Scientific paper

Determination of Nitrite and Nitrate in Freshwaters using Flow Injection Luminol Chemiluminescence Detection

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Abstract

A flow injection method for determination of nitrite and nitrate in freshwaters is described based on luminol–hypochlorite chemiluminescence (CL) system. Nitrate is reduced on-line with a cadmium reduction column to nitrite and its inhibition effect on luminol CL emission was measured. The effects of chemical and physical parameters such as buffer pH and concentration, luminol, sodium hypochlorite and sulfuric acid concentrations, flow rate, and sample volume were investigated. The calibration graphs were linear over the range 0.1–50 μM (R^2 = 0.9989 and 0.9984) for nitrite and nitrate respectively with a limit of detection (S/N = 3) of 4.0×10^{-8} M and a sample throughput of 120 samples per hour. The effect of foreign ions was studied and the method was successfully applied to the determination of nitrite and nitrate in water samples. The results obtained were in good agreement with those achieved by a spectrophotometric reference method at the 95% confidence level. Standard addition method was also applied to the freshwater samples and the recovery values were found in the range of 92–109% and 94–105% for nitrite and nitrate respectively.

Keywords: Flow injection analysis; chemiluminescence; nitrite; nitrate; freshwaters.

1. Introduction

Nitrite at elevated concentrations is harmful and toxic for human health. The toxicity of nitrite is primarily due to its interaction with iron(III) in hemoglobin in blood to produce methemoglobinemia which is a fatal disease. The reaction between nitrite and secondary or tertiary amine results in the formation of N-nitroso compounds, some of which are know to be carcinogenic, teratogenic and mutagenic.[1] Nitrate also at high concentrations can be considered as a pollutant since it can be reduced to nitrite; therefore food and drinking water with high concentrations of nitrate are also dangerous. It is due to this significant influence of nitrite and nitrate on human health and the environment that makes it important to monitor their concentration in drinking waters. The recommended maximum contaminant level (MCL) of nitrate and nitrite in drinking water in the USA is 10 mg/L and 0.06 mg/L respectively. In seawater, nitrate and nitrite are two forms of dissolved inorganic nitrogen with a concentration range of 1.0–500 and 0.1–50 μM respectively.[2]

Several analytical methods have been reported for the determination of nitrite and nitrate in environmental waters. These include spectrophotometry/colorimetry,[3–5] fluorimetry,[6] atomic absorption spectrometry,[7] capillary electrophoresis,[8] amperometry,[9] chemiluminescence,[10–12] ion chromatography,[13] and gas chromatography–mass spectrometry.[14] Moorcroft *et al.*[15] reported a comprehensive review on the advantages and disadvantages of the various techniques used for the determination of nitrite and/or nitrate in environmental waters and biological fluids.

Fanning[16] reported an excellent review addressing a number of reducing agents that have been identified to facilitate the conversion of nitrate to nitrite with copper coated cadmium,[17, 18] and hydrazine.[19] The copperised

cadmium column has been the most widely used for reduction of nitrate to nitrite and is essentially preferred when using flow-injection systems. Photo-induced reduction of nitrate to nitrite has also been applied with a conversion efficiency of 70–84%.[12, 20]

Flow analysis techniques are well-established tools for the automation and miniaturization of analytical methodologies, providing advantages such as: increased sample throughput, high versatility, high robustness, new analytical improvements based on operating modes under non-stationary conditions, decrease of the human exposure under hazardous chemical/ physical sample pretreatments, more environmentally friendly procedures obtained due to process downscaling and use of alternative detection systems with the concomitant simplification of the operating conditions. [21]

Luminol (5-Amino-2,3-dihydro-1,4-phthalazinedione) is the most widely used CL reagent. The CL emission of luminol is based on its oxidation by various oxidizing agents in the alkaline medium. The CL methods using luminol as a reagent have been reported for the monitoring of pesticides, pharmaceuticals,[22–25] and nitrate and nitrite[10–12, 20] at trace levels in different samples. The CL reaction of hypochlorite with luminol is a well known reaction,[26] and generates a strong CL in an alkaline medium. Nitrite can consume hypochlorite and thus reduces the background CL signal from the luminol-hypochlorite reaction when it is introduced in the reaction. This inhibition of CL is the basis of the determination of nitrite/nitrate in the concentration range of 0.1–50 μM. The reaction mechanism and kinetics of nitrite with hypochlorite have been reported elsewhere.[27] Nitrate is reduced quantitatively to nitrite on-line using a cadmium reduction column. The method was successfully applied to the determination of nitrate and nitrite in freshwaters with a sample throughput of 120 samples per hour.

2. Experimental

2. 1. Materials and Methods

All plastic ware used during the experiments was cleaned by soaking in nutrient free detergent (2% v/v, Neutracon, Decon Laboratories, UK), rinsed with ultra-high-purity (UHP) water (Elga, Purelab Option, UK) followed by soaking in 10% HCl (v/v) for 24 h, again rinsed with UHP water and stored in plastic bags. All reagents and standards were of analytical grade, unless stated otherwise and all solutions were prepared with UHP water.

Luminol (0.01 M) stock solution was prepared by dissolving an appropriate amount of luminol (5-amino-2,3-dihydro-1,4-phthalazinedione, Aldrich) in UHP water and stored in at 4 °C. A working solution was prepared by dilution of the stock solution with borate buffer (pH 10.5, 0.05 M). Nitrite and nitrate (0.01 M) stock solutions were prepared by dissolving the required amount of sodium nitrite and sodium nitrate in UHP water and stored at 4 °C. Working standard solutions were prepared by serial dilution of the stock solutions in UHP water. Hypochlorite (0.01 M) stock solution (10% w/v, Scharlau, Spain) was prepared with UHP water by diluting the required volume of sodium hypochlorite solution standardized by iodometry with sodium thiosulphate. Working standards were prepared by serial dilution of the stock solution in sulfuric acid (0.5 mM). Phosphate, silicate, chloride, fluoride, sulfate, bicarbonate, arsenate and ammonium stock solutions were prepared by dissolving the appropriate amount in UHP water from their respective salts. Working standards were prepared from these stock solutions for interference study.

2. 2. Cadmium Reductor Column

A cadmium reduction column was prepared according to the following procedure:[28] A saturated solution of cadmium acetate was prepared in UHP water and zinc filling (pre-washed with HCl, 0.5 M) was placed in it. Cadmium metal deposited on surface of the zinc fillings was removed. The process was continued until cadmium precipitation occurred. The cadmium metal precipitates were washed with UHP water, followed by HCl (0.5 M) and again with UHP water. The precipitates obtained were dried in an oven and ground to a powder. The cadmium powder was then washed with UHP water and packed in an acid washed Teflon tube (100 mm length 2 mm i.d.). The tube was plugged with cleaned cotton wool at both ends and connected to the flow manifold. The packed column was washed with a stream of water for 30 min. followed by HCl (0.5 M) for 2 h and finally with UHP water for 30 min to remove impurities.

2. 3. Flow System and Procedure

The flow injection-CL system used for the proposed work is shown in Fig. 1. A peristaltic pump (Ismatec, Switzerland) was used to deliver the sample carrier and reagent solutions (R_1) at a flow rate of 2 mL/min. A six-port rotary sample injection valve (Rheodyne 5020, Anachem, Luton, UK) was used to inject standards/sample into the water carrier stream passing through the chelating column (Chelex 100, iminodiacetate, sodium form, 50–100 mesh, Sigma, UK) and merges with a stream of hypochlorite in sulfuric acid (R_2). This stream was then merged with the luminol CL reagent stream (R_3) with a T-piece. The merged streams traveled through a glass spiral flow cell positioned directly in front of an end window photomultiplier tube (PMT, 9798B, Electron Tubes, Ruislip, UK). The PMT, glass coil and T-piece were enclosed in a light-tight housing and the PMT was attached to a 2.0 kV power supply (Electron Tubes, PM20SN, UK). The detector response was recorded in the

form of peaks on a chart recorder (Kipp & Zonen BD 11E. Holland). All manifold tubing was PTFE (0.75 mm i.d., Fisher, UK) except for the peristaltic pump tubing, which was flow-rated silicone (Elkay). The percent inhibition can be calculated by the formula 100(CLo – CLn)/CLo, where CLo is the peak height CL intensity in the absence of nitrite and CLn is the peak height CL intensity in the presence of nitrite.

For the determination of nitrate, a cadmium reduction column (100 mm length × 2 mm i.d.) was incorporated in the manifold after the chelating column. This stream was merged at a T-piece with acidic hypochlorite and then with the luminol stream for the CL inhibition measurement as described.

2. 4. Reference Method

To investigate the accuracy of the proposed method, freshwater samples were analyzed in parallel by the standard spectrophotometric method.[29] For the determination of nitrite, standard solutions containing nitrite in the range of 1.0–100 μM and freshwater samples were transferred into 25 mL volumetric flasks. A 0.5 mL of the sulphanilic acid (0.6 % in HCl, 2.4 M) was added to each solution and allowed to stand for 3 to 4 mins followed by an addition of 0.5 mL of α-naphthylamine (0.6% in HCl, 0.1 M) and 0.5 mL of sodium acetate solution (25%) to each standard and sample solutions. The total volume was made up to 25 mL with UHP water and allowed to stand for 10 min. The absorbance was monitored at 520 nm with a spectrophotometer (UV/Vis-spectrophotometer, Jenway, 6505, UK). The results obtained are given in the Table 1. For the determination of nitrate, an aliquot of standard solution containing nitrate in the range 1.0–100 μM and freshwater samples were passed off-line through the cadmium reduction column and then treated according to the procedure as described above.

3. Results and Discussion

3. 1. Optimization

The FI-CL manifold used for determination of nitrite and nitrate was optimized by examining the influence of variables on the analytical signal using a univariate approach. All studies were performed with nitrite (10 μM), sodium hypochlorite (10 μM), sulfuric acid (1.0 mM), luminol (5 μM) in borate buffer (0.05 M) solutions, sample loop (60 μL), flow rates (1.5 mL/min) and a PMT voltage (850 V). Each selected parameter was then used subsequently for the selection of other parameters.

The efficiency of luminol CL is highly dependent on the reaction pH and exhibits stronger emission under alkaline conditions. Therefore, the effect of borate buffer and sodium hydroxide solution in the range of 0.01–0.5 M was examined. Maximum CL response was observed with a borate buffer of 0.05 M as reported previously[30] hence, luminol was prepared in borate buffer (0.05 M). Further, the influence of borate buffer pH in the range of 9.5–11.5 was studied. Maximum CL inhibition with nitrite was observed at borate buffer (0.05 M) pH 10.5 and was therefore selected and used in subsequent studies (Fig. 2a). The influence of luminol concentration prepared in borate buffer (pH 10.5, 0.05 M) was examined in the range 1.0–50 μM. The maximum CL inhibition was observed at 10 μM luminol with further increase in luminol concentration resulting in non-reproducible CL signals with high blank values (Fig. 2b). Therefore, a 10 μM luminol concentration was selected and used for further experiments.

The effect of sulfuric acid was examined over the range of 0.1–5.0 mM. Maximum CL inhibition was obtained at 0.5 mM and a further increase in sulfuric acid resulted in a decrease in the CL response (Fig. 2c). Therefore, a 0.5 mM sulfuric acid solution was used for subsequent studies. The influence of hypochlorite concentration was studied over the range 5.0–150 μM prepared in sulfuric acid (0.5 mM). An optimum CL inhibition was observed at 50

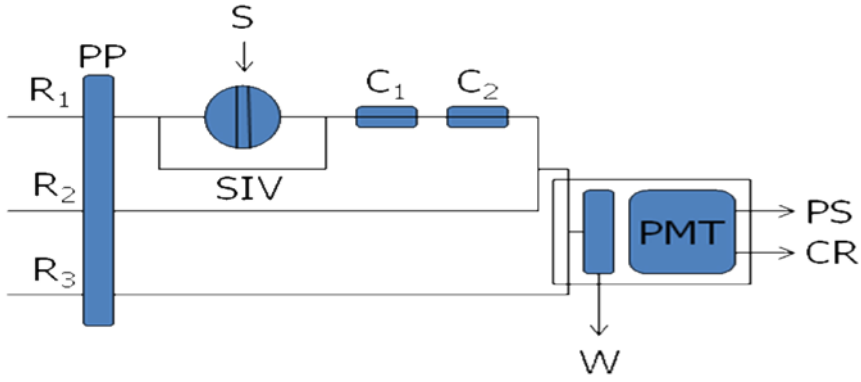

Figure 1. FI-CL manifold for the determination of nitrite and nitrate. R_1 = sample carrier stream (UHP water), R_2 = hypochlorite in sulfuric acid stream, R_3 = luminol CL reagent stream, PP = peristaltic pump, SIV = sample injection valve, S = standards/samples standards of nitrite and nitrate, C_1 = chelating column (40 mm length × 10 mm i.d), C_2 = cadmium reduction column (100 mm length × 2 mm i.d), PMT = photomultiplier tube, PS = power supply, CR = chart recorder and W = waste.

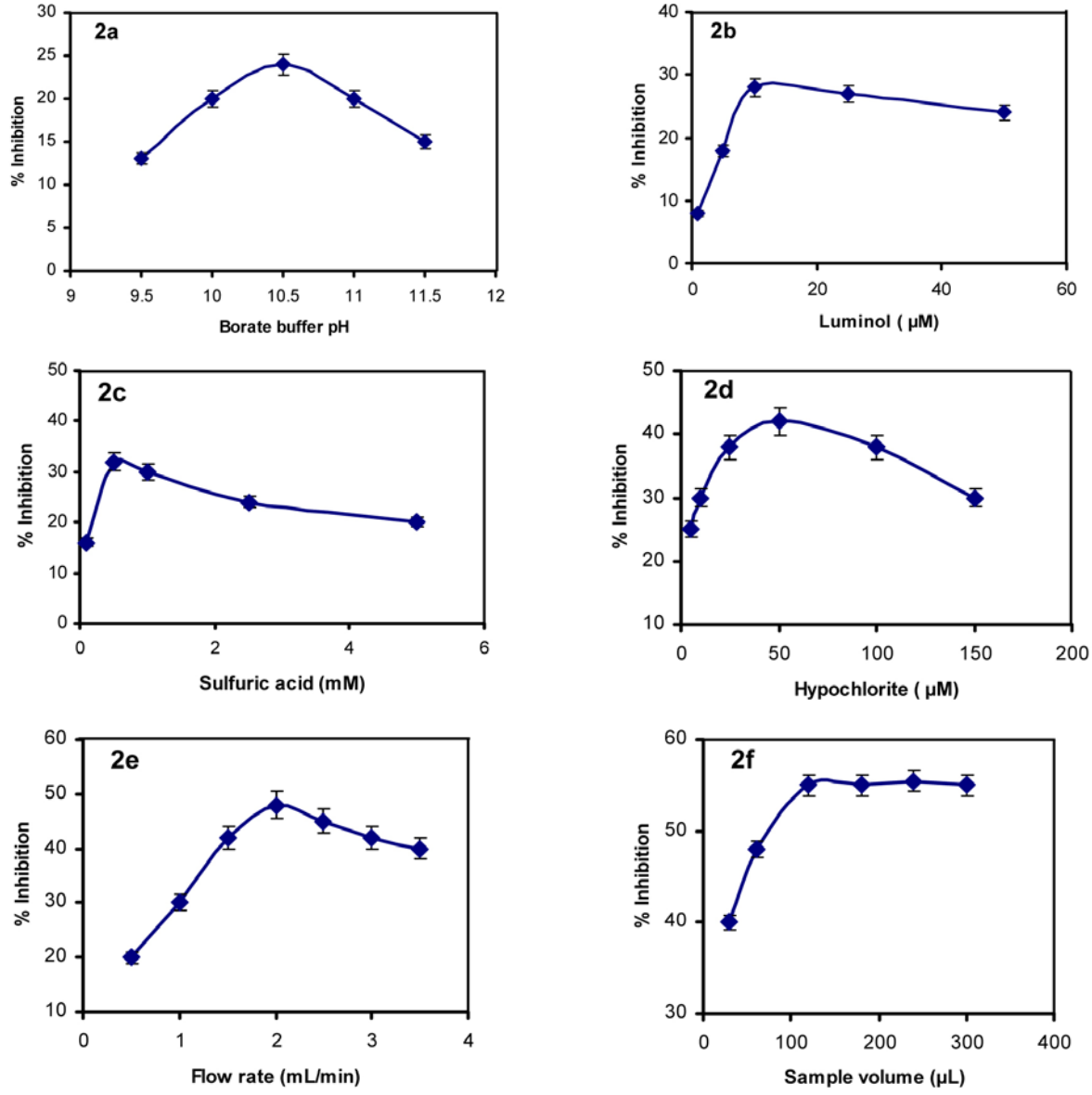

Figure 2. Effect of various chemical parameters on luminol-hypochlorite CL inhibition of 10 µM nitrite: (a) Borate buffer pH (b) luminol concentration (c) sulfuric acid concentration (d) hypochlorite concentration (e) flow rate and (f) sample injection volume.

µM hypochlorite as shown in Fig. 2d and therefore was selected and used for subsequent experiments.

The effect of flow rate was examined over the range 1.0–7.0 mL/min. A flow rate of 2.0 mL/min gave the optimal CL inhibition response (Fig. 2e) with a steady base line and reproducible peak heights and hence was used for all streams. The effect of sample injection volume was examined over the range 60–300 µL and optimal CL inhibition was obtained at 120 µL above which no appreciable change was observed, hence was used subsequently (Fig. 2f). The effect of the length of the cadmium reduction column over the range 50–150 mm × 2 mm i.d. was examined on reduction of nitrate to nitrite. Maximum CL inhibition was observed at cadmium reduction column length of 100 mm × 2 mm i.d with reproducible CL signals and therefore, was used for all subsequent study. The efficiency of the column for the reduction of nitrate to nitrite was investigated by determining the ratio of CL inhibition of nitrate and nitrite (10 µM). The reduction column efficiency was found to be greater than 90 %.

3. 2. Analytical Figures of Merit

Under the optimized conditions, the calibration graphs of CL inhibition versus concentration of nitrite and nitrate over the range 0.1–50 µM (R^2 = 0.9989 and 0.9984) were obtained respectively. The relative standard deviation (RSD) was 2.1% for 10 replicate analyses of 5 µM nitrite, with a limit of detection (S/N = 3) of 4.0×10^{-8} M and a sample throughput of 120/h.

Table 1. Analytical results of nitrite and nitrate in freshwater samples by proposed and reference methods.

Water samples	pH	Salinity (g/L)	Conductivity (μS at 25 °C)	Nitrite (mg/L) Proposed method (mean ± SD)	Nitrite (mg/L) Reference method[29] (mean ± SD)	Nitrate (mg/L) Proposed method (mean ± SD)	Nitrate (mg/L) Reference method[29] (mean ± SD)
1	7.7	0.21	418	0.24 ± 0.01	0.22 ± 0.02	2.6 ± 0.06	2.2 ± 0.02
2	7.9	0.25	499	0.31± 0.03	0.33 ± 0.06	1.5 ± 0.08	1.7 ± 0.01
3	8.1	0.16	319	0.48 ± 0.05	0.45 ± 0.07	4.8 ± 0.02	5.1 ± 0.06
4	8.31	0.4	786	0.41 ± 0.02	0.46 ± 0.05	6.4 ± 0.03	5.8 ± 0.02
5	7.8	0.47	946	0.35 ± 0.08	0.30 ± 0.02	4.5 ± 0.04	4.8 ± 0.06
6	7.4	0.24	468	0.19 ± 0.03	0.21 ± 0.05	9.4 ± 0.03	10.2 ± 0.08
7	7.3	0.29	573	0.29 ± 0.03	0.33 ± 0.02	8.7 ± 0.08	8.1 ± 0.05

$F_{table} = 4.28 > F_{calc.} = 1.02$ for nitrite and $F_{calc.} = 1.61$ for nitrate $t_{tab} = 2.4 > t_{calc.} = 0.7$ for nitrite and $t_{calc.} = 0.15$ for nitrate

3. 3. Interferences Study

The interference of foreign ions present in water at environmentally relevant concentrations was investigated by analyzing solutions containing 5.0 μM nitrite. The tolerable level of foreign species was taken as a relative error not greater than ± 5%. No interference could be found with 500-fold for Na^+, K^+, Ca^{2+}, Mg^{2+}, SO_4^{2-}, Cl^- and F^-, 100-fold for PO_4^{3-}, HCO_3^- and SiO_4^{4-}, 5-fold for SO_3^{2-} and S^{2-} on the determination of nitrite except ammonium ion which interfered at concentration level of >5.0 mg/L. The natural level in ground water is usually below 0.2 mg/L for ammonia, although surface waters may contain up to 12 mg/L.[31] Iron(II), cobalt(II) and vanadium(IV) enhanced the CL response due to their action as catalysts for luminol oxidation in the presence of molecular oxygen.[32–35] These ions were removed by incorporating an inline iminodiacetate chelating resin micro-column.[36] Sulfite and sulfide were also found to interfere in the determination. However, these ions are not present in freshwaters at interference level, and seawater samples may contains 1 mg/L of sulfide or more.

3. 4. Application

The proposed method was applied to the determination of nitrite and nitrate in freshwater samples (ground water for irrigation and tap water for drinking and domestic use). Samples were collected around Quetta valley, Pakistan, in acid washed HDPE bottles, filtered through a cellulose membrane filter (cellulose acetate, pore size 0.45 μm, 47 mm diameter, Whatman, Maidstone, UK) and analyzed. Samples were injected in the manifold, without and with cadmium reduction column for nitrite and ni-

Table 2. Results of the recovery test for the determination of nitrite and nitrate in freshwater samples.

Water Samples	Added Nitrite (mg/L)	Found Nitrite (mg/L)	Recovery (%)	Added Nitrate (mg/L)	Found (mg/L) Nitrate	Recovery (%)
1	0	0.41	–	0	3.5	–
	0.25	0.68	103	1	4.3	96
	0.5	0.98	108	5	8.4	99
	0	0.31	–	0	5.1	–
2	0.25	0.53	95	2.5	8.1	94
	0.5	0.80	99	5	9.8	103
	0	2.5	–	0	7.8	–
3	0.1	0.33	94	2.5	10.5	98
	0.25	0.52	104	5	13.1	98
	0	0.21	–	0	2.8	–
4	0.1	0.33	106	2.5	5.5	104
	0.25	0.48	104	5	8.1	96
	0	0.19	–	0	6.2	–
5	0.25	0.48	109	2.5	8.9	98
	0.5	0.71	103	5	11.5	97
	0	0.40	–	0	4.9	–
6	0.2	0.55	92	2.5	7.8	105
	0.5	0.84	93	5	10.1	102
	0	0.35	–	0	8.1	–
7	0.2	0.52	95	2.5	10.9	103
	0.5	0.81	95	5	13.9	99

trate+nitrite determination respectively. The nitrite concentration was subtracted from total nitrate/nitrite concentrations to get the nitrate concentration. The results are shown in Table 1 along with pH, salinity and conductivity of the water samples. These results show good agreement with the spectrophotometric reference method used[29] at the 95% confidence level (Statistical tests *F* and *t* were applied). Recovery experiments were performed using freshwater samples with recovery values of 92–109% for nitrite and 94–105% for nitrate obtained respectively as shown in Table 2.

4. Conclusion

The proposed FI-CL method is simple and rapid (120 per hour sample throughput) with a limit of detection of 4.0×10^{-8} M for nitrite and nitrate in freshwaters. Interferences from cations present in freshwaters were removed by an in-line chelating resin micro-column. The results obtained for freshwaters were in good agreement with a spectrophotometric reference method.

5. References

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Povzetek

Opisana je metoda za določevanje nitrita in nitrata v površinskih vodah, s pretočno injekcijsko analizo, ki temelji na kemiluminiscenčni reakciji luminol-hipoklorit. Nitrat se reducira v kadmijevi redukcijski koloni do nitrita. Meri se inhibicijski učinek kemiluminscenčne emisije luminola. Določeni so bili vplivi nekaterih kemijskih in fizikalnih parametrov kot so pH, koncentracije luminola, natrijevega hipoklorita, žveplove (VI) kisline, pretoka in volumna vzorca. Umeritvena krivulja je linearna v območju od 0,1 do 50 μM za nitrat in nitrit (R^2 = 0.9989 in 0.9984) , z mejo zaznave $4{,}0 \times 10^{-8}$ M. V eni uri lahko izmerimo do 120 vzorcev. Določeni so bili tudi vplivi nekaterih motečih ionov. Metoda je bila uporabljena za določevanje nitrata in nitrita v realnih vzorcih. Rezultati se dobro ujemajo z referenčno spektrofotometrično metodo pri 95 % meji zaupanja. Metoda je bila preverjena tudi z analizo vzorcev, ki so jim bile dodane standardne množine analitov. Izkoristki so bili med 92 in 109 % za nitrit in 94 in 105 % za nitrat.