's *t*-test for mean comparison indicates that there was no difference between the results obtained from both procedures (at the level of significance of 0.05 and four degrees of freedom).

The developed methodology was also applied to the analysis of six standard mixtures of nitrate and nitrite. The concentration of nitrite and nitrate in the spiked samples varied in the ranges of 0.5–2.5 mg L^{-1} and 0.5–4 mg L^{-1}, respectively. Recoveries within the ranges 97.3–99.5% and 96.3–106.7% were observed for nitrite and nitrate, respectively. From the recoveries obtained, it can be concluded that the method presents good accuracy.

Comparison with other systems

Only a few flow systems have been reported for the spectrophotometric determination of nitrate and nitrite using hydrazine sulphate as reducing agent. The LODs offered by these approaches [5, 18] are higher than the obtained in this work (Table 4). In this sense, the better LOD achieved by MPFS may be attributed

Table 4. Comparison with other systems

Parameter	MPFS (Present work)	FIA [18]	SIA [5]	SIA [19]	FIA [20]	FIA [22]	FIA [21]
Detection technique	Spec	Spec	Spec	Spec	Spec	CL	Biamp
Nitrate reduction	Homogeneous (Hy)	Homogeneous (Hy)	Homogeneous (Hy)	Heterogeneous (Cu–Cd)	Heterogeneous (Cu–Cd)	Homogeneous (Hy)	Photochemical reduction
Detection limit							
Nitrite (µg NO_2^- L^{-1})	13	92	70	4.6	69	0.5	25
Nitrate (µg NO_3^- L^{-1})	39	496	200	28	142	1.2	50
Working range							
Nitrite (mg NO_2^- L^{-1})	0.013–3	0.092–10.1	0.07–18.4	0.023–1.8	0.16–3.3	0.002–2.3	0.1–1.0; 2.0–5.0
Nitrate (mg NO_3^- L^{-1})	0.039–7	0.496–14.9	0.2–24.8	0.124–6.2	0.35–8.8	0.003–3.1	0.1–2.0; 6.0–16
Repeatability (%)							
Nitrite	0.5	1.5	1.5	0.8	3.2	3.2	1.2
Nitrate	1.3	2.3	3.1	1.3	4.0	3.2	1.6
Injection throughput (injections h^{-1})							
Nitrite	55	4	NR	30	30	26	27
Nitrate	27	4		30	30	26	25

Spec Spectrometry; *CL* chemiluminiscence; *Biamp* Biamperometry; *Hy* hydrazine; *Cu–Cd* copperized cadmium column; *NR* not reported.

to the pulsed flow of the micro-pumps that makes possible a better and faster reaction zone homogenisation [13]. Moreover, the proposed MPFS system provides 12 times less consumption of chromogenic reagent with regard to FIA [18].

On the other hand, the LOD obtained in this work can be comparable with the obtained using heterogeneous reduction (i.e. copperized cadmium) [19, 20] or biamperometry [21] as detection technique. As can also be seen in Table 4, the lowest LOD is achieved exploiting chemiluminiscence [22]. By contrast, the repeatability given by the aforementioned approach is not as good as the obtained using the system developed.

Conclusions

The results obtained show that the proposed MPFS method can be applied to the determination of nitrite and nitrate in water samples.

Each solenoid micro-pump acts individually in the insertion of the fluids so that reagents are propelled to the system only when necessary. For this reason, one of the main advantages of the developed methodology is its low reagent consumption with regard to FIA systems. The simplicity, robustness and versatility of this system should also be highlighted.

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