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Determination of ammonia and primary amine compounds and Kjeldahl nitrogen in water samples with a modified Roth's fluorimetric method

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Abstract

A method for the simultaneous determination of primary amino groups and ammonium ion has been proposed. The method is based in solution derivatization with *o*-Phthaldialdehyde/*N*-acetyl-cisteine (OPA/NAC) and fluorescence measurement of the formed isoindols. Analytical characteristics and description of the developed procedure have been provided. The calibration graphs for ammonium (up to 1.44 mg L^{-1} of N) and methylamine as primary amino model compound (up to 0.282 mg L^{-1} of N), were obtained. Bivariate and multivariate calibration models have been tested. The limits of detection were 0.07 mg L^{-1} of N and 0.004 mg L^{-1} of N for ammonium and amine, respectively.

The procedure was first applied directly to standard solutions containing ammonium and amines and secondly to digested solutions by Kjeldahl method. The results obtained allowed to establish the best digestion conditions in order to perform the total amine conversion into ammonium.

This procedure has been also applied to real samples (irrigation ditch, residual and fountain waters) and the concentrations of primary amine groups and ammonium have been evaluated. The results obtained after Kjeldahl digestion allowed to estimate the total Kjeldahl N contained in the samples. The samples were also analysed by Nessler method and similar results were obtained.

Keywords: Kjeldahl nitrogen; Roth's fluorimetric method; Ammonia and primary amines

1. Introduction

Ammonium is a micronutrient in water systems, which is an important link in the nitrogen cycle of aquatic ecosystems. However, its determination is still a delicate task, due to it is high susceptibility to contamination, and the classic methods do not appear to be well controlled in many laboratories [1]. Ammonium can be found in superficial, subterranean or marine waters at low concentrations, about 10 μg L^{-1}. In residual waters it can be found at higher concentrations, about 30 mg L^{-1} due to ammonification and nitrate reduction processes. Often, it can be found together with short-chain aliphatic primary amines (mainly methylamine) because they are widely distributed in the environment. Aliphatic amines are used in several chemical and manufacturing industries and are also common components of biological systems as degradation products of organic material such as amino acids and proteins. Besides hygienic problems due to the stinging smell, these compounds may be hazardous to human health. In addition, they can react with certain nitrogen-containing compounds to form nitrosamines, which are potentially carcinogenic substances [2]. Consequently, there is an increasing interest in the determination of aliphatic amines in various aqueous matrices. The tolerable limits for amines are regulated as Kjeldahl nitrogen (which includes ammonium and organic nitrogen), being between 15 and 85 mg L^{-1} for residual waters and 1 mg L^{-1} for tap water.

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Liquid chromatography combined [3] or not with derivatization [4], is generally used for the analysis of ammonium and aliphatic amines in aqueous media. Unbiased UV–vis or fluorimetric methods which could be used as screening sample methods in order to reduce costs and saving time in the environmental laboratory or in situ determinations are not found in the literature. The Roth's fluorimetric method [5–6] using a thiol and *o*-Phthaldialdehyde (OPA) as derivatizing reagents can be adapted to the determination of ammonium and there are already some publications in the bibliography [7]. Aminot et al [8,9] determined ammonia using OPA-sulphite reagent and Mana and Spohn [10] OPA-tioglycolate. In previous studies, we have proposed the use of OPA and the thiol *N*-acetyl-cisteine (NAC) reagents for the unbiased fluorescent ammonium determination in water samples [11] exciting at 415 nm. This procedure offers some advantages over ammonium reference methods, such as Nessler reagent method or the ammonium selective electrode method, the lower detection limit, the absence of any systematic error and amines interference and less toxicity of reagents.

Recently, we have reported the determination of primary amine groups in water samples, by performing amine derivatization on C18 cartridges and using OPA/NAC reagents [12,13] and 333 nm as excitation wavelength. The response of several primary amino groups in the OPA/NAC reaction was demonstrated to be similar in [12], being possible to estimate the total primary amine concentration in water employing the calibration graph of methylamine as model compound. By using this solid phase assisted derivatization procedure, ammonia at concentrations up to 1.5 mg L^{-1} was not an interference species.

In this paper, however, we have studied the simultaneous determination of ammonium and methylamine as model compound of primary amine groups by using solution derivatization with OPA/NAC reagent, two different excitation wavelengths and bivariate and multivariate calibration methodologies. Principal component regression (PCR) was tested using the spectra of emission obtained exciting at 333 and 415 nm.

The method has been applied before Kjeldahl sample treatment in order to estimate ammonia and primary amino compounds in the sample. The method has been also tested as an indicator of potential failures in the Kjeldahl conversion of the primary amine groups to ammonia and has been used for estimating Kjeldahl nitrogen after digestion sample treatment. Several real samples were processed and the results obtained have been compared with those provided by the Nessler reference method.

2. Experimental

2.1. Apparatus

All spectrophotometric and spectrofluorimetric measurements were made on a Hewlet-Packard (Avondale, PA, USA) HP 8452 diode array spectrophotometer furnished with a 1 cm path length, and a Hitachi F-4500 fluorescence spectrophotometer, respectively. The pH was measured with a Crison micropH 2000 pH-meter.

Digestion unit for Kjeldahl treatment was a Tecator Digestion system 6, 1007 Digestor (Höganäs, Sweden), and Distillation Unit was a Büchi 323 Distillation Unit (Switzerland).

2.2. Reagents and standard solutions

All solutions were prepared in nanopure water and all reagents were of analytical grade. Stock standard solutions of ammonium were prepared by dissolving ammonium chloride (Probus, Spain) in nanopure water (100 mg L^{-1} or 1000 mg L^{-1}). Standards solutions with variable amounts of ammonium were obtained by dilution. Methylamine (MA) solutions were prepared in the same way.

2.2.1. OPA/NAC method

OPA (Fluka chemika, Switzerland): NAC (Fluka chemika, Switzerland) reagent was prepared with a 1:1 ratio (8.8 mM in each component), dissolving OPA reagent with 5% of MeOH (Scharlau, Spain) and mixing it with NAC dissolved in water. Borate buffer (0.5 M), pH 10.8 was prepared by dissolving an adequate amount of boric acid (Scharlau, Spain) in water and then adjusting the pH with NaOH (Panreac, Spain). Ethylamine (EA), *N*-Propylamine (N-PrA), Isopropylamine (IPA), Butylamine (BA), Pentylamine (PeA), Hexylamine (HA), Dimethylamine (DMA), Diethylamine (DEA), and β-Phenylethylamine (β-FEA) (Sigma, Germany) solutions at 0.75 mg L^{-1} level were prepared. Other concentrations assayed appear in the text. Mixtures of amines: 0.75 mg L^{-1} EA and 0.75 DEA; 0.5 DMA, 0.5 N-PrA and 0.5 IPA; 0.75 BA and 0.75 PeA; 0.75 β-FEA and 0.75 HA were also used.

2.2.2. Nessler method [14]

Sodium–potassium tartrate solution (Panreac, Spain) was prepared by dissolving 50 g in 100 mL of nanopure water and sodium hydroxide (Panreac, Spain) by dissolving 12 g in 50 mL of nanopure water. Nessler reagent (NR) was prepared by adding the mercuric chloride solution (3 g of mercury (II) chloride, Merck, Germany) to the potassium iodide (Guinama, Spain) solution (10 g dissolved in the minimum amount of water). Forty millilitre of sodium hydroxide (18%p/v) were added to the HgI_4^{2-} solution; and the final solution was diluted up to 100 mL.

2.2.3. Kjeldahl treatment

For sample or standard digestion, red mercury (II) oxide (Panreac, Barcelona, Spain), potassium sulphate (Prolabo, Fontenay, France) and sulphuric acid (Fluka Chemie, Steinheim, Switzerland) were used. Distillation reagent was prepared by dissolving 25 g of sodium thiosulphate (Prolabo, Fontenay, France) and 500 g of sodium hydroxide (J.T.Baker, Deventer, Holland) together in 1 L of nanopure water.

2.3. Procedures

2.3.1. Standard solutions

2.3.1.1. OPA/NAC reaction. Variable volumes of ammonium and/or amine (alone or mixed) stock solutions and nanopure water when necessary, up to a volume of 1 mL, 0.1 mL of borate buffer 0.5 M (pH = 10.8) and 0.9 mL of OPA/NAC reagent were placed in a quartz cuvette. The reaction was assumed to start after the addition of the last drop of OPA/NAC reagent. Each experiment was assayed by recording spectra emission between 430 and 600 nm (λ_{exc} = 415) and between 380 and 610 nm (λ_{exc} = 333) over the reaction time ranged 0–300 s. Signal was obtained at 120 s for λ_{ex} = 333 nm, λ_{em} = 440 nm, and at 300 s for λ_{ex} = 415 nm, λ_{em} = 485 nm. All measurements were performed at 25 °C. The ammonium and methylamine concentrations ranged from 0 to 1.75 and 0 to 0.625 mg L^{-1}, respectively, following a 5^2 design (Table 1).

2.3.1.2. Nessler reaction. Variable volumes of ammonium stock solution, 10 μL of sodium–potassium tartrate (0.177 M), water up to a constant volume (2.4 mL), 0.1 mL of NaOH 6 M were placed in a plastic cuvette and 0.1 mL of Nessler reagent were added to the mixture. The reaction was assumed to start after the addition of the last drop of reagent. Each experiment was assayed by recording spectra between 300 and 700 nm at 30 s intervals over the reaction time ranged 0–600 s. Signal was obtained at 425 nm and 400 s. Absorbance signal was measured against water blank. All measurements were performed at 25 °C.

2.3.1.3. Kjeldahl treatment. For the application of Kjeldahl treatment [15], several digestion-distillation conditions were assayed. Different amounts of potassium sulphate (2.22–10.5 g), mercury (II) oxide (0.0332–0.525 g), concentrated sulphuric acid (3.39–12 mL) and times (90–120 min) were tested in the digestion step. The reagent mixture was added to 100 mL of standard solution or sample in order to perform the digestion at 370 °C. The digestion residue obtained was cooled and diluted up to 50 mL with nanopure water.

Distillation was performed by adding variable volumes (25–50 mL) of distillation reagent ($NaOH/Na_2S_2O_3$). Distilled ammonia was picked up on 25 mL of 0.04 N sulphuric acid. Collected solutions (80 mL) were diluted up to 100 mL. Before standard procedure the pH of the solutions was adjusted to 10.5.

Table 1
Code and concentration (mg L^{-1}) of standard calibration set (5^2 design)

NH_4^+\MA (mg L^{-1})	0	0.25	0.375	0.5	0.625
0	00	01	02	03	04
0.25	10	11	12	13	14
0.75	20	21	22	23	24
1.25	30	31	32	33	34
1.75	40	41	42	43	44

2.3.2. Water samples

Environmental water samples with unknown ammonium concentration were analysed. They were named as S1: irrigation ditch water, S2: residual water from a factory and S3: fountain water.

2.3.2.1. Sample treatment. Samples or standards were treated in order to eliminate residual chloride and cation interference, according to the reference standard method [16]. One millilitre of dechlorant sodium sulphite 7.14 mM and 1 mL of zinc sulphate heptahydrated 0.348 M were added to 100 mL of water (sample or standard). The formed precipitate was filtered and the first 25 mL of the sample were wasted, collecting the rest of the sample. The pH was adjusted to 2 with concentrated H_2SO_4 for sample conservation. Sample pH was adjusted to 10.5 before OPA/NAC or Nessler reactions.

2.3.2.1.1. OPA/NAC reaction. 1 mL of treated standards or sample (S1 and S3), and 0.1 mL diluted up to 1 mL for S2, were analysed by adding reagents as explained above for standards.

2.3.2.1.2. Nessler reaction. Treated standards or sample (S1 and S3) 2.4 and 0.1 mL diluted up to 2.4 mL for S2, were analysed by adding reagents as explained above for standards.

2.3.2.2. Kjeldahl treatment. Sample S1 and S3 100 mL or 7 mL of S2 diluted up to 100 mL were treated by Kjeldahl method in triplicate. One millilitre of digested sample (S1 and S3), and 0.3 mL of S2 diluted up to 1 mL were used for the OPA/NAC method. When Nessler method was applied, the volumes of digested sample employed were 2.4 mL for S1 and S3, and 0.5 mL diluted up to 2.4 mL for S2.

2.4. Statistical analysis

The data were aligned according to the maximum emission signal for PCR. For all calculations, Unscrambler (CAMO, Norway) and Matlab for Windows (Math Works, Natick, MA) were used. Two methodologies have been employed to perform the calibration step [17]: the most usual Top-Down selection (TDS), where principal components are entered in the calibration model in order of explained variance (from the first to the last), and the Best-Subset selection (BSS), where principal components are entered in order of prediction ability. In second step, original variable selection on the optimised model by using the uninformative variable elimination (UVE) approach has been used [18]. The elimination of uninformative original variables can improve the predictive ability and produce more robust calibration models (lower complexity). In the selected methodology, random error variables are added to the original data set, and the importance of the experimental variables is evaluated by comparing the obtained regression coefficients for both types of variables. The original variables with lower predictive ability than the added ones (noise) are eliminated.

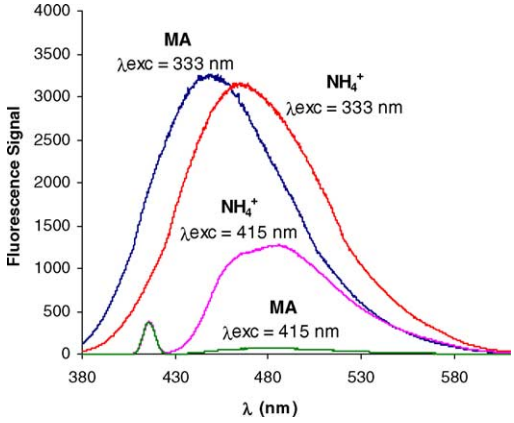

Fig. 1. Fluorescence emission spectra for a standard of 1.75 mg L^{-1} of ammonium and a standard of 0.375 mg L^{-1} of methylamine, both at λ_{exc} =333 nm and at λ_{exc}=415 nm.

Results were considered to be significant at the 5% critical level ($p < 0.05$)

3. Results and discussion

3.1. Calibration step

According to a previous work [12], standards of Methylamine, Ethylamine, *N*-Propylamine, Isopropylamine, Butylamine, Pentylamine, Hexylamine, and β-phenylethylamine provided similar response when their concentrations were expressed as mg L^{-1} of nitrogen, with percentage recovery respect to methylamine signal of 100, 93.5, 101.1, 93.4, 89, 100.4, 108.2 and 99% for each amine, respectively. The same emission maxima located at 440 nm were also obtained for all of them exciting at 333 nm (Fig. 1). The additivity between different amine signals was tested analysing mixtures of amines (see experimental section). Percentage recoveries obtained were between 81–88%. With these results, it was corroborated that the amine response in the OPA–NAC reaction was not dependent on the primary amine, and methylamine could be used as a representative compound.

In order to study the simultaneous determination of ammonium and primary amines (c.a. methylamine), a design 5^2 was assayed (Table 1). Both, Ammonium and primary amines, provide fluorescent signal exciting at 333 nm and measuring at λ_{em} 440, although the emission maximum for ammonium was 462 nm (Fig. 1**).** No significant difference was found at the 5% critical level (homogenous variance, $F_{cal} < F_{tab-\alpha=0.05}$, and comparable slopes, $t_{cal} < t_{tab-\alpha=0.05}$) between the amine calibration graphs obtained with different constant amounts of ammonium ranging from 0 to 1.75 mg L^{-1} (Table 2). The contribution of the ammonium to the total signal can be seen in the corresponding intercept values. This fact corroborates the additively of ammonium and amine signals. The same behaviour is obtained for ammonium slopes and intercepts in presence of different amine

Table 2
Analytical parameters (a: intercept, s_a: standard deviation of intercept, b: slope, s_b: standard deviation of slope, n: number of standards, r^2: correlation coefficient, $s_{y/x}$: standard deviation of calibration curve) and statistical results (F_{cal}, $F_{tab,\ \alpha=0.05}$, t_{calc}, $t_{tab,\ \alpha=0.05}$) for amine (methylamine-MA) in presence of constant ammonium concentration (ranged from 0.25–1.75 mg L^{-1}), and ammonium in presence of constant amine (MA) concentration (ranged from 0.25–0.625 mg L^{-1})

λ_{exc} (nm)	Analytes	Calibration curve $Y = (a \pm s_a) + (b \pm s_b)C$ (n, r^2, $s_{y/x}$)	F_{calc}	F_{tab} α = 0.05	Conclusion 1	t_{calc}	t_{tab} α = 0.05	Conclusion 2
333	MA	Y = (900 ± 300) + (7000 ± 600) C (7, 0.9663, 300)						
333	MA + 0.25 mg L^{-1} NH_4^+	Y = (1400 ± 500) + (6800 ± 1100) C (4, 0.9528, 300)	3.33	8.43	Homogenous variances	0.180	2.36	Similar slopes
333	MA + 0.75 mg L^{-1} NH_4^+	Y = (1810 ± 170) + (6500 ± 500) C (5, 0.9883, 200)	2.03	14.9	Homogenous variances	0.627	2.31	Similar slopes
333	MA + 1.25 mg L^{-1} NH_4^+	Y = (2100 ± 90) + (6600 ± 200) C (5, 0.9969, 100)	7.49	14.9	Homogenous variances	0.632	2.31	Similar slopes
333	MA + 1.75 mg L^{-1} NH_4^+	Y = (2840 ± 50) + (5760 ± 160) C (4, 0.9985, 60)	13.7	39.3	Homogenous variances	2.02	2.37	Similar slopes
333	NH_4^+	Y = (900 ± 300) + (1200 ± 200) C (3, 0.9698, 150)						
333	NH_4^+ + 0.25 mg L^{-1} MA	Y = (2500 ± 110) + (1050 ± 120) C (6, 0.9511, 190)	3.34	12.2	Homogenous variances	0.742	2.57	Similar slopes
333	NH_4^+ + 0.375 mg L^{-1} MA	Y = (3570±180) + (860 ± 160)·C (4, 0.9342, 200)	1.84	38.5	Homogenous variances	1.40	3.18	Similar slopes
333	NH_4^+ + 0.5 mg L^{-1} MA	Y = (4000±110) + (980 ± 110) C (5, 0.9630, 170)	3.89	17.4	Homogenous variances	1.06	2.78	Similar slopes
333	NH_4^+ + 0.625 mg L^{-1} MA	Y = (4200±400) + (1100 ± 300)·C (3, 0.9196, 400)	2.04	647	Homogenous variances	0.480	4.30	Similar slopes
415	NH_4^+	Y = (110 ± 50) + (680 ± 40) C (4, 0.9942, 50)						
415	NH_4^+ + 0.25 mg L^{-1} MA	Y = (110 ± 50) + (660 ± 50) C (5, 0.9853, 70)	1.59	39.2	Homogenous variances	0.372	2.57	Similar slopes
415	NH_4^+ + 0.375 mg L^{-1} MA	Y = (90 ± 40) + (640 ± 30) C (4, 0.9943, 50)	1.18	39.2	Homogenous variances	0.940	2.78	Similar slopes
415	NH_4^+ + 0.5 mg L^{-1} MA	Y = (110 ± 30) + (640 ± 30) C (4, 0.9956, 40)	1.49	39.2	Homogenous variances	0.790	2.78	Similar slopes
415	NH_4^+ + 0.625 mg L^{-1} MA	Y = (110 ± 30) + (640 ± 30) C (4, 0.9967, 40)	2.04	39.2	Homogenous variances	1.04	2.78	Similar slopes

Signal do no response to MA concentration at λ_{exc} = 415 nm.

Table 3
Characteristics of principal component regression models obtained for ammonium and methylamine by using top-down selection (TD), best subset selection (BBS) or uninformative variable elimination-best subset selection (UVE–BBS)

	Ammonium (λ_{exc} 415 nm)			Amine (λ_{exc} 333 nm)		
	TD	BSS	UVE–BSS	TD	BSS	UVE–BSS
Number of variables	231	231	32	180	180	29
Selected PCs	1,2	1,3	1,2	1,2,3	1,2,3	1,2
Explained variance, block X	99.999	99.999	99.999	99.999	99.999	99.999
RMSECV	0.089	0.051	0.052	0.034	0.037	0.040

amounts (Table 2). The mean slopes obtained at 440 nm for amine and ammonium were 6532 (s = 472, n = 5) and 1038 (s = 127, n = 5) and expressed as N 2950 (s = 213, n = 5) and 855 (s = 104, n = 5), respectively. These values indicated that the amine isoindols were 3.5 times more sensitive than ammonium isoindol exciting at 333 nm and measuring at 440 nm.

Fig. 1 shows the registers obtained for ammonium and methylamine exciting at 415 nm. Blank signal was obtained for the amine and a maximum at 485 nm for ammonium. As can be seen in Table 2 at the selective excitation wavelength for ammonium, that is λ_{ex} 415 nm (λ_{em} 485 nm**)** the same calibration graph was obtained in presence or absence of amine. In presence of different amounts of amine the intercepts were similar to that provided by the ammonium calibration graph, being its contribution due to the blank reagent. The ammonium calibration graph at 485 nm and the ammonium and amine calibration equations at 440 nm, exciting at 415 and 333 nm, respectively, were needed to estimate both analytes as demonstrated in the following.

The obtained emission spectra exciting at 415 and 333 nm for the standards (Table 1) were processed by using principal component regression (PCR), in order to develop a multivariate model to ammonium and amine, respectively. Top-down selection (TDS) and Best-subset selection (BSS) have been employed to perform the calibration step [17]. PCR models were obtained using as **X**-block the data (column centred). The number of variables, the percentage of explained variance, the number of factors and root mean squared errors of prediction (RMSECV) by leave-one-out cross-validation are given in Table 3. RMSECV values were calculated in order to evaluate the prediction ability of models. For methylamine there are no differences between the two options of selection, and a model with complexity three has been selected. For ammonium PC number 2 is not included in the model with BSS selection, and only PCs number 1 and 3 have been used (complexity two). Results obtained with variable selection on the optimised model by using the uninformative variable elimination (UVE) approach [18] have been also done in Table 3. Only 32 and 29 original variables have been used for methylamine and ammonium, respectively. In both cases a final model complexity two has been selected. The RMSECV values obtained are good as can be seen in Table 3 for all models, although UVE–BSS models were selected.

3.2. Accuracy and precision

Taking into account previous results, the standards were analysed. The measurements were performed exciting at 415 and 333 nm and using the ammonium calibration graph at 485 nm and the ammonium and amine calibration equations at 440 nm, respectively. Table 4 shows the ammonium and amine concentration found in the different mixtures assayed. Satisfactory results were obtained in all cases with relative errors for amine and ammonium contents between 7–8% and 4–24% and relative standard deviations between 6–13% and 1–9%, respectively.

Te same mixtures were analysed by using the PCR models. Improved results were obtained for accuracy of ammonium. The relative errors found were between 1 and 16%.

3.3. Kjeldahl method

The OPA/NAC proposed method was used for testing amine conversion into ammonium by use of the bivariate calibration model. Different Kjeldahl conditions were assayed in

Table 4
Found amine and ammonium concentration in standard solutions (mixtures of ammonium and Methylamine) and real samples by applying bivariate calibration graphs with standards or PCR

Samples	Added ammonium (mg L^{-1})	Found ammonium (mg L^{-1})		Added methylamine (mg L^{-1})	Found amine (methylamine) (mg L^{-1})	
		Bivariate Calibration	PCR		Bivariate Calibration	PCR
Standard	0.25	0.31 ± 0.03	0.29 ± 0.02	0.25	0.23 ± 0.03	0.26 ± 0.02
	0.75	0.89 ± 0.04	0.76 ± 0.06	0.375	0.35 ± 0.02	0.40 ± 0.06
	1.25	1.14 ± 0.03	1.23 ± 0.07	0.5	0.46 ± 0.06	0.50 ± 0.04
	1.75	1.68 ± 0.02	1.74 ± 0.06	0.625	0.58 ± 0.04	0.58 ± 0.09
Sample 1		n.d	n.d.		0.051 ± 0.007	0.086 ± 0.009
Sample 2		24.92 ± 0.10	32.2 ± 0.4		8.8 ± 0.4	10.25 ± 0.14
Sample 3		0.36 ± 0.03	0.29 ± 0.05		0.058 ± 0.013	0.082 ± 0.004

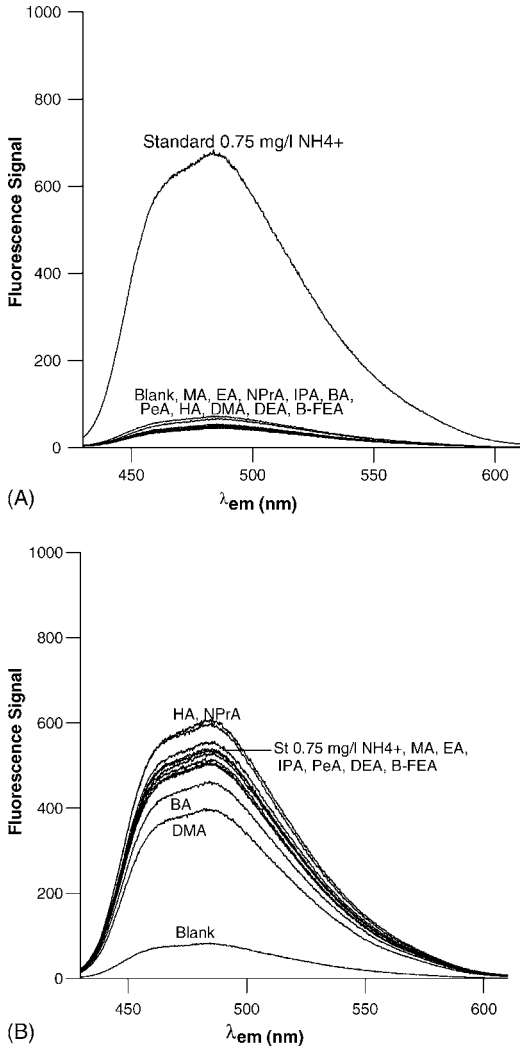

Fig. 2. Fluorescence emission spectra corresponding to different OPA/NAC isoindol derivatives at 485 nm (λ_{exc} = 415 nm) for blank, standard of 0.75 mg L^{-1} NH_4^+, 1.3 mg L^{-1} MA, 1.875 mg L^{-1} EA and DMA, 2.465 mg L^{-1} N-PrA and IPA, 3.05 mg L^{-1} BA and DEA, 3.625 mg L^{-1} PeA, 4.215 mg L^{-1} HA, and 5.05 mg L^{-1} β-FEA. (A) Measured before Kjeldahl digestion. (B) Measured after Kjeldahl digestion.

order to select the best one, which guarantee the total amine conversion (see Section 2). While the ammonium extraction from the sample was almost independent of the experimental conditions, the amine transformation into ammonium was dependent, and total transformation of MA into ammonium was obtained by digesting sample with 6.7 g of K_2SO_4, 0.525 g of HgO and 10 mL of H_2SO_4 for 120 min, and adding 30 mL of $NaOH/Na_2S_2O_3$ for its distillation. With these conditions, the methylamine conversion into ammonium was 109%. A similar value was obtained by the Nessler method.

Other primary and secondary amines, such as ethylamine, *n*-propylamine, isopropylamine, butylamine, pentylamine, hexylamine, dimetylamine, diethylamine and β-phenylethylamine, were also assayed in order to study its conversion into ammonium. In Fig. 2 are shown the fluorescence spectrum at λ_{exc} 415 of the OPA–NAC-ammonium and amine derivatives before and after Kjeldahl treatment. As can be seen the analytical signal of the amine derivatives before Kjeldahl digestion is similar to the blank (Fig. 2A). However, after performing the Kjeldahl digestion the amines are converted into ammonium (Fig. 2B). The ammonium measurement was performed by OPA/NAC procedure (λ_{exc} 415–λ_{em} 485 nm) and contrasted by the Nessler method; the results are shown in Table 5. As can be seen, comparable results were provided by both methods and recoveries nearly 100% were obtained in solutions with only one amine, as well as in amines mixture solutions.

3.4. Application to real samples

The procedure has been applied to the determination of ammonium and primary amines (expressed as methylamine) in real water samples (see Section 2). Three water samples of different nature were analysed: Ditch water, Industrial waste and Fountain water. As can be seen in Table 4, similar results were obtained by using both calibration systems, bivariate and PCR models. The paired *t*-test was not significant for both ammonium and methylamine determinations with values of α, 0.368 and 0.295, respectively.

Table 5
Percentage Ammonium recovery of different amine after Kjeldahl digestion, by applying Nessler or OPA/NAC method at 485 nm

Amine (Concentration before Kjeldahl digestion)	OPA-NAC Method (%ammonium recovery)	Nessler Method (%ammonium recovery)
EA (3.75 mg L^{-1})	101	110
N-PrA (4.93 mg L^{-1})	122.5	121.5
IPA (4.93 mg L^{-1})	107	103
BA (6.10 mg L^{-1})	88	82
PeA (7.25 mg L^{-1})	100	96
HA (8.43 mg L^{-1})	125	121
DMA (3.76mg L^{-1})	71.5	69
DEA (6.10 mg L^{-1})	112	101
β-FEA (10.10 mg L^{-1})	104	101
EA (1.878 mg L^{-1}) + DEA (3.047 mg L^{-1})	84	79
DMA (1.253 mg L^{-1}) + N-PrA (1.642 mg L^{-1}) + IPA (1.642 mg L^{-1})	82	81
BA (3.05 mg L^{-1}) + β-FEA (5.05 mg L^{-1})	100	85
PeA (3.622 mg L^{-1}) + HA (4.21 mg L^{-1})	85	92

(EA, ethylamine; N-PrA, N-propylamine; IPA, iso-propylamine; BA, butylamine; PeA, Pentylamine; HA, hexylamine; DMA, dimethylamine; β-PEA, β-phenylethylamine).

Sample 3 was also analysed by Nessler method and comparable results for ammonium were obtained: 0.32 ± 0.02 ($n = 3$).

The OPA/NAC procedure was applied to the water samples after Kjeldahl digestion. In this case, the analytical measurement of ammonium corresponds to the ammonium content plus the organic nitrogen. The results obtained have been compared with those obtained by Nessler method. The results of ammonium found were (0.19 ± 0.07), (213 ± 3) and (2.103 ± 0.018) mg L^{-1} ammonium for ditch, industrial waste and fountain waters, respectively, which were similar to those obtained by Nessler (*t*-test for paired samples $\alpha > 0.05$): (0.223 ± 0.018), (232 ± 3), (2.14 ± 0.02) mg L^{-1} ammonium respectively. These results are in agreement with the water nature, for natural water with low pollution, the *N*-Kjeldahl will not exceed 5 mg L^{-1}. For industrial waste water, this parameter can be higher than 200 mg L^{-1} of ammonium. Bearing in mind the results of Table 5, other amino compounds besides ammonium and primary aliphatic amines, were present in these waters.

4. Conclusions

In this article, the use of OPA/NAC reagent for the simultaneous determination of ammonium and primary aliphatic amines has been proposed. Bivariate calibration or multivariate Principal Component Regression yielded similar results. Methylamine can be used as model compound, because the response of each amine was the same when it was expressed in mg L^{-1} of nitrogen, and there was additivity in their signals when mixtures were processed.

Similar calibration curves were obtained for ammonium in presence of some fixed amine concentrations ranged from 0.25 to 0.625 mg L^{-1} methylamine exciting at 415 nm. No response for amines was obtained working at this wavelength. Slopes of the Methylamine calibration curves obtained in presence of some fixed ammonium concentration ranged from 0.25 to 1.75 mg L^{-1} were also all similar exciting at 333 nm. The signal of the ammonium affected intercept values in the last case.

Kjeldahl treatment was also tested obtaining similar slopes for ammonium calibration graphs before and after the treatment. Amine conversion into ammonium was near 100% for every amine studied alone or in mixtures. The method was a good indicator of failures in amine conversion by the Kjeldahl digestion method and can be used to estimate Kjeldahl nitrogen.

Real samples were analysed before and after Kjeldahl digestion. The application of Kjeldahl treatment to real water samples gave results in agreement of the water nature.

Nessler method was applied in order to validate accuracy of the proposed method, providing similar results to those obtained by the OPA/NAC method.

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