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Solid-Phase Test Reagent for Determination of Nitrite and Nitrate

Ekaterina P. Nesterenko[1], Brian Murphy[1,2], Eoin Murray[3], Breda Moore[3], Dermot Diamond[1]

1. *Insight Centre for Data Analytics, National Centre for Sensor Research, Dublin City University, Dublin 9, Ireland*

2. *School of Chemical and Pharmaceutical Sciences, Dublin Institute of Technology, Dublin, Ireland*

3. *T.E. Laboratories Ltd. (TelLab), Tullow, Carlow, Ireland*

Abstract – A non-toxic solid-phase test reagent for rapid determination of nitrite and nitrate was developed. Powdered reagent utilised azo- and diazo-components (*p*-nitroaniline and chromotropic acid, respectively), solid organic acid acidifier (malonic, maleic or oxalic acid), zinc dust (for nitrate determination), catalyst and masking agent. Effects of solid acidifier and the amount of Zn reducing agent were evaluated. The optimal reagent formulation was established in terms of dynamic range and response kinetics, and validated by parallel measurements in split samples using an accredited ion-chromatographic method. The optimal reagent contained malonic acid as acidifier and 1.0% of Zn reducing agent, and showed good sensitivity for the determination of nitrite (LOQ of 0.03 mg/L) and nitrate (LOQ of 0.17 mg/L), and fast analysis time of under 6 min. These reagents were shown to be stable for at least 3 months with RSD of <3%.

Keywords: nitrite, nitrate, solid-phase reagent, colourimetry, water analysis

1. Introduction

Monitoring the content of nitrite and nitrate in natural and drinking waters, food (where nitrates are often used as preservatives), forage and feed additives, as well as soil and other environmental objects is one of the major analytical tasks due to wide and often uncontrolled usage of nitrogen fertilisers and the ability of plants to accumulate nitrate in their tissues.[1,2] Leaching of large amounts of nitrates into groundwater and water reservoirs

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leads to accelerated growth of algae, and as a consequence, to the depletion of water oxygen and subsequent death of fish and other underwater fauna.[3,4]

The toxicity of nitrate itself is quite low, however, the product of its reduction, nitrite is highly toxic.[5] Nitrite in the bloodstream is involved in the oxidation of haemoglobin (Hb) to methaemoglobin (metHb), a molecule that lacks the capacity to bind and transport oxygen to tissues. The resulting condition is called methaemoglobinaemia. Nitrite may also react in the stomach with nitrosatable compounds (e.g. secondary and tertiary amines or amides in food) to form carcinogenic N-nitroso compounds.[6] According to EU regulation, the accepted daily intake of NO_2^- and NO_3^- is 0-0.07 mg/kg and 0-3.7 mg/kg of body weight, respectively.[7,8] Thus, determination of nitrite and nitrate in food, drinking water, feed and water used in agriculture and marine industries becomes of primary importance.

Although methods for the determination of nitrite and nitrate are well developed and include both classical chemical and physical-chemical analytical techniques, the majority of these methods are laborious and require time-consuming sample preparation due to sample matrix complexity, and some of them may have quite low sensitivity.[9,10] In some cases, the set analytical problem can be solved by using test methods based on the formation of coloured products. Photometric and colorimetric methods for the determination of nitrate and nitrite are based on reactions of formation of nitroso-compounds; nitration or oxidation reactions; formation of ion pairs, extractable with organic solvents, and formation of azo-compounds (Griess reagent and its modifications).[11-13]

Griess method, based on diazotisation and azocoupling reaction, is considered to be the most suitable test method and is currently remaining the official method of analysis of the Association of Official Analytical Chemists (AOAC) for the determination of nitrate and nitrite.[14] However, despite its popularity and variety of types of Griess reaction, it has a number of disadvantages: diazotisation and azocoupling are reactions that are conducted at different acidity (strongly acidic for diazotisation and weakly basic for azocoupling); 1-naphtylamine that is typically used for azocoupling is easily oxidised and is strongly carcinogenic, also the colour change of the tested solution is from colourless to purple, making it very hard to visually determine the change of colour at low concentration of analytes without using spectrophotometric instrumentation. All of this makes this reagent

less user-friendly for use by untrained public, such as aquarium owners and farmers – individuals, who need a close monitoring of nitrite/nitrate contents in water, as well as for field tests.

Currently there are a number of nitrite/nitrate instrument-less test kits in liquid, solid, or immobilised form (onto paper or plates), produced by Merck,[15-19] Macherey-Nagel,[20-22] Hach[23-26] (Table 1). However, these methods are not free from disadvantages either. They are either time consuming (i.e. up to 40 min, Hach), use multiple reagents and vials (i.e. Merck), or have quite a high LOD, especially for NO_2^-. Some of these methods utilise concentrated acids/bases as reactions have to be carried out at different pH, or cadmium powder for NO_3^- reduction to NO_2^-, the introduction of which can be challenging. Also high sensitivity of reactive indicator papers leads to the problems of their preparation and storage. Thus, despite the variety of test methods available, there is still a need for the development of simple, user-friendly and safe test kits for the determination of NO_2^- and NO_3^-. Pelleted or powered reagents are a promising form, however, despite a number of such test-methods being reported over the last decade, in many cases they still lack sensitivity, or are time consuming.[12,27,28] The aim of this work was to improve the sensitivity of the determination of nitrite and nitrate ions through a method based on utilising pre-mixed solid reagents or pellets.

Table 1. LOQ values for some commonly used commercial nitrite/nitrate tests of different formulation.

Test for nitrite	LOQ NO_2^-, mg/L	Ref	Test for nitrate	LOQ NO_3^- mg/L	Ref
Merck Millipore					
MQuant: test strips	100	[17]	MQuant: test strips	10	[19]
Spectroquant: photometric test, liquid reagents	0.007	[18]			
MColortest NO_2^-: colorimetric with color card and sliding comparator	0.025	[15]	MColortest NO_3^-: colorimetric with color card and sliding comparator	10	[16]
Macherey-Nagel					
Quantofix: test strips	1	[20]	Quantofix: Test strips	10	[20]

Nanocolor: photometric test, liquid reagents	0.3	21	Nanocolor Nitrate Z: photometric test, liquid reagents	0.1	22
Hach					
Nitrite: test strips	1	23	Nitrate: test strips	1	23
Nitrate-Nitrite Test Kit, Model NI-12: photometric test, powder reagent	0.01	24	Nitrate-Nitrite Test Kit, Model NI-12: photometric test, powder reagent	0.01	24
NitriVer 3: Powder pillows	0.002	25	Nitrogen-Nitrate: Powder pillows	0.01	26

2. Experimental

Reagents. All chemicals used within this study were of analytical grade purity. *p*-Nitroaniline, chromotropic acid, potassium chloride, potassium bromide, Na_2EDTA, oxalic acid, malonic acid, maleic acid, acetone and zinc dust (5-10 µm) were all purchased from Sigma-Aldrich (Gillingham, UK). Standard solutions containing 1000 mg/L of NO_3^- or NO_2^- were kindly provided by T.E. Laboratories (Ireland). Working solutions in the concentration range from 0.05 to 200 mg/L for NO_3^- and from 0.05 to 50 mg/L for NO_2^- were prepared via serial dilutions with deionised water. Deionised water (18.2 MΩ) for the preparation of standard solutions, samples and the mobile phase was supplied from a Milli-Q system (Millipore, Bedford, MA, USA).

Instrumentation: Absorbance was measured using Shimadzu UVmini 1240 (Shimadzu, Japan). Kinetic studies were carried out using a Perkin Elmer Lambda900 UV-VIS-NIR spectrometer (Santa Clara, USA). Ion-chromatographic determination of NO_3^- was performed using an isocratic Dionex DX-120 Ion Chromatograph (Dionex, Sunnyvale, USA), equipped with autosampler and anion self-regenerating suppressor Dionex AERS 500 (4 mm) for suppressed conductivity detection.

Method: For the determination of target analytes, a 5 mL sample aliquot was added to a 100 ± 1 mg portion of the powdered reagent and left to react. Upon the addition of the NO_2^- (or NO_3^- for nitrate reagent) - containing solution, the reagent powder dissolved within 10-15 sec and colour started to develop within 1 min. At low concentrations of NO_2^- or NO_3^- (0.05 mg/L and 0.5 mg/L respectively), a colour change of the solution was from pale hay-

yellow to ochroid-yellow, and at high concentration of NO_2^- or NO_3^- (over 20 mg/L and 100 mg/L respectively), colour changed to ripe cherry red hue. Intermediate hues were orange-yellow, orange, orange-red and red (Figure 1), and were well differentiated visually, making the method suitable for both spectrophotometric and, importantly, visual colorimetric detection.

Ion-chromatographic analysis: Prior the analysis all sample were filtered through a membrane filter with a pore size of 0.2 μm in order to remove debris. A 3.5 mM Na_2CO_3/ 1.0 mM $NaHCO_3$ solution was used as a mobile phase at a flow rate of 1.2 mL/min. An IonPac AS14 (250 x 4 mm I.D.) anion exchange column (Dionex, Sunnyvale, USA) was used. Analysis was carried out in the accredited laboratory according to an ISO 17025 method.
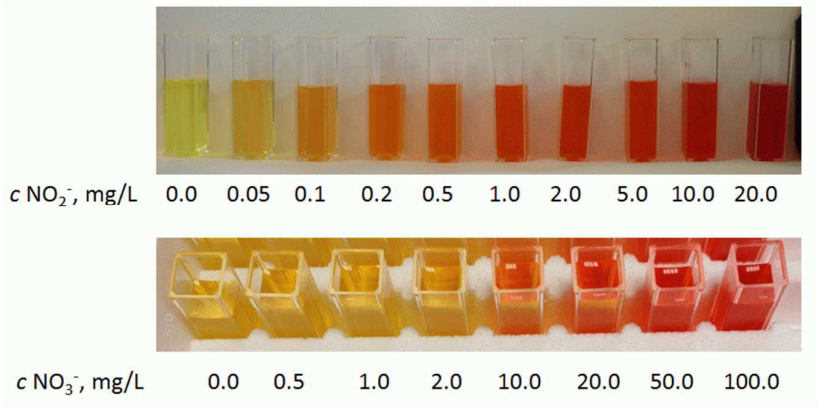

Figure 1. General reagent colour change scale.

3. Results and Discussion

Powdered reagents for the determination of nitrite, based on Griess-Ilosvay reaction and utilising tartaric acid as acidifier have been used for over 30 year.[29] Lampe's work was also amongst first attempts to move away from using carcinogenic 1-naphtylamine. Later a number of works were devoted to the development of a modified reaction, where *o*-nitroaniline,[30] *o*-, *m*-, *p*-nitroaniline and 2-nitro-4-aminotoluene[31] were used as diazo-components, and pyrocatechol, resorcinol and chromotropic acid[31,32] were used as azo-components.

The reaction of diazotisation of *p*-nitroaniline and its azocoupling with chromotropic acid is a well-studied reaction (the reaction mechanism is presented in ESI, Figure S1), the reagents used are non-toxic, and in different modifications it has been applied to analysis of various samples.[27] However, methods, developed based on this reaction are not free from disadvantages. Thus, solid-form reagent employing inorganic and organic supports to facilitate pellitisation has a high LOQ (30 mg/L for colorimetric detection),[12] while the procedure, utilising liquid reagents with subsequent sorption has a low LOD (0.6 µg/L), but is time consuming as solutions have to be held for 45 min.[27]

In the present work, a method described in [33] was chosen, as it provided rather fast analysis time, however, albeit with rather low sensitivity. Therefore our goal in this research was to combine the rapid analysis potential of this approach with significantly improved sensitivity. The solid-phase reagent, described in [33] consists of p-nitroaniline as diazo-component and chromotropic acid as azo-component, KCl and KBr that catalyse the reaction, as well as EDTA for masking heavy metal cations that might be present in analysed samples, and finally, oxalic acid as acidifier.

In their work Knyazev *et al.*[12] showed that the sensitivity of determination of NO_2^- depended on pH and decreased in the order pH 2> pH 3 >pH 1. The importance of acidity places special requirements on the choice of acidifying agents: they have to be readily soluble, non-hygroscopic, colourless, non-toxic acids with pK_a in the range of 1.5 to 3.5.

Here, the choice of the acidifying agents was determined by the specifics of the task: if the developed reagents were to be used for nitrite/nitrate analysis of samples with high content of Ca^{2+}, Mg^{2+} and/or heavy metals, the use of tartaric or oxalic acids (previously studies as solid acidifiers[12]) would lead to the formation of poorly soluble, or insoluble tartrates and oxalates.

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For this study malonic, maleic and oxalic acids were chosen as acidifying agents, with the latter taken for comparison. These acids satisfy the requirements listed above, and their pK_a values are as follows: malonic acid – pK_{a1} = 2.83, pK_{a2} = 5.69; oxalic acid - pK_{a1} = 1.25, pK_{a2} = 4.14; maleic acid - pK_{a1} = 1.90, pK_{a2} = 6.07. Importantly, these acids have chelating properties, thus facilitating masking possible hindering metal cations.

Reagent for the determination of nitrite.

The solid-phase reagent for nitrite determination contained *p*-nitroaniline (2 mass %), chromotropic acid (2 mass %), KCl (4 mass %), KBr (12 mass %), Na_2EDTA (0.4 mass %) and 79.6 mass % of solid acidifier (malonic, maleic or oxalic acid). For the preparation of the reagents to be tested, all the components (except for Zn dust, which is added to the mixture at the latest stage for nitrate test reagent only) were weighed out, thoroughly mixed together and placed into the agate mortar and wetted with a small amount of acetone. Then the mixture was ground with the pestle until all acetone evaporated and the resultant powder was thin and dry. The colour of the reagent with malonic or maleic acid as acidifier was lemon-yellow with a green hue, while for the reagent with oxalic acid as acidifier, the colour was light moss-green. The resultant reagent was stored in a sterile high-density polypropylene vial with a tight lid, which was placed in a dry and dark location at ambient temperature.

To identify the minimum time required for the completion of the reaction, a series of experiments was carried out for reagents with different acidifiers. For this, a 5 mL aliquot of a nitrite solution of varied concentration was added to a 100 ± 1 mg portion of a reagent that was placed into the measurement UV-Vis cuvette equipped with a lid. The stopwatch was started at the same time as the addition of the solution was made. After the solution was added, the mixture was vigorously shaken for 5 s and spectrophotometric measurements commenced immediately afterwards. The absorbance change was measured at λ = 515 nm with reference to water, and recorded for 20 min. Such wavelength was chosen, as the maximum of absorbance of final product was lying in the range of 510-530 nm. Measurements for each NO_2^- concentration (ranging from 0.05 to 20 mg/L) were repeated in triplicate for each of three reagents studied. The resultant graphs for each of the reagents studied can be seen in Figure 2(top, a-c). It was found that the reagents with malonic and maleic acids provided the shortest reaction time, as maximum absorption for

each of the NO_2^- concentrations studied was reached within 2.5 – 4 min, while for oxalic

acid-containing reagent maximum absorption of the final product was observed after 8-10

minutes. Importantly, it was shown that the use of the reagent, utilising malonic acid as

acidifier resulted in a stronger absorbance of the final solution. For 2 mg/L NO_2^- sample the

absorbance of the final solutions was found to be 1.4 AU for malonic acid, 0.95 AU for

maleic acid and 0.62 AU for oxalic acid containing reagents. Such change in sensitivity is

attributed to the pH of the solutions, which was pH 1.84, pH 1.12 and pH 1.44 for malonic

acid, maleic acid and oxalic acid containing reagents, respectively. This finding is in line with

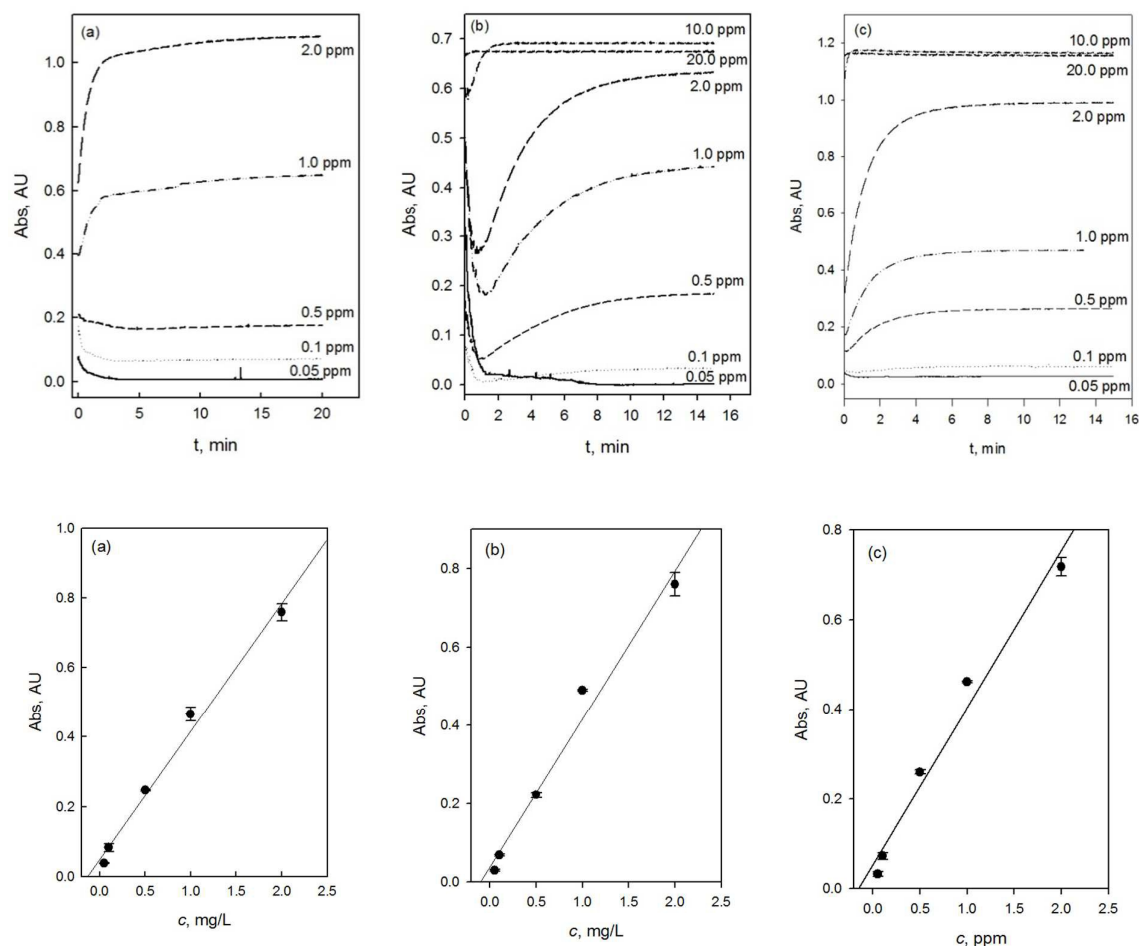the results shown in the earlier study.[12]

Figure 2. Kinetic plots (top figures) and calibration plots (bottom figures) for reagents with

(a) malonic, (b) oxalic and (c) maleic acids as acidifiers. Measurements for calibration plots

were made from the kinetic plots after 3 min, 10 min and 4 min, respectively.

Analytical parameters, such as calibration plot linearity range, limit of detection (LOD) and limit of quantification (LOQ) were found for each of the reagents. For plotting calibration curves (Fig. 2), NO_2^- solutions of varied concentrations (from 0.05 to 50 mg/L) were added to powdered reagent as described above and left to react for 3 min, 10 min or 4 min for malonic, oxalic, or maleic acid containing reagent, respectively. For each nitrite concentration $n = 9$ parallel tests were performed. The equations for each calibration plot are presented below:

Abs(AU) = 9.768 10^{-3} + 0.497 c NO_2^- (mg/L); r^2 = 0.993 − malonic acid containing reagent

Abs(AU) = 7.692 10^{-3} + 0.473 c NO_2^- (mg/L); r^2 = 0.993 − oxalic acid containing reagent

Abs(AU) = 0.023 + 0.446 c NO_2^- (mg/L); r^2 = 0.996 − maleic acid containing reagent

The LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively, where σ is the standard deviation of the blank ($n = 30$) and S is the slope of the calibration plot. LOD, LOQ and linearity range are presented in Table 2.

Table 2. Comparison of analytical parameters of reagent based nitrite test using malonic, oxalic and maleic acids as solid acidifying agents.

	Malonic acid	Oxalic acid	Maleic acid
Reaction time, min	2.5-3	8-10	4-5
Linearity range, orders of magnitude	2.9	2.9	2.9
LOD, mg/L	0.009 ± 0.002	0.008 ± 0.002	0.008 ± 0.001
LOQ, mg/L	0.031 ± 0.006	0.024 ± 0.001	0.025 ± 0.005

Additionally batch-to-batch reproducibility and reagent stability over time were evaluated. For batch-to-batch reproducibility calibration curves were plotted for each of the batches, and then statistically compared. It was found that RSD% was 5-8% for reagents with malonic or oxalic acids. For the reagent stability over time test, one batch of the reagent was used for the period of four months the RSD% for nitrite measurements were found to be 2.1% for malonic acid reagent, and 2.9% for oxalic acid one. Overall, it was shown that all reagents

have almost the same LOD, LOQ and linearity range values, however, colour-changing reaction was faster in case of malonic acid containing reagent.

Reagent composition for the determination of nitrate.

For the nitrate test reagent, once the base reagent for the determination of nitrite was prepared, an amount of Zn dust was added relative to the amount of solid base reagent, and the resulting mixture thoroughly shaken and vortexed until the colour of the mixture attained a uniform grey hue. Reagent versions with malonic, oxalic and maleic acids as acidifying agents, and varied concentrations of Zn dust were studied. Zn dust was chosen as a reducing agent due to its significantly lower toxicity, compared to the commonly used Cd reducing agent.

To identify the minimum time required for the completion of the reaction, experiments were carried out as described above for reagents containing $0.50 \pm 0.02\%$, $1.0 \pm 0.1\%$ and $2.0 \pm 0.1\%$ of Zn dust. Measurements for each NO_3^- concentration (ranging from 0.05 to 20 mg/L) were repeated in triplicate for each of the three reagents studied. The resultant graphs for malonic acid containing reagent with varied concentration of Zn dust are shown in Figure 3 (top). It was found that the reaction was completed faster when higher concentration of Zn was used: 1.5-3 min for 2% Zn versus 6 min for 0.5% Zn concentration in the reagent. Importantly, the use of the reagent containing 2% Zn dust resulted in a stronger absorbance of the final solution. This was related to a higher conversion rate of NO_3^- to NO_2^- by the reagent containing a higher level of reducing agent. Conversion rates were estimated using calibration plots for nitrite determination using corresponding reagent without Zn additive. The conversion rates can be found in Table 3.
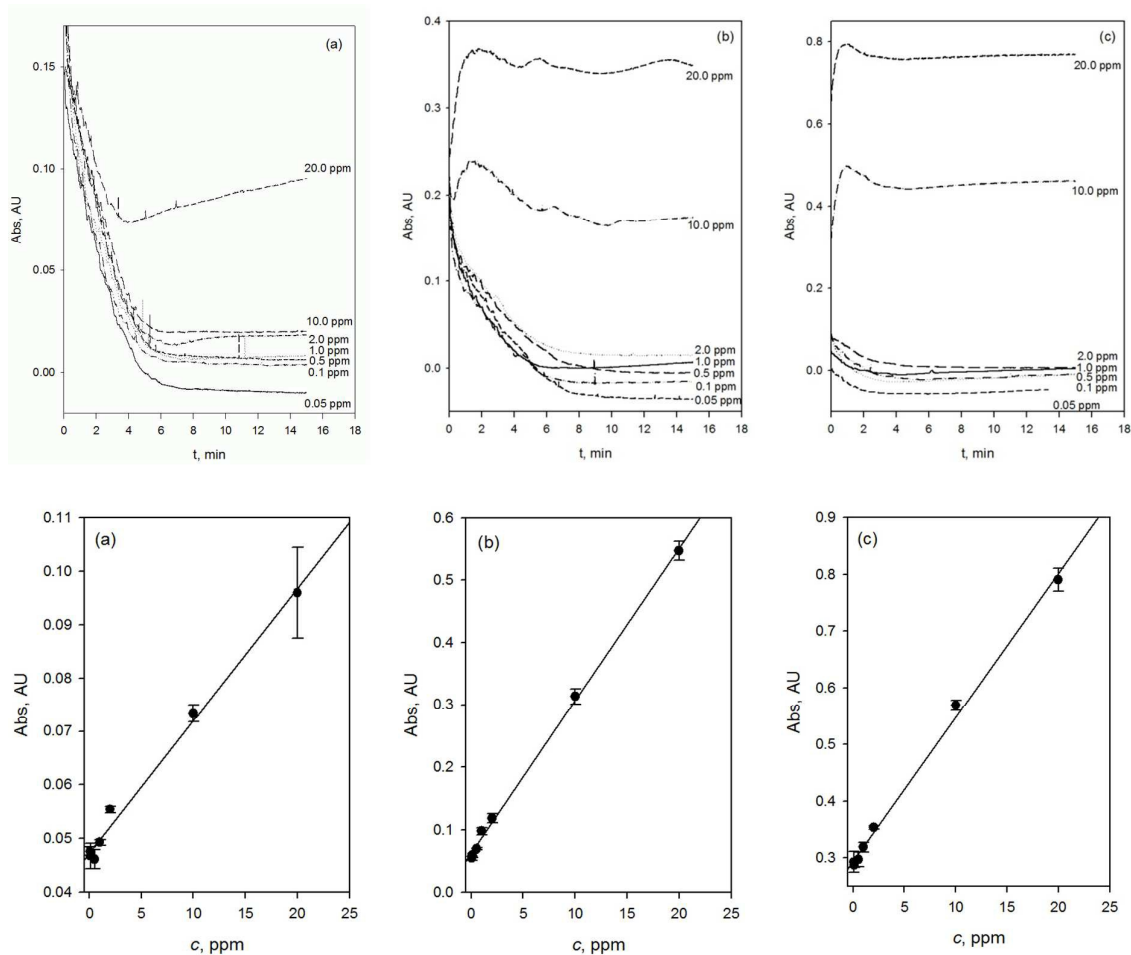
Figure 3. Kinetic plots (top figures) and calibration plots (bottom figures) for nitrate reagent with malonic acid as acidifier and (a) 0.5%, (b) 1.0% and (c) 2.0% Zn dust additive. Measurements for calibration plots were made from the kinetic plots after 6 min, 6 min and 3 min, respectively.

The same experiment series was carried out for reagents with oxalic and maleic acids as acidifiers. For maleic acid containing reagent no colour changing reaction was observed for over 30 min study at any concentrations used, so any further study was not carried out and this reagent was disregarded. Results for the reagents based on oxalic acids are presented in Figure 4 (top). Similarly to the reagent with malonic acid, here the reaction was completed faster when higher concentration of Zn dust was used. Thus, for the reagent, containing 0.48% Zn reaction was completed in over 20 min, for 1.1% Zn – in 15 min and for 1.9% Zn – in 9-10 min. Although in both cases the reaction was the fastest with approximately 2% of Zn dust in the reagent, it was observed that when such reagents were used, a fine

precipitate was formed, increasing turbidity of solutions. Based on these observations it was

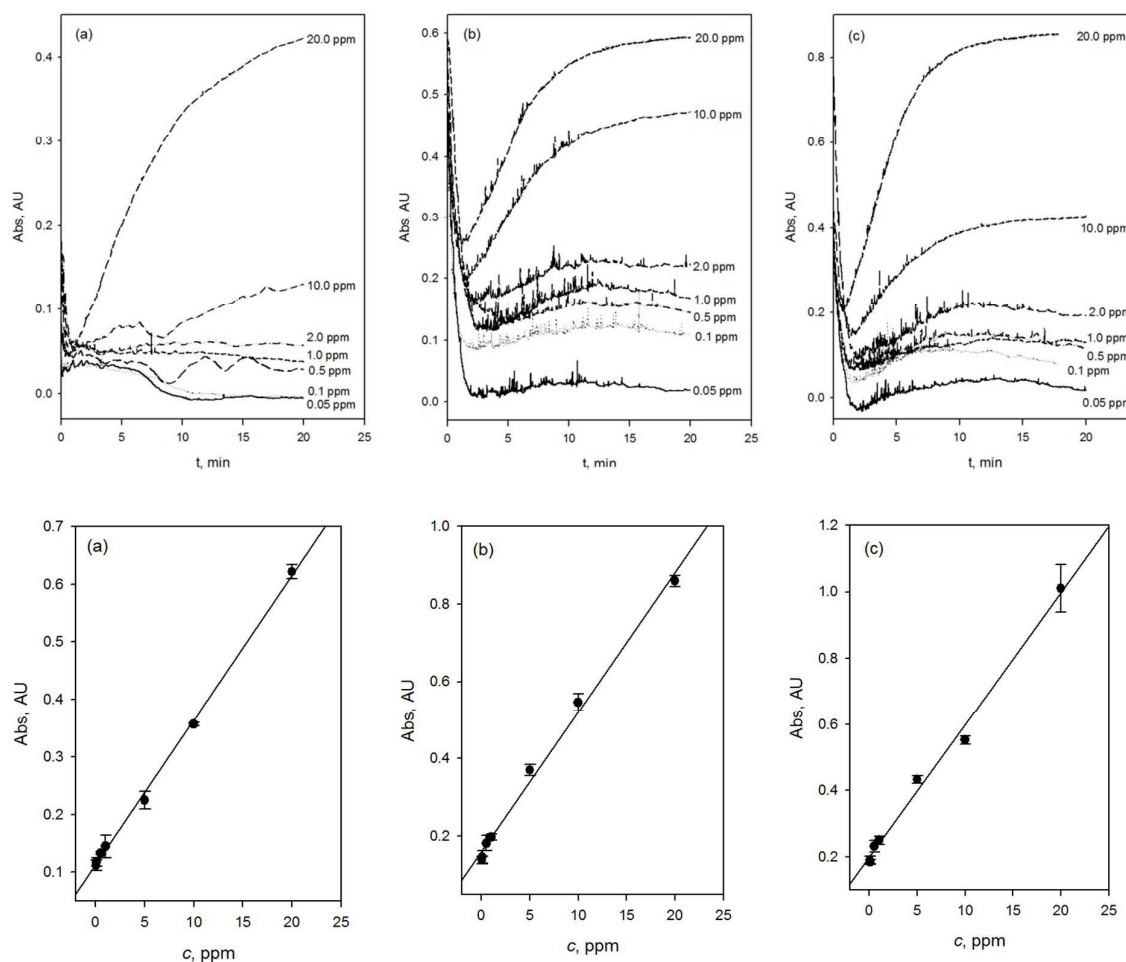suggested that 1% Zn additive was an optimal amount.

Figure 4. Kinetic plots (top figures) and calibration plots (bottom figures) for nitrate reagent with oxalic acid as acidifier and (a) 0.48%, (b) 1.1% and (c) 1.9% Zn dust additive. Measurements for calibration plots were made from the kinetic plots after 25 min, 15 min and 10 min, respectively.

Analytical parameters, such calibration plot linearity range, LOD and LOQ were found for each of the reagents. For plotting calibration curves (Fig. 3, bottom) NO_3^- solutions of varied concentrations (from 0.5 to 200 mg/L) were added to powdered reagent as described above and left to react for 6 min, 6 min or 3 min for reagents with malonic acid as acidifier and 0.5%, 1.0% or 2.0% Zn dust. For each nitrate concentration $n = 9$ parallel tests were

performed. The equations for each calibration plot are presented below for reagents with malonic acid:

$Abs(AU)=0.047+0.003\,c\ NO_3^-$ (mg/L); r^2=0.991 – for the reagent with 0.5% Zn;

$Abs(AU)=0.063+0.025\,c\ NO_3^-$ (mg/L); r^2=0.998 – for the reagent with 1.0% Zn;

$Abs(AU)=0.289+0.028\,c\ NO_3^-$ (mg/L); r^2=0.998 – for the reagent with 2.0% Zn.

For plotting calibration curves (Fig. 4, bottom) for the reagent utilising oxalic acid as acidifier, same conditions, as described above were employed, with the exception of hold times which were 25 min, 15 min or 10 min for reagents with 0.48%, 1.1% or 1.9% Zn dust. The equations for calibration plots for each of the reagent with oxalic acid are presented below:

$Abs(AU)=0.113+0.025\,c\ NO_3^-$ (mg/L); r^2=0.998 – for the reagent with 0.48% Zn;

$Abs(AU)=0.161+0.036\,c\ NO_3^-$ (mg/L); r^2=0.993 – for the reagent with 1.1% Zn;

$Abs(AU)=0.198+0.040\,c\ NO_3^-$ (mg/L); r^2=0.992 – for the reagent with 1.9% Zn.

Table 3 Comparison of reagents with different acidifiers and Zn content.

	Malonic acid			Oxalic acid			Maleic acid		
%Zn dust	0.5	1.0	2.0	0.48	1.1	1.9	0.7	1.0	2.0
Reaction time, min	6	2-6	1.5-3	>20	15	9	>30 min		
LOD, mg/L	0.563 ± 0.205	0.056 ± 0.014	0.123 ± 0.042	0.162 ± 0.029	0.208 ± 0.085	0.201 ± 0.034	n/a		
LOQ, mg/L	1.705 ± 0.622	0.169 ± 0.044	0.374 ± 0.126	0.491 ± 0.088	0.612 ± 0.258	0.610 ± 0.102	n/a		
Linearity range, orders of magnitude	1.96	2.95	2.61	2.49	2.39	2.38	n/a		
Conversion rate,%*	74 (0.5 mg/L) to 1 (20 mg/L)	99 (0.5 mg/L) to 6 (20 mg/L	100 (0.5 mg/L) to 11 (20 mg/L	53 (0.5 mg/L) to 6 (20 mg/L	74 (0.5 mg/L) to 9 (20 mg/L	95 (0.5 mg/L) to 10 (20 mg/L	n/a		

* Conversion rate was measured for a range of NO_3^- concentrations as of calibration curve. The conversion rate linearly decreased with increase of NO_3^- concentration.

LOD, LOQ and linearity range for calibration plots were calculated as described above and presented in Table 3. The highest sensitivity and calibration plot linearity range were observed for the reagent with malonic acid and 1.0% of Zn dust reducing agent. Importantly, this reagent also showed a high nitrate-nitrite conversion rate and fast reaction time. One of the key advantages of this reagent over currently existing solid-phase nitrate reagent is a fast reaction time of 2-6 min versus 20-30 min for some of the reagents.[26]

Overall, it was found that amongst reagents for nitrite and nitrate determination that were studied within this work, a reagent, utilising malonic acid as acidifier exhibited best sensitivity and reaction time both for nitrite and nitrate determination (in latter case upon addition of 1% Zn dust. As a result, these reagents were used for further determination of nitrite and nitrate in natural waters samples.

The stability of nitrite/nitrate reagents.

The stability of selected optimal reagents for the determination of nitrite and nitrate was studied over a period of three months. Every four weeks, a five-point calibration (concentration range from 0.05 mg/L to 2 mg/L for NO_2^-, and from 0.5 mg/L to 20 mg/L for NO_3^-) was plotted, and at the end of the study, all plots were overlayed and a standard deviation for the slope obtained. The RSD% for the NO_2^- calibration plot slope was found to be 2.92%, while for the NO_3^- calibration plot slope, the RSD% was 1.1%. As the reagents exhibited minimal evidence of degradation over the 3 month study, it is reasonable to infer that the stability is likely to be significantly longer than this.

The performance of nitrate reagent for the analysis of high salinity samples.

The performance of the reagent for determination of nitrate was investigated for high salinity samples. For this, a seawater sample with confirmed absence of NO_3^- was spiked with 100 mg/L or 1000 mg/L NO_3^- standard solution to introduce a known amount of NO_3^- (from 0 mg/L to 70 mg/L). Subsequently, all spiked samples were analysed using the method developed herein utilising optimal nitrate reagent. The results are presented in Table 4. The correlation between added and found amounts is satisfactory; therefore the developed method can be used for the determination of nitrate in high salinity samples within the studied concentration range.

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Table 4. Added-found concentrations of NO_3^- in spiked seawater samples, n = 3.

Added NO_3^- in sample, mg/L	Found NO_3^-, mg/L	RSD%	Recovery, %
no spiking	0.0 *	-	-
5.0	5.13 ± 0.39	7.6	102 ± 11
10.0	9.51 ± 0.81	8.5	101 ± 13
50.0	54.30 ± 4.30	7.9	104 ± 11
75.0	67.70 ± 6.12	9.0	100 ± 15

* Determined value was below LOD, thus the sample was considered as containing no NO_3^-.

Sample analysis for the determination of nitrate/nitrite in natural waters

Water samples were received from T.E. Laboratories and analysed on the same day. Until the analysis samples were kept at 4 ˚C in HDPP containers. Generally samples did not require any pre-treatment with exception of one, which contained significant amount of brick-coloured precipitate and particle suspension. In order to avoid the interference with the reagent and turbidity effect on the final results of the measurement, this sample was filtered through 1 layer of the Whatman Grade 595 filter paper (thickness 150 μm, pore size 4-7 μm). The pH of samples varied from pH 6.93 to 8.12. First, the nitrite test had to be carried out as it is the interfering component during determination of nitrate if it is present in the sample. Its content has to be determined and subtracted from the nitrate results. Each measurement was performed n = 9 times. It was shown that samples No.: 4, 5, 7-9 (Table 5) contained the amount of NO_2^- close to limit of detection, and in sample No.: 1 the concentration of nitrite was low and so its presence was not affecting determination of nitrate, as the determined concentrations were less than standard deviation for the Nitrate method. For the determination of nitrate, a reagent, containing 1% of Zn dust as a reducing agent was utilised, and concentrations of NO_3^- ranging from 1.76 mg/L to 62.95 mg/L were determined.

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Table 5. Comparison of NO_2^- and NO_3^- concentrations in various sample media obtained using the solid-state reagent method and ion-chromatography.

ID #	Sample Characteristics	IC mg NO_3^-/L	Developed NO_3^- reagent mg NO_3^-/L	Developed NO_2^- reagent, mg NO_2^-/L
1	Borehole	3.95	1.76±1.41	0.16±0.005
2	10 mg/L reference standard	9.15	8.90±1.05	-
3	Effluent	11.61	12.40±1.35	-
4	Process Water	11.82	14.43±0.51	0.02±0.004
5	Stream	19.11	19.00±1.65	0.02±0.001
6	Drinking water (1)	37.47	35.35±2.30	-
7	Drinking water (2)	37.87	38.99±0.09	0.03±0.001
8	Aut Even Effluent	49.41	62.95±3.85	0.01±0.005
9	Potable water	50.92	54.80+4.40	0.01±0.002

A graphical comparison of the nitrate results (Table 5) obtained via developed method and ion-chromatography is presented in Figure 5. The correlation between the two methods is satisfactory (r^2=0.973) with a slope of 1.03, which is very encouraging, given the wide variety of sample types screened. The t-test was applied to compare two sets of data, and the results are presented in the ESI Table S1.
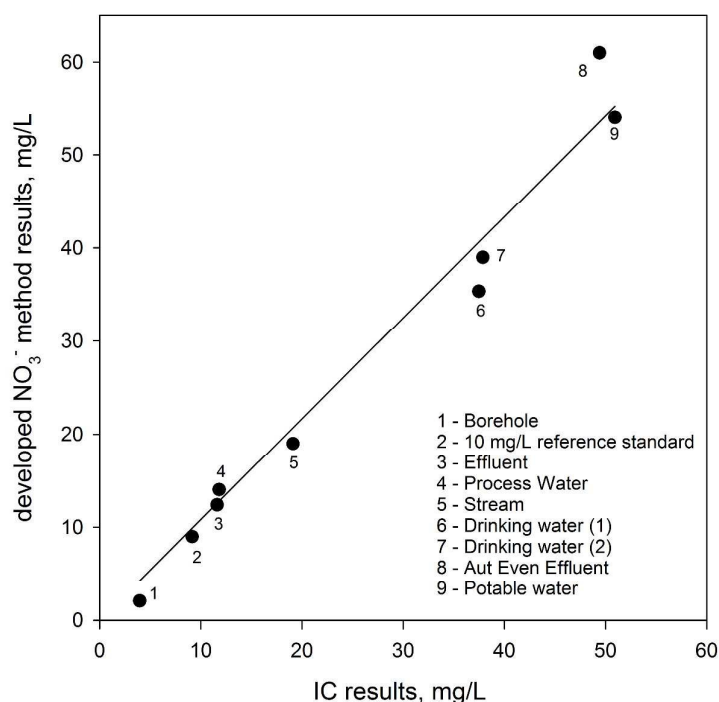
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Figure 5. The comparison of nitrate analysis results using the developed test method and the ion-chromatography reference method for the samples in Table 5.

Conclusions. A rapid non-toxic solid-phase test reagent for the determination of nitrite and nitrate was developed. Powdered reagent for nitrite determination utilised *p*-nitroaniline (azo-component), chromotropic acid (diazo-component), KCl and KBr (catalysts), Na$_2$EDTA (masking agent) and solid acidifier (malonic, maleic or oxalic acid). Effects of solid acidifier and the amount of Zn reducing agent (for the determination of nitrate) were evaluated. It was shown that the optimal reagent contained malonic acid as acidifier and 1.0% of Zn reducing agent. These reagents provided good sensitivity for the determination of nitrite (LOQ of 0.03 mg/L) and nitrate (LOQ of 0.17 mg/L) and fast analysis time of under 6 min. It was shown that the developed reagent can be used for the analysis of high salinity samples. These reagents were shown to be stable for at least 3 months, with %RSD values for the calibration plot slope for $NO_2^- = 2.92\%$, and $NO_3^- = 1.1\%$.

These solid-phase reagents can be easily handled, or pre-packed in vials and utilised for low-cost, accurate, selective and sensitive in-situ analysis of nitrite and nitrate in a wide variety of environmental or industrial water samples. The proposed approach can be coupled with various measurement tools, including visual (by eye) comparisons, light-emitting diodes/photodiodes, spectroscopic analysis, and digital colour imaging and analysis, and extended to other analytes for which similar reagent based methods exist.[34-36]

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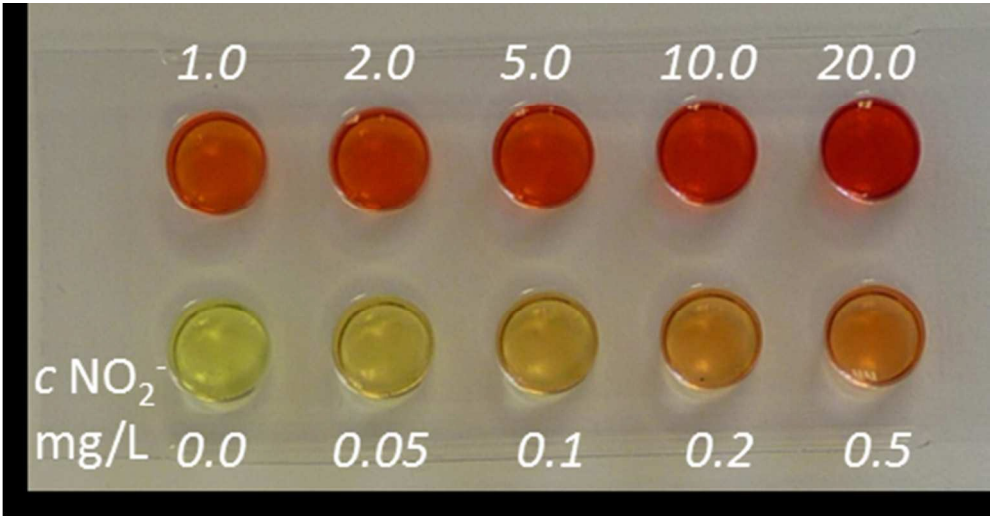
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A non-toxic solid-phase test-reagent for rapid determination of nitrite/nitrate, consisting of azo-/diazo-components, solid organic acid acidifier, zinc dust (for nitrate test), was developed and validated.

83x43mm (150 x 150 DPI)