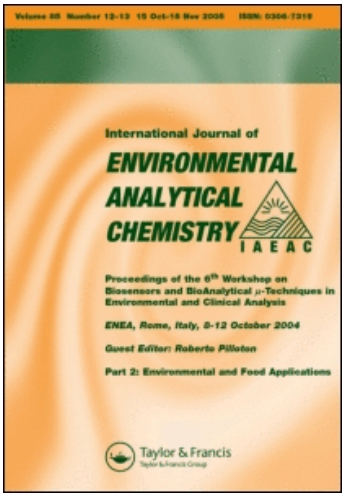

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A new approach to indophenol blue method for determination of ammonium in geothermal waters with high mineral content†

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The aim of the present work is the modification of the standard indophenol blue method in order to be feasible the determination of ammonium in geothermal water samples with high mineral content. Several pretreatment techniques were studied and evaluated in order to neutralise the pH of all solutions (blank, sample, standards) and minimise the interferences from salts. The Ultrasonic bath followed by mechanical stirring and vacuum filtration seems to be the most efficient pretreatment technique with almost 100% recovery of NH_4^+. Also there was an optimisation in the main procedure of the method in order to gain in sensitivity. The proposed method was compared with the standard ion chromatography and spectrophotometric indophenol blue methods and a *t*-test was performed. The RSD% values ($n = 4$) of the proposed spectrophotometric method were below 3.5% and the LOD of NH_4^+ was $0.026\,mg\,L^{-1}$. The proposed method was applied to several geothermal water samples from regions of Greece.

Keywords: ammonium; geothermal water; ion chromatography; indophenol; spectrophotometry

1. Introduction

Total ammonia is a critical parameter to evaluate the drinking water safety and possible environmental pollution. Methods for the determination of total ammonia (including ammonium) were reviewed in a paper by Seale [1]. Nessler's method was used but this method lacks sensitivity and requires the use of toxic mercury compound. Consequently, methods based on Berthelot reaction between ammonia, chlorine and phenolic compounds to form indophenol dyes, have become more popular [2]. Although it is more sensitive than Nessler's method, its sensitivity is not sufficient for determination of ammonia in saline and seawater samples. The Berthelot method is more difficult to apply in seawater than in freshwater due to buffering capacity problems. The pH shift in seawater analysis has been demonstrated and a shift to lower pH may result in lower sensitivity and slower reaction rate. Precipitation of magnesium as hydroxide in alkaline environment is another difficult point [3].

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Existing electroanalytical methods for ammonia include catalytic cathodic stripping voltammetry and potentiometry [4,5]. These methods are susceptible to interferences by amines and the direct analysis may require long equilibration time. Flow injection chemiluminescence analysis has also been used in rainwater [6] but with low sensitivity. Ion chromatography (IC) is particularly useful for separation and quantification of ammonium at $\mu g\,L^{-1}$ levels [7–9]. The main problem of the method becomes evident in determination of ammonium traces in presence of large concentration of Na. Aqueous samples with $NH_4^+ : Na^+$ ion concentration ratios up to 1 : 10,000 are difficult to be analysed by IC. Moreover, the analysis of a large number of high mineral samples may cause (i) memory effect of the IC column, (ii) drift in the background, and (iii) the necessity for flushing the column after each run. Applying a column-switching system to change the order of carboxylated and sulfonated stationary phase columns followed by suppressed conductivity detection or with post-column derivatisation enables the determination of ammonium traces in samples with high concentration of Na^+, Ca^{2+}, etc. but the drawbacks are the total cost of analysis and the time needed [10,11]. Thomas *et al.* studied the use of high-capacity cation-exchange column in IC for ammonium determination in environmental waters. The resolution of trace ammonium from sodium is feasible with this method in ratios $NH_4^+ : Na^+$ almost up to 1 : 10,000. However, the time for each run can be up to 30 min [12]. Kuo *et al.* developed a fluorimetric post-column derivatisation method for the determination of trace amounts of ammonium ion (at $\mu g\,L^{-1}$ level) in matrices with high concentrations of sodium and amino acids. In this method, ammonium ion was determined by ion chromatography combined with fluorimetric detection (IC-FL) in less than 16 min [13]. The IC-FL method with a post-column fluorimetric derivatisation allows the determination of ammonium ion in matrices where the ratios of ammonium to sodium are up to 1 : 28,000. More recently, Wang *et al.* developed a novel purge and trap preconcentrator for the determination of trace amounts of ammonium in high-salinity water samples by ion chromatography (IC) [14]. Despite the fact that these methods are highly sensitive, reproducible and selective for ammonium ion determination the cost of the IC apparatus needed is higher than that of a spectrophotometer [11].

Geothermal waters contain substances at different concentration levels, depending on the temperature, the age of the hydrothermal system, and other geological factors. The main cations found in geothermal waters are Na^+, K^+, Ca^{2+}, Mg^{2+}, Li^+, Sr^{2+}, Mn^{2+} and Fe^{2+}, while the most common anions are Cl^-, HCO_3^-, SO_4^{2-}, F^- and Br^-. Geothermal waters in some cases resemble freshwater but in other cases they have high salinity as seawater. The determination of ammonia in geothermal waters is difficult with the above methods mainly due to high concentration of ionic species like HCO_3^-, Ca^{2+}, Mg^{2+} and Na^+.

Accordingly the objective of this work was to overcome the interferences occurring during the determination of ammonia in geothermal waters when these methods are being applied [2,4–9]. In order to overcome problems that appear in the determination of ammonia in such samples IC and UV-VIS spectrophotometric methods were compared, and a slightly modified indophenol blue method is proposed and validated as well. In the pH range of most natural waters, ammonia exists mainly as NH_3^+. Both forms are easily converted into the other, with the ratio of ammonia to ammonium largely depending on pH, salinity and temperature. Ammonium is predominant species at pH lower than 8.75, while ammonia is predominant at pH higher than 9.75 [11].

2. Experimental

2.1 *Apparatus*

An Ion chromatograph (Shimadzu, Japan) coupled to a conductivity detector (CDD-6A) and a Hitachi U-2001 (Tokyo, Japan) double beam spectrophotometer with a 10-mm glass cell were used for measurements. A WTW 315i pH-meter was employed for pH monitoring. The pretreatment techniques were accomplished with the following devices: Ultrasonic bath, model FS-28 (62 kHz, Fisher-Scientific UK), heating-magnetic stirrer model ARE (VELP Scientifica, It), and vacuum filtration system with glass fibre filters 0.45 μm type GF 52 (Schleicher & Schuell MicroScience). The specifications of the IC columns (Alltech) were Universal Cation, Conventional Stainless Steel, 100×4.6 mm, Metal- Free Peek, 100×4.6 mm, Universal Cation Guard Cartridge Kit (1 holder & 3 cartridges), Universal Cation Guard Cartridges, Scavenger Column, 30×4.6 mm. All samples were injected via a 6-port valve.

2.2 *Reagents*

All reagents were of the highest available purity and the demineralised water of Milli-Q quality was obtained from Millipore (Bedford, MA, USA) R060/Super-Q water system. Methanesulfonic acid 3 mmol L^{-1}, and oxalic acid 2.5 mmol L^{-1} were used for the IC method. These eluents were obtained from Sigma-Aldrich. All eluents were filtered through a 0.45 μm filter to ensure that all the solvent was degassed and that particular matter was not present. Phenol crystallised (C_6H_6O), trisodium citrate 2 Hydrate ($C_5H_5Na_3O_7 \cdot 2H_2O$), sodium hypochlorite 10% (NaClO), sodium hydroxide (NaOH) were purchased from Panreac. Sodium nitroprusside ($Na_2[Fe(CN)_5NO]$ $2H_2O$), sodium chloride (NaCl), potassium chloride (KCl), ammonium chloride (NH_4Cl), calcium chloride ($CaCl_2 \cdot 2H_2O$), magnesium sulphate ($MgSO_4$ 7 H_2O), sodium bicarbonate ($NaHCO_3$), and ferrous sulphate ($FeSO_4 \cdot 7H_2O$) were obtained from Riedel de Haen. Ethanol solution pro analysis from Merck was used to liquefy the crystallised phenol. The following solutions were prepared for the experiments. 11.1 mL liquified phenol (≤89%) was mixed with ethyl alcohol 95% v/v to a final volume of 100 mL. The phenol solution was prepared weekly. The nitroprusside solution 0.5% w/v was prepared by dissolving 0.5 g sodium nitroprusside in 100 mL deionised water. The solution was stored in amber bottle for 1 month. The alkaline citrate solution was prepared by dissolving 200 g trisodium citrate and 10 g sodium hydroxide in deionised water and diluting to a final volume of 1 L. Sodium hypochlorite was diluted to 5%. 100 mL of alkaline citrate solution was mixed with 25 mL sodium hypochlorite to prepare the oxidising solution [2]. Ammonium standard solutions were prepared in appropriate concentrations from the stock ammonium solution (1000 mg L^{-1}). Mixed standard solutions of cations with ammonium were prepared in different concentrations in order to study the interference of cations in ammonium determination. All samples were collected in 100-mL bottles without airspace and they were stored at 4°C. All analyses were performed in the same day.

2.3 *Procedure for ion chromatography*

The eluent solutions were transferred in a degassing glass bottle and flashed with helium for 15 min. The oven temperature was 40°C and the system was let for 50 minutes to be stabilised in order to start measurements of conductivity signals. The flow rate was

adjusted to 1.0 mL min^{-1} for both eluents. The capacity of the injector loop was 100 μL and the injection was made by the use of a 250 μL micro-syringe. The sample was transferred from the injector loop to the column by the mobile phase through a 6-port injection valve. The column contained 7-μm silica polybutadiene/maleic acid copolymer. The conditions for IC were set as follows: autostop 12 min, gain 0.1 μS cm^{-1}, response slow, range 1 bipolar 1250 mV, pressure 550 psi.

2.4 *Indophenol blue method*

2.4.1 *Procedure for standard indophenol blue method*

A sample volume of 25 mL was transferred into a 50-mL ehrlenmeyer flask, then 1 mL phenol solution, 1 mL sodium nitroprusside solution and 2.5 mL oxidising solution were added with thorough mixing after each addition. The samples were covered with plastic wrap or Parafilm and kept in the dark at room temperature (22 to 25°C) for at least 1 h. The absorbance was measured at 640 nm. Six standards were used to prepare the calibration graph. The blank was treated like the standards. The results are expressed in NH_4^+ mg L^{-1}, in order to be comparable to the results of IC method.

2.4.2 *Applying a pretreatment technique before the indophenol method*

In order to overcome the extra buffering capacity produced by the Mg–citrate system and eliminate the pH difference between freshwater and geothermal water, several pretreatment techniques were performed and the comparative results are illustrated in Table 1.

When the sample was heated in temperatures higher than 40°C the loss of ammonium was observed. The recovery tests show that a vigorous stirring at 1000 rpm can be an efficient way to create precipitation. After the vacuum filtration the ammonium recovery can be close to 98%. The time for stirring or ultrasonic bath is not a critical parameter, although 10 minutes are required in order to eliminate CO_2 from the sample and equalise the pH between samples, standards and blank. Also the formation of the precipitate reaches a maximum after 5 minutes. The filtration or stirring at 600 rpm and filtration pretreatment techniques are not suitable for the limitation of interferences due to low recovery 70–88%.

Table 1. Effect of various pretreatment techniques on the recovery of ammonium in standards with similar matrix as geothermal waters.

Pre-treatment technique	Conditions	Recovery (%)
Vacuum filtration	Glass fibre filter 0.45 μm	73
Stirring and vacuum filtration	Stirring for 10 min at 25°C at 600 rpm	78
Ultrasonic bath and vacuum filtration	Ultrasonic power 80% for 10 min	87
Heating-stirring	Heating at 40°C for 10 min with stirring at 600 rpm	88
Ultrasonic bath-stirring-vacuum filtration	Ultrasonic power 80% for 10 min followed by stirring for 5 min at 25°C. Finally vacuum filtration performed	100
Vigorous stirring-vacuum filtration	Stirring for 10 min at 25°C with rate 1000 rpm	98

The pretreatment technique was applied to blank, standards and samples solutions in order to eliminate the high concentrations of HCO_3^-. Also the concentration of calcium, magnesium and iron was decreased. According to the recovery tests which are given in Table 1, the best pretreatment technique seems to be ultrasonic bath for 10 min following by vigorous stirring 5 min and filtration. The pretreatment technique was used for balancing the pH of blank and standard solution with that of the samples, just before adding the reagents, to eliminate the concentration of dissolved CO_2. After this step turbidity may occur and filtration is required. The pH of the final solutions after applying the pretreatment technique must be within the range 7.1 ± 0.1. If it is lower, an alkaline medium must be added.

2.4.3 *Proposed approach for the determination of NH_4^+ in geothermal water samples*

The sample was placed in an ultrasonic bath for 10 minutes, then the sample was stirred for 10 minutes and finally it was filtered. In this step HCO_3^- concentration was eliminated and the pH value must be 7.1 ± 0.1. Then 25 mL sample is transferred into 50-mL Erlenmeyer flask and the following reagents are added with thorough mixing after each addition: 1.0 mL phenol solution, 1.0 mL sodium nitroprusside solution, and 3.0 mL oxidising solution. Then 1.0 mL solution of $0.5\,mol\,L^{-1}$ NaOH is added in the geothermal water sample. After the addition of all reagents the final pH must be around 10.4 ± 0.1. The final solutions are covered with plastic wrap and are measured at 640 nm after 60 min in 10-mm glass cell.

3. Results and discussion

3.1 *Determination of ammonium by ion chromatography*

Potential interferences from the presence of high concentrations of alkali and alkaline earth cations such as Na^+, K^+, Ca^{2+}, Mg^{2+} in the determination of ammonium were studied. In geothermal waters the concentration of cations: K^+, Ca^{2+}, Mg^{2+} are expected to be in the same range from 10^1 up to $10^3\,mg\,L^{-1}$. The concentration of Na^+ could be up to $10^4\,mg\,L^{-1}$ while the concentration of NH_4^+ ranges from 10^{-1} up to $10^1\,mg\,L^{-1}$. Due to the fact that the standard IC method uses eluent of $pH < 4$ acidity, the predominant species is NH_4^+. Standard solutions were prepared in order to study the interference of cations on ammonium determination. Also standard solutions of HCO_3^- were prepared in order to check the affection of HCO_3^- in the pH of the IC eluents. In concentrations up to $2\,g\,L^{-1}$ there was no change in the pH of the eluents.

The chromatographs in Figure 1 illustrate the maximum concentration ratio of NH_4^+: Na^+ where the determination is feasible. Potential interferences caused by direct injection into the column of samples containing high concentration of common analytes were also studied. Some of the samples were injected into the column without dilution. In that case the column was filled up and the baseline was not steady for a long time.

At first, the interference of Na^+ in the determination of ammonium using methysulfonic acid as eluent was investigated. The analytical conditions are described in paragraph 2.3 above. As it can be seen in Figure 1, the retention times of Na^+ and NH_4^+ cations are very close to each other, thus the two peaks are not well resolved. The chromatogram in Figure 1(c) shows that determination of ammonium is feasible in samples with concentration ratios NH_4^+: Na^+ below 1 : 15. In chromatogram Figure 1(d) it

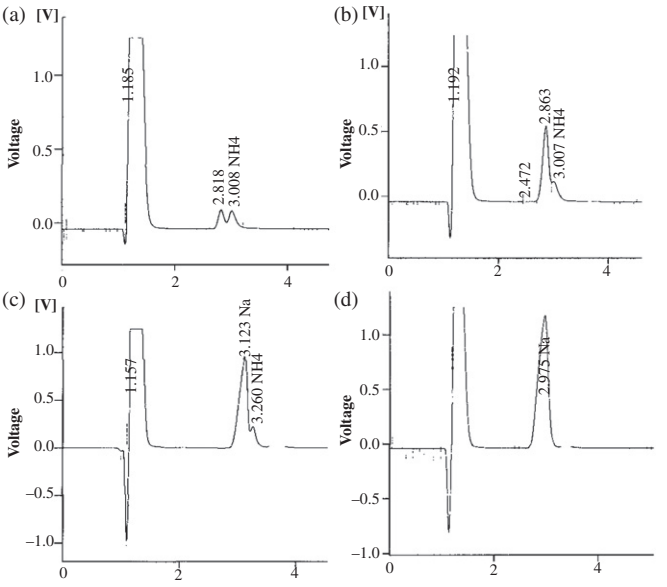

Figure 1. Ion chromatographs of standard solutions NH_4^+, Na^+ in different concentration NH_4^+: Na^+ ratios (a) 1 : 1; (b) 1 : 10; (c) 1 : 15; (d) 1 : 25.

is shown that Na^+ interferes strongly the determination of NH_4^+ at concentration ratios $NH_4^+ : Na^+ = 1 : 25$ or higher. The quantification of the analytes was achieved by using peak height instead of peak area because the partial overlapping affects more the peak area quantification. As it was mentioned above, the concentration ratios $NH_4^+ : Na^+$ in geothermal water can be up to 1 : 10,000, so the determination of ammonium in such cases is not possible with these analytical conditions.

No significant interferences from other cations were observed because the peaks were sufficiently resolved without overlapping. A standard solution containing Na^+ 10 mg L^{-1}, NH_4^+ 1 mg L^{-1}, K^+ 2 mg L^{-1}, Mg^{2+} 1 mg L^{-1} and Ca^{2+} 5 mg L^{-1} was prepared in order to check the retention time of the cations and to predict possible peak interference. The chromatograms in Figure 2 show that there is an observable interference only from Na^+. Accordingly, the rest of common cations do not interfere with the determination. The retention times for all cations are: 3.123 min (Na^+), 3.260 min (NH_4^+), 3.625 min (K^+), 8.060 min (Mg^{2+}) and 8.667 min (Ca^{2+}), respectively. Other cations except Na^+ do not interfere due to the sufficient difference in the retention times.

Subsequently, it was examined in which concentration level these cations could potentially interfere with the ammonium peak. Table 2 shows the effects of foreign cations on the determination of ammonium. The results showed that there is no significant interference of these in the determination of ammonium.

Table 3 presents the data obtained from analysing samples with the optimum conditions. The method of standard addition was used in order to obtain more accurate results. The determination of ammonium in low salt content samples was feasible with recovery close to 100%. However, for high salt content samples (conductivity $> 1,780\,\mu S\,cm^{-1}$), low NH_4^+ concentrations were not sufficiently recovered.

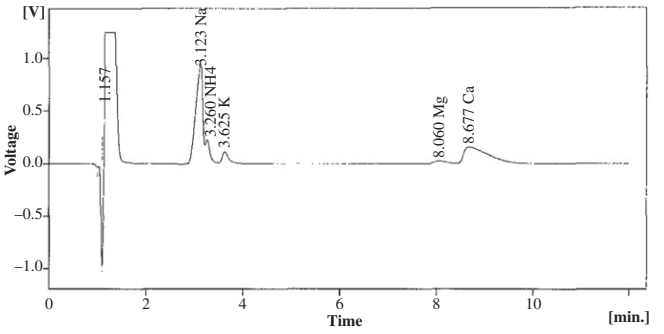

Figure 2. Standard solution containing cations with various concentrations: Na^+ 10.0 mg L^{-1}, NH_4^+ 1.00 mg L^{-1}; K^+ 2.00 mg L^{-1}; Mg^{2+} 1.00 mg L^{-1}; Ca^{2+} 5.00 mg L^{-1}.

Table 2. Effect of several cations on ammonium determination by IC (NH_4^+: 0.010 mg L^{-1} $n = 5$).

Ion	Added as	Concentration, mg L^{-1}	Ammonium recovery ± RSD,%
Na^+	NaCl	0.12	97.3 ± 3.0
K^+	KCl	4.0	99.0 ± 3.6
Ca^{2+}	$CaCl_2$	10	99.5 ± 2.1
Mg^{2+}	$MgCl_2$	15	99.3 ± 2.7

Table 3. Recovery tests for NH_4^+ with the optimum conditions.

Sample	Conductivity (μS cm^{-1})	Spiked (mg L^{-1})	Found (mg L^{-1})	Recovery (%)	RSD% ($n = 4$)
Ultrapure water	0.2	0	n.d.	–	–
		0.5	0.51 ± 0.01	102	1.7
Mineral water (Souroti, Thessaloniki)	1460	0	0.54 ± 0.02	–	3.2
		0.5	1.01 ± 0.03	97	2.7
Mineral water (Doubia, Chalkidiki)	1780	0	1.21 ± 0.02	–	1.6
		0.5	1.72 ± 0.03	101	1.6
Geothemal water (Nigrita, Serres)	3000	0	n.a.	–	–
		0.5	n.a.		
Geothermal water (Thermi, Thessaloniki)	10,000	0	n.a.	–	–
		0.5	n.a.		

Notes: n.d.: not detected; n.a.: not analysed.

The determination of NH_4^+ in samples with conductivity $>2{,}000$ μS cm^{-1} is not efficient under these analytical conditions due to the fact that the high content of salts affects dramatically the overall performance. A possible way to analyse samples that contain NH_4^+ and Na^+ in ratio 1 : 10,000 is to use high capacity ion exchange columns. The total analysis time for each sample is 30 min [12].

3.2 *Determination of ammonium by spectrophotometric indophenol method*

An intensely blue compound, indophenol, is formed by the reaction of ammonia, hypochlorite and phenol, catalysed by sodium nitroprusside. Complexing magnesium and calcium with citrate buffer eliminates interference produced by precipitation of these ions at high pH. Also the difference between pH values of blank and samples affect the time of the final colour development. So the absorbance is strongly pH dependent. Due to the fact that the pH of geothermal waters is usually <9, the predominant species is NH_4^+. In order to compare the results from both techniques the data from indophenol blue method are expressed as NH_4^+.

3.2.1 *Effect of cations in the determination of ammonium with indophenol method*

In order to find out in which concentrations the ions Na^+, K^+, Mg^{2+}, Ca^{2+}, Fe^{2+}, Mn^{2+} and HCO_3^- interfere the determination of ammonium, standards solution were analysed. The recovery tests from ammonium standard solutions NH_4^+ ($0.300\,mg\,L^{-1}$) are demonstrated in Table 4.

From the results given in Table 4 it is obvious that Mg^{2+} and Ca^{2+} cations interfere with the determination of NH_4^+ when their concentration exceeds $800\,mg\,L^{-1}$. These cations cause turbidity because the concentration of trisodium citrate as complexing agent is not enough. Moreover, there is not significant interference from Na^+ ions. The ammonium recovery is 102% even in high concentrations of sodium (Table 4). It is clear that analysis of these samples is satisfactory with the proposed indophenol method.

3.2.2 *Mechanism of the reaction*

The indophenol method for determining ammonia in water samples is based on the formation of an indophenol blue pigment during the reaction of phenol and hypochlorite in the presence of ammonia. In alkaline medium (pH = 8–11.5) a chloramine is first produced. This reacts with the surplus hypochlorite and with phenol forms quinone chloramine in the presence of catalytic quantities of sodium nitroprusside. Quinone chloramines further reacts with surplus phenol to produce indophenol. The blue coloration is due to the dissociated form of indophenol. The prime task of the trisodium citrate buffer used here is to bind magnesium and calcium ions into complexes,

Table 4. Interfering concentrations for the determination of NH_4^+ ($0.300\,mg\,L^{-1}$ $n = 5$).

Ions	Concentration ($mg\,L^{-1}$)	Recovery ± RSD (%)
Na^+	20,000	102 ± 4
K^+	1500	101 ± 3
Mg^{2+}	1000	82 ± 4
	800	90 ± 1
Ca^{2+}	1000	82 ± 3
	800	92 ± 2
Fe^{2+}	15	88 ± 4
Mn^{2+}	5	101 ± 3
HCO_3^-	2000	100 ± 3

thus preventing the precipitation of their hydroxides and carbonates in the alkaline reaction medium. The pH of the reaction medium consists of the most critical parameter for ammonium determination with the indophenol blue method. At pH value below 8, the reaction fails to start at all. On the other hand, at pH over 11.5 the oxidation of ammonia to nitrite is not complete. The latter show a greenish coloration instead of a clear blue one.

3.2.3 *The optimum pH value in indophenol reaction*

A standard solution containing NH_4^+ 0.385 mg L^{-1} was used in order to find the optimum pH value for the indophenol method. The final pH value was adjusted at different values (Figure 3) in order to obtain the maximum absorbance. A pH value ranging from 10.4 to 10.5 is optimum for indophenol reaction.

3.2.4 *Problems of the indophenol method in standard solutions with matrix similar to high mineral geothermal waters*

Standard solutions with concentration levels similar to geothermal waters were prepared. Some problems resulted when applying the indophenol method in these matrices. The final pH does not reach the ideal pH value 10.4 ± 0.1. The pH values are different between the standards solutions and the unknown samples. Also a delay in colour growth was observed. In addition, an increase of the turbidity in the solution of sample with high concentration of calcium and magnesium was observed.

3.2.5 *The Mg–citrate buffer system*

In high minerality waters there is a hidden buffer system that causes problems in pH adjustment and interfere strongly the indophenol reaction during ammonium determination. A similar problem was observed for the determination of ammonium in geothermal waters. An examination of the buffer systems in these kinds of samples show that there is

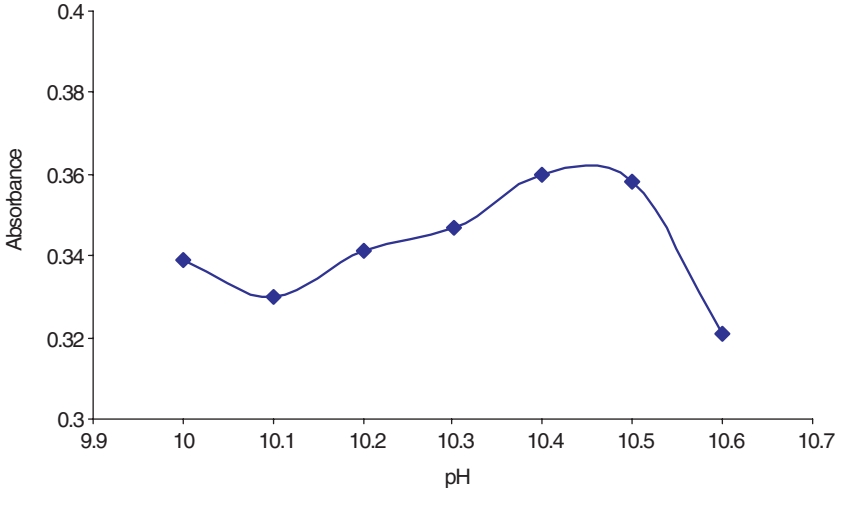

Figure 3. Effect of pH on absorption of standard solution of NH_4^+ containing 0.385 mg L^{-1}.

a type of chemical equilibrium which can affect significantly the buffering capacity [3]. Hydrolysis of a species (marked as L) occurs that binds a hydroxide ion ($L + OH^- \rightarrow LOH^-$), so that addition of hydroxide ion does not increase the pH as expected. Accordingly, the system acts like buffer. In the present case the most probable system would be the hydrolysis of $MgCIT^-$ between pH 9 and 11.

$$MgCIT^- + OH^- \rightarrow [Mg(OH)CIT]^{2-}$$

A further addition of a defined amount of NaOH (at pH > 11) destroys the complex due to the competition for OH^- and magnesium hydroxide (pKs = 10.47) is formed according to the reaction:

$$[Mg(OH)CIT]^{2-} + OH^- \rightarrow Mg(OH)_2(s) + CIT^{3-}.$$

3.3 *Optimisation of the proposed method*

The pH is the most critical parameter of the method. The main issue of the standard indophenol blue method is the slow reaction rate and the low ammonium recovery that is caused from different or lower than optimum pH values. Although the pH range 8.0–11.5 is considered as optimum, a more precise study reveals that close to pH = 10.4 the sensitivity is maximum. The first task was to balance the pH values of the standard ammonium solutions and blank. The increment of the alkaline buffer volume to 5 ml was enough to balance the pH values of blank and standards but far away from the optimum pH value of 10.4 ± 0.1. The recovery of ammonium standard solution ($0.300\ mg\ L^{-1}$) was up to 101% and the optimum pH value was achieved, 10.45 (Table 5). Also the use of the pretreatment technique is necessary for balancing the pH of the blank and standards with the pH of the geothermal samples.

It should be noted that geothermal water samples usually have pH values ranging from 5.5 to 7.0 and concentration ratios Mg^{2+}: NH_4^+ or Ca^{2+}: NH_4^+ even higher than 1000 : 1. Consequently, to avoid pH differences and/or precipitation, the citrate must be in excess to ensure that Mg^{2+} ions are quantitatively transformed in soluble complexes. Initially, pH measurement is required to the unknown samples in order to identify the initial acidity of them and then all solutions should be neutralised before the application of the indophenol blue method. The use of a pretreatment method almost neutralise the solutions and

Table 5. Effect of pH on the determination of ammonium in standard solutions of (NH_4^+ $0.300\ mg\ L^{-1}$ $n = 5$).

	Oxidising solution (mL)	pH	Found $mg\ L^{-1}$	Recovery (%)
Without pretreatment	2.5	9.25	0.216	72
	3.0	9.45	0.231	77
	4.0	9.55	0.254	85
	5.0	9.65	0.279	93
Ultrasonic bath-striring-filtration	2.5	9.89	0.285	95
	3.0	10.05	0.297	99
	4.0	10.15	0.297	99
	5.0	10.45	0.303	101

increase the pH value in a range 7.1 ± 0.1 due to the removal of CO_2 and precipitation of bicarbonates. Then, 5.0 mL buffer/oxidising is added to blank and standards in order to increase the pH up to 10.4.

This amount of the buffer/oxidising solution can not overcome the capacity of the formed hidden magnesium–citrate buffer in geothermal water samples and achieve the optimum pH value for indophenol reaction. Consequently, an additional alkaline reagent is needed for those samples in order to obtain a $pH = 10.4$. Thus, *ca.* 1 mL of NaOH 0.5 mol L^{-1} is added and this amount should be kept to the minimum required in order to limit the possibility of precipitate formation in samples with unknown concentration of Mg ions. With these modifications the final pH value of all the solutions fall within the optimum $pH = 10.4 \pm 0.1$ which is optimum for the indophenol reaction.

According to the definition, the limit of the detection (LOD, c_L) is a number expressed in units of concentration or amount of an element that can be identified as statistically different from an analytical blank. LOD was statistically evaluated after a series of 10 replications of a procedural blank (Milli-Q water with addition of reagents), after which the standard deviation of the measurements was calculated. The detection limit of the method was $LOD = 0.026$ mg L^{-1}. The equation of the calibration graph was linear though zero. The slope was 1.209 and $R^2 = 0.9998$.

Independent standard solutions of ammonium ion were analysed over low (< 0.1 mg L^{-1}) and high concentration range (> 2 mg L^{-1}). Each sample was analysed in 10 replicates. The relative standard deviation within the low concentration range of ammonia was 5.0% while at the high concentration range it was 0.7%.

3.4 *Comparison of the proposed method with the standard IC and indophenol blue methods. Application to geothermal and mineral water samples*

A comparison was made to test for possible differences between techniques as listed in Table 6. Ammonium was determined in a series of samples by ion chromatography, indophenol blue spectrophotometric method and the proposed indophenol method. The proposed method is applicable even in samples with high conductivity $> 2{,}000$ μS cm^{-1}. From the results which are listed in Table 6, it is obvious that there is a good agreement between the proposed method and ion chromatography for the samples

Table 6. Comparison of techniques for the determination of ammonium (mg L^{-1}) in geothermal waters, including test for significant difference.

	Mean ± SD		
Samples[a]	IC	Indophenol standard method	Proposed modified indophenol method
Mineral water (Souroti, Thessaloniki)	0.54 ± 0.02	0.35 ± 0.03	0.53 ± 0.02
Mineral water (Doubia, Chalkidiki)	1.21 ± 0.02	1.06 ± 0.03	1.18 ± 0.03
Geothemal water (Nigrita, Serres)	n.a.	0.42 ± 0.02	0.62 ± 0.02
Geothermal water (Thermi, Thessaloniki)[b]	n.a.	8.12 ± 0.06	10.1 ± 0.08
		$t_{exp} = 9.32 > t_{crit,\ 95\%} = 3.18$	

Notes: [a]The conductivity of each sample is given in Table 3.
[b]Values of this sample were subdivided for the paired *t*-test.

that have been measured. On the other hand, the proposed method showed higher results over the standard method and a paired *t*-test between the two series of results obtained showed significant difference.

4. Conclusions

The main problem for the determination of ammonium in geothermal water samples by the indophenol blue method is the matrix of the sample. The hydrolysis of the magnesium–citrate complex, acts as a buffering system in the sample which changes the final pH and interferes with the colour formation reaction. The use of a pretreatment technique is an alternative way to minimise possible interferences. This step is very important because the reaction and formation of the indophenol blue complex is pH-dependent. After the neutralisation, all solutions (blank, standards and samples) obtain almost the same pH values. At this point the addition of reagent is recommended in order to start the reaction from the same point. Also in the proposed method it is recommended to change the volumes of the oxidising reagent. The quantity of the tri-sodium citrate buffer was increased in order to ensure the transformation of Mg^{2+} ions into soluble complexes. Moreover, addition of an extra alkaline reagent like NaOH is required in special water samples to overcome the buffering capacity. The determination of ammonium in geothermal water samples seems to be efficient with the proposed modified indophenol method and the results are in good agreement with those obtained by the ion chromatographic method. Some good features of the proposed method are that (i) it is a simple method and it needs only a spectrophotometer, (ii) it provides acceptable analytical performance characteristics, (iii) the overall time for the analysis of large number of samples is less than ion chromatography, (iv) the method is more tolerant to high minerality geothermal waters, and (v) it can be automated. Due to these advantages, the proposed technique can be applied as a screening method for such sample matrices.

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