Microliter Operation for Determination of Nitrate-nitrogen via Simple
Zinc Reduction and Color Formation in a Well Plate with a Smartphone

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Abstract

We propose a simple greener colorimetric method for the determination of nitrate-nitrogen by operating on a 96-well microplate and using a smartphone camera as a simple detector. A slurry containing 0.3 mg zinc was used for reduction of nitrate to nitrite, the reduction solution was transferred to 96-well microplate to react with Griess reagent to form pink azo dye product. The color product image was captured and processed by the smartphone camera and ImageJ software, respectively. The limit of detection and limit of quantitation were 0.04 mg/L and 0.10 mg/L nitrate-nitrogen, respectively for the smartphone camera. Application on real samples was demonstrated. The proposed method results showed no significant difference (at 95% confidence) with hydrazine reduction method. The proposed methods could be used as an alternative method for on-site analysis due to being portable and rapid as duplicate run of 20 samples could be run simultaneously in 12 min.

**Keywords:** zinc reduction, nitrate determination, well microplate, smartphone camera
Introduction

Nitrate nitrogen (NO$_3$-N) is one of the pollutants most found in ground water and surface water. Nitrate contamination has been increasingly affected water quality around the world mainly due to fertilizer misuse in agriculture. Nitrate leaching has caused a huge damage in ecosystem and also posed several health risks. Determination of nitrate has been investigated with various analytical techniques such as spectrophotometry,\textsuperscript{1,2} colorimetry,\textsuperscript{3,5} potentiometry,\textsuperscript{3} conductometry,\textsuperscript{6} amperometry,\textsuperscript{7,8} ion chromatography,\textsuperscript{9} etc. Colorimetric method is one of effective methods for nitrate analysis. Nitrate is reduced to nitrite with suitable reducing agents, for example, cadmium,\textsuperscript{3} copperised cadmium,\textsuperscript{4,5,10,11} zinc,\textsuperscript{12,13} hydrazine sulfate,\textsuperscript{14} vanadium (III),\textsuperscript{15,16} etc.

A copperised cadmium is a highly effective reductant with reduction efficiency of over 90%.\textsuperscript{17} However, cadmium is a toxic heavy metal which is harmful to human and environment.\textsuperscript{18} Several researchers have used zinc metal as a reductant because zinc is less toxic although has lower efficiency in reduction.\textsuperscript{6,12,13,18} As a reductant, an excess amount of zinc is often used resulting in zinc waste,\textsuperscript{18} so the optimization of zinc is important.

It has been previously reported that an increase in salinity or ionic strength significantly controlled the over-reduction of nitrate. Wu \textit{et al.} proposed the use of NaCl to adjust the ionic strength of sample water to improve reduction rate and stability of reduction via modified zinc-cadmium method.\textsuperscript{13} Ryabenko \textit{et al.} reported that adding salt (NaCl) to sample solution could improve the efficiency in reduction of nitrate to nitrite by cadmium. During reduction of nitrate in the absence of NaCl, cadmium could successively reduce nitrate to nitrogen gas. Whereas, in the presence of NaCl, the nitrate reduction could be effectively stopped at nitrite.\textsuperscript{19} Despite of the interference of chloride ion in nitrate determination, the previous study has demonstrated that chloride ion was utilized as a catalyst for reduction of nitrate to nitrite in resorcinol method.\textsuperscript{20} For nitrite product determination, the Griess reagent is a well-known chromogenic reagent that consists of sulfanilamide and N-(1-naphthyl)-ethylenediamine under an acidic condition.\textsuperscript{17} The
nitrite product reacts with sulfanilamide via diazotization reaction and coupling with N-(1-naphthyl)-ethylenediamine to form an azo dye.\textsuperscript{21}

The colorimetric method is widely used for nitrate determination but it requires a large volume of sample/reagent (~25 mL) and it is time-consuming due to one by one measurement.\textsuperscript{13,22} In order to overcome this, a well microplate has been increasingly utilized in development of downscaling chemical analysis. An array of microliter wells is served as a platform for simultaneous multi-sample detection, resulting in less analysis time as well as reduction of chemical consumption and waste generation. The use of sample and reagent volumes could be reduced to ~0.3 mL and 3 mL for 96-well and 24-well microplates, respectively.\textsuperscript{23}

Also, many imaging devices have been applied for capturing an image of colorimetric reaction such as a flatbed scanner,\textsuperscript{24-26} webcam camera,\textsuperscript{27,28} digital camera\textsuperscript{27} and mobile phone camera/smartphone camera, etc. \textsuperscript{18,29-32,34} The latter is commonly used as part of everyday life and provides more benefits, such as portable and relatively cost-effective, compared to the others.

This work aims to develop microliter operation for determination of nitrate-nitrogen via simple zinc reduction and color formation in a well microplate with a smartphone as a detector. The captured image was digitized using image-processing software resulting in image color information and analyte concentration. The reduction step and diazotized coupling reaction with Griess reagent were operated in each well of microplate, with minimal amount of zinc slurry aiming for less-toxic nitrate reduction, together with addition of sodium chloride. The developed method was demonstrated for on-site application to real samples.

\textbf{Experimental}

\textit{Reagents and chemicals}

All reagents used were of analytical grade and deionized (DI) water were used in all experiments. The stock standard solution of nitrate-nitrogen and nitrite-nitrogen (1000 mg/L) were prepared by dissolving potassium nitrate (dried at 105°C for 6 hrs.) (Merck, Germany) and
sodium nitrite (QRëC, New Zealand), respectively, in deionized water. Working standard solutions were freshly prepared by diluting the stock standard solution of nitrate-nitrogen and nitrite-nitrogen with DI water.

The Griess reagent was prepared by dissolving 1.0 g of sulfanilamide (PanReac AppliChem, Germany) with 80 mL of DI water then 10 mL of 85% phosphoric acid (RCI Labscan, Thailand) was added to completely dissolve the sulfanilamide. After that, 0.10 g of N-(1-naphthyl)-ethylenediamine dihydrochloride, NED (PanReac AppliChem, Germany) was added to the previous solution and adjusted to 100 mL in the volumetric flask with DI water. This solution can be used for 1 month while kept in the dark place and stored at < 4 °C. The zinc slurry was prepared by mixing 0.240 g of zinc dust (Ajax Finechem, Australia) with 2.00 g of sodium chloride (Merck, Germany) in 10 mL of glycerol (Merck, Germany), unless stated otherwise.

**Apparatus**

UV-vis spectrophotometer (UV-1800, Shimadzu, Japan) with a narrow-width plastic cuvette (Brand 759210, Germany) was used for conventional measurement. A smartphone camera (ASUS, Zenfone 6) was also used for capture the color image of product solution in 96-well microplate (Corning, USA) following by image processing with ImageJ software.

**Procedure**

For nitrate-nitrogen determination, one drop of zinc slurry (16.2±0.8 mg, see supporting information, Table S1) was dropped using a disposable hypodermic syringe and needle (0.9 mm O.D.) in each well of 24-well microplate (Nunc, China). The nitrate-nitrogen standard solutions (1,000 µL) were added to zinc slurry. The convection allowed the mixing. It was then left for 4 minutes for reduction of nitrate to nitrite, unless stated otherwise. For spectrophotometric detection, after reduction step, the clear Zn-reduced solution (800 µL) was transferred to another 24-well microplate by a micropipette. The Griess reagent (20 µL) was added. An absorbance of pink solution product was measured against blank solution without zinc using a
spectrophotometer at wavelength of 543 nm. For colorimetric measurement reduced product with a smartphone camera, the clear Zn-reduced solution (200 µL) was transferred to 96-well microplate following by adding Griess reagent (5 µL) before capturing image in the house-made light control box (18 cm x 18 cm x 25.5 cm, see supporting information Fig. S1).

The color image of the product solutions on 96-well microplate was processed to get the image information (RGB, red, green, blue). The measurement of nitrite-nitrogen was performed by pipetting nitrite-nitrogen standard solutions into the proper wells. The Griess reagent was added to react with nitrite and then the product solution was detected using the above-mentioned detectors. The relation of RGB intensity of the color image obtained from a smartphone camera with nitrate-nitrogen/nitrite-nitrogen concentration was studied.

The optimization of all parameters was studied using spectrophotometric method. The reduction rate (\%reduction) of nitrate to nitrite by zinc slurry can be calculated by the ratio of the actual absorbance values of nitrate-nitrogen after reduction to the actual absorbance value of nitrite-nitrogen at the same concentration.\textsuperscript{13}

**Results and Discussion**

*Conditions of reduction with zinc*

In the first step of nitrate-nitrogen determination, the reduction of nitrate with zinc was studied for reduction efficiency. The involved parameters of interest were optimized including zinc content, addition of sodium chloride and reduction time.

a) Effect of zinc amount

The effect of zinc amount on reduction of nitrate was studies by varying mass of zinc. A series of the slurries with zinc contents of 0.1 to 0.5 mg per drop was used to reduce 0.025 and 0.25 mg/L of nitrate-nitrogen concentrations. The results are illustrated in Fig. 1. An increase in mass of zinc from 0.1 to 0.3 mg per drop gave a rise in absorbance of azo dye product, indicating an increase in the nitrate reduction. When > 0.3 mg masses of zinc were used, the absorbance
gradually decreased. This could be due to possible mixture of the nitrite product and other products such as N\textsubscript{2} and NH\textsubscript{4}\textsuperscript{+} from excess zinc\textsuperscript{26,33} Therefore, the 0.3 mg of zinc was selected for further studies.

Fig. 1 is here

b) Effect of sodium chloride

Effect of sodium chloride was studied by adding in the reduction process for the stability of nitrate reduction reaction by zinc, as proposed previously\textsuperscript{19}. A series of NaCl solutions (0-10 g NaCl /L of 1000 µL final solution) was examined for reduction of nitrate-nitrogen solutions of 0.010 to 1.0 mg/L. As stated earlier, %reduction was obtained by ratios of absorbances of the products due to nitrate/nitrite. The effect of sodium chloride on nitrate reduction is shown in Fig. 2. The results indicated that the addition of sodium chloride enhanced the reduction rate and stability, consistent with a previous study\textsuperscript{13,19,20}. The highest reduction rate and reduction stability was observed with 2.5 g/L sodium chloride concentration (26±3%). Thus, sodium chloride concentration of 2.5 g/L was selected for optimum condition.

Fig. 2 is here

c) Effect of reduction time

The effect of reduction time on %reduction of nitrate determination was studied by varying the contact time of zinc slurry with 0.25 mg/L of nitrate-nitrogen for 2-10 min. It was found that %reduction increased rapidly from 1 to 4 min of reduction time then dropped gradually as shown in Fig. 3. The decrease in %reduction after 4 min was likely due to further reduction of nitrate to lower oxidation number of nitrogen species\textsuperscript{26,33}. Therefore, 4 min was selected for further experiments.

Fig. 3 is here
Conditions for the diazotization and coupling reactions

The formation of color product via diazotization and coupling reactions was studied. In order to optimize the reaction, an amount of reagent and color development time were investigated.

a) Color development time

The color development time of azo dye product was studied by measuring absorbance of azo dye product. Nitrite-nitrogen (0.25 mg/L) reacted with the Griess reagent from 1 to 15 min. The relation of absorbance versus the color development time was plotted (see supporting information, Fig. S2). The relation curve displayed that the absorbance of azo dye gradually increased before becoming constant after 5 min. So, color development time was chosen at 5 min or longer.

b) Concentration of Griess reagent

The Griess reagent used in colorimetric standard procedure for nitrite analysis consists of sulfanilamide, NED and phosphoric acid. However, NED used in the Griess reagent is carcinogenic agent. Concentration of Griess reagent containing 3.86 mmol/L of NED was optimized. Various concentration of NED (0-428 µmol/L) were used to react with 800 µL of the clear Zn-reduced solution of 0.25 mg/L NO$_3$-N and the absorbance was measured at 543 nm. The highest absorbance was obtained using amount of NED at 94 µmol/L (20 µL of Griess reagent) (see supporting information, Fig. S3). Therefore, 20 µL of Griess reagent was applied for the proposed method using spectrophotometer as a detector.

Downscaling and smartphone-based detection

From the above results, for downscaling operation with a smartphone camera as a detector, conditions for reduction steps were similar to that for the conventional spectrophotometric measurement. For color development steps, instead of 24-well microplate, the pink azo dye product was formed on 96-well microplate and the volume of the reduction product solution and
Griess reagent were deceased by 4 folds, i.e. 200 µL of the reduction product solution was transferred to 96-well microplate and 5 µL (94 µmol NED /L) of the Griess reagent was added. The color image of pink azo dye solution in a 96-well microplate was captured by the smartphone camera for image processing. It was found that the well plate-based method with smartphone detection could be operated with analysis throughput at 48 samples per the 96-well microplate per 12 min as due to effect of light on the wells at the edges of the plate.

**Analytical Characteristics**

Under the optimum conditions, the proposed method using spectrophotometric detection was found to be linearly related to the concentration of nitrate-nitrogen from 0.01-2.5 mg/L, RSD <5% with $R^2=0.9960$ (n=3) as shown in Fig. 4(a). For smartphone camera measurement, it was found that the relative G intensity of azo dye product (obtained by subtracting G intensity of blank solution with G intensity of sample solution) had a linear relation with nitrate-nitrogen concentration in range of from 0.1-2.5 mg/L, RSD <4% with $R^2=0.9989$ (n=3) as shown in Fig. 4(b). The limit of detection (LOD) and limit of quantitation (LOQ) of this method obtained by the spectrophotometer are 0.004 mg/L and 0.010 mg/L nitrate-nitrogen (n=7), respectively. For the smartphone camera detection using 96-well microplate, LOD and LOQ are 0.04 mg/L and 0.10 mg/L nitrate-nitrogen (n=3), respectively. The higher limit of quantitative obtained using a smartphone camera than that of spectrophotometer because of the difference in detection modes (reflection/absorption).

**Fig. 4 is here**

**Application**

The proposed method was applied to real samples. Concentrations of nitrate in surface water samples collected from 5 locations around Chiang Mai, Thailand were determined by (a) the proposed method (using a smartphone), (b) another using the developed reduction step using a spectrophotometer and (c) the standard method using hydrazine reduction. The results are
represented in Table 1. By the paired t-test, it was found that no statistically significant difference at 95% confidence level, for (a) and (c), (t stat = 1.29, t critical = 2.78, df = 4) and for (b) and (c), (t stat = 2.57, t critical = 2.78, df = 4).

Table 1 is here

Conclusions

In summary, we have developed an alternative colorimetric method which is convenient, portable and low cost for nitrate-nitrogen determination. The mass of less zinc with sodium chloride addition was used as a reducing agent for nitrate-nitrogen reduction. The step of reduction and diazotized coupling reaction can be operated on a well microplate.

A smartphone used commonly in our daily life can be employed as a portable detector and image processing was done with the image processing software/application. The advantages of using well microplate are simultaneous analysis, high-throughput of the sample and less waste generation. The proposed method with smaller amounts of reagents under effective reduction using 96-well microplate with a smartphone camera is an alternative method for on-site analysis due to being portable and rapid (duplication run of 20 samples in 12 min.) The proposed method was successfully applied for the determination of nitrate-nitrogen in surface water samples.

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References

1. M. Monteiro, F. Ferreira, N. De Oliveira and A. Avila, *Anal. Chim. Acta*, **2003**, 477, 125.

2. R. Burakham, M. Oshima, K. Grudpan and S. Motomizu, *Talanta*, **2004**, 64, 1259.

3. J. H. Margeson, J. C. Suggs and M. R. Midgett, *Anal. Chem.*, **1980**, 52, 1955.

4. M. F. Giné, H. Bergamin F, E. A. G. Zagatto and B. F. Reis, *Anal. Chim. Acta*, **1980**, 114, 191.

5. K. Nagashima, X. Qian and S. Suzuki, *Anal. Sci.*, **1987**, 3, 179.

6. X.-L. Su, P. Chen, X.-G. Qu, W.-Z. Wei and S.-Z. Yao, *Microchem. J.*, **1998**, 59, 341.

7. S. A. Glazier, E. R. Campbell and W. H. Campbell, *Anal. Chem.*, **1998**, 70, 1511.

8. V. Mori and M. Bertotti, *Anal. Lett.*, **1999**, 32, 25.

9. L. A. Silva, M. Korn and J. B. de Andrade, *Ultrason. Sonochem.*, **2007**, 14, 275.

10. C. L. Pasquali, P. F. Hernando and J. D. Alegria, *Anal. Chim. Acta*, **2007**, 600, 177.

11. M. D. Patey, M. J. Rijkenberg, P. J. Statham, M. C. Stinchcombe, E. P. Achterberg and M. Mowlem, *TrAC, Trends Anal. Chem.*, **2008**, 27, 169.

12. Y. Kiso, Y.-J. Jung, K. Kuzawa, Y. Seko, Y. Saito, T. Yamada and M. Nagai, *Chemosphere*, **2006**, 64, 1949.

13. J. Wu, Y. Hong, F. Guan, Y. Wang, Y. Tan, W. Yue, M. Wu, L. Bin, J. Wang and J. Wen, *Sci. Rep.*, **2016**, 6, 20165.

14. C. A. Shand, B. L. Williams and G. Coutts, *Talanta*, **2008**, 74, 648.

15. D. C. Woollard and H. E. Indyk, *Int Dairy J.*, **2014**, 35, 88.

16. E. Metzger, A. Thibault de Chanvalon, F. Cesbron, A. Barbe, P. Launeau, D. Jézéquel and A. Mouret, *Environ. Sci. Technol.*, **2016**, 50, 8188.

17. American Public Health Association, M. A. H. Franson, American Water Works Association and Water Pollution Control Federation, “*Standard methods for the examination of water and wastewater*”, **1989**, American Public Health Association, Washington, D.C.
18. E. Murray, E. P. Nesterenko, M. McCaul, A. Morrin, D. Diamond and B. Moore, *Anal. Methods*, 2017, 9, 680.

19. E. Ryabenko, M. A. Altabet and D. W. Wallace, *Limnol. Oceanogr. Methods*, 2009, 7, 545.

20. J.-Z. Zhang and C. J. Fischer, *Mar. Chem.*, 2006, 99, 220.

21. R. B. R. Mesquita, M. T. S. O. B. Ferreira, R. L. A. Segundo, C. F. C. P. Teixeira, A. A. Bordalo and A. O. S. S. Rangel, *Anal. Methods*, 2009, 1, 195.

22. M. Sohail and S. B. Adeloju, *Talanta*, 2016, 153, 83.

23. A. Durand, Z. Chase, T. Remenyi and F. Quéréué, *Front Microbiol.*, 2013, 3, 437.

24. S. A. Klasner, A. K. Price, K. W. Hoeman, R. S. Wilson, K. J. Bell and C. T. Culbertson, *Anal. Bioanal. Chem.*, 2010, 397, 1821.

25. L. Feng, Y. Zhang, L. Wen, Z. Shen and Y. Guan, *Talanta*, 2011, 84, 913.

26. B. M. Jayawardane, S. Wei, I. D. McKelvie and S. D. Kolev, *Anal. Chem.*, 2014, 86, 7274.

27. A. Choodum, P. Boonsamran, N. NicDaeid and W. Wongniramaikul, *Sci Justice.*, 2015, 55, 437.

28. J. L. O. Santos, O. D. Leite, A. D. Vieira, D. S. Jesus and M. Y. Kamogawa, *J. Braz. Chem. Soc.*, 2016, 27, 70.

29. N. Lopez-Ruiz, V. F. Curto, M. M. Erenas, F. Benito-Lopez, D. Diamond, A. J. Palma and L. F. Capitan-Vallvey, *Anal. Chem.*, 2014, 86, 9554.

30. J. Saez, G. Arana, L. Fernandez-Cuadrado and F. Benito-Lopez, *Procedia Eng.*, 2016, 168, 518.

31. X. Wang, F. Li, Z. Cai, K. Liu, J. Li, B. Zhang and J. He, *Anal. Bioanal. Chem.*, 2018, 410, 2647.

32. X.-X. Zhang, Y.-Z. Song, F. Fang and Z.-Y. Wu, *Anal. Bioanal. Chem.*, 2018, 410, 2665.

33. L. Limousy, P. Dutournie and D. Hadjiev, *Water Environ. Res.*, 2010, 82, 648.

34. Z. Wan, L. Zhong, Y. Pan, H. Li, Q. Zou, K. Su and P. Wang, *Anal. Sci.*, 2017, 33, 1291.
Figure Captions

Fig. 1  Effect of zinc amount on reduction of nitrate-nitrogen (n=3).
Experimental condition: 1 drop of Zn slurry without sodium chloride (0.1-0.5 mg per drop), 0.9 mm O.D. needle; NO$_3$-N solution 1000 µL, 0.025 and 0.25 mg/L; reduction time 3 min; the clear Zn-reduced solution 800 µL; volume of Griess reagent 50 µL; color development time 5 min; absorbance at 543 nm.

Fig. 2  Effect of sodium chloride on reduction efficiency of nitrate-nitrogen (n=3).
Experimental condition; 1 drop of Zn slurry (0.3 mg Zn), 0.9 mm O.D. needle; sodium chloride concentration, 0-10 g/L; NO$_3$-N solution 1,000 µL, 0.010-1.0 mg/L; reduction time 4 min; the clear Zn-reduced solution 800 µL; volume of Griess reagent 20 µL; color development time 5 min; absorbance at 543 nm.

Fig. 3  The reduction time on %reduction of nitrate-nitrogen determination (n=3).
Experimental condition: 1 drop of Zn slurry (0.3 mg Zn and 2.5 g/L NaCl); 0.9 mm needle; NO$_3$-N 1000 µL, 0.25 mg/L; reduction time 2-10 min; the clear Zn-reduced solution 800 µL; volume of Griess reagent 50 µL; color development time > 5 min; absorbance at 543 nm.

Fig. 4  Calibration graphs for nitrate-nitrogen determination; (a) using spectrophotometer, (b) 96-well microplate with the smartphone camera (n=3).
Table 1 Determination of nitrate in water sample obtained via the proposed method and hydrazine reduction method (n = 3)

| Sample            | Nitrate found, mg/L NO$_3$-N | Proposed method | Hydrazine reduction method*** |
|-------------------|------------------------------|-----------------|------------------------------|
|                   |                              | Smartphone camera* | Spectrophotometer**          |                              |
| Surface water 1   | 0.80 ± 0.00                  | 0.69 ± 0.09      | 0.63                         |
| Surface water 2   | 0.11 ± 0.01                  | 0.15 ± 0.00      | 0.16                         |
| Surface water 3   | 0.23 ± 0.03                  | 0.27 ± 0.01      | 0.18                         |
| Surface water 4   | 0.53 ± 0.05                  | 0.53 ± 0.06      | 0.44                         |
| Surface water 5   | 0.17 ± 0.01                  | 0.21 ± 0.01      | 0.19                         |

Correlation coefficient (r); *0.9937, **0.9865 with respect to ***
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Experimental condition: 1 drop of Zn slurry without sodium chloride (0.1-0.5 mg per drop), 0.9 mm O.D. needle; NO₃⁻-N solution 1000 µL, 0.025 and 0.25 mg/L; reduction time 3 min; the clear Zn-reduced solution 800 µL; volume of Griess reagent 50 µL; color development time 5 min; absorbance at 543 nm.
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