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Sequential injection system for on-line analysis of total nitrogen with UV-mineralization

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Abstract

An automatic method for the determination of total nitrogen in wastewater by sequential injection analysis and mineralization with UV radiation has been developed. The method is based on the mineralization of the samples with sodium persulphate in basic medium under UV radiation. Small volumes of sample and reagents are firstly aspirated into a single channel and then propelled by flow reversal to the UV reactor and then to the detector. The organic and inorganic nitrogen compounds are oxidized to nitrate that is then measured at 226 nm. The sequential injection procedure has been optimized and the factors affecting the efficiency of the oxidation have been studied with a number of test substances with different chemical structures and properties. Solutions in the concentration range 1–56 g l^{-1} of nitrogen can be analyzed with the described procedure. The sample rate is of 30–40 samples h^{-1}. The LOD is 0.6 mg l^{-1} N and the reproducibility is 1.8% (28 mg l^{-1} N). Organic carbon in the form of glucose was added to a number of test solutions to study the potential interference of organic matter.

The method was compared with the Kjeldahl digestion method by analyzing 15 wastewater samples with both methods. The nitrate and nitrite content of the non-oxidized samples were subtracted from the corresponding nitrogen content determined after photo-oxidation and the value compared with the Kjeldahl nitrogen content.

Keywords: Sequential injection analysis; SIA; Total nitrogen; Waters

1. Introduction

Dedicated procedures have been described for the determination of specific organic nitrogen compounds such as urea, aliphatic and aromatic amines, amino acids or nitrophenols, usually involving either enzyme reactions [1–3] or complex chromatographic separation [4–6]. However, for monitoring purposes or routine analysis, the determination of total organic nitrogen (TON) is preferred as it provides more information on the system under study.

The first step for the determination of organic nitrogen is the digestion of the sample in order to convert organic compounds into an inorganic form of nitrogen mainly nitrate, nitrite or ammonium. Also referred to as mineralization, it is the most tedious and time-consuming step and at the

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same time the greatest source of errors in the analytical determination.

Recent developments in organic nitrogen determination are focused mainly in the automation of digestion procedures and in continuous digestion and analysis using either UV radiation for photo-oxidation or microwave radiation combined with the use of flow injection techniques (FIA) [7–9].

In photo-oxidation based methods decomposition of organic matter takes place under UV radiation in the presence of small amounts of oxidant. As described in a review on photochemical methods [10], the photo-oxidation reactions are complex and depend on several factors such as pH and the concentration of the oxidant.

Potassium persulphate has been proposed as oxidizing agent for the determination of total nitrogen (TN) because the UV radiation at 254 nm catalyses the decomposition of this compound in sulphate and other reactive species, such as oxygen and hydroxyl radicals that in turn oxidize the organic compounds, ammonium and nitrite to nitrate [10,11] which can be easily quantified by molecular spectrophotometry.

On-line sample UV-oxidation has been described using FIA systems [12–14] and segmented flow [15–20] by integrating them in nitrate analyzers in which the formed nitrate is determined either by direct measurement in ultraviolet [12], or indirectly by reduction to nitrite using copperized cadmium [13,15,17,18] or hydrazinium sulphate [19,20] or reduction to ammonium [16].

The sequential injection analysis (SIA) approach has important advantages over conventional FIA, as a single SIA configuration can be adapted for multi-reagent techniques and multidetection systems without the need of changing the manifold. Additionally it is simpler in equipment, the manifold does not have to be changed if flow parameters or injection volumes are modified and sample and reagent volumes required are very small. In SIA systems the sample and reagent zones are first sequentially aspirated and stacked into a channel and then the flow is reversed in order to transport them into the detector. A multiposition selector valve is used as distributor and either a sinusoidal flow pump [21,22], a peristaltic pump [23] or an automatic burette [24] have been used as liquid drivers.

Since it was firstly introduced by Ruzicka et al. [25,26] as a further development of FIA, SIA has been applied in a wide range of analytical applications and detection systems including ion selective electrodes [27], atomic absorption [28] and spectrophotometry [28–31] alone or combined with additional units such as gas diffusion cells or reduction columns thus confirming the potential of this technique.

In the system proposed here we combine the SIA system with a UV reactor for on-line digestion and spectrophotometric detection and apply the method to the determination of TN in wastewater samples.

2. Experimental

2.1. Apparatus

The sequential injection manifold is shown in Fig. 1. The sampler, the burette and the selector valve are integrated into a Crison Compact Titrator (Alella, Spain). The photo-oxidation reactor consists of a 15-W mercury lamp ($\lambda = 254$ nm) placed inside an aluminium cylinder, a Teflon tube (150 cm × 1.5 mm internal diameter) coiled around it and a cooling fan. The detector is a Hewlett Packard 8452A diode array spectrophot-

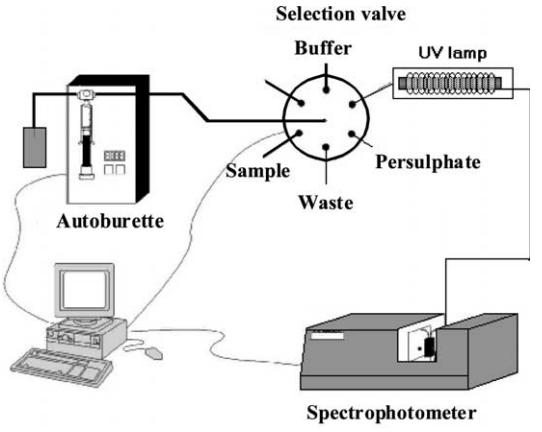

Fig. 1. SIA manifold for on-line UV mineralization and analysis of TN.

ometer. Absorbance is measured at 226 and 320 nm, the latter for the correction of the refractive index variations.

2.2. Reagents

Stock solution of test substances were prepared to contain 140 mg l^{-1} N and working solutions were prepared by proper dilution of them with distilled water. The test substances were: $NaNO_3$, NH_4Cl, urea, glycine (GLY), aspartic acid (ASA), barbituric acid (BBA), EDTA, 4-aminophenol (*p*-AP), 4-nitrophenol (*p*-NP) and 2,5-pyridil dicarboxilic acid (PDA). These compounds were chosen to have a wide variety of nitrogen compounds.

The oxidant reagent was potassium persulphate (stock solution 40 g l^{-1}, working solutions 0–6 g l^{-1}) in sodium tetraborate buffer (7 g l^{-1}, pH 9). The buffer solution was also sodium tetraborate 7 g l^{-1}.

2.3. Procedure

First, at 2.5 ml min^{-1},100 μl of buffer are aspirated followed by 30 μl of oxidant, 50 μl of sample, 30 μl of oxidant and finally, 100 μl of buffer. Thus, the sample (50 μl) is sandwiched between two reagent zones (2×30 μl) and two buffer solution zones (2×100 μl) to ensure the excess of reagent and buffer. Then the flow direction is reversed, and also at 2.5 ml min^{-1}, the sample and reagents are mixed and driven to the photoreactor where the oxidation of nitrogen compounds to nitrate occurs due to the combined effect of UV radiation and persulphate excess. The flow is then stopped for a certain irradiation period (3–5 min) after which the mineralized sample is propelled to the detector at 2.5 ml min^{-1}. Absorbance is measured at 226 and 320 nm, the latter to correct variation of the refractive index. Two ml of distilled water are used in order to propel the sample to the detector and also to clean the reaction coil thus leaving the system prepared for a new analysis cycle.

The concentration of nitrate in the mineralized sample can be then calculated from the absorbance at 226 nm and from that, the TN content in the sample. The difference between the TN and the sum of the concentrations of nitrate and nitrite before the photo-oxidation is the so-called 'Kjeldahl' nitrogen content. The TON is calculated as the difference between TN and the sum of the concentrations of nitrate, nitrite and ammonium.

Each sample is injected by triplicate and also three blanks are irradiated and the corresponding signal used to correct the signal for the test solutions and samples. Sample throughput is 30–40 h^{-1}, depending on the irradiation time

3. Results and discussion

The oxidation of the nitrogen test substances is dependent on the concentration of the oxidant reagent, the nitrogen content of the sample, the irradiation time, the pH and the type of nitrogen compound. These experimental parameters are closely related and may even have opposite effects on the analytical performance of the method. Therefore the optimal parameters have been fixed taken into account these parameters simultaneously and also taking into account the results obtained by the authors in the development of a FIA system for the mineralization of nitrogen compounds

3.1. Irradiation time

The irradiation time is dependent on the intensity of the UV radiation. As the mineralization step is achieved in a stopped flow mode, its duration can be easily adjusted without any change in the manifold. Test solutions containing 28 mg l^{-1} N in the form of NH_4Cl, urea, BBA and the blank were irradiated for 0–5 min and analyzed. The concentration of persulphate was fixed at 4 g l^{-1} and the pH was 9 based on the previous FIA results [14]. After 80 s under UV radiation, the photo-oxidation is nearly 100% for these test compounds except for BBA, which is nearly 80% (Table 1).

However due the excess of non-decomposed persulphate, the blank signal represents approximately 50% of the analytical signals (for 28 mg l^{-1} N). For longer irradiation periods the analytical signals remain constant and the blank decreases

Table 1
Influence of irradiation time on the oxidation of NH_4^+, urea and BBA

Test compound (28 mg l^{-1} N)	Photo-oxidation rate (%) vs. irradiation period					
	0 s	20 s	80 s	140 s	200 s	320 s
Blank[a]	0.0±0.2	0.0±0.3	27±1	47±1	61.5±0.5	84±2
NH_4^+	0.5±0.3	59±1	102±2	105±3	99.0±0.9	97±2
Urea	0.6±0.4	50.7±0.6	95±2	92±2	95.8±0.4	86±3
BBA	16.7±0.6	86±3	79±1	88±1	90±1	83±2

$[Na_2S_2O_8] = 4$ g l^{-1}; sodium tetraborate 0.02 M, pH 9. Sample volume: 50 μl, reagent volume: 2 × 30 μl, buffer solution: 2 × 100 μl.
[a] Based on 100% oxidation of $Na_2S_2O_8$ (4 g l^{-1}) after 10 min.

thus improving the sample to blank ratio. However another problem arises for very long irradiation times and it is the formation of air bubbles that interfere with the spectrophotometric determination. Irradiation for 3 min was chosen as a compromise between conversion efficiency, low blank signal and no bubble formation.

3.2. pH

Test compounds are then analyzed at different working pH with 3 min irradiation time. The pH for the photo-oxidation is adjusted using the following buffers: acetate (0.1 M, pH 4.5), HPO_4^{2-} (0.5 M, pH 7.5), tetraborate (0.02 M pH 9) and carbonate (0.025 M pH 11). The recovery of 28 mg l^{-1} N–NH_4 is quantitative at pH equal to or higher than 7.5 while the curve for urea and BBA has a maximum at pH 9 (Fig. 2) coincident with the optimal pH in FIA systems. The recoveries for all the test substances are also between 85–100% at pH 9 (Table 2) and therefore this pH was the selected value for all the experiments.

3.3. Concentration of persulphate

Several solutions containing sodium tetraborate 0.02 M and sodium persulphate in the range 2–6 g l^{-1} (0.007–0.022 M) are tested in order to be used as oxidizing reagent. In all cases pH is 9 and irradiation time is 3 min. Under these conditions, the efficiency of the oxidation step for NH_4Cl, GLY and urea (28 mg l^{-1} N each) is nearly 100% even with lower persulphate concentrations (2 g l^{-1}) but for the other tested substances in general, raising the concentration of $Na_2S_2O_8$ increased the N recoveries (Fig. 3). The lower recoveries obtained at low concentrations may be due to insufficient excess of oxidizing reagent for a fast oxidation reaction. The effect is more evident with test substances with higher organic carbon content, which may compete for the O_2 available. Persulphate concentrations higher than 4 g l^{-1} are needed for a quantitative recovery of most of the test substances. Also for the analysis of more concentrated sample solutions a persulphate concentration (6 g l^{-1}) is required. For solutions containing 56 mg l^{-1} N the efficiency of the oxidation is still nearly 100% for ammonium, urea, ASA and *p*-NP and lower for BBA, EDTA, *p*-AP, and PDA (Table 2).

3.4. Linear range, detection limits and reproducibility

Ten injections of a standard solution 28 mg l^{-1} NO_3^- are analyzed with the described procedure. The standard deviation is ±0.005 mg l^{-1} N, which represents a variation of ±1.8%. The calibration graph is linear up to 60 mg l^{-1} N and the detection limit ($3\sigma_{n-1}$) is 0.5 mg l^{-1} N. It is noteworthy that this detection limit is limited by the method used to determine the resulting nitrates. By using a more sensitive method (e.g. the reduction by hydrazine), lower detection limits can be achieved but also the linear range will be narrower. This approach is noteworthy for the analysis of TN in natural water samples but the UV detection at 226 nm is more adequate for

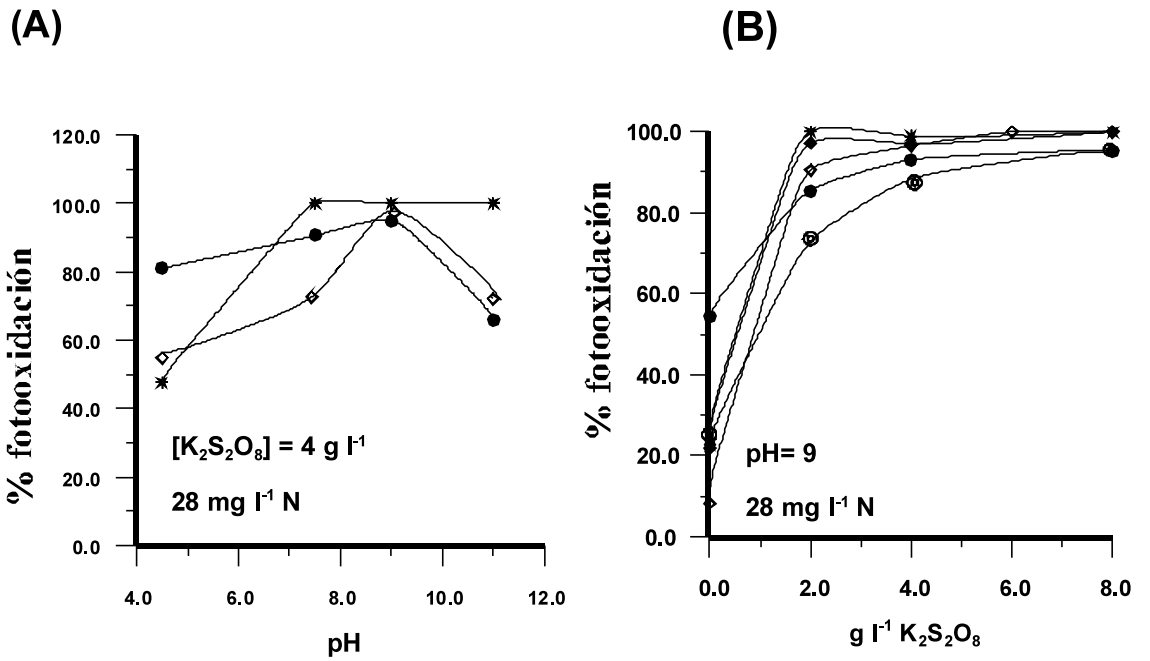

Fig. 2. Influence of: (A) pH and (B) of the oxidant concentration on the oxidation rates of (*) ammonium chloride; (◇) urea; (●) BBA; (◆) GLY and ((✇)) EDTA.

wastewater samples with nitrogen content around 5–50 mg l^{-1}.

3.5. Interference from organic matter

Organic matter may interfere with the UV-persulphate method described here in two ways. Its oxidation may consume the oxygen available and also, the formation of carbon dioxide may lower the pH. In any case the result is a decrease in the oxidation rate of the nitrogen compounds. These potential interfering effects were studied by analyzing several standards containing a fixed amount of tested substances (nitrate, ammonium and urea) and increasing amounts of organic material in the form of glucose. The nitrogen content of the standard solutions were 28 mg l^{-1} N and 56 mg l^{-1} and the concentration of organic carbon was varied between 1 and 40 mmol l^{-1}, which is equivalent to a chemical oxygen demand (COD) of 32–1280 mg l^{-1}.

Table 2
Effect of concentration on recovery of nitrogen using persulphate 4 g l^{-1} and tetraborate buffer 7 g l^{-1}, pH 9

Test substance	% Photo-oxidation vs. N-content in the sample	
	28 mg l^{-1} N	56 mg l^{-1} N
NH_4Cl	99±0.9	101±1
GLY	97±1	93.2±0.8
Urea	94±1	100±1
p-NP	98.4±0.3	104±2
ASA	96.8±0.4	93.9±0.7
BBA	94.5±0.5	73.8±0.3
PDA	84.5±0.5	61.6±0.9
EDTA	88.3±0.5	75.8±0.3
p-AP	87.4±0.4	86±2

Sample volume: 50 μl, reagent volume: 2 × 30 μl, buffer solution: 2 × 100 μl.

The results are given in Fig. 4. With 4 g l^{-1} of sodium persulphate, there was a decrease in the oxidation rate in the presence of even moderate amounts of organic carbon (COD 128 mg l^{-1}). The increase in the irradiation time did not improve the efficiency. The oxidation rate was improved by increasing the concentration of persulphate; with 20 g l^{-1} of persulphate, a COD of 640 mg l^{-1} could be tolerated. Unfortunately, other problems arose with such a large

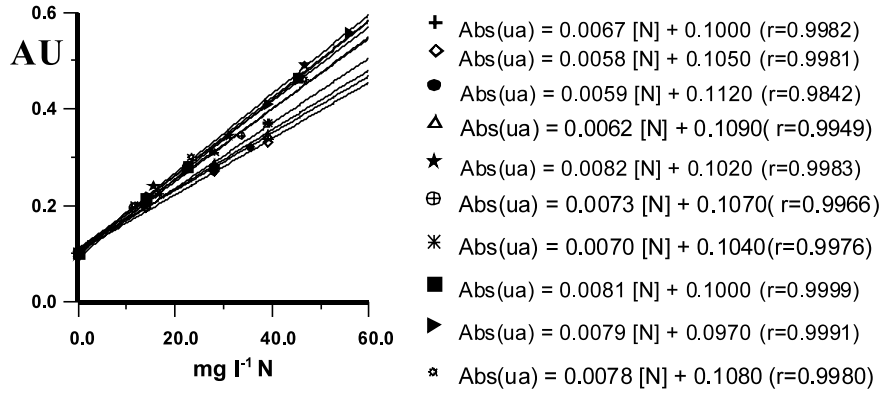

Fig. 3. Calibration curves obtained in optimal conditions ([$K_2S_2O_8$]: 6 g l^{-1}; pH 9; digestion time: 3 min with solutions of (■) sodium nitrate, (★) ammonium chloride, ((¤)) urea, (⊕) BBA, (►) GLY, (*) ASA, (+) *p*-NP, (●) PDA, (△) EDTA and (◇) *p*-AP.

concentration. On one side the blank signal is dependent on the content of organic matter in the sample, which is theoretically unknown. Several attempts were made of applying multicomponent analysis to discriminate between the contribution of the nitrate and the persulphate to the measured signal. However, there was no success because of the very close overlap of the signals and the difficulties in reproducibility of the results obtained for triplicate spectra obtained in the wavelength range 210–300 nm. In addition, the amount of gas bubbles formed by self-decomposition of the persulphate interfered with the measurement of the analytical signal. The addition of sodium metabisulphite was not effective either to destroy the excess of persulphate. This was better achieved by increasing the irradiation time although in this case the amount of persulphate (and consequently the maximum COD tolerated) must be lowered to avoid problems derived from gas bubbles in the

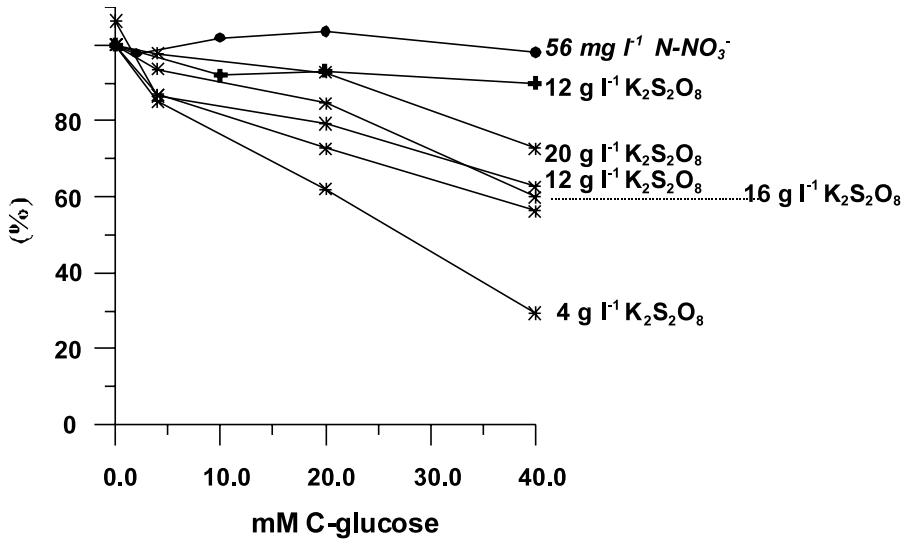

Fig. 4. Influence of the organic carbon content on the recoveries of (*) 56 mg l^{-1} and (+) 28 mg l^{-1} N-urea according with the quantity of potassium persulphate added to solution R1.

Table 3
Comparison of the results obtained with the Kjeldahl method and the SIA–UV method proposed when applied to real wastewater samples

Sample	N-Kjeldahl mg l^{-1}	$NO_2^- + NO_3^-$ mg l^{-1} N	(NK + $NO_2^- + NO_3^-$) mg l^{-1} N	SIA–UV mg l^{-1} N	Difference mg l^{-1} N
ALA-2E	19.6	35.2	54.8	50±3	+5
ALA-2S	32.2	10.8	44.0	53±3	−9
ALA-3E	39.4	–	39.4	33±2	+6.4
ALA-3S	–	34.1	34.1	37±2	−3
ALA-4S	15.7	3.9	19.6	24.6±0.9	−5
ALA-5S	16.5	–	16.6	17±2	+0.6
E2295	45.8±0.5	–	–	49±3	−3
P2295	58.5	–	–	54±3	+4
S2295	50.4	–	–	51±1	−0.6
E1295	58.8	–	–	62±2	−3
LAG-1	17.1	0.3	18.3	17.4	+0.9
LAG-2	12.6	0.5	14.3	13.1	+1.2
LAG-3	8.7	5.3	14.1	14.0	+0.1

flow line. A concentration of 12 g l^{-1} of sodium persulphate and irradiation with the UV lamp for 5 min was chosen as a compromise. Under these conditions, up to 28 mg l^{-1} N can be analyzed provided the COD is lower than 1000.

3.6. Analysis of wastewater samples

Thirteen wastewater samples were analyzed with the Kjeldahl method and with the SIA–UV method. The nitrate and nitrite content of the non-oxidized samples were determined using the reduction by hydrazine and the Griess-Ilosvay reagent. The values obtained were added to the Kjeldahl nitrogen value and the results were compared with the SIA–UV value (Table 3). The mean difference between the two sets of results was −0.42 mg l^{-1} N with a standard deviation of the difference being 4.20 mg l^{-1}. The t-test was carried out to check out the correlation between the results given by both methods being $t = 0.36$. It is lower than the critical value for a 99% confidence (3σ), which confirms that there are no significant differences between the values found with both methods.

4. Conclusion

The method can be applied to the determination of TN in wastewater samples. For samples with a high value of COD, the mineralization is complete up to 56 mg l^{-1} N when using a persulphate concentration as high as 20 g l^{-1}. This causes a high blank signal that can be eliminated with longer irradiation times (10 min) but in that case the gas bubbles formed by the decomposition of persulphate may interfere with the spectrophotometric measurement and they should be first eliminated.

Another possibility is to use a more diluted oxidant (12 g l^{-1}) and an irradiation period of 5 min. In that case the method is linear up to 28 ppm N with a COD lower than 1200. That means that the wastewater samples should be diluted before the analysis.

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