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Direct determination of nitrate in small volumes of natural surface waters using a simple spectrophotometric method

Abstract: A simple, sensitive and selective spectrophotometric method is proposed for rapid and direct determination of nitrate in small volumes of natural surface waters (maximum 2.0 ml), and optimal experimental conditions, along with other analytical parameters, have been evaluated. The method is based on the reaction between nitrate and chromotropic acid, in concentrated sulfuric acid media (95%), when a yellow colored product is obtained. The visible spectra of this reaction product, recorded against a blank solution, show a maximum at 412 nm, and the absorbance remains stable for at least 24 h. The method allows the nitrate determination over the range 0.50–11.00 mg l⁻¹, with a molar absorptivity of 3.837×10^4 l mol⁻¹ cm⁻¹ and a detection limit of 0.12 ppm. The interferences caused by several common ions (K⁺, NH₄⁺, Na⁺, Ca²⁺, Mg²⁺, Al³⁺, Cl⁻, NO₂⁻, HCO₃⁻, CO₃²⁻, H₂PO₄⁻, HPO₄²⁻) which are present in most natural surface waters were determined. The validation of the spectrophotometric method was done by comparing the nitrate content obtained by the proposed method with those obtained by using the standard method with sulphosalicylic acid, for all studied types of waters (drinking water, tap water, river water and sea water). The results show that the proposed method can be successfully used for direct determination of nitrate in small volumes of natural surface waters (maximum 2.0 ml). The main advantage of this method is that the colored reaction product appears immediately after mixing the reagents; other intermediary steps are not necessary.

Keywords: chromotropic acid; natural surface water samples; nitrate determination; spectrophotometric method.

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Introduction

The contamination of natural surface waters with nitrate is an environmental problem in most developed and developing countries, because this is one of the principal nutrients that stimulate the growth of macrophytes and phytoplankton, causing the eutrophication of aqueous environments (Camargo et al. 2005). The nitrate content in natural waters is rising at an alarming rate, mainly due to the anthropogenic sources, such as extensive utilization of fertilizers in agriculture, or insufficient treatment of domestic and industrial wastewaters (Lohumi et al. 2004).

Although not itself harmful [only a high intake of nitrate can cause abdominal pains and diarrhea (Standard Methods for the Examination of Water and Waste Water, 1995)], the toxicity of nitrate to humans is attributable to its reduction to nitrite. Thus, concentrations higher than 50 mg l⁻¹ of nitrate in drinking water have been known to cause methemoglobinemia in children, due to the reduction of nitrate to nitrite by intestinal bacteria (Fewtrell 2004). For this reason, the European Community has established that the drinking water must contain no more than 25 mg l⁻¹ of nitrate, while in the surface waters, the nitrate concentration should be lower than 50 mg l⁻¹ (Directive 2000/60/EC). Under these conditions, the rapid and direct quantitative determination of nitrate concentration in natural surface waters is still a subject of interest, since this is an important indicator of water quality, and a high concentration of this can be a potential risk to human health.

In nitrate determination, different types of methods (Moorcroft et al. 2001), including ion-chromatography (Miura and Hamada 1999, Stalikas et al. 2003, Michalski and Kurzyca 2006), gas chromatography-mass spectrometry (Kage et al. 2002), capillary electrophoresis (Fukushi et al. 1999), potentiometry (Ruzicka and Marshall 1990, Badea et al. 2001), and luminescence (Jobgen et al. 2007) have been used. Most of these methods are either time consuming, or require sample pre-treatment or expensive instruments, which limits

their applications in laboratory practice. Spectrophotometry is adequate for the development of a rapid, simple and inexpensive analytical method. The simplicity of the system design, low cost, easy automation and feasibility of wide-range determination are the main favorable characteristics of spectrophotometric methods.

Various spectrophotometric methods are available for the determination of nitrate in natural surface waters, both directly and indirectly, after its reduction to nitrite using metallic granules. Thus, phenyl-disulfonic acid, sulfosalicylic acid, dimethyl-2,6-phenol, brucine, xylenol, resorcinol, etc. (Andrews 1964, Murphy 1991, Dean 1995, Shrimali and Singh 2001) have been studied as reagents for direct spectrophotometric determination of nitrate. However, in most cases, the methods require careful control of acidity, the experimental procedures are time-consuming and laborious, and the sensitivity and precision are low. By contrast, the indirect methods are based on the reaction of nitrite with different organic reagents, such as 2,3-diaminonaphthalene, 5-aminofluoresceine, tetra-substituted amino aluminum phthalocyanine, 5,6-diamino-1,3-naphthalene disulfonic acid, etc. (Axelrod and Engel 1975, Wang et al. 2000, Zhang et al. 2001). However, the utilization of these methods has several disadvantages like toxicity of organic reagents, low reproducibility of experimental measurements, long-time procedures, a narrow detection range, or the necessity of synthesis and purification of organic reagents, which are not always commercially available (Biswas et al. 2004).

In this study, the reaction between nitrate and chromotropic acid, in concentrated sulfuric acid media, was spectrophotometrically studied. The yellow reaction product is stable and has a good behavior from the point of view of the spectrophotometric study. This method is a modification of a previous method with chromotropic acid (West and Ramachandran 1966), aiming at simplifying the experimental procedure for nitrate determination, and thus improving the analytical performances of the method. The proposed method is simple, sensitive and selective, requires no control of temperature and can be a good alternative for rapid and direct determination of nitrate in small volumes of water surface samples. In addition, the chromotropic acid is a common chemical reagent, the use of which does not require additional precautions, which implies that the proposed method does not present a significant risk to human beings. In order to emphasize its applicability, the proposed method was evaluated for nitrate determination in different types of natural surface waters.

Experimental

Reagents

All the reagents were of analytical reagents grade and were used without further purifications. Double distilled water was used for the preparation of aqueous solutions, and to avoid contamination, this was freshly prepared.

A stock solution of 500 mg l⁻¹ nitrate was prepared by dissolving sodium salt (purchased from Reactivul, Bucharest, Romania) in double distilled water. Working solutions of nitrate were prepared from stock solution by dilution with double distilled water. The reagent solution was obtained by dissolving 0.05 g of chromotropic acid disodium salt dihydrated (C₁₀H₆Na₂O₈S₂·2H₂O, from Merck, KGaA, Darmstadt, Germany) in 50 ml of concentrated sulfuric acid (95%). The required reaction media was obtained using concentrated sulfuric acid (95%), purchased from Aldrich (Sigma-Aldrich, St. Louis, MO, USA).

Apparatus

Spectrophotometric measurements were performed using a JASCO V-550 spectrophotometer, with a 1.0 cm glass cell. The ChemSketch software provided by ACD Labs (MH Electronics Inc., Washington, USA) was used to measure and record visible absorption spectra.

General procedure

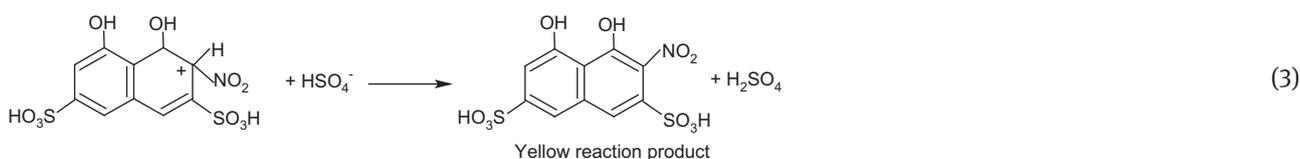
Volumes of 0.1–1.2 ml of 46.5 mg l⁻¹ nitrate aqueous solution were transferred in dry glass flasks and a final volume of 2.0 ml was adjusted with double distilled water. Chromotropic acid solution (1.0 ml, 0.05%) and 3.0 ml of concentrated sulfuric acid (95%) were added carefully, drop by drop. The flasks were gently stirred to mix the solution and let stand for 30 min, to reach room temperature. The absorbance was measured at 412 nm, in a 0.5 cm glass cell against a blank solution prepared similarly, but without nitrate. The nitrate concentration in an unknown sample was determined using a prepared calibration graph, obtained as described above.

The selectivity coefficients ($\alpha_{NO_3^-,j}$) were calculated as the ratio between nitrate concentration and the interfering ion concentration, which gives a 5% absorbance change in a reference solution. It should be noted that in case Na⁺, in the calculation of the selectivity coefficient was considered only the ion concentration added to the reference solution.

The validation of this method was done using two types of drinking waters (commercially available in Romania), tap water (obtained from the laboratories of the Technical University Gheorghe Asachi of Iași), river water (from the Bahlui River, Iași, Romania) and sea water sampled from the Black Sea (Romanian coast). In this case, the nitrate analysis was performed according to the procedure described above, and the obtained results were compared with those obtained by using the standard spectrophotometric method with sulfosalicylic acid (Water Quality 2000). In addition, the river and sea water samples were

This position is preferred, both due to the high electron density created by the hydroxyl group (which is an electron donor group), and from geometrical considerations. Breaking of the electron delocalization in the aromatic ring requires energy, and for this reaction to occur efficiently, heating is needed. The required heating is obtained at the dilution of concentrated sulfuric acid with an aqueous solution of nitrate.

(iii) The HSO_4^- ion acts as a base and removes the proton from the intermediate, so the electron delocalization in the aromatic ring is restored:

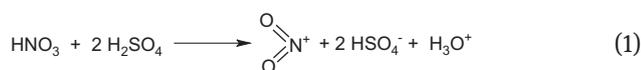


filtrated before utilization, through a glass filter crucible of $0.45 \mu\text{m}$ size, in order to remove solid particles.

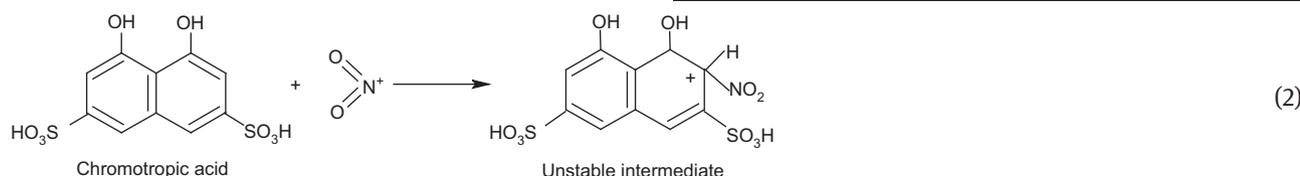
Results and discussion

The reaction between nitrate ions and chromotropic acid was used first for qualitative analysis of nitrate from the aqueous solution (Ripan et al. 1963), when it was observed that the yellow color of the reaction product appears only in the presence of concentrated sulfuric acid. Starting from this observation, the possible reaction mechanism can be described as an electrophilic substitution (Nenitescu 1980), which involves the following steps:

(i) in the presence of sulfuric acid, the nitrate ions are transformed in nitric acid. By contrast, the sulfuric acid determines the protonation of the hydroxyl group from the formed nitric acid and forms a reactive electrophilic nitronium ion:



(ii) The electrophilic nitronium ion attaches to chromotropic acid at the *ortho* position towards the hydroxyl group and forms an intermediate that is unstable due to breaking of the electron delocalization of the aromatic ring:



The yellow color of the reaction product appears immediately and remains stable for at least 24 h. However, in order to reach room temperature, the absorbance measurements should be performed after 30 min of standby. Although, the molecular structure of the reaction product was not identified in this study, the formation of a mononitro derivate instead of di- or poly-nitro derivatives can be assumed, because the chromotropic acid is added in excess to ensure the complete consumption of nitrate ions from aqueous solutions.

Influence of sulfuric acid concentration

As shown before, sulfuric acid has a double role in carrying out the reaction between nitrate and chromotropic acid, namely: (i) generating the nitronium ion, and (ii) providing the heat which accelerates the color reaction. However, sulfuric acid is a dangerous reagent, especially in concentrated solutions, and must be used with precautions. For this reason, the effect of sulfuric acid concentration on spectrophotometrical characteristics of the colored reaction product should be studied, in order to find the optimal conditions.

The influence of sulfuric acid concentration on the reaction was studied by varying the amount of concentrated sulfuric acid (95%) added to a series of mixtures

prepared from 2.0 ml of aqueous solution ($0.5\text{--}9.80\text{ mg l}^{-1}\text{ NO}_3^-$) and 1.0 ml of chromotropic acid (0.05%), following the general procedure described in the experimental section. The obtained results are illustrated in Figure 1. No detectable color was observed when the addition of sulfuric acid was $<0.5\text{ ml}$. After this value, the absorbance increased with increasing sulfuric acid volume, until 3.0 ml, when a maximum of absorbance was obtained. Higher sulfuric acid concentrations caused a decrease of measured absorbance, which was more evident when the nitrate concentration was higher. On the basis of these observations, an addition of 3.0 ml of concentrated sulfuric acid (95%) to a mixture of 2.0 ml of aqueous solution and 1.0 ml of chromotropic acid solution (mixing ratio aqueous solution: sulfuric acid=1:2) was considered as the optimum for spectrophotometric determination of nitrate with chromotropic acid.

Influence of chromotropic acid quantity

The optimal quantity of chromotropic acid was studied by varying the volume of chromotropic acid solution (0.05%) used as a color reagent. A series of mixtures were prepared by adding different volumes of chromotropic acid solution (0.2–1.4 ml) to 2.0 ml of aqueous solution containing 6.25 or 9.80 $\text{mg l}^{-1}\text{ NO}_3^-$ and 2.6–3.8 ml of concentrated sulfuric acid (95%). The effect of chromotropic acid concentration used in the reaction on the obtained absorbance, measured at 412 nm against a suitable blank solution (prepared similarly, but without nitrate) is shown in Figure 2.

As can be seen from Figure 2, the measured absorbance gradually increased with increases in the chromotropic acid volume and reached a maximum at 1.0 ml of

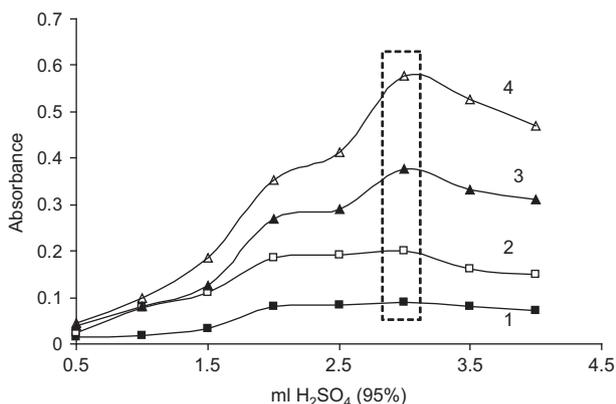


Figure 1 Effect of sulfuric acid concentration (ml) used in reaction on the absorption signal: (1) 1.55 $\text{mg NO}_3^- \text{l}^{-1}$, (2) 3.10 $\text{mg NO}_3^- \text{l}^{-1}$, (3) 6.25 $\text{mg NO}_3^- \text{l}^{-1}$, and (4) 9.80 $\text{mg NO}_3^- \text{l}^{-1}$.

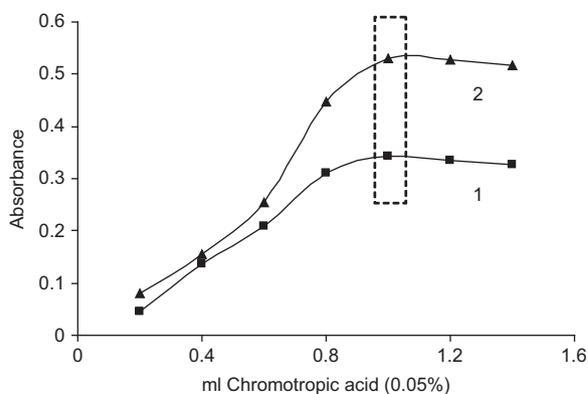


Figure 2 Effect of chromotropic acid concentration used in the reaction on the absorption signal [aqueous solution with nitrate concentration (1) 6.25 mg l^{-1} , and (2) 9.80 mg l^{-1}].

chromotropic acid solution. After this value, no significant changes of the measured absorbance were observed. This means that 1.0 ml of chromotropic acid solution (0.05%) is sufficient for total transformation of nitrate in the yellow reaction product, and this value was chosen as the optimal volume of reagent solution.

Analytical characteristics of proposed spectrophotometric method

The absorption spectra of the yellow reaction product, recorded against a blank solution, for different nitrate concentrations, are presented in Figure 3. It can be observed that in comparison with the reagent solution (Figure 3 – inside figure), which has an absorption maximum at 351 nm (in the UV spectral domain), the reaction product shows a distinctive peak in the visible region, with a maximum of absorption at 408 nm. However, in order to eliminate errors in the absorbance measurements caused by differences in the density of solutions, the absorption spectra should be recorded against a blank solution, prepared similarly as described in the general procedure, but without nitrate. Under these conditions, the maximum of absorption is obtained at 412 nm, and can be successfully used for quantitative determination of nitrate in aqueous solutions. The chromotropic acid reacts immediately with nitrate, forming a yellow colored reaction product, in concentrated sulfuric acid media, and the absorbance, measured after 30 min of standby, remains stable for at least 24 h.

The calibration curve was obtained as described in the general procedure using six standard solutions, with concentrations of nitrate included in the linear dynamic range

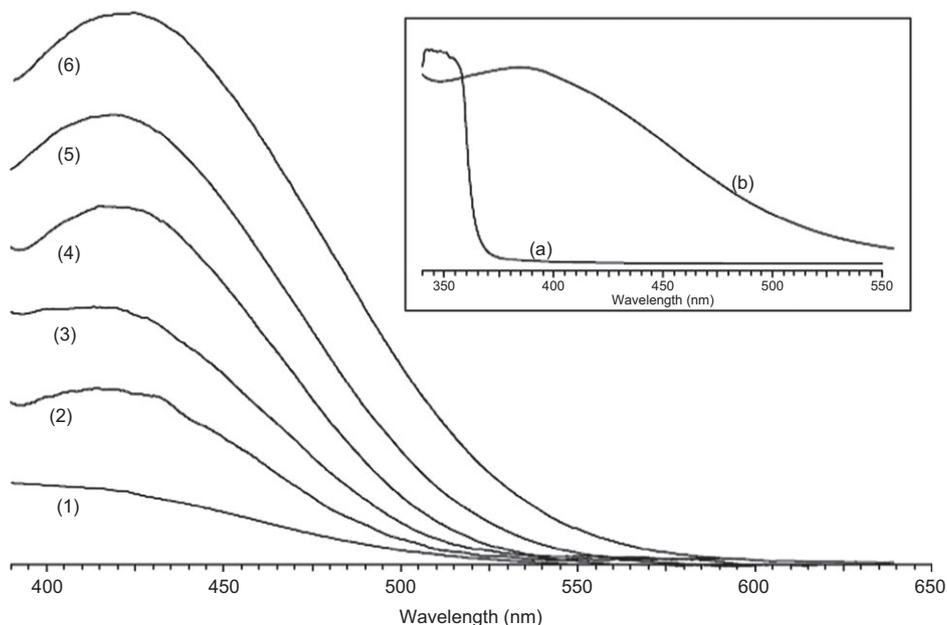


Figure 3 Absorption spectra of the yellow reaction product at different concentrations of nitrate in mg l^{-1} [(1) 0.51, (2) 1.55, (3) 3.10, (4) 6.25, (5) 9.3, and (6) 10.85], recorded against a blank solution. Inside – the absorption spectra of chromotropic acid (a) and reaction product (b) recorded against double distilled water.

(Table 1), and the linear regression equation obtained by using the mean values of 10 replicate measurements is:

$$y(\text{absorbance})=0.0573 \cdot x+0.007; R^2=0.9996 \quad (3)$$

where x is the nitrate concentration (mg l^{-1}), and R^2 is correlation coefficient.

The Lambert-Beer law is obeyed from 0.5 to 11.0 mg l^{-1} of nitrate, with a molar coefficient of $3.837 \cdot 10^4 \text{ l mol}^{-1} \text{ cm}^{-1}$, at 412 nm against a blank solution. The detection limit calculated as three times the standard deviation (σ) of 10 replicate measurements, the calibration sensitivity obtained from the slope of the calibration curve, the precision (RDS%) as well as other analytical characteristics of the spectrophotometric method, are summarized in Table 1.

Analytical parameter	At 412 nm, against blank solution
Molar absorptivity	$3.83 \cdot 10^4 \text{ l mol}^{-1} \text{ cm}^{-1}$
Correlation coefficient	0.9996
Calibration sensitivity	0.05 l mg^{-1}
Limit of detection (3σ)	0.12 ppm
Limit of quantification (10σ)	0.42 mg l^{-1}
Linear dynamic range	$0.49\text{--}11.01 \text{ mg l}^{-1}$
RDS (%)	1.58%

Table 1 Analytical characteristics of the proposed spectrophotometric method. σ , standard deviation; 0.5 cm glass cell; $n=10$; approximately 23°C .

On the basis of analytical characteristics presented in Table 1, the proposed spectrophotometric method is rapid, sensitive and precise, has a reasonable linear dynamic range, does not require expensive reagents and can be a good alternative for direct quantitative analysis of nitrate in small volumes of natural surface water samples.

Effect of interfering ions

The effect of possible interfering ions has been quantified by the selectivity coefficients ($a_{\text{NO}_3, i}$) defined as the ratio between nitrate concentration (c_{NO_3} , mg l^{-1}) and interfering ions concentration (c_i , mg l^{-1}) which gives a 5% absorbance change in a reference solution. In this study, the interfering ions were added to a reference solution with a constant nitrate content of 3.10 mg l^{-1} , until a 5% change in absorbance was obtained. It should be noted that in case Na^+ , in the calculation of the selectivity coefficient was considered only the ion concentration added to the reference solution. The calculated values of the selectivity coefficients are given in Table 2.

The obtained values of the selectivity coefficients indicate that the proposed method is selective for nitrate in the presence of several common ions which are fairly abundant in the natural surface water samples. Thus, it can be observed from Table 2 that K^+ , NH_4^+ , Na^+ , Ca^{2+} , Mg^{2+} , Al^{3+} , Cl^- , HCO_3^- , CO_3^{2-} , H_2PO_4^- , HPO_4^{2-} ions practically do not

Interfering ion, j	$\log a_{NO_3^-,j}$	Interfering ion, j	$\log a_{NO_3^-,j}$
K ⁺	-2.37	NO ₂ ⁻	-0.42
Na ⁺	-3.83	Cl ⁻	-2.98
NH ₄ ⁺	-2.68	HCO ₃ ⁻	-3.80
Ca ²⁺	-2.71	CO ₃ ²⁻	-3.79
Mg ²⁺	-2.99	H ₂ PO ₄ ⁻	-3.33
Al ³⁺	-2.92	HPO ₄ ²⁻	-3.17

Table 2 The values of selectivity coefficients.

Note: The $a_{NO_3^-,j}$ values were determined in a 3.10 mg NO₃⁻ l⁻¹ reference solution, and calculated for a 5% changing in absorbance (n=3; approx. 23°C).

interfere in the determination of nitrate. Another important fact is the selectivity of this method for nitrate in the presence of chloride ions, which are present in various natural surface waters and interfere in most of the nitrate analysis method (Zhang and Fischer 2006). On the basis of obtained values of the selectivity coefficients, it is noted that the proposed method can be used for quantitative determination of nitrate in saline media.

Only nitrite ions give a positive interference in the nitrate determination when their concentration is two times higher than the concentration of nitrates. However, in natural surface waters, concentrations higher than micro-molar levels of nitrite are present only at the base of the eutrophic zone, as result of biological activity. On the surface, most of nitrite ions are oxidized by oxygen dissolved from water and converted to nitrate (Zhang et al. 2000), which makes that the interference of nitrites in nitrate determination using this method is insignificant. In samples which contain significant nitrite concentrations, this should be removed prior to nitrate analysis by this method, which can be performed by reduction of nitrite to nitrogen, with sulfamic acid (Hartley and Asai 1963).

Validation of proposed method

The proposed method was validated by the determination of nitrate in five categories of natural surface waters: two types of drinking waters (commercially available in Romania), tap water from laboratories at our University, river water from Bahlui River (Iasi, Romania) and sea water (with relative high salinity) from the Black Sea (Romanian coast). In all cases, the water samples were analyzed according to the general procedure, and 2.0 ml from each water sample was used to perform the measurements. The nitrate content was determined using a prepared calibration graph and absorbance measurements were carried out

Water sample	Proposed method	Standard method (Water Quality, 2000)
Drinking water (type 1)	2.56±1.67	2.51±1.43
Drinking water (type 2)	2.09±1.21	2.12±2.05
Tap water	7.09±1.87	7.03±1.53
River water	22.19±1.12	22.26±1.69
Sea water	21.14±2.34	21.07±2.41

Table 3 Nitrate content of studied natural surface water samples obtained by a proposed spectrophotometric method and standard method with sulfosalicylic acid.

Note: Average of three replicate determinations ±S%, calculated on the basis of calibration graph (approx. 23°C).

at 412 nm, against a blank solution. The values of nitrate contents obtained by the proposed method were compared with those obtained by using the standard spectrophotometric method with sulphosalicylic acid (Water Quality, 2000) (Table 3). A good agreement was obtained for all types of water samples. Therefore, the proposed method can be a good alternative device for direct nitrate analysis in natural surface water samples. In addition, this method required small volumes of water samples, is advantageous in terms of accuracy and simplicity, is cheap and is readily available in most laboratories of water analysis.

Conclusions

The proposed method using chromotropic acid as a spectrophotometric reagent for direct determination of nitrate in different types of surface waters is simple, selective, reproducible and requires small volumes of water samples (maximum 2.0 ml). The yellow reaction product is formed in the presence of concentrated sulfuric acid (95%), and its absorbance (measured at 412 nm against a blank solution) remains stable for at least 24 h. The method allows the determination of nitrate over the range 0.5–11.0 mg l⁻¹, with a molar absorptivity of 3.837×10⁴ l mol⁻¹ cm⁻¹ and a detection limit of 0.12 ppm. The good selectivity of the proposed method to determine nitrate in the presence of several ions which are fairly abundant in natural waters, pointed out the applicability of this, and showed that the constituents of natural surface waters do not interfere significantly with the determination of nitrate. The method is rapid, requires low-cost equipment and therefore can be a good alternative for the direct determination of nitrate in natural surface waters.

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