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Influence of the presence of surfactants and humic acid in waters on the indophenol-type reaction method for ammonium determination

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Abstract

This work has evaluated the influence of humic acid and/or surfactants in the quantification of ammonium in waters with the indophenol-type reaction method. Thymol has been employed with the colorimetric method for sample ammonium concentrations between 0.25 and 1 mg L^{-1}. In addition, SPE/diffuse reflectance method has been used for quantification of ammonium at low concentrations (between 0.025 and 0.25 mg L^{-1}). Matrix effect owing to humic substances were observed with the colorimetric method when the concentration was equal or higher than 25 mg L^{-1}. Lower concentrations of humic compounds produced matrix effects with the more sensitive SPE/diffuse reflectance method. Generalized H-point standard addition method (GHPSAM) was employed for evaluating the effect of humic acids and for eliminating the bias error produced by humic acids. Real water samples of different types were analyzed and accurate results for ammonium concentration were obtained with both procedures in presence or absence of humic acids. Cationic and anionic surfactants inhibited the derivatization reaction at percentages up to 0.001% and 0.5%, respectively, and non-ionic surfactants at percentages of 5.5%

Keywords: Surfactants; Humic acid; Ammonium; Water

1. Introduction

The most widely accepted method for the determination of ammonium in natural waters is based on the Berthelot reaction [1]. In this method the samples are treated with phenol and an oxidizing agent, typically chlorine, to transform ammonium into indophenol blue, which is then determined colorimetrically. The original method has been subjected to numerous modifications, for example the replacement of phenol by less toxic reagents such as thymol or salicylate, and the addition of a catalyst [2,3]. We have recently described a modification for improving the sensitivity based on isolation onto SPE membranes of the indothymol blue formed and subsequent measurement of the diffuse reflectance [4]. Interferences in the determination of ammonium by the indophenol-type reactions caused by potential interferents such as aliphatic amines, peptides, aminoacids and metals have been systematically evaluated. Table 1 shows the interferents studied in literature [2,4–6].

On the other hand, nowadays, surfactants are used in a wide range of applications. They are used in agriculture, households and different industries (metal working, mining, cosmetics, etc.). Thus, they could be present in natural [7], waste [8] and coastal [9] waters samples. They also has been proposed to remove humic acids from water samples [10,11], so they could be present in water after water-treatment plants. In addition they could interfere in the determination of some organic compounds [12] by interacting with the reagents, by masking the analyte, by modifying the signal or by reducing the recovery of the analyte.

Humic substances (HA) are the most abundant dissolved organic compounds present in natural waters. The normal content of these compounds could vary from 1 to 20 mg L^{-1} [13,14]. However, in some cases higher concentrations can be found [15]. Humic compounds are formed in soil and in aquatic systems during the degradation of plant and animal derived organic matter, and they play an important role in several environmental and geochemical processes [16]. They are generally characterized as being yellow to black in color. The structure of humic substances is believed to be a crosslinked network of organic chains (from 52% to 62% of carbon) containing functional groups such

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Table 1
Studied interferents for the indophenol-type reaction method in the literature

Reagent	Sudied interferents	References
Salicylate/dichloroisocyanurate	Primary amines Aminoacids Peptides	[5]
Thymol/HClO	Cations: Na^+, K^+, Ca^{2+}, Mg^{2+}, Al^{3+}, Fe^{3+}, Cu^{2+} Anions: $SO_4{}^{2-}$	[2]
Thymol/HClO	Cations: Na^+, K^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Al^{2+}, Fe^{3+}, Cu^{2+} Anions: Cl^-, $SO_4{}^{2-}$, $NO_3{}^-$, $NO_2{}^-$, $PO_4{}^{3-}$	[6]
Thymol/HClO	Cations: Na^+, K^+, Mn^{2+}, Hg^{2+}, Fe^{3+}, Cu^{2+} Anions: Cl^-, $SO_4{}^{2-}$, $NO_3{}^-$ Primary amines Secondary amines	[4]

as carboxylic, aliphatic, aromatic, hydroxyl and amino, with empty spaces that can trap and bind other organic and inorganic compounds present in aquatic system [17]. For this reason the determination of several compounds potentially present in natural waters may be affected by their possible association with humic substances. For example, an important fraction of metals is complexed by humic substances, which should be considered especially in the determination of metals at trace levels [18].

Different strategies have been proposed for eliminating the negative effect produced by humic substances on the determination compounds of environmental relevance such as pesticides [19,20], polycyclic aromatic hydrocarbon [14] and several metals. Most of those methods are based on the isolation or the degradation of humic matter to release the target compounds during the sample treatment [21]. However, the presence of humic compounds has been not taken in consideration in many other determinations of environmental relevance. This is the case of ammonium which is an important indicator of water quality.

To our knowledge, no similar studies have been undertaken with the humic substances and surfactants. Therefore, in this work we evaluated the effect of humic substances and surfactants present in water samples in the quantification of ammonium at levels between 0.025 and 1.5 mg L^{-1}. SPE–diffuse reflectance approach has been used in order to study the low concentrations of ammonium tested.

2. Experimental

2.1. Apparatus

For UV–vis measures a HP-8453 UV–vis spectrophotometer from Hewlett-Packard (Palo Alto, CA, USA) furnished with 1-cm pathlength quartz cells were employed, absorption spectra were registered from 400 to 800 nm. Humic acid spectra were recorded from 200 to 900 nm.

Reflectance measures were done with the UV–vis spectrophotometer combined with a Labsphere RSA-HP-8453 reflectance spectroscopy accessory (d/8°) from Labsphere (Hewlett-Packard). Diffuse reflectance spectra were collected from 400 to 800, using a certified reflectance standard USRS-99-010 from Labsphere (Hewlett-Packard)

2.2. Chemicals and solutions

All the reagents were of analytical grade, and all solutions were prepared in nanopure water from a Nanopure II system (Barnstead, USA). Ammonium chloride was purchased from Probus (Badalona, Spain). Humic acid was obtained from Aldrich (Steinheim, Germany). Stock standard solution of ammonium and humic acids (100 mg L^{-1}) were prepared by dissolving the pure reagents in water. Working standard solutions were prepared by diluting the stock solutions with water.

Sodium hypochlorite 15% was obtained from Probus. Solution of 3% and 5% (active chlorine) were prepared by diluting the adequate amount with water. Thymol (Riedel-de Häen, Seelze, Germany) 0.8 M was prepared dissolved the adequate amount in 2 M NaOH (J.T. Baker, Deventer, Holland). Nitroprusside (Probus) at a concentration 0.1 M was used as a catalyst in the thymol method.

Carbonate buffer 0.05 M was prepared by dissolving the appropriate amount of sodium carbonate (Panreac, Barcelona, Spain) and sodium hydrogencarbonate (Probus) to obtain pH 9.02, 10.7 and 11.06. Sodium chloride was obtained from Prolabo. Surfactants cetyl trimethyl ammonium chloride (CTAB), benzalkonium chloride, nonylphenol and sodium dodecil benzenesulfonate (SDS) were obtained from Fluka (Steinheim, Switzerland).

2.3. Procedures

2.3.1. Colorimetric indothymol method [2]

Two milliliters of ammonium working standard solutions at concentrations ranging from 0.25 to 1 mg L^{-1} were mixed with 40 μL of carbonate buffer, 5 μL of hypochlorite 5% and 20 μL of nitroprusside. The mixture was shacked for 1 min and then, 0.1 mL of thymol (0.8 M) was added. The absorbance of the solution was registered after 5 min of reaction between 600 and 900 nm. Room temperature was used in all steps.

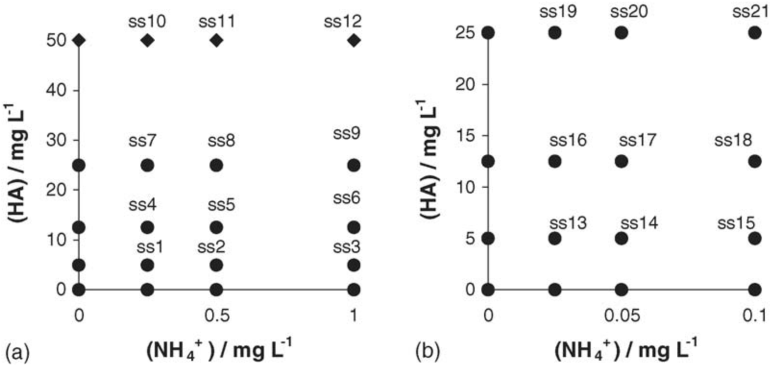


Fig. 1. Concentrations of ammonium and humic acid in synthetic samples.

All the measurements were done in triplicate unless stated otherwise.

2.3.2. *SPE/diffuse reflectance method*

Thymol derivates were formed by mixing 10 mL of ammonium working standard solution (at concentrations ranging from 0.025 to 0.25 mg L^{-1}) with 200 μL of carbonate buffer, 25 μL of HClO 3% and 100 μL of nitroprusside. The mixture was shacked for 1 min, and then 0.5 mL of thymol was added. After 5 min the resulting solution was passed through a quarter part of C18 Empore extraction disks (Varian, St. Paul, USA), previously conditioned with 2 mL of MeOH and 0.5 mL of CTAB 1% (m/v). Samples, reagents and standards were passed through the extraction membranes by means of a solvent filtration unit at a flow rate of 2 mL min^{-1}. Room temperature was used in all steps. Reflectance measurements were done between 600 and 900 nm. Measurements were done in duplicate, unless state otherwise.

2.4. *Synthetic water samples*

Synthetic samples named ss1–ss21 were done adding different concentrations of humic acid to ammonium solutions, as can be seen in Fig. 1a and b for the colorimetric and SPE/reflectance methods, respectively. Concentrations of ammonium varied from 0.25 to 1 mg L^{-1} in the colorimetric method and from 0.025 to 0.1 mg L^{-1} in the SPE/diffuse reflectance method. Concentrations of humic acid in the samples were 5, 12.5, 25 and 50 mg L^{-1}. Blanks containing humic acids were also processed. Derivatization was effected with thymol as derivatizing reagent.

2.5. *Water samples*

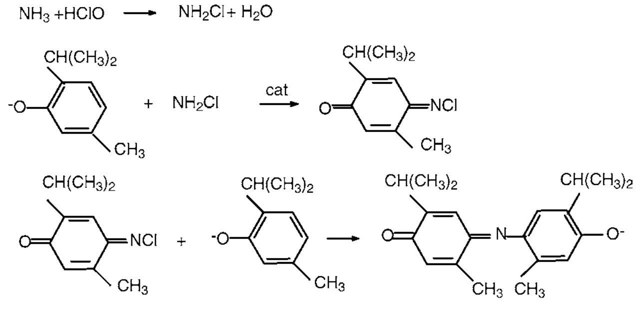
Thymol was used as derivatizing reagent and the colorimetric as well as the reflectance procedures were applied. Samples rs1 (lake), rs2 (river), rs3 (dam), rs4 (irrigation ditch), rs5 (irrigation ditch) and rs6 (muddy puddle) were natural waters. Sample rs7 was obtained from a water-treatment plant. Samples rs8, rs9, rs10 and rs11 were washing waters from a detergent factory. All these samples were from Comunidad Valenciana (Spain). They were analyzed with the colorimetric or SPE/reflectance methods depending on the concentration of ammonium.

3. Results and discussion

3.1. *Preliminary studies*

The mechanism of the indothymol blue formation is given in Fig. 2. The reaction conditions for indothymol method were selected according to Ref. [4]. However, in this study, the dependence of sensitivity as function of pH was evaluated. The slopes of the calibration curves were 0.150, 0.260 and 0.514 mg L^{-1} at pH 9.02, 10.7 and 11.06, respectively. Sensitivity was maximum at 11.06. Therefore, pH 11.06 was selected for further studies. Table 2 shows some analytical data obtained by the colorimetric method and by the SPE/diffuse reflectance method. The SPE/reflectance based method also provided better sensitivity at pH 11.06 than that obtained previously at pH 10.7 [4].

The spectra of humic acid solutions within 5 and 100 mg L^{-1} concentration range were directly measured in order to obtain a calibration curve of humic substances. Calibration curve at 300 nm was: $y = (0.0172 \pm 0.004)x + (0.06 \pm 0.02)$, $R^2 = 0.9961$ (determination coefficient) and $S_{xy} = 0.0459$ (standard deviation of the regression). These calibration parameters were used to calculate the amount of humic substances in real water samples.



Cat: $Na_2((Fe(CN)_5)NO)$

Fig. 2. Mechanism of the indothymol blue formation.

Table 2
Analytical parameters for ammonium determination in the presence of different amounts of humic acid

Method	Analytical parameters							
	Humic acid (mg L^{-1})	$a \pm s_a$	$b \pm s_b$ (L mg^{-1})	R^2	n	$S_{x/y}/b$	LOD (mg L^{-1})	R.S.D. (%)
Colorimetric	0	0.060 ± 0.020	0.587 ± 0.014	0.998	11	0.036	0.160	2.7
	5	0.008 ± 0.006	0.490 ± 0.010	0.995	4	0.029		
	12.5	0.062 ± 0.004	0.501 ± 0.008	0.997	4	0.019		
	25	0.023 ± 0.007	0.430 ± 0.010	0.994	4	0.033		
	50	0.094 ± 0.006	0.380 ± 0.010	0.993	4	0.030		
SPE/reflectance	0	56.1 ± 0.7	−185 ± 13	0.9902	5	0.005	0.011	7.0
	5	43.6 ± 0.8	−225 ± 1	0.991	4	0.005		
	12.5	46 ± 1	−234 ± 24	0.979	4	0.008		
	25	46 ± 1	−295 ± 22	0.989	4	0.005		

3.2. Influence of surfactants in the derivatizing reaction

The effect of surfactants was evaluated by measuring blanks and ammonium solution (0.5 mg L^{-1}) in the presence of different percentages of benzalkonium chloride (cationic), sodium dodecil benzenesulfonate (anionic) and nonylphenol (non-ionic). Fig. 3 shows the effect of surfactants in the ammonium absorbance signal. As can be seen nonylphenol was not an interferent at percentages lower than 5.5%. Dodecil benzenesulfonate in the range 0.2 to 4% was studied. The results showed that percentages higher than 0.5% interfere in the proposed method. The formation of the indothymol blue derivate was not possible if benzalkonium chloride was present at percentages higher than 0.001%. Therefore, the proposed procedure could not be applied to samples with those contents of anionic, cationic and non-ionic surfactant because they inhibit the formation of the derivate.

The assayed concentration for non-ionic surfactant was five orders of magnitude higher than the normal content in environmental water samples and for anionic surfactant three orders of magnitude higher. Therefore, those compounds are not interferents in the ammonium determination in environmental water samples. In the case of cationic surfactants, the concentration assayed was approximately five times higher than the normal content, so that, this kind of surfactants could be interferents in highly polluted water samples [22].

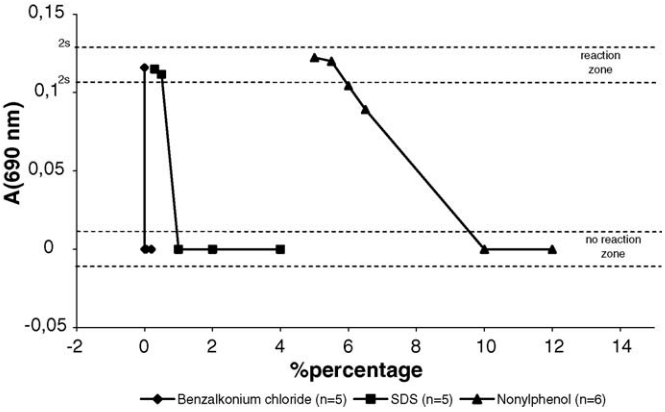


Fig. 3. Effect of the cationic, anionic and non-ionic surfactants tested in the absorbance signal (A) of ammonium.

3.3. Influence of humic acid

3.3.1. Ammonium concentrations between 0.25 and 1 mg L^{-1}

Fig. 4 shows the slopes of the calibration curves obtained for ammonium by the colorimetric method within the tested wavelength interval in the presence of different amounts of humic acid. The slopes obtained for ammonium were only markedly modified in the presence of humic acid at concentration equal or higher than 25 mg L^{-1}.

Table 2 shows the calibrations equations obtained in presence of different amounts of humic acid at 690 nm. The application of a t-test demonstrated that the slopes of the calibrations curves from standards containing between 5 and 12.5 mg L^{-1} of humic acid were statistically comparable to that obtained in absence of humic acid. At concentrations of 25 and 50 mg L^{-1} the values obtained were different. The matrix effect increased as a function of the concentration of humic acid in the samples. An increase of the ordinate value was obtained in reference to that

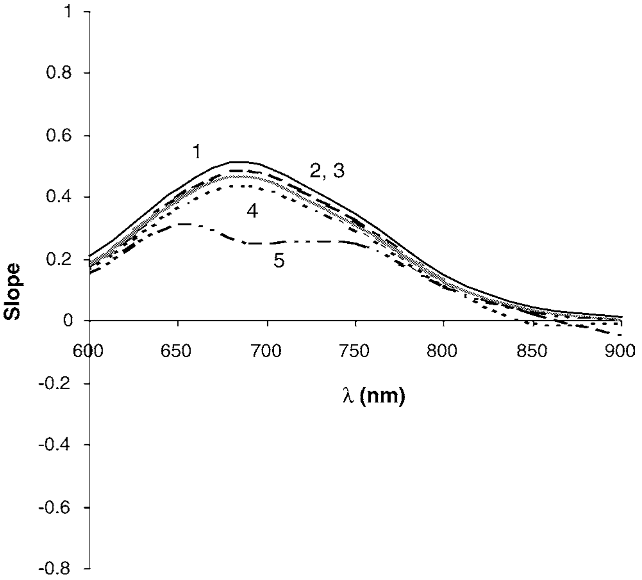


Fig. 4. Slopes of the linear calibration curves as function of wavelength obtained with the colorimetric based method for ammonium determination: (1) without HA, (2) HA 5 mg L^{-1}, (3) HA 12.5 mg L^{-1}, (4) 25 mg L^{-1} and (5) 50 mg L^{-1}.

achieved in absence of humic acid when humic acid was present at 50 mg L^{-1} due to its absorbance contribution.

The solutions were stored and measured every day during 7 days in order to study the effect of time in the possible absorption or liberation of ammonium from humic acid. No significative differences were found in the slopes of ammonium calibration curves. The %R.S.D. calculated from the seven slopes of the ammonium calibration curves were 5%, 4% and 4% for standards containing humic acid at 5, 12.5 and 25 mg L^{-1}, respectively.

3.3.2. Ammonium concentration between 0.025 and 0.25 mg L^{-1}

Fig. 5 shows the slopes of the calibration lines obtained for ammonium by the SPE/reflectance diffuse method within the tested wavelength range in the presence of different amounts of humic acid. The slopes of the calibration curves were affected by the presence of humic acid at 5, 12.5 and 25 mg L^{-1}.

The equations obtained for ammonium (Table 2) showed matrix effect (slopes statistically different to those obtained in absence of humic acid) even for the lowest concentration of humic acid assayed (5 mg L^{-1} HA). The ordinate was also modified with respect to that obtained in absence of humic acid (Table 2).

3.4. Analysis of samples

Several synthetic samples and eleven real water samples were analyzed. The colorimetric method was used for samples with high ammonium contents and the SPE/reflectance method for samples with low contents of ammonium.

The colorimetric method was used for synthetic samples (ss1–ss9) (see Fig. 1a) and SPE/reflectance method was used for synthetic samples from ss10 to ss18 (see Fig. 1b). Ammonium concentration in samples ss1, ss2, ss3, ss4, ss5 and ss6 was determined with the calibration curves obtained with standards, because no influence of humic acid was observed. For samples ss7, ss8 and ss9 until ss21, the generalized H-point standard addition method (GHPSAM) was employed to determine the concentration of ammonium because the humic acid introduced bias error as it has been demonstrated in the previous section.

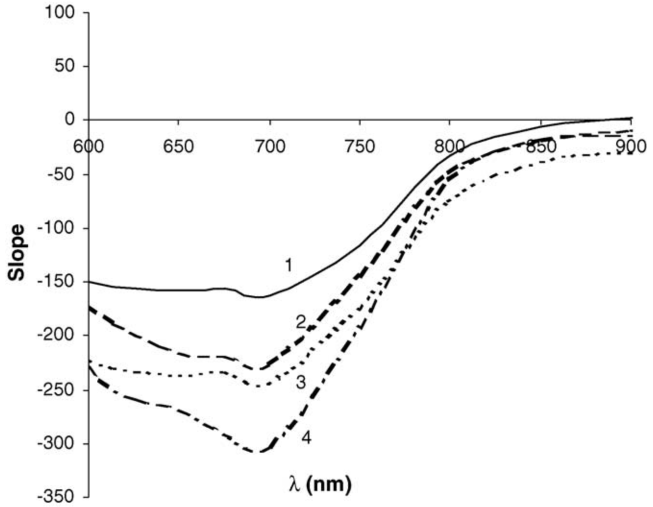


Fig. 5. Slopes of the linear calibration curves as function of wavelength obtained with the SPE/reflectance method for ammonium determination: (1) without HA, (2) HA 5 mg L^{-1}, (3) HA 12.5 mg L^{-1} and (4) 25 mg L^{-1}.

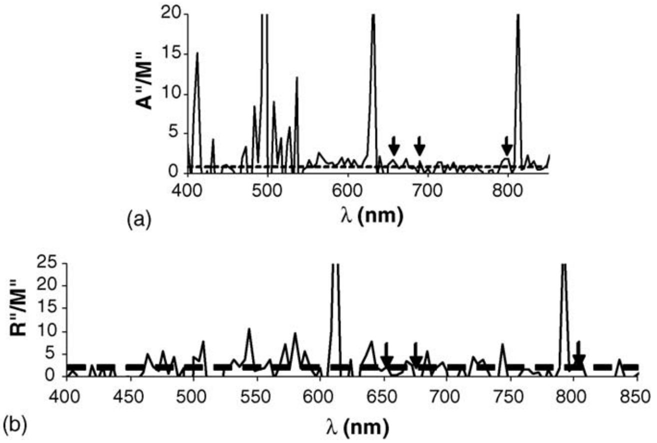


Fig. 6. Graphics (a) A''/M'' vs. λ for synthetic samples ss8 and (b) R''/M'' vs. λ for real water sample rs6 (see text for more details).

The GHPSAM [22,23] method chooses the spectral zones where the interference is linear and avoids its contribution by working with trios of wavelengths (j, k, l) and also corrects the matrix effect. As two examples, Fig. 6 shows the graphics A''/M'' or R''/M'' versus λ for samples ss8 and rs6. These graphics show the zones of the spectra where the interference can be considered linear. The working wavelengths selected from A''/M'' or R''/M'' versus λ plots for the colorimetric method were 650, 690 and 800 nm and for SPE/reflectance, the wavelengths were 650, 675 and 800 nm. GHPSAM calculates the analyte concentration by Eq. (1).

$$-c_{\mathrm{H}} = \frac{q\,\Delta A_{S,k,j} - p\,\Delta A_{S,l,k}}{q\,\Delta M_{k,l} - p\,\Delta M_{l,k}} \qquad (1)$$

where $p = (\lambda_k - \lambda_j)/(\lambda_l - \lambda_j)$ and $q = (\lambda_l - \lambda_k)/(\lambda_l - \lambda_j)$. $\Delta A_{S,k,j}$ and $\Delta A_{S,l,k}$ are the increments of the sample absorbance at wavelengths k, j and l, k. $\Delta M_{k,l}$ and $\Delta M_{l,k}$ are the increments of the slopes of the calibrations graphs at the three wavelengths: j, k and l (650, 675 and 800 nm, and 650, 675 and 800 nm for colorimetric and SPE/reflectance methods, respectively).

Tables 3 and 4 show the measured concentrations of ammonium in the synthetic samples (from ss1 to ss21) and their recoveries. When the GHPSAM was used (see Table 3) the values of M_j, M_k and M_l for applying Eq. (1) were the slopes of the calibration graphs obtained with standards containing the corresponding humic acid concentration.

Different water samples were processed by the colorimetric method or by the SPE/diffuse reflectance method and, if possible, by both methods. Firstly, the content of humic acids was tested by registering the spectrum between 200 and 900 nm. The spectra for samples rs1, rs2, rs3, rs4, rs5, rs8, rs9, rs10 and rs11 indicated the absence of humic acid.

Table 3
Ammonium concentration and %Er for synthetic and real water samples

	Influence of humic acid, GHPSAM	MOSA	Ammonium concentration ± s; relative error (%Er)	
			Colorimetric method ($n = 3$)	SPE/reflectance method ($n = 3$)
Synthetic samples				
ss1	–	–	0.26 ± 0.01; 4	–
ss2	–	–	0.47 ± 0.03; −6	–
ss3	–	–	1.01 ± 0.03; 1	–
ss4	–	–	0.28 ± 0.04; 12	–
ss5	–	–	0.47 ± 0.02; 6	–
ss6	–	–	1.02 ± 0.02; 2	–
ss7	X	–	0.27 ± 0.02; 8	–
ss8	X	–	0.52 ± 0.01; 4	–
ss9	X	–	1.0 ± 0.1; 0	–
ss10	X	–	0.25 ± 0.01; 0	–
ss11	X	–	0.53 ± 0.03; 6	–
ss12	X	–	0.97 ± 0.04; −3	–
ss13	X	–	–	0.027 ± 0.002; 8
ss14	X	–	–	0.060 ± 0.005; 20
ss15	X	–	–	0.096 ± 0.008; −4
ss16	X	–	–	0.026 ± 0.002; 4
ss17	X	–	–	0.060 ± 0.005; 20
ss18	X	–	–	0.11 ± 0.01; 10
ss19	X	–	–	0.020 ± 0.002; −20
ss20	X	–	–	0.042 ± 0.005; −16
ss21	X	–	–	0.094 ± 0.009; −6
Real samples				
rs1	–	–	0.93 ± 0.13	1.15 ± 0.03[a]
rs2	–	X	3.9 ± 0.1	3.78 ± 0.01[a]
rs3	–	X	0.52 ± 0.05	0.56 ± 0.01
rs6	X	–	0.50 ± 0.06	0.44 ± 0.02
rs7	X	–	0.58 ± 0.08	0.59 ± 0.03
rs8	–	–	0.63 ± 0.01	0.67 ± 0.03
rs11	–	–	8.9 ± 0.2	–

[a] Diluted samples.

The ammonium concentrations in samples rs1, rs8 and rs11 were calculated from the standard calibration graphs (Table 2) because matrix effect was absent. Spikes with known ammonium concentrations of rs1 and rs8 samples were also analyzed and good recoveries were obtained (see Fig. 7). Sample rs11 was only analyzed by the colorimetric method due to its high level of ammonium.

Samples rs2 and rs3 were investigated by the standard addition method (MOSA) method because matrix effect other than those of humic acid and surfactants were found in these samples. By using the slope of the MOSA calibration curve and considering the signal corresponding to the spike of ammonium added, good recovery values were obtained as can be seen in Fig. 7.

Table 4
Recoveries for synthetic samples obtained with the GHPSAM with both methodologies

Humic acid ($\mathrm{mg\,L^{-1}}$)	Colorimetric method		SPE/reflectance method	
	[NH$_4^+$] ($\mathrm{mg\,L^{-1}}$) added	%Recovery	[NH$_4^+$] ($\mathrm{mg\,L^{-1}}$) added	%Recovery
5	0.25	104 ± 5	0.025	100 ± 9
	0.5	94 ± 6	0.05	120 ± 10
	1	101 ± 3	0.1	96 ± 8
12.5	0.25	113 ± 2	0.025	106 ± 9
	0.5	94 ± 4	0.05	120 ± 10
	1	102 ± 2	0.1	115 ± 10
25	0.25	107 ± 8	0.025	82 ± 7
	0.5	104 ± 2	0.05	84 ± 8
	1	101 ± 10	0.1	94 ± 9
50	0.25	101 ± 3	–	–
	0.5	106 ± 6	–	–
	1	98 ± 4	–	–

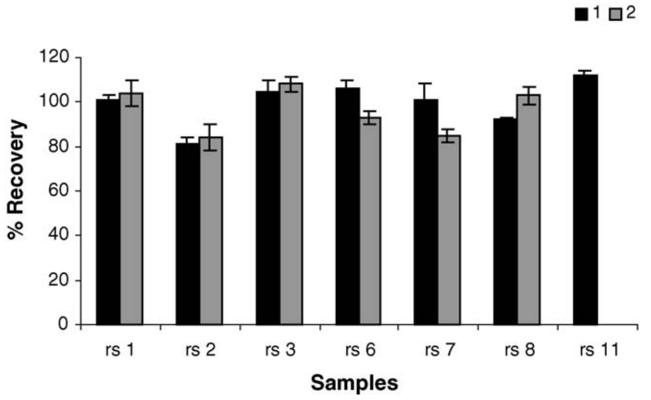


Fig. 7. Recoveries for water samples spiked with ammonium 0.25 mg L^{-1} with (1) colorimetric and 0.025 mg L^{-1} with (2) SPE/reflectance methods.

For samples rs4 and rs5 the SPE/diffuse reflectance method established ammonium concentrations below detection limit (0.011 mg L^{-1}).

Samples rs6 and rs7 presented black and yellow appearance, respectively, owing to humic acids. The concentration of humic acid was calculated from the humic acid calibration curve at 300 nm. The values obtained were 15 ± 1 and 4.6 ± 0.1 mg L^{-1} for samples rs6 and rs7, respectively. The application of GHPSAM was necessary for these samples when using SPE/diffuse reflectance. Table 3 gives the values of the found concentration of ammonium in those samples. The GHPSAM was used to estimate the ammonium concentration and to calculate the recovery for spiked rs6 and rs7 samples, measured with SPE/reflectance. As M_j, M_k and M_l values in Eq. (1) were used the slopes of the MOSA method at wavelengths 650, 675 and 800 nm, respectively. Fig. 7 shows that the recoveries achieved were adequate.

Samples rs8, rs9, rs10 and rs11 were samples polluted with surfactants. rs8 and rs11 contained surfactants below the permitted concentration and rs9 and rs10 showed amounts of SDS and benzalkonium chloride, respectively, higher than those permitted by the indothymol method. Samples rs8 and rs11 were spiked with 0.25 mg L^{-1} of ammonium. Fig. 7 shows the recoveries achieved, as can be seen, there was not matrix effect, as percentages were near 100%. Therefore, ammonium concentrations in samples rs8 and rs11 were calculated with the calibration curve obtained with standards. Table 3 shows the results obtained. Any blue derivate was obtained for samples rs9 and rs10 due to the presence of detergents at amounts producing the inhibition of the reaction.

4. Conclusions

In this work we have evaluated the effect of humic substances and surfactants on the determination of ammonium by a method based on the indophenol-type reaction. Two approaches have been tested and compared, the classical colorimetric method for ammonium concentrations between 0.25 and 1 mg L^{-1}, and the previously developed method [4] which uses SPE and diffuse reflectance spectroscopy for ammonium concentrations between 0.025 and 0.25 mg L^{-1}.

The effect of surfactants in the formation of the derivate was evaluated. In general, they inhibit the formation of the thymol-ammonium derivate. The limiting percentages of surfactants are 0.5% for anionic, 0.001% for cationic and 5.5% for non-ionic surfactants. Since the contents of surfactants in real water samples are generally lower than these values, both the colorimetric and the SPE indothymol methods are still satisfactory in ammonium determination.

Humic substances cause a negative effect on the response obtained for ammonium, but in the colorimetric method the effect is negligible for concentrations of humic acid lower than 25 mg L^{-1}. Since in most natural waters the expected level for humic substances is lower than 6 mg L^{-1}, the determination of ammonium would be not affected if present between 0.25 and 1 mg L^{-1}. For samples with high contents of humic acid, this interference can be corrected by applying the GHPSAM method.

In the analysis of samples containing ammonium at lower concentration levels (between 0.025 and 0.25 mg L^{-1}) the SPE/diffuse reflectance method has to be applied. This method showed less tolerance to humic acids. Nevertheless, in the presence of such substances, the GHPSAM can be used to eliminate the error due to the absorbent blank.

The influence of surfactants and humic acid on the quantification of ammonium was observed in some of the real water samples tested. The GHPSAM was necessary for two samples which contained humic acid at significative levels. If there are other types of matrix effect, rather than that of the humic acid and surfactants, involved in the ammonium measurement, such as the two real water samples rs2 and rs3, MOSA, method needs to be used in order to correct these effects.

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