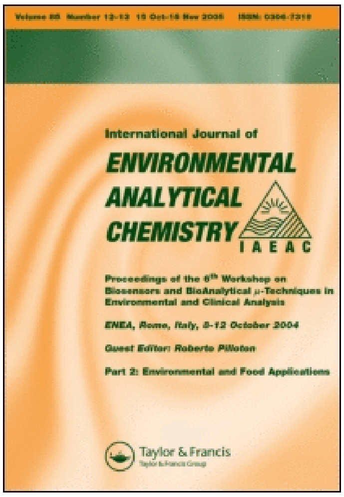

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Ammonium Determination in Water Samples by Using Opa-Nac Reagent: A Comparative Study with Nessler and Ammonium Selective Electrode Methods

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AMMONIUM DETERMINATION IN WATER SAMPLES BY USING OPA–NAC REAGENT: A COMPARATIVE STUDY WITH NESSLER AND AMMONIUM SELECTIVE ELECTRODE METHODS

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A selective and sensitive method based on the ammonium derivatisation with *o*-phthaldialdehyde (OPA) and *N*-acetyl-cysteine (NAC) has been developed for ammonium determination in real water samples. The proposed procedure has been compared with ammonium reference methods such as Nessler reagent method and ammonium selective electrode. All procedures have been chemometrically tested and compared in terms of the main analytical properties. These procedures have been used to determine ammonium in unknown water samples. The OPA–NAC reagent method does not present any systematic error (proportional or constant), while Nessler reagent presents both of them for some samples assayed. The ammonium selective electrode is free of corrigible systematic errors, however presents amine interference. The OPA–NAC ammonium method is able to achieve a detection limit (LOD) of 0.07 mg/L in the sample, with a linear dynamic range up to 1.4 mg/L of ammonium.

Keywords: Ammonium; OPA–NAC reagent; Real water samples; Nessler method; Selective electrode method

INTRODUCTION

Ammonium is a micronutrient in water systems. It has an important role in nitrogen cycle. When it is at high concentrations, the amount of nutrients increases in water systems and then there is an increase of biological activities, which lead to algae growth, turbidity, odour, bad taste and toxicity problems. Ammonium can be found in superficial, subterranean or marine waters at low concentrations, about 10 μg/L. In residual waters it can be found at high concentrations, about 30 mg/L due to ammonification and nitrate reduction processes.

Because of the importance of ammonium in natural processes in waters, it is necessary to have selective and sensitive methods for this determination. Standard

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reference methods such as the photometric detection of ammonia with Nessler reagent, indophenol reaction or ammonium selective electrode are widely applied [1]. Moreover, new methods have been developed in recent years. In Table I some analytical characteristics are summarised, such as detection limits, linear dynamic range and the technique employed, for some methods published in the literature. FIA methods have been the most outstanding in the last years, usually combined with another systems like flux collector [7], a liquid core waveguide fluorescence detector [9], chemiluminescence systems [10], fluorimetric detection based on the complexation of ammonium with *o*-phthaldialdehyde and thioglycolate [13], and photometric diode array detector with Nessler reagent [4]. Methods using ion chromatography [6,8], and ammonium selective electrodes [2,3,11,14] have also been described.

In this article, a new procedure based on the derivatisation of ammonia with *o*-phthaldialdehyde (OPA) and *N*-acetyl-cysteine (NAC) under alkaline conditions have been proposed, based on our previous studies over the reaction of amines with OPA–NAC [15–18]. The OPA reagent has been also mixed with substances like thioglicolate, mercaptoethanol or mercaptopropianate, in order to form isoindole derivatives under alkaline conditions. Other substances such as sulfite have been employed but more complex systems have been obtained [19]. The formed isoindole derivative with OPA–NAC is detected fluorimetrically at $\lambda_{excitation} = 415$ nm and $\lambda_{emission} = 485$ nm or $\lambda_{excitation} = 333$ nm and $\lambda_{emission} = 462.4$ nm. The optimal conditions have been established in order to obtain the highest sensitivity and selectivity. The results obtained

TABLE I Summary of some procedures described in the literature for ammonium determination

Author/Year	*Technique*	*LOD* (μg/L)	*Linear interval* (mg/L)
[H. Hara, 87] [2]	A washing method for flow-through determination of ammonium with an ion gas electrode.	–	0.1–10
[H. Hara, 88] [3]	Continuous flow determination of ammonium with a gas electrode detector.	3.85	3.857e-3–1.2857
[F. Cañete, 88] [4]	FIA system that inludes three detectors: potentiometric, conductimetric and photometric (DAP). Nessler reagent.	–	0.05–0.9
[Menezes-Santos 92] [5]	FIA system employing pre-concentration with cation-exchange resin. Based on Nessler reaction.	3	0.05–0.5
[H. Shen, 98] [6]	Ion chromatography with sensor array detector.	18	0.36–18
[Z. Genfa, 98] [7]	Fluorimetric flow injection analysis with flux collector. Based on OPA/sulfite/NH_4^+ reaction and pre-concentration.	0.054	9e-5–0.135
[Y. Huang, 00] [8]	Column switching ion chromatography	12.8	0.05–5
[J. Li, 99] [9]	Fluorimetric flow injection analysis with liquid core waveguide. Based on OPA/sulfite/NH_4^+ reaction	1.87	0.0107–0.0535
[W. Qin, 99] [10]	Chemiluminiscence flow system. Based on luminol reaction.	7.2	0.0535–5.35
[J.P. Hart, 99] [11]	Amperometric ion biosensor, based on a modified carbon electrode.	107	0.107–1.3375
[A. Walcarius, 99] [12]	Flow-injection indirect amperometric detection of ammonium ions. Clinoptilolite-modified electrode.	90	0.36–18
[H. Mana, 00] [13]	Fluorimetric flow injection analysis. Based on OPA/thioglycolate reaction.	–	2.675e-3–5.35
[Schwarz, 00] [14]	Ammonium selective electrodes.	0.155	0.18–1.8

are compared with those provided by Nessler reaction and ammonium selective electrode. Previously reviews of the different parameters involved in these reference methods have been performed.

The three methods assayed have been chemometrically tested in order to evaluate the presence or absence of bias error and to improve their uncertainties in unknown samples. The advantages and disadvantages of each one have been showed in terms of sensitivity, selectivity, accuracy and precision.

EXPERIMENTAL

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 pathlength, and a Hitachi F-4500 fluorescence spectrophotometer, respectively. Potential measurements were made on a Crison pH-meter furnished with a Crison gas-sensing ammonia electrode (Barcelona). The pH was measured with a Crison micropH 2000 pH-meter.

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 or 1000 mg/L).

OPA–NAC method: 8.8 mM OPA (Fluka chemika, Switzerland)/NAC (Fluka chemika, Switzerland) (1 : 1) ratio was prepared by dissolving previously OPA reagent with 5% of MeOH (Scharlau, Spain). Other solutions were prepared with a 10 : 1 ratio: 3.7–0.37 mM and 1.7–0.17 mM OPA–NAC 1.4% MeOH. 0.5 M Borate buffer solution at pH 8, 10.2, 10.6, 10.8 and 11.1 was prepared by dissolving an adequate amount of boric acid (Scharlau, Spain) in water and then adjusting the pH with NaOH (Panreac, Spain). Methylamine (Sigma, Germany), Ethylamine (Sigma, Germany), Isopropylamine (Baker, Holland) and β-phenylethylamine (Sigma, Germany) were also used.

Nessler method: Sodium–potassium tartrate solution (Panreac, Spain) was prepared by dissolving 50 g of the compound in 100 mL of nanopure water. Sodium hydroxide (Panreac, Spain) was prepared by dissolving 12 g of the compound in 50 mL of nanopure water. Nessler reagent (NR) was prepared by dissolving 10 g KI (Guinama, Spain) in the minimum amount of nanopure water. This solution was added to 0.22 M (50 mL) mercury chloride (Merk, Germany) solution until a red precipitate appeared. The resulting solution was decanted; and 40 mL of 4.5 M NaOH were added to the HgI_4^{2-} solution and diluted up to 100 mL. Two different reagent solutions were prepared: NR1 (with excess of KI solution [1]); NR2 (KI solution is added up to a red precipitate appeared [20]). Other analytical reagents used were EDTA (Probus, Spain), polyvinyl alcohol (Probus, Spain), Triton X-100, sodium sulfite (Probus, Spain) and zinc sulfate heptahydrate (Merk, Germany).

Selective electrode method: NaOH (Panreac, Spain) and the same amines used in OPA–NAC method were used.

Procedures

Standard Solutions

OPA–NAC reaction: In a quartz cuvette were placed variable volumes of ammonium stock solution, 0.9 mL of OPA–NAC reagent, 0.1 mL of 0.5 M borate buffer (pH = 10.8) and water up to constant volume of 2 mL. Based on our previous studies, concentrations 3.7 mM (1 : 1), 8.8 mM (1 : 1), 1.7 : 0.17 mM and 3.77 : 0.377 mM of OPA : NAC were selected as initial reaction conditions. A factorial design 3^2 was carried out at two pHs: 10.6(−) and 10.8(+); two OPA concentrations: 8.8 mM(−) and 15 mM(+); and two NAC concentrations: 8.8 mM(−) and 60 mM(+). 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 400–600 nm ($\lambda_{exc} = 415$ [13]) and 380–610 nm ($\lambda_{exc} = 333$) over a reaction time ranged 0–300 s. Signal was obtained at 120 s for $\lambda_{ex} = 333$ nm $\lambda_{em} = 462.4$ nm, and at 300 s for $\lambda_{ex} = 415$ nm $\lambda_{em} = 485$ nm. All measurements were performed at 25°C.

Nessler reaction: In a plastic cuvette were placed variable volumes of ammonium stock solution, 10 μL of 0.177 M sodium–potassium tartrate and water up to constant volume (2.4 mL). 0.1 mL of Nessler reagent was added to the mixture, and 0.1 mL of NaOH was added when NR2 was used. 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. Absorbance signal was measured against water blank. All measurements were performed at 25°C.

Selective electrode method: 0.25 mL of 10 M sodium hydroxide was added to 25 mL of standard or sample. Potential measurement was registered when potential value remains constant one minute with time. Ammonium calibration curve was registered between 0 and 100 mg/L.

Water Samples

The performance of the method was tested with unspiked and spiked nanopure water and water samples with unknown ammonium concentration. The environmental water samples were named as S1: Irrigation ditch sample, S2: Residual water from a factory, S3: Fountain water.

Sample treatment: The samples were treated according to the reference standard method [1]. A 100 mL portion of water (real or nanopure) was subjected to sample treatment by adding 1 mL of dechlorant 7.14 mM sodium sulphite and 1 mL of 0.348 M zinc sulphate heptahydrated. The precipitate formed was filtered and the first 25 mL were wasted. The mixture pH was adjusted to 2 with concentrated H_2SO_4. When OPA–NAC method was applied, the sample pH was adjusted to 10.5.

For selective electrode method, direct measures were carried out. Variable sample volumes (7–20 mL) were diluted up to 25 mL with nanopure water. The final volume was processed following the procedure described for standard solutions.

Standard Addition and Youden Methods

OPA–NAC reaction: Volumes of treated sample (0.8 mL) were used for the Standard Addition Method (MOSA) [21] to which variable volumes of ammonium standard solution were added. These volumes were processed as it was described for standard solutions. (Ammonium added concentration in the final volume (2 mL) was between 0 and 1.4 mg/L).

For the Youden Method [22], volumes of water sample ranged from 0.6 up to 1 mL were processed. Small volumes were employed (0.05–0.125 mL) for samples with high ammonium concentration.

Nessler reaction: Volumes of sample (treated or not) of 1.5 and 2.4 mL were used for the Standard Addition Method (MOSA), to which variable volumes of ammonium standard solution were added. These volumes were processed as it was described for standard solutions. (Ammonium concentration in the final volume was between 0 and 2.5 mg/L.) The two volumes used for MOSA were also employed for the Youden Method [22].

Small sample volumes were processed (between 0.05 and 0.2 mL) in samples with high ammonium concentration (*ca.* S2).

Selective electrode method: MOSA method was applied to samples with very low ammonium concentration (*ca.* S1).

RESULTS AND DISCUSSION

OPA–NAC Method

Fluorescence emission spectra of a standard ammonium and blank solutions at (a) $\lambda_{ex} = 415$ nm or (b) $\lambda_{ex} = 333$ nm are shown in Fig. 1. At $\lambda_{ex} = 415$ nm $\lambda_{em} = 485$ nm, signal increases as time increases and reaches a plate at 5 min, meanwhile at $\lambda_{ex} = 333$ nm $\lambda_{em} = 462.5$ nm signal decreases as time increases (Fig. 2). From this study it can be concluded that the wavelength selected is an important parameter. In order to obtain higher sensitivity, five minutes for $\lambda_{ex} = 415$ nm and two minutes for $\lambda_{ex} = 333$ nm were selected for further experiments. The optimal conditions (8.8 mM OPA–NAC (1 : 1) ratio and pH = 10.8) were obtained from the results of the factorial design (see Tables II and III) carried out at two pHs: 10.6(−) and 10.8(+); two OPA concentrations: 8.8 mM(−) and 15 mM(+); and two NAC concentrations: 8.8 mM(−)

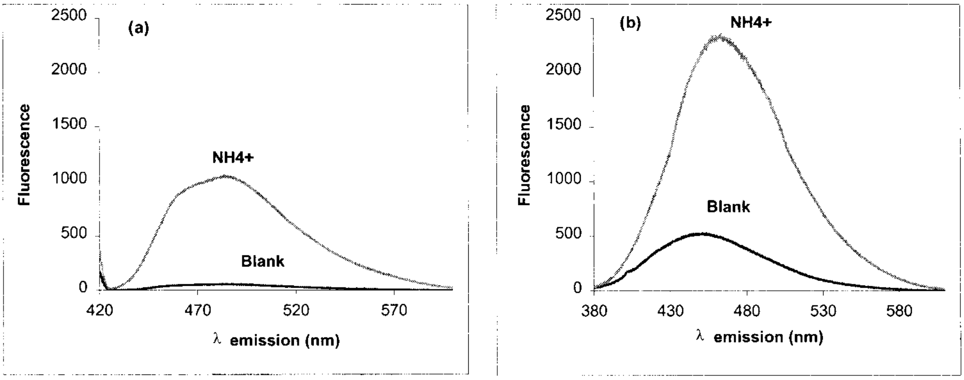

FIGURE 1 Fluorescence emission spectra for a blank and standard ammonium solution at (a) $\lambda_{ex} = 415$ nm and (b) $\lambda_{ex} = 333$ nm. Conditions: 8.8 mM OPA–NAC (1 : 1) in borate buffer pH 10.8.

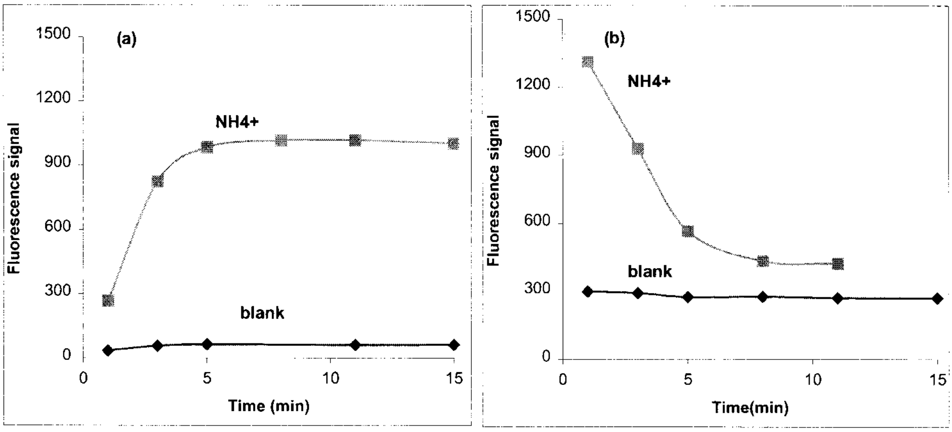

FIGURE 2 Fluorescence signal *versus* time for blank and standard ammonium solution at (a) $\lambda_{ex} = 415\,nm$ $\lambda_{em} = 485\,nm$; (b) $\lambda_{ex} = 333\,nm$ $\lambda_{em} = 462.4\,nm$. Conditions: 8.8 mM OPA–NAC (1 : 1) in borate buffer pH 10.8.

TABLE II Factorial design to obtain the optimum pH, OPA and NAC concentration

	PH	*OPA*	*NAC*
1	+	−	−
2	+	+	−
3	+	+	+
4	+	−	+
5	−	−	−
6	−	+	−
7	−	+	+
8	−	−	+

and 60 mM(+). The importance of the factors was: [NAC] > [OPA] > pH. The results for factors interaction were: OPA–NAC > pH–NAC. pH–OPA interaction was not relevant.

In Table IV are summarised the analytical parameters corresponding to ammonium determination. In order to register the two spectra from the same sample, 2 min were selected at $\lambda_{ex} = 333\,nm$ $\lambda_{ex} = 462.4\,nm$, and 5 min at $\lambda_{ex} = 415\,nm$ $\lambda_{em} = 485\,nm$.

Due to the amine reactivity with OPA–NAC reagent, the interference of these compounds was studied. Aliphatic amines such as methylamine, ethylamine, isopropylamine and alquilaryl amines as β-phenylethylamine were mixed with the reagent following the procedure described above. At $\lambda_{ex} = 415\,nm$, none of them gave an important fluorescence signal. As an example, the calibration curve of ammonium in presence of methylamine (0.1 mg/L) using $\lambda_{ex} = 415\,nm$, $\lambda_{em} = 485\,nm$ ($t = 5\,min$) is shown in Table IV. However, at $\lambda_{ex} = 333\,nm$, $\lambda_{em} = 462.4\,nm$ ($t = 2\,min$), the calibration curve obtained has similar slope but different ordinate to that obtained for ammonium. For high amine concentrations (*ca.* 15 mg/L for methylamine, and 30 mg/L for ethylamine and isopropylamine), did not find relevant signals at 415 nm.

TABLE III Importance of factors and interaction between pH–OPA and NAC concentration (reference value: 14.88)

Factor of interaction	*D value*	*Conclusion*	
PH	32.82	$D_{pH} > D_{reference}$	Three important factors: [NAC] > [OPA] > pH
OPA	58.65	$D_{OPA} > D_{reference}$	
NAC	976.50	$D_{NAC} > D_{reference}$	
pH–OPA	11.26	$D_{pH\text{–}OPA} < D_{reference}$	Not important interaction
pH–NAC	32.17	$D_{pH\text{–}NAC} > D_{reference}$	Two important interactions: OPA–NAC > pH–NAC
OPA–NAC	58.34	$D_{OPA\text{–}NAC} > D_{reference}$	

TABLE IV Ammonium calibration curves and its figures of merit by using OPA–NAC reagent. (**) Ammonium calibration in presence of 1 mg/L of methylamine. Conditions: 8.8 mM OPA–NAC (1 : 1) in borate buffer pH 10.8

Conditions	*Time* (min)	$a \pm s_a$	$b \pm s_b$ $(n, r^2, s_{y/x})$	*DL* (mg/L)	*QL* (mg/L)	*SDP* $(s_{y/x}/b)$
OPA–NAC 8.8 mM (1 : 1), $\lambda_{ex} = 415$ nm	5	48 ± 11	759 ± 7 (10, 0.9983, 17)	0.07	0.2	0.02
OPA–NAC 8.8 mM (1 : 1), $\lambda_{ex} = 333$ nm	5	610 ± 30	360 ± 40 (6, 0.943, 60)	0.5	1.6	0.16
OPA–NAC 8.8 mM (1 : 1), $\lambda_{ex} = 333$ nm	2	450 ± 50	1380 ± 70 (6, 0.9899, 90)	0.19	0.6	0.06
(**) OPA–NAC 8.8 mM (1 : 1), $\lambda_{ex} = 415$ nm	5	60 ± 11	737 ± 16 (5, 0.9985, 18)	–	–	–
(**) OPA–NAC 8.8 mM (1 : 1), $\lambda_{ex} = 333$ nm	2	1380 ± 110	1380 ± 160 (5, 0.962, 180)	–	–	0.13

Confirmation Studies about OPA–NAC Method *vs* Nessler Reagent Method and Ammonium Selective Electrode Method

Nessler reagent method for standards: Although the use of the *Nessler reaction* is a well-established method for ammonium determination, the parameters that can affect the reaction were previously reviewed. According to the literature, the Nessler reagent was prepared by two different ways (see experimental section). Working with standard solutions, no differences were obtained between them, however 0.1 mL of NaOH 6 N was added when NR2 was used. Variable amounts of Nessler reagent (50–300 μL) were assayed and 100 μL of Nessler reagent was chosen. Absorbance values increase as time increases and became stable from 200 to 600 s.

According to the literature [1], EDTA is added in order to avoid interferences. However, the absorbance signal disappeared when this reagent was added to the reaction mixtures with standards. In presence of other recommended agent, such as sodium–potassium tartrate, the absorbance signal remains stable. The influence of tartrate addition to the mixture reaction in the range 0.709–14.17 mM was studied and the addition of 10 μL (0.177 M tartrate) was selected as optimum amount.

For high ammonium concentrations (*ca.* 2.5 ppm) the base line drift due to the appearance of turbidity. In order to eliminate the precipitate formed, the addition of polyvinylalcohol or Triton X-100 was studied, but any of them could make the precipitate disappeared.

Table V shows the figures of merit for five different conditions and different reaction times. Similar detection limit, quantification limit and standard deviation of procedure were obtained in all the studied conditions.

Selective electrode method for standards: The ammonium calibration curve was linear when E(mV) was represented in front of LOG($C_{NH_4^+}$) (Fig. 3(a)-1). The effect of amine compounds on the signal E(mV) was studied. The calibration curve of each amine in the concentration range 0–15 mg/L was obtained. As can be seen in Fig. 3(b), the amine calibration curves followed a polynomic behaviour when E(mV) was represented in front of LOG[NH_4^+]. This behaviour was different for each amine. The amine calibration curve in presence of a constant amount of ammonium (1 mg/L) was also performed. The slope obtained in presence of ammonium was different to that obtained for amine standard solutions (Fig. 3(c)).

As can be seen in Fig. 3(a)-2, different behaviour was also observed when a constant amine concentration (methylamine *ca.* 1 mg/L) was added to the calibration ammonium solutions. In this case, the results were adjusted to a polynomial function. Based on these results we conclude that the ammonium selective electrode method shows bias error in presence of amines (even at low level of 1 ppm).

Analysis of Water Samples

Water samples from different environment were processed following the procedures describe above. The samples were previously treated according to the procedure described in the experimental section [1].

TABLE V Ammonium calibration curves and its figures of merit by using Nessler reagent method

Conditions	*Time* (s)	$(b \pm s_b)$	$(a \pm s_a)$	$s_{y/x}$	r^2	*n*	*DL* (mg/L)	*QL* (mg/L)	*SDP* $(s_{y/x}/b)$
100 µL NR1, without tartrate	600	(0.216 ± 0.003)	(0.041 ± 0.006)	0.013	0.9972	9	0.3	1.1	0.11
100 µL NR1, 0.0708 M tartrate	0	(0.123 ± 0.003)	(0.044 ± 0.008)	0.014	0.9967	8	0.4	1.2	0.12
	210	(0.123 ± 0.003)	(0.049 ± 0.008)	0.015	0.9967	8	0.4	1.2	0.12
	390	(0.123 ± 0.003)	(0.052 ± 0.007)	0.014	0.9970	8	0.3	1.1	0.11
	600	(0.123 ± 0.003)	(0.054 ± 0.007)	0.013	0.9973	8	0.3	1.1	0.11
100 µL NR1, 0.708 mM tartrate, treated nanopure water	0	0.130 ± 0.007	0.096 ± 0.009	0.014	0.9922	5	0.3	1.1	0.11
	120	0.125 ± 0.006	0.105 ± 0.008	0.012	0.9939	5	0.3	1.0	0.10
50 µL NR1, 0.708 mM tartrate	600	(0.1018 ± 0.0011)	(0.0266 ± 0.0017)	0.003	0.9998	4	0.08	0.3	0.03
100 µL NR2, 100 µL NaOH, 0.708 mM tartrate	0	(0.125 ± 0.003)	(0.010 ± 0.009)	0.014	0.9978	5	0.3	1.2	0.12
	210	(0.131 ± 0.006)	(0.018 ± 0.015)	0.02	0.9945	5	0.6	1.8	0.18
	390	(0.132 ± 0.006)	(0.018 ± 0.015)	0.02	0.9944	5	0.6	1.9	0.19
	600	(0.132 ± 0.06)	(0.018 ± 0.015)	0.02	0.9942	5	0.6	1.9	0.19
100 µL NR2, 100 µL NaOH, 0.708 mM tartrate, treated nanopure water	0	0.1196 ± 0.0017	0.011 ± 0.005	0.007	0.9993	5	0.18	0.6	0.06
	210	0.125 ± 0.005	0.020 ± 0.012	0.019	0.9961	5	0.5	1.5	0.15
	390	0.126 ± 0.005	0.022 ± 0.013	0.02	0.9957	5	0.5	1.6	0.16
	600	0.127 ± 0.005	0.023 ± 0.013	0.02	0.9957	5	0.5	1.6	0.16
100 µL NR2, 100 µL NaOH, 0.708 mM tartrate, 100 µL PVA	0	(0.111 ± 0.007)	(0.062 ± 0.019)	0.03	0.9880	5	0.8	2.7	0.27
	210	(0.117 ± 0.004)	(0.089 ± 0.011)	0.017	0.9964	5	0.4	1.5	0.15
	390	(0.116 ± 0.004)	(0.098 ± 0.011)	0.019	0.9958	5	0.5	1.6	0.16
	600	(0.115 ± 0.005)	(0.103 ± 0.013)	0.02	0.9948	5	0.5	1.8	0.18

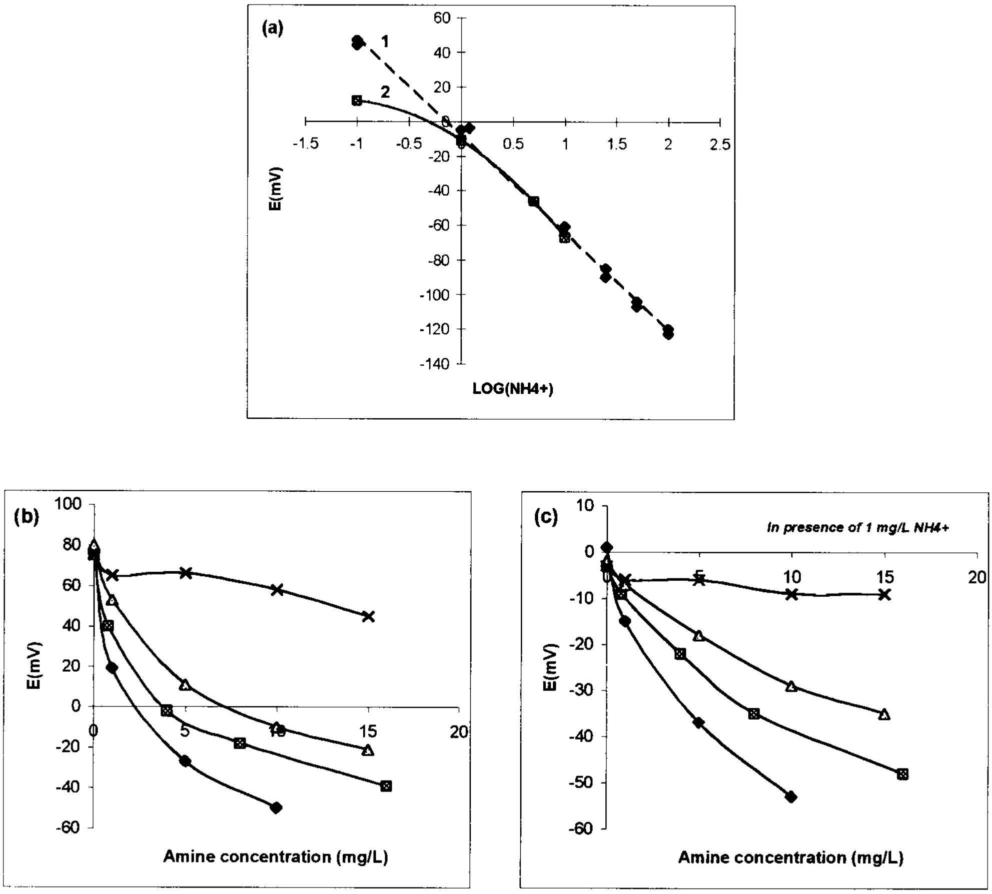

FIGURE 3 Calibration curves: E(mV) *vs* LOG(C_{con}) (a) Ammonium (1) and Ammonium in presence of constant amount of methylamine (1 mg/mL) (2). (b) Amine calibration curves (◆ methylamine, ■ ethylamine, ▲ isopropylamine, × β-phenylethylamine). (c) Amine calibration curves in presence of constant amount of ammonium (1 mg/L) (◆ methylamine, ■ ethylamine, ▲ isopropylamine, × β-phenylethylamine).

Application of Nessler reaction: The effect of sample treatment was studied. This treatment does not affect the results in *nanopure water* samples. A *t*-test reveals that there are not any meaningful difference between these slopes and the slopes of the calibration graph with standards (see Table V) of ammonium because α was higher than 0.05.

The MOSA and Youden methods were applied to the samples in order to evaluate the presence or absence of systematic errors.

The MOSA slopes obtained for all real water samples assayed were statistically different to that obtained with ammonium standard solutions. In all cases the *t*-test gave $\alpha < 0.05$, which indicated that slopes were different, so a matrix effect was present in all samples. The slopes of the MOSA were dependent on the sample volume processed, 1.5 or 2.4 mL, as can be seen in Fig. 4.

In order to evaluate the matrix constant error, Youden method [22] was applied. Sample S2 showed Youden effect and *t*-test revealed that ordinate was statistically different from zero from $t = 210$ to 390 s being $\alpha < 0.05$. The Youden graph is shown

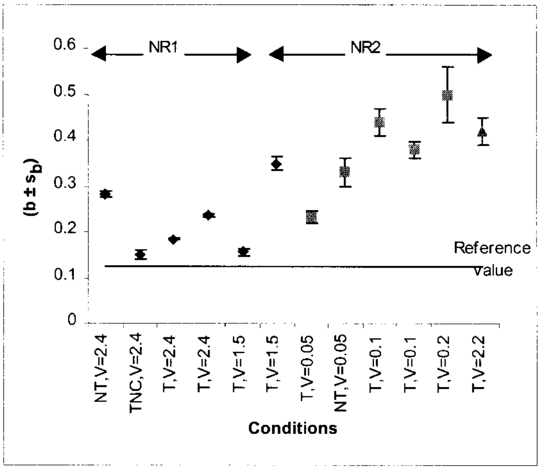

FIGURE 4 Slopes ($b \pm s_b$) of calibration curves applying Nessler reagent method *versus* different conditions studied. (◆) Sample 1 (▲) Sample 2 (■) Sample 3. Conditions: NR1 or NR2 (Nessler reagent 1 or 2); T: treated sample; NT: non-treated sample; TNC: treated without dechlorant; V: Sample volume (mL); Total volume = 2.5 mL.

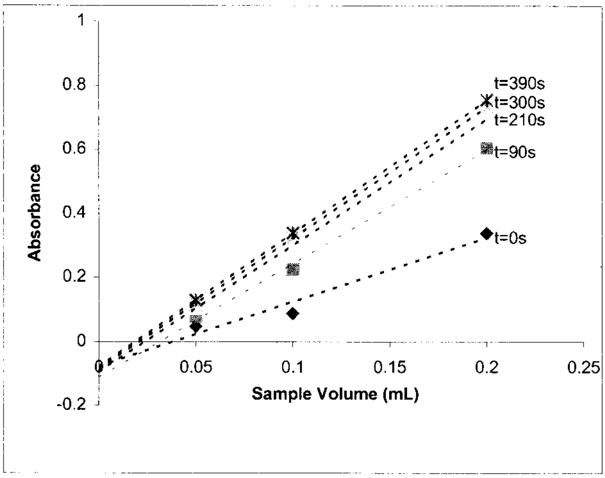

FIGURE 5 Youden graph at different times (0–390 s). Conditions: Variable sample volume, Nessler reagent (NR2) 0.1 mL, 6 N NaOH 0.1 mL, 0.177 M tartrate 0.01 mL and water sample up to 2.5 mL.

in Fig. 5. The TYB (Total Youden Blank) value obtained was taken into account in the ammonium concentration determination.

The ammonium concentration in the samples must be calculated by applying MOSA or alternatively by GHPSAM [23] that allows to determine the analyte concentration in unknown samples (see appendix). Table VI shows the found concentrations in the different samples by applying MOSA and GHPSAM, and similar results were obtained by using both methods. Then, only the matrix effect is present and other kind of interference is absent.

TABLE VI Found ammonium concentration in real samples by applying different methods

Sample	*Method*	*By*	*Time* (s)	*Conc* (mg/L)	*Concentration* ± *s*
S1	Selective electrode	Direct measurement	–	0.09012	(0.089 ± 0.002)
		Direct measurement	–	0.09026	
		Direct measurement	–	0.08665	
	Nessler	Standard addition	210	0.154499	(0.166 ± 0.010)
		Standard addition	300	0.172495	
		Standard addition	390	0.172089	
		GHPSAM	210	0.17333	(0.19 ± 0.03)
		GHPSAM	300	0.22533	
		GHPSAM	390	0.17333	
	OPA–NAC	Standard addition ($\lambda_{ex} = 415\,nm$)	300	0.19844	(0.18 ± 0.02)
		Standard addition ($\lambda_{ex} = 333\,nm$)	120	0.1688	
S2	Selective electrode	Direct measurement	–	15.6375	(16.8 ± 1.0)
		Direct measurement	–	16.96178	
		Direct measurement	–	17.66537	
	Nessler	Standard addition	210	17.35917	(18.0 ± 0.6)
		Standard addition	300	18.10647	
		Standard addition	390	18.57086	
		GHPSAM	210	16.75	(17.1 ± 0.5)
		GHPSAM	300	–	
		GHPSAM	390	17.5	
	OPA–NAC	Standard addition ($\lambda_{ex} = 415\,nm$)	300	14.21945	(14.10 ± 0.17)
		Standard addition ($\lambda_{ex} = 333\,nm$)	300	13.97394	
S3	Selective electrode	Direct measurement	–	0.42287	(0.45 ± 0.02)
		Direct measurement	–	0.45867	
		Direct measurement	–	0.45867	
	Nessler	Standard addition	210	0.301386	(0.323 ± 0.019)
		Standard addition	300	0.337916	
		Standard addition	390	0.329671	
	OPA–NAC	Standard addition ($\lambda_{ex} = 415\,nm$)	300	0.3842	(0.37 ± 0.02)
		Standard addition ($\lambda_{ex} = 333\,nm$)	120	0.3523	

Application of Selective Electrode Method

The concentrations found in the real samples are shown in Table VI. For all samples the ammonium concentration could be determined by direct measurement. MOSA method was applied to irrigation ditch water (S1) due to its low concentration in ammonium ion and no matrix effect was observed. The slope obtained by applying MOSA (slope $= -56.3 \pm 0.5$) was statistically equal to that obtained by applying calibration graph (slope $= -56.6 \pm 1.2$) with ammonium standards. Then, the selective electrode provided smaller ammonium concentration for sample S1 than Nessler method, probably due to the presence of interferent species. For sample S2 the results were similar and for sample S3 slightly higher.

Application of OPA–NAC Method

In order to determine whether the TYB values were zero consistent, student's *t* was calculated. For the samples S1, S2 and S3 the application of Youden Method gave an intercept statistically equal to zero at $\lambda_{ex} = 415\,nm$ $\lambda_{em} = 485\,nm$. At $\lambda_{ex} = 333$ $\lambda_{em} = 462.4\,nm$ sample S2 showed a TYB value.

The MOSA application (Table VII) indicated the absence of matrix proportional bias error in S1 and S3 samples, because *t*-test reveal that slopes are meaningfully similar to that of the calibration curve with standards. Based on these results, for these samples, the ammonium concentration can be calculated by using the calibration graph with standards. *Residual water* (S2) presented matrix effect at $\lambda_{ex} = 333\,\text{nm}$ but not at $\lambda_{ex} = 415\,\text{nm}$, then, sample S2 can be analysed by using $\lambda_{ex} = 415\,\text{nm}$ without bias error and using also the calibration graph obtained with standards. Ammonium concentrations found in the samples are shown in Table VI.

A paired *t*-test was applied in order to compared the results obtained by the different methods. In all cases the t_{cal} were lower than the t_{tab} and the α values were > 0.05, being 0.213, 0.190 and 0.113 for OPA–NAC *vs* Electrode selective, OPA *vs* Nessler, and Electrode selective *vs* Nessler, respectively. Therefore, it can conclude that there are not meaningful differences between the three methods studied. The precision are comparable with RSD values ranged from 15 to 3%, 16 to 5% and 18 to 1% for Nessler, ammonium selective electrode and OPA–NAC methods, respectively.

In Table VIII the aim properties of each method are shown. The detection and quantification limits obtained by using OPA–NAC methods are lower than that obtained by Nessler or selective electrode methods. The dynamic ranges for the three methods are also shown in Table VIII. Nessler reagent method has the disadvantage of the formation of a precipitate when we are working with the real samples, and systematic error is detected (constant and proportional). So, the MOSA or GHPSAM methods are required in order to calculate the ammonium concentration. In presence of constant error the TYB value must be also evaluated. Meanwhile in OPA–NAC method has not matrix effect, so ammonium concentration can be calculated from the calibration graph with standards. Ammonium selective electrode allows determining ammonium by direct measurement of the sample, however it has the disadvantage of amine interference with non-additive signals. Furthermore, OPA–NAC method has resulted

TABLE VII Standard addition curves for real samples by using OPA–NAC method. Conditions: 8.8 mM OPA–NAC (1 : 1) in borate buffer pH 10.8

Sample	λ_{ex}(nm)	$a \pm s_a$	$b \pm s_b$ (n, r^2, $s_{y/x}$)
S1	415	103 ± 17	710 ± 20 (6, 0.9958, 30)
S1	333	550 ± 50	1380 ± 70 (6, 0.99, 90)
S2	415	466 ± 4	688 ± 5 (6, 0.9997, 7)
S2	333	3999 ± 16	480 ± 20 (5, 0.9943, 30)
S3	415	62 ± 12	803 ± 17 (6, 0.9983, 20)
S3	333	540 ± 40	1440 ± 60 (6, 0.9932, 80)

TABLE VIII Some analytical properties of Nessler reagent, selective electrode and OPA–NAC methods. The higher the number of asterisks (*), the higher is the characteristic

Analytical properties	*Methods*		
	Nessler	*Selective electrode*	*OPA–NAC*
Dynamic range	0.85–5 mg/L	5–100 mg/L	0.2–1.4 mg/L
Detection limit	0.6 mg/L	1.6 mg/L	0.07 mg/L
Reproducibility	**	**	**
Selectivity	Selective method	Amine interference	Selective method
Cost	**	*	**
Rapidity	4 sample/h	7 samples/h	7 samples/h

selective at $\lambda_{ex} = 415\,nm$ without having registered any interference, and ammonium concentration can be calculated directly form the calibration graph with standards.

In the three methods, the analytical signals vary with time. In selective electrode method, signal stabilisation is faster when high ammonium concentrations are being measured; therefore, potential value was registered when signal remains stable for a minute. In Nessler reagent method, the signal is registered at ten minutes in order to obtain stable signals; in OPA–NAC method, five minutes have been selected as reaction time. Taking into account all these considerations, it seems that OPA–NAC reagent can be used for ammonium determination, providing a rapid, selective and sensitive procedure. The detection limit reached by this procedure is lower than that required by the Official Journal of the European Communities for quality of water for human consumption.

CONCLUSIONS

In this article has been proposed the use of the mixture OPA–NAC as derivating reagent for ammonium. The highly fluorescent isoindol formed is detected selectively in presence of amines. The optimised procedure has been compared with Nessler reagent, which have been previously reviewed, and with selective electrode method.

The application of Nessler reagent to real samples offered some problems, such as precipitation formation and matrix effect. In order to calculate the ammonium concentration, the MOSA or GHPSAM was required. On the other hand, the application of selective electrode method to real samples offers the problem of the non-additive interference of amines.

However, by using the OPA–NAC procedure, ammonium concentration can be calculated from the calibration graph with standards, taking into account the TYB value (only when it will be present). This procedure has demonstrated advantages over other procedures and could be suitable for ammonium determination in water samples.

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APPENDIX A: GHPSAM THEORETICAL BACKGROUND

The GHPSAM makes it possible to estimate the concentration of an analyte in presence of an unknown interferent. The first step to apply the GHPSAM is the location of linear intervals in the interferent spectrum.

Let us assume that X is the analyte or the selected form of it to be determined and Z is the unknown global interference. If the spectral behaviour of the interferent $Z(A_{Zj})$ in the range of wavelengths $\lambda_l - \lambda_m$, can be described as a straight line with an a intercept and a b slope then it can be written:

$$A_{Z,j} = a + b\lambda_j \quad \lambda_j \in [\lambda_l, \lambda_m] \tag{A.1}$$

The absorbance of the sample S at each wavelength in the interval selected will be the sum of the absorbance of X at a concentration c_X and of Z:

$$A_{S,j} = A_{X,j} + A_{Z,j} = M_{X,j}c_X + a + b\lambda_j \tag{A.2}$$

where $A_{X,j}$ is the absorbance at λ_j of X and M_j is the molar absorption coefficient and the optical path product (or related measure) at λ_j of the analyte X.

The second derivative absorbance of the sample with regard to the wavelength in this interval is:

$$A''_{S,j} = \frac{d^2 A_{X,j}}{d\lambda^2} + \frac{d^2 A_{Y,j}}{d\lambda^2} = M''_j c_X \tag{A.3}$$

Equation (A.3) can be re-written as:

$$\frac{A''_{S,j}}{M''_j} = c_X \tag{A.4}$$

Thus, when plotting the values of the ratio $A''_{S,j}/M''_j$ *vs* λ_j constant values equal to the analyte concentration will be obtained in those intervals where the interferent spectrum presents a linear behaviour.

Three wavelengths λ_j, λ_k and λ_l within the interferent linear interval [λ_l, λ_m] must be selected to calculate the concentration of the analyte. The absorbance of the sample at those wavelengths, considering that the standard addition method has been followed, can be written as:

$$\begin{aligned}A_{S,j} &= M_{X,j}\, c_X^0 + M_{X,j}\, c_X^i + a + b\lambda_j \\ A_{S,k} &= M_{X,k}\, c_X^0 + M_{X,k}\, c_X^i + a + b\lambda_k \\ A_{S,l} &= M_{X,l}\, c_X^0 + M_{X,l}\, c_X^i + a + b\lambda_l\end{aligned} \tag{A.5}$$

where c_X^0 is the analyte concentration in the sample, c_X^i is the analyte added concentration (the i superscript denotes the different standard additions) and $M_{X,j}$, $M_{X,k}$ and $M_{X,l}$ are the slopes of the calibration lines (or the molar absorption coefficients or related measure) at λ_j, λ_k and λ_l of the analyte X.

Two parameters p and q, can be defined as:

$$p = \frac{\lambda_k - \lambda_j}{\lambda_l - \lambda_j} \qquad q = \frac{\lambda_l - \lambda_k}{\lambda_l - \lambda_j} \tag{A.6}$$

and also two lines can be defined as the weighted differences between $A_{S,j}$ and $A_{S,l}$ and between $A_{S,j}$ and AS,k.

$$\begin{aligned}q\Delta A_{S,k,j} &= q(A_{S,k} - A_{S,j}) = q\Delta M_{k,j}\, c_X^0 + q(A_{Z,k} - A_{Z,j}) + q\Delta M_{k,j} c_X^i \\ p\Delta A_{S,l,k} &= p(A_{S,l} - A_{S,k}) = p\Delta M_{l,k}\, c_X^0 + p(A_{Z,l} - A_{Z,k}) + p\Delta M_{l,k} c_X^i\end{aligned} \tag{A.7}$$

These two lines permit the calculation of the concentration of the analyte from the abscissa of their intersection point, the so-called H point $(-c_H, \Delta A_H)$, where c_H is equal to c_X^0, the analyte concentration in the sample:

$$-c_H = \frac{q\Delta A_{S,k,j} - p\Delta A_{S,l,k}}{q\Delta M_{k,l} - p\Delta M_{l,k}} = \frac{A_{X,k}^0 - qA_{X,j}^0 - pA_{X,l}^0}{qM_j + pM_l - M_k} \tag{A.8}$$

From this expression we can optimise the wavelengths (λ_j, λ_k and λ_l) to be those that make bigger the denominator in Eq. (A.8), in order to obtain the most accurate results.