



## Fluorimetric determination of nitrite in water using a novel fluorescent dye



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### ABSTRACT

A simple, sensitive and selective fluorimetric method for the determination of trace amounts of nitrite in different water samples (e.g., underground water, geothermal waste water, domestic waste water and drinking water) was proposed. The fluorimetric determination is based on the diazotization reaction of nitrite with a fluorescent dye, tetraamino zinc (II) phthalocyanine in an acidic medium. Under the optimum experimental conditions, nitrite can be determined in the range  $10^{-6}$ – $10^{-4}$  mol·L<sup>-1</sup> with the detection limit  $2.04 \times 10^{-8}$  mol·L<sup>-1</sup>. The proposed method has been successfully applied to the determination of nitrite in various water samples. The results compared favorably with the results from a standard isocertified method.

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### 1. Introduction

Nitrite is a very important substance that has been used widely in food, industrial and agricultural production. Together with sewage treatment plants and industrial discharges, agriculture, forestry and freshwater aquaculture are the most important sources responsible for water and soil pollution, and farming activities represent one of the most important pollution sources in Europe. With the currently high dose application of nutrients, with overloading and inefficient use of these resources, agricultural run-off becomes a major contributor to the eutrophication of fresh water rivers and lakes. As a result nitrite is ubiquitous as fertilizing-by products from nitrate, crop productions, crop residues, manures etc. and eventually becomes a hazard as nitrite pollution into different water areas (agricultural water catchments, waste waters, underground water, lakes, fresh water and drinking water) [1,2]. Furthermore the excessive intake of nitrite from food products and from drinking water is hazardous to human health and can result in many diseases with the formation for example of highly carcinogenic nitrosamine substances [3]. Several international organizations (World Health Organization, WHO) [4] and EU policies (European Drinking Water Directive) [5] are involved with environmental

issues or with human health, are in place to improve one or several aspects of drinking water nutrient efficiency and some have imposed strict limits regarding the maximum quantity of contaminants that can be found in drinking water. In this regard EPA (United States Environmental Protection Agency) [6] has set an enforceable standard called maximum concentration level (MCL) of 1 mg·L<sup>-1</sup> (1 ppm) for nitrite [EPA 2002; EPA 2012], 9 Aug 2016 and the European Union of 0.50 mg·L<sup>-1</sup> for nitrite in drinking water.

The presence and level of nitrite in water pollution is therefore of great concern and various techniques having been developed over the years to detect and reduce the content in different water areas [7–11]. Most of the common methods used [8,9] include chromatography: high performance liquid chromatography [12–14], ion chromatography [15,16], electrochemical detection [17–20], flow-injection with chemiluminescence detection [21,22] or spectrophotometric methods [10, 11,23–26] (e.g. the Griess reaction). Various comparative tables with different methodologies for the determination of nitrite are outlined by Moorcroft et al. [7] and Wang et al. [8].

Besides these well-known detection techniques we developed a simple, sensitive and selective fluorimetric method for the determination of nitrite. The fluorimetric determination is based on the diazotization reaction of nitrite with a fluorescent dye, tetraamino zinc (II) phthalocyanine (ZnTAPc) in an acidic medium. The proposed method has been successfully applied to the determination of nitrite in different water samples and the results obtained compared favorably with the results from standard isocertified methods.

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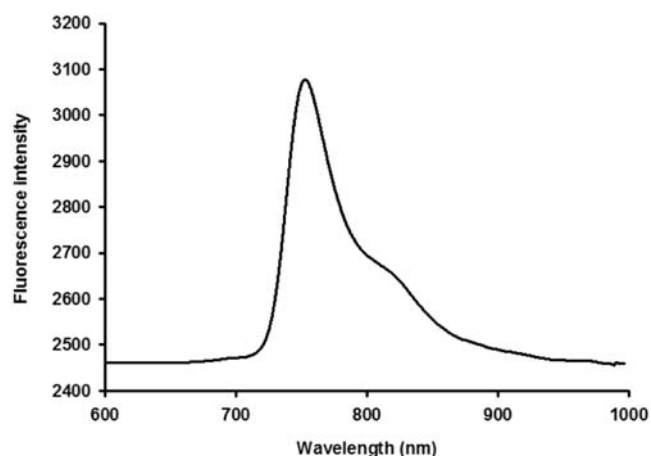


Fig. 1. Fluorescence spectrum for the Zinc tetraamino phthalocyanine in DMF. ( $10^{-4} \text{ mol} \cdot \text{L}^{-1}$ ).  $\lambda_{\text{max}} = 754 \text{ nm}$ .

## 2. Experimental

### 2.1. Reagents

All chemicals were of analytical reagent grade, and deionized water was used for preparation of all solutions. Deionized water was obtained from a Direct-Q 3 Water Purification system (Millipore Corporation, France).

A standard stock solution of  $10^{-1} \text{ mol} \cdot \text{L}^{-1}$  nitrite was prepared by dissolving 0.3450 g of sodium nitrite (purchased from Sigma-Aldrich) in 50 mL deionized water using the same precautions as described previously [10,11] and stored in a refrigerator.

Tetraamino zinc(II) phthalocyanine was synthesized and purified as previously described [27]. The stock solution of Zinc tetraamino phthalocyanine ( $10^{-3} \text{ mol} \cdot \text{L}^{-1}$ ) was prepared by dissolving 0.0127 g of ZnTAPc in 20 mL *N,N*-Dimethylformamide (DMF). Working solutions of ZnTAPc ( $10^{-4} \text{ mol} \cdot \text{L}^{-1}$ ) was prepared by dilution of the stock solution with DMF.

A hydrochloric acid (HCl) (obtained from Sigma-Aldrich) solution of  $6 \text{ mol} \cdot \text{L}^{-1}$  was prepared by diluting 9.85 mL of concentrated HCl (37%) to 20 mL with deionized water. Working solutions were prepared daily by appropriate dilution of the stock solution with deionized water.

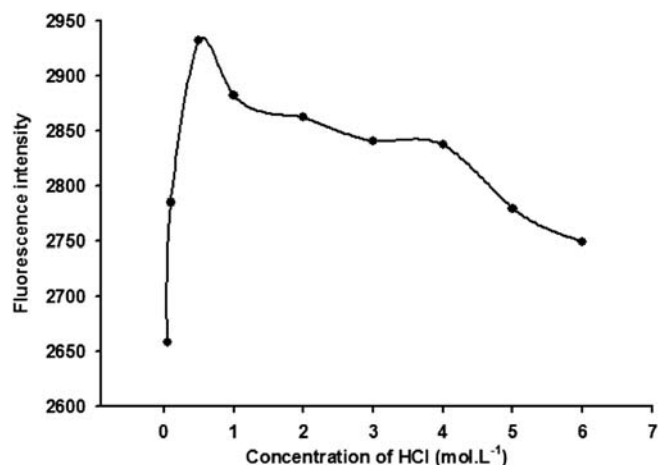


Fig. 2. Effect of HCl concentration on the fluorescence intensity. Conditions: [Nitrite] =  $10^{-4} \text{ mol} \cdot \text{L}^{-1}$ ; [ZnTAPc] =  $10^{-4} \text{ mol} \cdot \text{L}^{-1}$ .

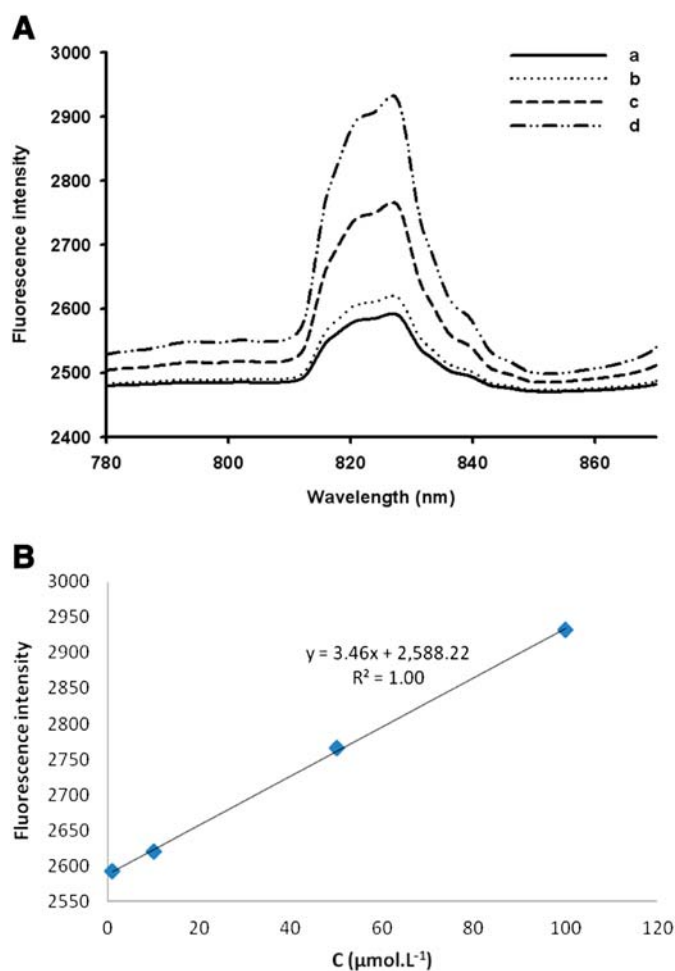


Fig. 3. A. Fluorescence spectra of Zinc tetraamino phthalocyanine. (a) ZnTAPc + HCl +  $\text{NO}_2^-$  ( $10^{-6} \text{ mol} \cdot \text{L}^{-1}$ ), (b) ZnTAPc + HCl +  $\text{NO}_2^-$  ( $10^{-5} \text{ mol} \cdot \text{L}^{-1}$ ), (c) ZnTAPc + HCl +  $\text{NO}_2^-$  ( $5 \times 10^{-5} \text{ mol} \cdot \text{L}^{-1}$ ), and (d) ZnTAPc + HCl + NO ( $10^{-4} \text{ mol} \cdot \text{L}^{-1}$ ). Conditions: [ZnTAPc] =  $10^{-4} \text{ mol} \cdot \text{L}^{-1}$ , [HCl] =  $0.5 \text{ mol} \cdot \text{L}^{-1}$ ,  $\lambda_{\text{max}} = 827 \text{ nm}$ ; B. Calibration curve.

### 2.2. Apparatus

The fluorescence measurements were done with a spectrometer QE65000 from Ocean Optics (Dunedin, Florida) equipped with a xenon lamp (HPX 2000). The HPX-2000 Xenon Light Source is a high-power, high-intensity source that is especially useful for fluorescence applications and for other applications where a high-intensity lamp is

**Table 1**  
Tolerance limits of foreign ions in the determination of  $10^{-5} \text{ mol} \cdot \text{L}^{-1}$  nitrite.

Foreign ions	Tolerance limit (molar ratio)
$\text{Ba}^{2+}$	1000
$\text{Fe}^{2+}$	1000
$\text{CH}_3\text{COO}^-$	1000
$\text{Co}^{2+}$	500
$\text{K}^+$	500
$\text{CO}_3^{2-}$	500
$\text{Cu}^{2+}$	100
$\text{Ni}^{2+}$	100
$\text{Pb}^{2+}$	100
$\text{Br}^-$	10
$\text{Ca}^{2+}$	10
$\text{Cl}^-$	10
$\text{Mn}^{2+}$	10
$\text{Mg}^{2+}$	10
$\text{Zn}^{2+}$	10

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