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Quantitative Determination of Subnanomolar Concentration of Nitrite in Natural Water by High-Performance Liquid Chromatography

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A new chromatographic method is presented that successfully determines nirite in natural water at subnanomolar concentration. The procedure was based on converting the nitrite to a derivative using 2,4-dinitro phenyl hydrazine followed by separation using High-Performance Liquid Chromatography (HPLC). The method is simple, rapid, and requires minimal sample preparation. The 2,4-dinitro phenyl azide formed is stable for at least 5 weeks under cold and dark conditions. The accuracy of the method was checked using the standard colorimetric technique in several natural water types. The detection limits of nitrite in three types of natural water were found to range from 0.1 to 0.2, with relative standard deviations for the complete procedure varying between 4 and 8%. The method has recovery range from 95 to 100% with relative standard deviations between 2 and 5%. Applications of the HPLC method to study the speciation of nitrite in natural waters in Gaza Strip are also presented.

INTRODUCTION

The nitrogen cycle in natural water plays an important role in the chemistry and biology of the water system. Eutrophication is a phenomenon caused by the overloading of nutrients in water. Traditionally, it is known that the autotrophic nitrifiers, predominantly *Nitrosomonas* (an ammonium oxidizer) and *Nitrobacter* (a nitrite oxidizer), were responsible for converting ammonium (NH_4^+) to nitrite (NO_2^-) and nitrite (NO_2^-) to nitrate (NO_3^-)[1] respectively. This process called nitrification is aerobic[2]. A second group of highly diverse heterotrophic microorganisms is responsible for conversion of the nitrite to nitrogenous gases[3]. Recently, it was realized that the nitrogen cycles in water were more complex than originally thought[4]. Nitrite is a relatively stable intermediate in these processes, its determination indicates the level of eutrophication and pollution of natural waters.

The common analytical methods for the determination of nitrite in aqueous solutions are colorimetric[5], fluorometry[6], chemiluminescence[7] and Raman

spectroscopy[8]. The standard colorimetric technique based upon diazotizing with sulfanilamide and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly colored azo dye which is measured colorimetrically, has a detection limit of 30–50 nM. Thus, this method is not sensitive enough to detect nitrite levels in over 99% of the water systems[8]. The fluorometry, chemiluminescence, and Raman methods are sensitive for the determination of nitrite in water. However, these methods are tedious, expensive, need extensive sample preparation and require sophisticated instrumentations.

In this study, we introduce a new approach to determine nitrite in natural waters. The method is based on the known reaction of nitrite with 2,4-dinitrophenyl hydrazine in acidic media to form 2,4–dinitrophenyl azide derivative, which is separated from the interfering substances and quantified by HPLC. The method is simple, rapid, extremely sensitive and has undetectable blanks.

EXPERIMENTAL

Instrumentation

High-Performance Liquid Chromatography: All chromatographic experiments were carried out on a High-Performance Liquid Chromatography (Perkin Elmer) consisting of an HPLC pump (Series 200 LC pump, Perkin Elmer), an automated gradient controller, a variable-wavelength UV detector (Model 785A Programmable Absorbance Detector, Perkin Elmer) at 307 nm. The column was high-speed cartridge column of 5 cm length (4.6 mm i.d.) C18 reversed-phase packing. The column was used at ambient temperature. Sample injector was Rheodyne 7725I (stainless steel) supplied with a 20 μL loop (Perkin Elmer).

Fourier Transform Infrared: Fourier Transform Infrared (Paragon 500 FT, IR Spectroscopy, Perkin-Elmer) was used for 2,4–dinitrophenyl azide strucure conformation.

UV-Vis Spectroscopy: UV-Vis spectroscopy (Perkin-Elmer, Lambda 20) was used for colorimetric determination of nitrite at 543 nm absorbance.

Chemicals and reagents

2,4-Dinitrophenyl hydrazine, HPLC grade acetonitrile, hydrochloric acid, carbon tetrachloride, sulfanilamide, phosphoric acid and N-(1-naphthyl) ethylene diamine hydrochloride were purchased from Merck.

Procedure

The 2,4-dinitrophenyl hydrazine (2,4-DNPH) was recrystallized twice from a mixture of acetonitrile/water (70 : 30, v/v) followed by final recrystallization from pure acetonitrile. The 2,4-DNPH was dried under vacuum and stored in dark in airtight container. A reagent from 2,4-DNPH was prepared by dissolving 15 mg of recrystallized 2,4-DNPH in a 15 mL solution prepared from concentrated HCl, water, and acetonitrile in a ratio of 2 : 5 : 2 (v/v/v). Because of the possibility of presence of nitrite impurities in the 2,4-NPH reagent, it was

extracted with 20 mL of carbon tetrachloride (CCl_4) using a wrist-action shaker (Stuart Flask Shaker, made in Great Britain) for 10 min. The mixture was centrifuged at 2000 rpm (Megafuge 1.0, Heraeus Sepatech) for 5 min, in order to separate the two phases. The organic layer was removed *via* a 5 mL pipette, and reagent solution was reextracted as described above. The purified reagent was prepared immediately prior to use.

For colorimetric determination of nitrite, to approximately 500 mL of distilled water add 200 mL of concentrated phosphoric acid (sp. gr. 1.834), 10g sulfanilamide ($H_2NC_6H_4SO_2NH_2$) followed by 1.0 g N-(1-naphthyl) ethylene diamine dihydrochloride. The solution was diluted to 1 L water and stored in a dark bottle in the refrigerator. This solution is stable for 1 month. 4 mL of filtered nitrite standard or natural water were treated with 0.3 mL of the reagent in the cuvette of a spectrophotometer. After the colour developed the absorbance was measured at 543 nm using UV/VIS is spectroscopy (Perkin-Elmer, Lambda-20).

RESULTS AND DISCUSSION

2,4-Dinitrophenyl azide structure assurance: 2,4-dinitrophenyl azide produced from the reaction between 2,4-DNPH and nitrite in natural water under acidic condition was extracted and purified from 2,4-DNPH-treated natural water sample. The Fourier transform infrared spectra of the isolated azide was compared to authentic azide spectra (synthesized from the reaction of an aqueous solution of $NaNO_2$ with 2,4-DNPH in H_2SO_4). Both spectra are identical, and show a very intense azide functionality band at 2136 cm^{-1}. The UV spectra of both azide samples in acetonitrile are identical and have UV maximum at 307 nm. All spectra recorded were also identical to the 2,4-dinitro phenyl azide spectra recorded in the literature[9, 10].

Derivative formation and detection: Three types of natural water were analyzed for nitrite concentration; ground, pond and seawaters. The water samples was collected from Gaza ground water (Islamic University tap water), Wadi Gaza pond water and the seawater was collected from Gaza Beach. Precautions was undertaken in handling and storing the collected natural water samples to prevent contamination or alteration of the samples. The water samples were filtered through a 47 mm glass fiber filter. 5 mL aliquots were analyzed for nitrite by adding 5 mL of 2,4-DNPH reagent in 25 mL round bottom flasks (fitted with ground stoppers). The flasks were shaken and the derivatization reaction allowed to proceed at ambient temperature. 1 mL aliquot was taken from the flasks and injected into the sampling loop. Figures 1 and 2 show typical chromatograms of the purified 2,4-DNPH (dissolved in distilled water) and the azide derivative, respectively. The detector and integrator for the chromatogram were set to their most sensitive setting. It is clear from Figures 1 and 2 that no peak in the blank chromatogram was detected in the area where the nitrite derivative elutes. The azide derivative peak has retention time of 2.4 min. It is important to note that when 2,4-DNPH is properly purified, nitrite is not detected in blanks. Background contamination by nitrite is one of the limitimg

factors reported by Zafariou *et al*[11]. in their chemiluminsent analysis of nitrite in seawater.

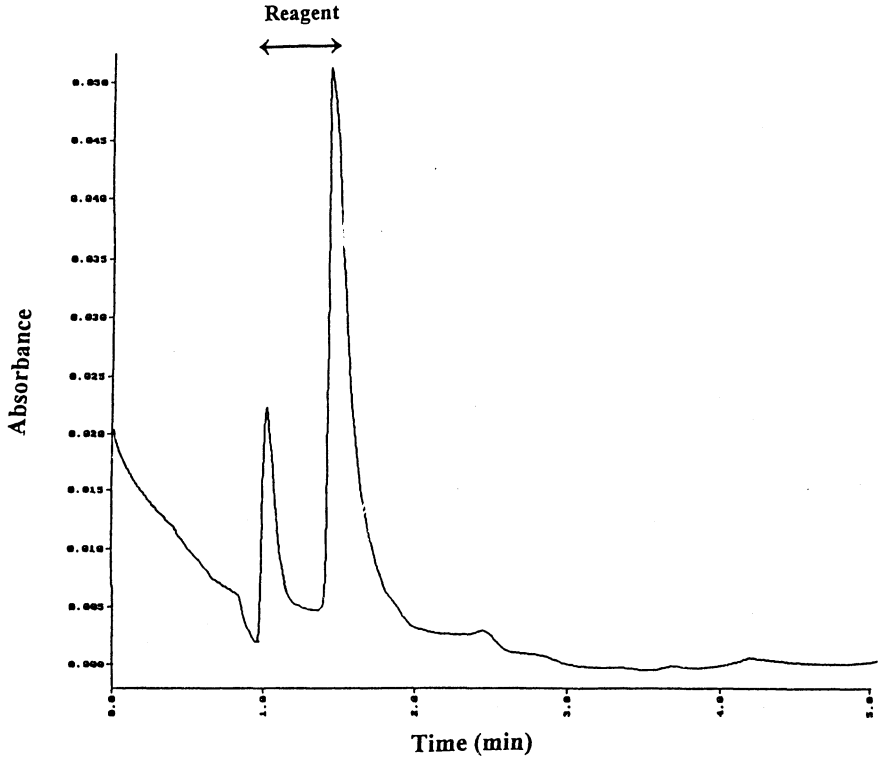

Fig. 1 Typical HPLC chromatogram of system blank prepared by injection 1 mL of 2,4-dinitrophenyl hydrazine (dissolved in distilled water) into 20 μL sample loop of the HPLC system.

Time profiles of derivative formation:Time profile studies were carried out to determine equilibration time for complete derivatization. Figure 3 shows that 90% derivitizations occur after 5 min at ambient temperature. Reaction times greater than 10 min practically do not affect the sharpness or the intensity of the azide derivative signal for the three types of natural waters, and therefore very reproducible peaks are obtained after 10 min.

Stability of the derivative: It should be pointed out that the stability of the derivative was checked by storage in dark condition at 4°C. The stability test was done at various time intervals throughout a 1 month period. As shown in Figure 4, the azide derivative is stable over the studied period. It should be noted that the stability of the derivative is extremely useful because it allows the sample to be collected, derivatized, and stored in relatively short time period for later chromatographic analysis.

Calibration plot and detection limit: The calibration plots were constructed using analyte concentration *vs.* peak areas of the signal response of the 2,4-dinitro phenyl azide. The results have shown that the calibration plot is linear

over the concentration range of 0.5–1000 nM. The correlation coefficients of linear regression analytes over five data points were greater than or equal to 0.997. The detection limit of nitrite was examined in three types of natural water. The results had shown that the method had a detection limit (signal/noise) range from 0.1 to 0.2 with a 20 μL sample loop. This detection limit could be greatly improved by increasing the injected sample size. It should be pointed out that the purity of derivatizing agent from nitrite impurity is very important when measuring detection limit. Moreover, an undetectable blank dictates the limit of detection, which is measured here to be 0.1 nM, the lowest reported for routine determination of nitrite in oligotrophic natural water[12, 13].

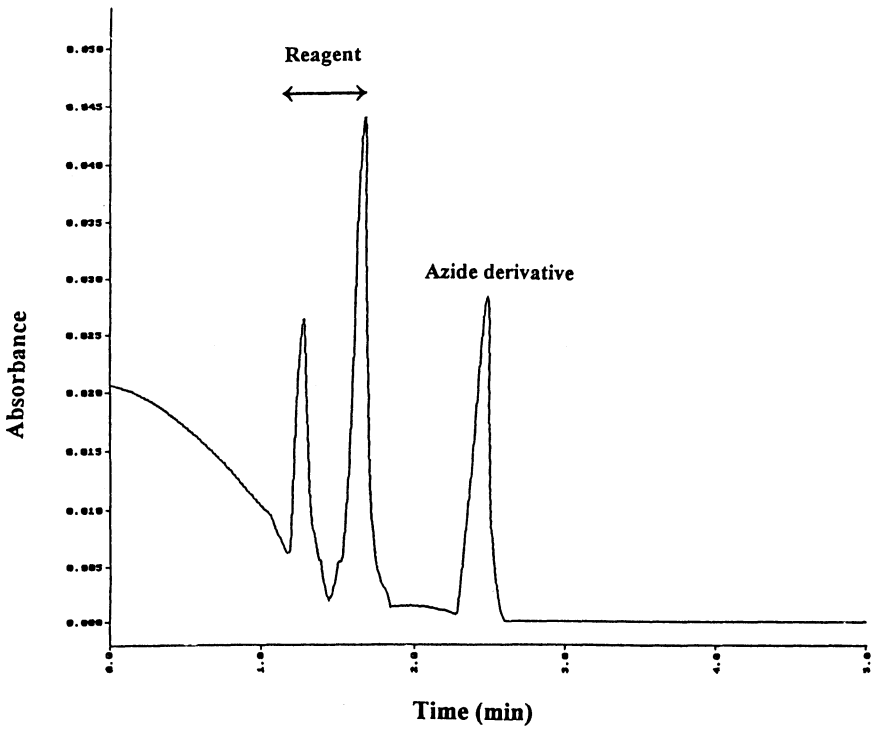

Fig. 2 Typical chromatogram of the 2,4-dinitrophenyl azide derivative, prepared by injection of 1 mL of the derivatized ground water into the 20 μL sample loop of the HPLC systm.

Comparison of the determination of nitrite by HPLC method and the standard colorimetric method: A variety of natural waters were collected, filtered, spiked with NO_2^- concentrations of 10.0, 50.0, and 100.0 nM. The results of the analysis of nitrite obtained using the HPLC method and the standard colorimetric method are listed in Table-1. The values obtained from the HPLC results agree well with the spiked concentration at the 95% confidence at all the tested concentrations. They were also in agreement (95% confidence interval) with those obtained using the standard colorometric method at 50 and

100 nM nitrite concentrations. However, lower recoveries were generally observed by use of the colorimetric method at a spiked concentration of 10 nM. The reproducibility (%RSD) of the HPLC method is in the range 2–5%, while that of the colorimetric method is in the range 3–8%.

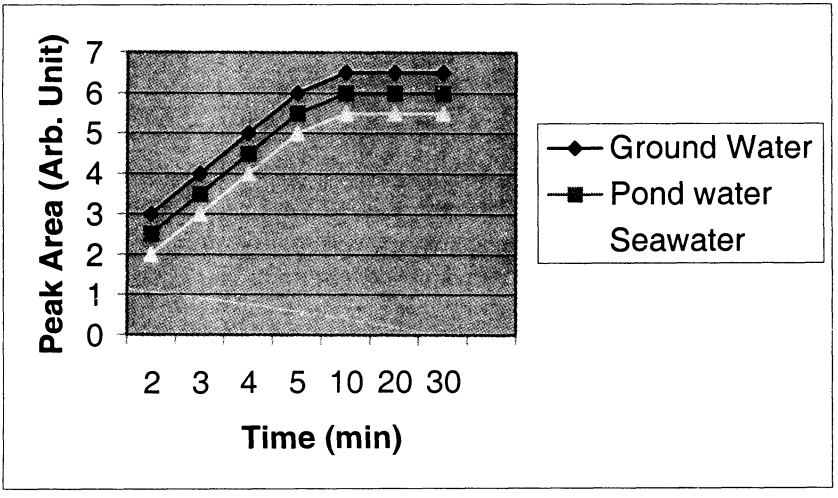

Fig. 3 The azide derivative peak area as a function of the time allowed for derivatization for ground, pond, and seawater. Each data point is the mean of five measurements.

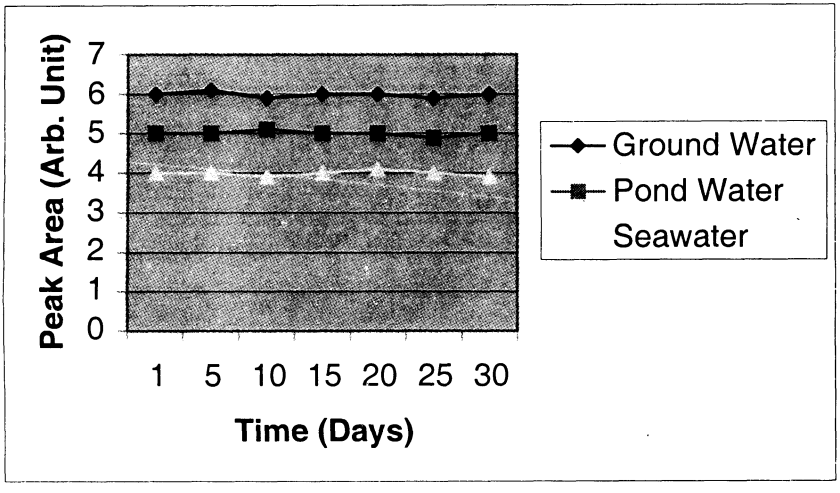

Fig. 4 Test of stability of the 2,4-dinitrophenyl azide for ground, pond, and seawater. Each time point represents the mean of four measurements on a given day.

Recoveries from natural water: In Figure 5 we compared recoveries of 200 nM nitrite from three types of natural water using the HPLC and colorimetric methods. The results indicated that the HPLC method recoveries range from 95 to 100%, with RSDs between 2 and 5% (n = 4). Lower recoveries were gener-

ally obtained by colorimetric method, range from 89 to 93%, with RSDs between 3 and 10% (n = 4). The data indicates that there is generally lower recovary from pond water than from ground or seawater. This observation suggests that the fulvic and humic materials, which result from the decomposition of organic materials in pond water, interfere in the analysis.

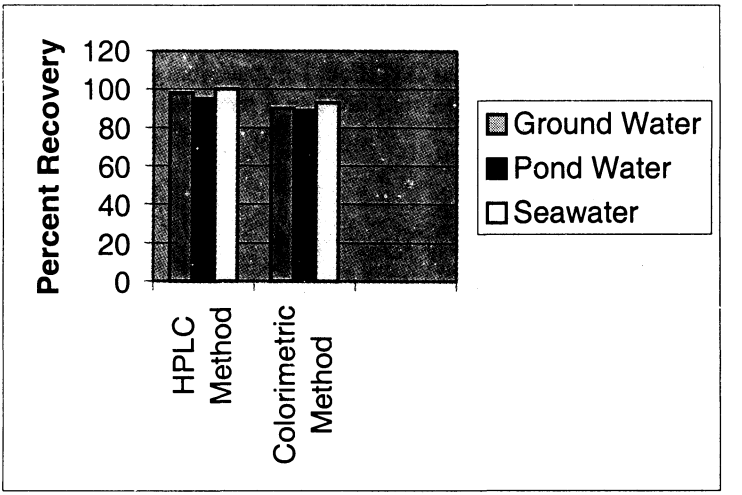

Fig. 5 Comparison of recoveries of 200 nM of nitrite from ground, pond and seawater by HPLC and colorimetric methods. Results presented are the mean of four replicates.

TABLE -1
COMPARISON OF NITRITE DETERMINATION BY THE HPLC AND THE STANDARD COLORIMETRIC METHODS

Water sample type	Spiked value nM (%RSD)	Determined by HPLC method, nM (%RSD)	Determined by colorimetric method, nM (%RSD)
Ground water	010	9.6 (2.1)	7.8 (4.5)
	050	49 (5.0)	46 (3.0)
	100	98 (3.3)	91 (7.1)
Pond water	010	9.0 (6.0)	7.5 (3.7)
	050	46 (5.2)	44 (6.2)
	100	94 (3.6)	89 (8.3)
Coastal seawater	010	9.9 (3.0)	8.0 (4.5)
	050	50 (2.3)	46 (3.5)
	100	100 (4.7)	93 (7.4)

[a] The reported results are the mean of at least four measurements.

TABLE -2
THE CONCENTRATIONS OF NITRITE DETERMINED IN A VARIETY OF GAZA STRIP NATURAL WATERS USING HPLC METHOD[a]

Water sample type (place of collection)	Nitrite concentration (nM)	% RSD
Ground water (Gaza city)	312	4.5
Ground water (Bit Lahia)	240	3.1
Ground water (Bureij Camp)	275	2.5
Ground water (Khanyunis)	482	6.8
Ground water (Rafah)[b]	268	5.2
Pond water (Wadi Gaza)	336	6.0
Coastal sea water (Gaza Beach)	21.5	3.8

[a] The reported results are mean of at least four measures
[b] All the ground waters mentioned above are used as drinking water.

Environmental Analysis: The concentration of nitrite in different types of Gaza Strip natural water was tested. The ground water samples were collected from different places in Gaza Strip, the pond water was collected from Wadi Gaza, and the seawater was collected from Gaza Coastal seawater. The results of the analysis were summarized in Table-2. It was noted that the concentrations of nitrite in ground and pond waters were the highest whereas the coastal seawater from Gaza beach was always observed to contain the lowest nitrite concentration level. This result is consistent with nitrite concentration levels reported in the literature[12, 13].

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