Spectrophotometric monitoring of nitrite in seawater after liquid microextraction of its derivative with 2,3-diaminonaphthalene
Mir Mahdi Zahedi, Amir Hosein Amiri and Mahmoud Nasiri

ABSTRACT
A simple dispersive liquid–liquid microextraction based on solidification of floating organic droplets coupled with spectrophotometric detection was developed for the determination of nitrite in Chabahar Bay seawater. In the preparation procedure, 2,3-diaminonaphthalene as derivatization reagent reacts with nitrite in acidic medium to form a photometric center of 1-[H]-naphthotriazole (NAT). Product material (NAT) was extracted by dispersive liquid–liquid microextraction with 1-dodecanol as extraction solvent and after centrifugation, the floated droplet was solidified in an ice bath and was easily removed for analysis at \( \lambda_{\text{max}} = 358 \) nm. Several important factors affecting the microextraction efficiency were optimized in artificial seawater as a best simulation media. Under the optimum conditions, the absorbance of NAT was linear with nitrite concentrations ranging from 0.1 to 11 \( \mu \)g/mL in seawater. Figures of merit of method such as enrichment factor (52), limit of detection (0.094 \( \mu \)g/mL), and repeatability (N = 6, %RSD = % 5) were evaluated as appropriate.

Determination of nitrite in Chabahar coastal zone showed that nitrite concentration varied in the range of 0.77–1.76 \( \mu \)g/mL with an increase of concentration from South to the North of Bay.

Key words | nitrite, seawater, solidification of organic droplet (DLLME-SFO), spectrophotometric detection, 2,3-diaminonaphthalene

INTRODUCTION
Nitrite is a naturally occurring anion in environmental waters and plays an important role in the analysis of seawater. The main anthropogenic source of nitrogen compounds in water are fertilizers which are being used in agriculture, mainly containing nitrate, ammonia, ammonium, urea and amines which are finally drained in water bodies (Malerba et al. 2012). The flux of these compounds is increased as a result of livestock excrements, urban and organic wastes from chemical industries, power stations, domestic wastes and septic tank effluents, and application of pesticides and herbicides in agriculture (McDonald 1981). Nitrite is one of the limiting nutrients and its concentrations in aquatic environments are used for assessment of water quality and evaluation of marine phytoplankton growth as well (Sciandra & Amara 1994). The eutrophication of these nutrients in water (Smith et al. 1999) can lead to algal blooms as widespread and critical problems in pollution of natural waters that causes hypoxia situation for living organisms, and blocks sunlight penetration into seawater. These situations have harmful effects on water quality and marine organisms (Landsberg 2002).

In the marine environment, nitrite is a natural compound and intermediate component in bacterial nitrification and denitrification processes of the nitrogen cycle (Ward 1996; Zehr & Kudela 2011) and its presence in the ambient marine environment have relatively toxic effects on aquatic animals (Lewis & Morris 1986; Jensen 2003). A high concentration of nitrite has a great impact on the intensive culture of commercial fish species (Svobodova et al. 2005). Fish absorb ions mainly through their gills, where the active uptake mechanisms
cause the accumulation of ions in the ion-transporting cells (Maetz 1971). The blood is the primary affected media of the nitrite so it diffuses into blood pigment and oxidises iron (III) of the haemoglobin (Hb) and form methemoglobin, thereby reducing the capacity of oxygen binding (Bodansky 1957; Kiese 1974). Moreover, nitrite can react with secondary amines and amides in human dietary components, resulting in the formation of carcinogenic nitrosamine and nitrosamides (Cox & Frank 1982; Akyüz & Ata 2009).

Therefore, the determination and control of nitrite is of great importance in the analysis of biological samples due to health problems posed by increased levels of this ion in water bodies. At present, a large number of analytical methods are available for the analysis of nitrite (Moorcroft et al. 2001) such as chromatographic (Jobgen et al. 2007), elec-
trometric (Perez-Olmos et al. 1998) and chemiluminescence methods (Garside 1982). Also, spectroscopic methods based on the reaction of nitrite with various reagents such as 3-aminonaphthalene-1,5-disulphonic acid (Motomizu et al. 1986), 5-aminofluorescein (Axelrod & Engel 1975), 5,6-diamino-1,3-naphthalene disulfonic acid (Wang et al. 2000), aniline (Masserini & Fanning 2000) and 4-hydroxyxoumarin (Ohta et al. 1986) were introduced for its determination.

Derivatization-based reactions for spectrophotometric determination of nitrite are the most commonly employed technique (Bendschneider & Robinson 1952). These are based on a well-known reaction, namely acidification of nitrite to form the nitrosium ion and its subsequent combination with a primary aromatic amine to form a diazonium ion (Seyhan 1989). The diazonium ion is then coupled with another aromatic compound to form an azo dye of which the absorbance is measured. Classically, nitrite has been determined by the Griess method, in which nitrite is diazotised with sulphhalimide and then reacted with N-(1-naphthyl)ethylenediamine to form a colored product (Indyk & Woollard 2001). This method suffers from low sensitivity, low specificity and interference by other ions, oxidants, colored matter and turbidity, especially in biological samples (Damiani & Burini 1986).

2,3-Diaminonaphthalene (DAN) is a highly selective and suitable reagent for spectroscopic determination of nitrite in biological samples. This reagent has some advantages such as high sensitivity, high specificity, simplicity in both sample preparation and derivatization, stable derivatives, linearity with a wide range of nitrite levels, low cost, universality, and lack of interferences when sufficiently dilute samples are used (Jobgen et al. 2007). Wiersma (1970) described a DAN procedure for spectrophotometric and fluorometric determination of nitrite. In this method the formed derivative was extracted from aquatic solution by double extraction steps with solvent-like 1,1,2,2-tetrachloroethane. Thereafter, Sawicki (1971) employed Wiersma’s procedure with a minor modification to develop a fluorometric method for the determination of nitrate in aqueous solution using a mixture of 1,2-dichloroethane and chloroform for extraction.

Here, compared to toxic chlorinated solvents which have been used for the extraction in earlier reports, we employed dispersive liquid–liquid microextraction based on solidification of organic droplet (DLLME-SFO) method (Rezaee et al. 2010; Abadi et al. 2012). This method permits high enrichment and extraction of trace level of nitrite from the complex matrix of seawater using low toxicity organic solvents such as 1-dodecanol, which are lighter than water. To the authors best knowledge there is no report on DLLME-SFO and spectrophotometric measurement of nitrite in seawater as yet.

MATERIAL AND METHODS

Apparatus

A Centurion Scientific K3 series K241R centrifuge was used to accelerate phase separation. A TPS WP-80 digital pH meter was used for pH adjustments. A 2 mL Hamilton syringe (Hamilton Company, Nevada) was used for injection of mixed volume of extraction solvents. Spectrophotometry studies were accomplished by an UNICO UV-Vis spectrophotometer model s2100 equipped with quartz microcell.

Reagents

All reagents were of analytical grade and obtained from Merck (Darmstadt, Germany), Fluka (Buchs, Switzerland) and Sigma–Aldrich (Steinheim, Germany) and used without further purification. All solutions were prepared with doubly distilled water (DDW). Stock nitrite solution (100 ppm) was prepared by dissolving 15 mg of sodium nitrite (previously dried for 4 hours at 110 °C) in 100 mL of DDW, followed by adding 10 drops of chloroform and a pellet of sodium hydroxide as preservative.
in order to prevent bacterial growth. More dilute solutions were prepared daily by diluting of stock solution with DDW. DAN reagent was prepared daily by dissolving 5 mg of DAN in 100 mL of 0.62 M hydrochloric acid, stored in a brown glass bottle at –18 °C. Artificial seawater was prepared by dissolving the following salts in 1 L of doubly deionized water: NaF (3 mg), KBr (100 mg), Na₂SiO₃·9H₂O (20 mg), SrCl₂·2H₂O (20 mg), KCl (3 mg), MgCl₂·6H₂O (10.780 g), NaHCO₃ (200 mg), H₃BO₃ (30 mg), CaCl₂·2H₂O (1.470 g) and NaCl (23.500 g) (Nassiri et al. 2014; Zahedi & Rezaei 2016).

Derivatization and DLLME-SFO procedure

Under optimum conditions of DLLME–SFO, 10 mL seawater sample was filtered through a 0.20 μm membrane filter, and its pH reset by an adding adequate amount of hydrochloric acid to 1.5 (as optimal pH for derivative reaction of nitrite with DAN reagent and further DLLME–SFO procedure). The reaction of nitrite with DAN was performed by adding 1.0 mL of DAN reagent to the sample, mixed and let stand for 5 minutes. A UV-vis spectrum of nitrite derivate with DAN (NAT: 2,3-naphthotriazole) in extraction solvent and its kinetic cure are shown in Figure 1(a) and 1(b). Then, aqueous sample was placed in a 15 mL screwcap glass tube and the microextraction procedure was carried out by rapid injection of a mixed solution of 250 μL 1-dodecanol (extraction solvent) and 1.2 mL methanol (dispersive solvent) into the sample which leads to a cloudy solution being formed. This sample was centrifuged for 5 minutes at 3,000 rpm and then placed in an ice bath. The organic solvent was solidified in 5 minutes and transferred into a conical vial to melt. Forty microliters of methanol was added to the extractant and mixed. Finally, 70 microliters of the mixture were used for determination of nitrite at \( \lambda_{\text{max}} = 358 \text{ nm} \).

RESULTS AND DISCUSSION

Effect of DAN concentration

To optimize the concentration of the DAN derivatizing agent a series of 10 mL aliquots of nitrite (10 μg/mL) were treated with 1 mL of DAN solutions with concentrations ranging from 10 to 80 μg/mL in 0.62 M hydrochloric acid. As shown in Figure 2 (upper curve), the increasing concentration of DAN within the range of 10–50 μg/mL resulted in the highest absorbance. There were no more absorbance changes in the upper concentration of DAN. Based on these results, the 50 μg/mL of DAN

![Figure 1](https://iwaponline.com/wqrj/article-pdf/52/1/11/378560/wqrjc0520011.pdf)

![Figure 2](https://iwaponline.com/wqrj/article-pdf/52/1/11/378560/wqrjc0520011.pdf)
concentration was determined as the optimal amount for reaction with nitrite (Sawicki 1971; Damiani & Burini 1986).

**Effect of pH**

The effect of pH in spectrophotometric determination of nitrite in seawater has two aspects that should be investigated. The first aspect is finding the optimal pH associated with the reaction of nitrite ion with DAN in seawater medium in which the derivative was formed with the highest absorbance. As seen in Figure 2 (lower curve), the pH of 1.5 gave the best result and was chosen as the optimum reaction pH and adjusted to this point using 0.62 M hydrochloric acid.

The second aspect is the effect of pH on the microextraction procedure, which was investigated by extracting prepared solution of seawater samples, varying with pH from acidic to basic in the range of 2.0–9.5 and not higher, because at high pH values, Mg and Ca precipitate out of seawater. This was due to the precipitation of metal ions available in artificial seawater with OH⁻ in basic condition. Based on the results obtained, the absorbance was independent of pH in DLLME-SFO procedure. Hence we decided to extract samples at pH = 1.5, the best conditions for derivatization of nitrite with DAN.

**Selection of extraction and dispersing solvent**

Selecting a suitable extraction solvent is of great importance in the DLLME-SFO method. It should have low solubility in water, high solubility in the disperser solvent, low volatility, low toxicity, low melting point near room temperature, lower density than water and high affinity to analyte (Rezaee et al. 2010; Abadi et al. 2012). Also, dispersing solvents should be miscible with both water and the extraction solvents and form a cloudy state when injected with the extraction solvent into aqueous samples. Based on these criteria, three possible organic solvents were investigated for use as the extraction solvent, including 1-undecanol, 1-dodecanol, and n-decanol in combination with methanol, ethanol, acetonitrile and acetone as dispersing solvents. Complete cloudy conditions did not exist by using acetone as the dispersing solvent. The best extraction results were obtained when using 1-dodecanol (melting point: 22– 24°C) and 1-undecanol (melting point: 13– 15°C) as extraction solvent, in combination with methanol as dispersing solvent. Ultimately, 1-dodecanol with a melting point close to room temperature, which made it easier to remove the solidified drop, was chosen as the extracting solvent. The results of these studies are shown in Figures 3 and 4.

**Effect of extraction solvent volume**

To study the effect of extraction solvent volume on the DLLME-SFO procedure, a series of seawater samples (treated under previously optimized conditions) were extracted with different volumes of 1-dodecanol (50, 100, 150, 200, 250, 300, 350 μL) as extracting solvent and 1.0 mL fixed volume of methanol as dispersing solvent. As shown in Figure 5 (lower curve), when the volume of 1-dodecanol was increased...
(from 50 to 250 μL) the extraction efficiency was increased accordingly. The upper volume of solvent extraction led to a slight decrease in the extraction efficiency of NAT. This may be explained by the dilution effect of NAT in the extracted organic phase. Therefore, 250 μL of 1-dodecanol was chosen as the optimum volume of extraction solvent.

**Effect of dispersing solvent volume**

The influence of dispersing solvent volume on the extraction efficiency was investigated. A series of treated seawater samples was extracted with various volumes of methanol (0.5, 0.7, 1.0, 1.2, 1.5, 1.7, 2.0 mL) as the disperser solvent. The results shown in Figure 5 (upper curve) indicate that the extraction efficiency was lower when the volume of methanol was less than 1.2 mL, because at volumes lower than 1.2 mL, the cloudy state could not be formed well, and the extractant could not be dispersed well in the sample solution. Conversely, increasing the volume of methanol from 1.2 to 2.0 mL led to a slight decline in absorbance due to enhanced solubility of 1-dodecanol in aqueous solution containing a high percentage of methanol. Therefore, 1.2 mL volume of methanol was selected as optimum.

**Effect of salt addition and extraction time**

Generally, the increase of ionic strength can cause a decrease in the solubility of the analytes in sample solution, and enhance the extraction efficiency. The effect of increasing ionic strength of the seawater sample on the DLLME-SFO efficiency was studied by adding NaCl (0–10% w/v) into the sample. DLLME-SFO experimental conditions were the same as those optimized before. No significant effect on the absorbance of NAT was found when different amounts of NaCl were added into the aqueous solution. However, this tendency could be predictable due to the presence of large quantities of salt components in the seawater matrix.

The effect of extraction time on the extraction efficiency was examined in the range of 0–10 minutes. No significant difference between extraction times was found. It has been proved (Zahedi et al. 2014) that the surface areas between extraction solvent and sample solution are infinitely large after forming the cloudy solution. Therefore, the analyte mass transfer into the extraction solvent is so fast that the extraction equilibrium can be achieved in a short time and the time of extraction is shortened considerably (Xu et al. 2009). Consequently, the extraction time of 1 minute was chosen as optimal for application in further experiments.

**Application and analytical performance**

Under the optimal experimental conditions, figures of merit for the suggested method such as enrichment factor, linear range, limit of detection (LOD; 3 σ/m where σ is standard deviation, m is the slope of the calibration curve) were estimated for the determination of NAT in seawater samples. The results are listed in Table 3. The LOD was calculated to be 0.05 μg/L, which satisfies the proposed limits for seawater quality standards. The enrichment factor and linear range were calculated to be 100 and 0.1–20 mg/L, respectively, which are within the acceptable range for DLLME-SFO methods.

![Figure 5](https://iwaponline.com/wqrj/article-pdf/52/1/11/378560/wqrjc0520011.pdf)

**Figure 5** Effect of extraction solvent volume (lower curve) and dispersing solvent volume (upper curve) on the DLLME-SFO procedure for spectrophotometric determination of NAT.

![Figure 6](https://iwaponline.com/wqrj/article-pdf/52/1/11/378560/wqrjc0520011.pdf)

**Figure 6** Locations of coastal Chabahar sampling sites.
deviation of background absorbance for seven replications and m is slope of the calibration line), limit of quantification (LOQ: 10 σ/m) and repeatability were evaluated. The calibration curve was linear in the range of 0.1–11 μg/mL with a coefficient of determination of 0.9974. Characteristics of the method for nitrite determination such as enrichment factor (52), LOD (0.094 μg/mL), LOQ (0.314 μg/mL), and repeatability, (N = 6, %RSD = % 5) were evaluated as appropriate.

In order to evaluate the quantitative performance of the method, several surface seawater samples from eight sites of Chabahar Bay were taken in January 2015 (Figure 6). Samples were kept in ice boxes and transported to the laboratory and stored at −18 °C in order to stabilize the nitrite content of the seawater until the analysis time (Procter 1962). Before analysis, collected samples were filtered using a 0.20 μm membrane filter to eliminate suspended matter. Then these real samples were treated by the DLLME-SFO procedure for photometric analysis. The results of this analysis are listed in Table 1. The nitrite concentration in Chabaher Bay increased from south to the north of the bay and comparison of these results with other work (Akyüz & Ata 2009) illustrates that Chabaher Bay has a low concentration of nitrite.

### CONCLUSIONS

In the present study, a novel dispersive liquid–liquid microextraction based on solidification of floating organic droplet method (DLLME-SFO) coupled with photometric detection has been successfully developed to analyze nitrite in seawater samples. The number of liquid–liquid microextraction methods for the determination of non-metals is low and few of them are devoted to the determination of nitrite. Only one procedure for the determination of inorganic anions by SFOD method has been published to date. We employed the DLLME-SFO method which was simulated for the seawater matrix and has numerous advantages such as simplicity of operation, rapidity, low cost, low toxic, high efficiency and high preconcentration factor.

### REFERENCES


Akyüz, M. & Ata, S. 2009 Determination of low level nitrite and nitrate in biological, food and environmental samples by gas
Spectrophotometric monitoring of seawater nitrite


First received 15 September 2016; accepted in revised form 14 December 2016. Available online 19 January 2017.