Published in final edited form as:

Talanta. 2015 October 1; 143: 234–239. doi:10.1016/j.talanta.2015.05.073.

Modified graphene oxide sensors for ultra-sensitive detection of nitrate ions in water

Wen Ren^a, Stefania Mura^b, and Joseph M.K. Irudayaraj^{a,*}

^aDepartment of Agricultural and Biological Engineering, Bindley Bioscience Center, Purdue University, West Lafayette, Indiana 47907, United States

^bUniversity of Sassari, Porto Conte Ricerche, Loc. Tramariglio, 07041, Alghero, Italy

Abstract

Nitrate ions is a very common contaminant in drinking water and has a significant impact on the environment, necessitating routine monitoring. Due to its chemical and physical properties, it is hard to directly detect nitrate ions with high sensitivity in a simple and inexpensive manner. Herein with amino group modified graphene oxide (GO) as a sensing element, we show a direct and ultra-sensitive method to detect nitrate ions, at a lowest detected concentration of 5 nM in river water samples, much lower than the reported methods based on absorption spectroscopy. Furthermore, unlike the reported strategies based on absorption spectroscopy wherein the nitrate concentration is determined by monitoring an increase in aggregation of gold nanoparticles (GNPs), our method evaluates the concentration of nitrate ions based on reduction in aggregation of GNPs for monitoring in real samples. To improve sensitivity, several optimizations were performed, including the assessment of the amount of modified GO required, concentration of GNPs and incubation time. The detection methodology was characterized by zeta potential, TEM and SEM. Our results indicate that an enrichment of modified GO with nitrate ions contributed to excellent sensitivity and the entire detection procedure could be completed within 75 min with only 20 μ l of sample. This simple and rapid methodology was applied to monitor nitrate ions in real samples with excellent sensitivity and minimum pretreatment. The proposed approach paves the way for a novel means to detect anions in real samples and highlights the potential of GO based detection strategy for water quality monitoring.

Graphical abstract

*Corresponding author. Tel: +1 765 494 0388; fax: +1 795 496 1115. josephi@purdue.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

Graphene oxide; nitrate ions; gold nanoparticles; absorption spectroscopy; high sensitivity

1. Introduction

Nitrate ions are widely found in the environment due to its high solubility. It is known that contamination of water by nitrate ions due to agricultural and industrial waste can pose a threat to environmental water quality [1, 2]. High concentration of nitrate ions in drinking water may cause serious illnesses such as birth defects, spontaneous abortions, intrauterine growth restriction and potential cancer risk [3-6]. A recent investigation for the presence of nitrate ions by the Environmental Protection Agency (EPA) indicated that the maximum contamination level (MCL) was exceeded in the self-supplied water consumed by more than 4 million Americans, necessitating advanced and sensitive methods to detect ions in water [4, 7]. Further, long-term accumulation of nitrate ions in the environment may be a potential risk to animals. Thus, there is growing demand for the development of simple and cost-effective methods for routine monitoring of nitrate ions with high sensitivity for both human and animal health and environment. Until now, various analytical techniques, such as chromatography, electrochemical method, Raman spectroscopy and fluorescent spectroscopy, have been used for nitrate ion detection, but these methods require complex and expensive instrumentation [8-15]. Besides, colorimetric and absorption spectrum-based methods that utilize gold nanoparticles (GNPs) have also been reported [4, 16], to provide a more convenient and economical route for routine monitoring of nitrate ions. In the past methods, nitrate ions or its reduced product, nitrite ions, cross-links the modified GNPs to generate a signal and the concentration of nitrate ions was determined by monitoring the increase in aggregation of GNPs. However, this approach is not sensitive because at lower concentrations, nitrate ions cannot trigger an observable aggregation of GNPs, and hence not detectable [17]. Meanwhile, it is known that some of the common anions in real water samples such as Cl^- , S^{2-} and SO_4^{2-} can also induce the aggregation of GNPs, and thus can contribute to non-specific signals and distort the detection of nitrate ions in real sample such as surface water or ground water. Therefore the reported methods based on absorption spectroscopy usually require complex pretreatment of real samples to minimize the influence from other materials when testing real samples. Furthermore, although some commercial instruments for nitrate ions detection are available, their sensitivity is in the range between 322-1046.5 μM (20-65 ppm). Hence, a rapid and facile method for the detection of nitrate ions at low concentration in real samples is still a challenge.

Graphene and graphene oxide (GO) have drawn enormous attention due to their excellent physical and chemical properties since its discovery in 2004 [18]. The electronic and chemical properties of graphene and GO have been investigated by various groups. A notable characteristic of graphene and GO is their interaction with other molecules. It has been demonstrated that graphene and GO can be used to load and deliver drugs such as nanocarriers [19, 20], because of the capability of graphene or GO to enrich drug molecules. When this enrichment is utilized in an analytical application, the sensitivity of detection (ie., the limit of detection, LOD) using graphene or GO can be greatly improved, because the enrichment procedure can increase the analyte concentration on the graphene or GO substrate in solution [21, 22]. For instance, Wen et al. has reported an ultra-sensitive surface enhanced Raman scattering approach to monitor folic acid based on GO/Ag nanoparticles hybrids. Here they show a dramatic improvement in sensitivity due to the enrichment capability of GO sheet to folic acid in solution; meanwhile the enrichment of GO also improved the detection of folic acid in serum, suggesting that an interaction between GO and target molecules could improve the detection sensitivity of nitrate ions in real samples [23]. Recent report on the absorption of nitrate ions by graphene sheet in water further highlights the enrichment potential of graphene sheet to nitrate ions [24], further implying the possibility of enhancement sensitivity of nitrate detection utilizing GO. Besides, the large surface and its low manufacturing cost can make GO an excellent choice for analytical applications. Further, compared with graphene, GO could be a better choice for the development sensors due to its high solubility and stability in water.

In this effort we report on an ultra-sensitive nitrate ion detection method based on the absorption spectrum utilizing GO and GNPs as substrates. Our approach can directly detect nitrate without the reduction step, thereby making the detection procedure simple and direct while avoiding the use of toxic chemicals. Different from the previous works based on GNPs [4, 16], in our scheme, GNPs interacts with amino group modified GO instead of directly interacting with nitrate ions because very low concentration of nitrate ions cannot trigger the aggregation of GNPs which has been a key limitation to detecting at low levels in prior works [17]. In our detection procedure, amino group modified GO can cause aggregation of GNPs, while the interaction between nitrate ions and modified GO will reduce this aggregation. Hence, based on the reduction of GNPs aggregation measured by the absorption spectrum, the concentration of nitrate ions can be determined. Because the most common anions also induce aggregation of GNPs and can influence the detection by contributing to non-specific aggregation, the previous detection approaches for nitrate detection based on the increased aggregation levels of GNPs was not sensitive and furthermore, these methods required complex pretreatment. Compared with the previous approaches, our method based on monitoring the reduction in GNP aggregation showed better potential to detect nitrate ions in real samples. Based on the demonstrated adsorption potential of GO sheet to nitrate ions and the interaction between amino groups and nitrate ions [16, 24], the enrichment of nitrate ions in the sample with amino group modified GO sheets contributed to a highly sensitive nitrate ion detection protocol as demonstrated from our experiments. With serial optimizations of detection conditions the lowest detected concentration of nitrate ion is 5 nM in deionized water, which is much lower than the methods based on absorption spectroscopy reported by previous work (the limit was 161

μM) [4]. Using water samples obtained from the local river and purposefully contaminating it with a known concentration of nitrate ions a similar sensitivity was demonstrated with minimum pretreatment. The developed method thus shows strong potential for nitrate ion monitoring in the environment in a simple and cost-effective manner. We believe that the developed analytical strategy based on monitoring the reduction in aggregation of GNPs can be used to detect other ions with high specificity and sensitivity.

2. Material and methods

2.1 Chemicals and reagents

Sodium nitrate (NaNO_3) was purchased from JT Baker Chemical Co. Sodium acetate (NaAc), sodium carbonate (Na_2CO_3) and sodium chloride (NaCl) were obtained from Mallinckrodt Chemicals, HAuCl_4 , sodium citrate, ethylenediamine ($\text{NH}_2\text{-CH}_2\text{-CH}_2\text{-NH}_2$), sodium bromide (NaBr), sodium iodide (NaI), sodium sulfide (Na_2S) and sodium sulfate (Na_2SO_4) were from Sigma Chemical Co. GO was purchased from Cheap Tubes Inc. All reagents are used as received without further purification.

2.2 Gold nanoparticles preparation

Glasswares used in the following procedures were cleaned in a bath of freshly prepared 3:1 $\text{HCl}:\text{HNO}_3$ (aqua regia) and rinsed thoroughly in milli-Q grade water prior to use. Colloidal GNPs were prepared according to the reported method [25]. Briefly, 1 ml of 1% HAuCl_4 was added to 100 ml H_2O . The obtained solution was heated to boiling under stirring, followed by injection of 2 ml of 1% sodium citrate solution. After continued boiling for 10 minutes, the solution was cooled to room temperature to obtain a wine red color. For detection, 4 ml of GNPs was centrifuged at 8000 rpm for 10 min and washed with H_2O , and then redispersed in 0.2 ml H_2O . The concentration of GNPs was determined by the reported method [26].

2.3 Graphene oxide modification

GO modification with amino groups was carried out based on the method reported by Lee [27]. Generally, GO is dissolved in ethanol at a concentration of 1 mg/ml. 12 μl of ethylenediamine is added to 0.3 ml of 1 mg/ml GO ethanol solution and the obtained mixture was stirred slowly at room temperature overnight. The product was then washed with ethanol and water 3 times respectively, and redispersed in water at a concentration of 0.5 mg/ml.

2.4 Detection of nitrate

During a typical detection procedure, 20 μl of 0.5 mg/ml amino group modified GO solution was mixed with 20 μl of nitrate ions in deionized water and the mixture was incubated for 1 hour at room temperature. Then 5 μl of this mixture was added to 5 μl of concentrated GNPs. After 5 min, 266 μl of H_2O was added and the UV-vis spectra was recorded.

To prepare real samples, river water (pH was 7.0) was obtained from a local river and centrifuged at 10000 rpm for 10 min to remove large precipitates such as floating sediments. The samples were then purposefully contaminated with nitrate ions to serial concentrations.

To detect nitrate ions, 20 μ l of 1.0 mg/ml amino group modified GO solution was mixed with 20 μ l of the sample solution. After 1 hour of interaction of modified GO with nitrate ions in river water at room temperature, 266 μ l of diluted GNPs is added. After 5 minutes the UV-vis spectra of the samples were collected.

2.5 Characterization

UV-vis spectra are recorded with a Jasco V570 UV/Visible/NIR spectrophotometer (Jasco, Inc., Easton, MD). Transmission electron microscopy (TEM) images of the samples were collected with a FEI Tecnai G2 20 in operation voltage at 100 kV. Scanning electron microscopy images are measured with a FEI NOVA nanoSEM field emission SEM, (FEI Co., Hillsboro, OR) operating at 5.0 KV. Zeta potential measurements were performed with a Zetasizer (Nano ZS, ZEN3600, Malvern, UK).

3. Results and discussion

3.1 Detection procedure

Until now, several simple and economical detection methods that utilize the absorption properties of GNPs have been reported based on the increased aggregation level of GNPs due to the interaction between nitrate ions and recognition groups modified on GNPs [4, 16]. However the sensitivity of these schemes are unsatisfying, because nitrate ions in low concentration cannot trigger the efficient aggregation of GNPs to generate a detectable spectral signature [17]. Herein, to overcome this limitation, we use amino group modified GO to trigger the aggregation of GNPs which will be proportionately reduced when nitrate ions are adsorbed on the modified GO. Then the concentration of nitrate ions were determined based on the reduction in aggregation. The detection procedure is shown in Scheme 1. After modification with ethylenediamine, amino groups are functionalized onto GO sheet. When mixed with GNPs which are negatively charged due to the capping agent, sodium citrate, the amino group modified GO can trigger the aggregation of GNPs due to the positive charge bearing amino groups. In the presence of nitrate ions, the amino groups will first interact with nitrate ions in solution as reported before [16], and this interaction will neutralize the positive charge due to amino groups. Therefore the presence of nitrate ions will reduce the aggregation of GNPs in proportion to the concentration of nitrate ions. It should be noted that the GO sheet can also interact with nitrate ions. It is reported that graphene can be used to adsorb nitrates in water [24], while it is demonstrated that nitrate ions can interact with π aromatic clouds [28], both of which suggest that the GO sheet could be used to enrich nitrate ions in solution. Benefiting from the large area of GO sheet, this enrichment can increase the nitrate ion concentration on modified GO sheets to improve detection sensitivity.

3.2 Optimization of detection

To achieve the best detection sensitivity for nitrate ions, serial optimizations were performed and R is evaluated as a measure of response, where,

$$\Delta R = (R_{\text{blank}} - R_{\text{nitrate}}) / R_{\text{blank}},$$

Herein, R_{blank} is a ratio of absorption at 620 nm to absorption at 520 nm in the UV-vis spectra of blank samples, and R_{nitrate} is the ratio of absorption at 620 nm to absorption at 520 nm in the UV-vis spectra of samples with nitrate ions. The peak located at 520 nm in the UV-vis spectra is assigned to the surface plasmon band of 13 nm GNPs. [29] Because of the aggregation of GNPs, there is a systemic increase in the absorption at 620 nm, a decrease in the absorption at 520 nm. [30] In our past works on colorimetric detection based on the aggregation of GNPs, the ratio of absorption at 620 nm to absorption at 520 were usually used to evaluate the aggregation of GNPs. [31, 32] It is clear that a higher value of R indicates a more obvious difference between the blank and nitrate samples, and therefore higher sensitivity.

The sensitivity of the proposed nitrate ion detection strategy depends on the amount of amino group modified GO. Fig. 1A shows the R obtained from different concentration of modified GO in the presence of 50 nM nitrate ions. As the concentration of modified GO increased (from 1.51 $\mu\text{g/ml}$ to 4.53 $\mu\text{g/ml}$ in the final solution), an increase in R was also observed. Upon further increasing the amount of modified GO to 6.04 $\mu\text{g/ml}$, a reduction in R was noted, indicating a decrease in response. According to the optimized results, the best detection sensitivity was achieved in the presence of 4.53 $\mu\text{g/ml}$ of modified GO. In the proposed detection process, increasing the concentration of modified GO will result in a greater aggregation of GNPs and a higher R_{blank} value, thus a better sensitivity can be achieved. However, higher levels of modified GO ($> 4.53 \mu\text{g/ml}$) can trigger more aggregation of GNPs in the sample in the presence of nitrate, therefore a smaller difference between R_{blank} and R_{nitrate} was observed, resulting in smaller R and lower sensitivity. While a lower amount of modified GO would not induce noticeable aggregation of GNPs and hence it is not possible to detect nitrate ions in low concentration. Therefore, optimization of the amino group modified GO shows that 4.53 $\mu\text{g/ml}$ of modified GO provided the best sensitivity.

Besides the amount of modified GO, the concentration of GNPs is also optimized and the results are as shown in Fig. 1B. It can be seen that R is amplified in proportion to the increase in concentration of GNPs from 0.2 nM to 1 nM; however, further increase in GNPs reduced the R , suggesting a decrease in detection sensitivity. It was observed that 1 nM of GNPs is the best concentration to detect nitrate ions at lower concentrations. As reported, the aggregation behavior of GNPs depends on the concentration: higher concentration of GNPs facilitates aggregation. Therefore it is believed that better detection sensitivity for nitration ions will be achieved with higher concentration of GNPs because of the more obvious R_{blank} and R_{nitrate} signals based on the aggregation of GNPs with less nitrate ions. However, because too much aggregation of GNPs will reduce the signal difference between blank and nitrate ion samples, increasing the concentration of GNPs further will negatively influence the detection sensitivity.

To achieve a saturated state interaction between nitrate ions and amino group modified GO, the effect of incubation time was investigated. To optimize the incubation time, R with different incubation periods was recorded and the results are shown in Fig. 1C. It can be observed that for the samples with 50 nM nitrate ions at 30 min incubation the value of R is negative, indicating an increase in aggregation of GNPs compared to the blank sample,

possibly due to the incomplete interaction between nitrate ions and modified GO. It is known that free nitrate and modified GO can both cause aggregation of GNPs, resulting in increased aggregation than that with modified GO only as shown at 30 min. According to the reported work, complete adsorption of nitrate ions by graphene sheet requires ~45 min [24]. In our optimization steps, R increased with the incubation period and achieved a max after 1 hour, suggesting that the adsorption of nitrate ions on modified GO is almost saturated. In this optimization step, lower concentration of nitrate ions resulted in a prolonged interaction time, longer than the reported time of 45 min. According to our optimization, an incubation time of 1 hour was necessary to achieve best sensitivity.

3.3 Characterization of detection process

The amino modification and detection processes were investigated by zeta potential measurement. The results of zeta potential are shown in Table 1. The zeta potential of the original GO is -29.4 mV, which is attributed to those oxygen containing functionalities. After ethylenediamine modification, the zeta potential of the modified GO increased to -18.9 mV, indicating that amino groups have been immobilized onto GO sheet. Meanwhile, it is also noticed that the net charge of modified GO is still negative, which is assigned to the ethylenediamine modification which only occurs with epoxide carbon while other oxygen containing functionalities such as carboxyl groups remain. After incubation with nitrate ions, the zeta potential of modified GO is reduced to -26.6 mV, implying that the positive charge of amino group was neutralized by the negative charge of nitrate ions after the adsorption of nitrate ions on modified GO. This decrease in zeta potential is a clear evidence of the interaction between nitrate ions and modified GO sheet.

To further understand the detection process, SEM and TEM images were recorded to provide visible results as shown in Fig. 2. According to the SEM images, it can be clearly seen that the aggregation of GNPs in the sample with no nitrate is more obvious than that of the samples with 50 nM of nitrate. Correspondingly, TEM images show similar results: the nitrate ions will reduce the aggregation of GNPs in our detection. Furthermore, unlike the works of GO sheet modified with nanoparticles, TEM images show that most of the GNPs are not anchored to the modified GO sheets, indicating that in our nitrate detection process amino modified GO triggers the aggregation of GNPs.

3.4 Calibration curve of nitrate ion detection

Our detection method shows excellent sensitivity to detect nitrate ions based on the absorption spectrum. Fig. 3 shows the UV-vis spectra and the ratios of absorption at 620 nm to absorption at 520 nm in UV-vis spectra (A_{620}/A_{520}) from the samples with different concentration of nitrate ions in deionized water. It can be seen that the lowest detected concentration is 5 nM, which is about 30,000-fold lower than the previously reported (161 μ M) methods that use absorption spectrum analysis [4]. It can also be noted that the A_{620}/A_{520} of the samples decreased when the nitrate concentration increased. In the concentration range from 0 to 50 nM, the A_{620}/A_{520} ratio decreased rapidly, suggesting an unsaturated interaction between the added amino group modified GO sheets and nitrate ions at concentration less than 50 nM. When the nitrate concentration is higher than 50 nM, the A_{620}/A_{520} ratio did not decrease as much as the results in the lower concentration range,

implying that the adsorption of nitrate with modified GO is nearly saturated. It can therefore be inferred that an increase in amino group modified GO can extend the detection range, but at the same time the detection sensitivity will decrease.

3.5 Selectivity of the detection

The selectivity of our detection method is assessed by using common anions in surface water and ground water such as CO_3^{2-} , acetate, Cl^- , Br^- , I^- , S^{2-} and SO_4^{2-} as controls, and the results of A_{620}/A_{520} are shown in Fig. 4. It can be noted that compared to blank, only nitrate ions can decrease the absorption ratio, A_{620}/A_{520} , of the samples. In contrast, other anions amplified the A_{620}/A_{520} ratio. Nitrate ions can interact with modified GO to result in reduced aggregation than that caused by modified GO only. However, this type of interaction may not exist between modified GO to other anions. In the previous analytical strategies based on absorption spectroscopy for nitrate detection based on the aggregation of GNPs [4, 16], a concern exists when detecting nitrate ions in real sample because on non-specific aggregation of GNPs induced by other ions which will result in false signal and influence the detection reliability and sensitivity. Unlike reported methods, in our scheme the nitrate ions in the sample will reduce the aggregation and therefore it will be easier to recognize the decrease in signal representing the concentration of nitrate ions in real samples, resulting in a simpler pretreatment of the real sample, and detection specificity and sensitivity.

3.6 Detection of nitrate ions in purposefully contaminated river water samples

River water was obtained from a local river and the samples were purposefully contaminated with serial dilutions of nitrate ions after baseline correction. As show in Fig. 5, similar to the results obtained from the sample of nitrate ions in deionized water, a reduction of A_{620}/A_{520} with respect to the nitrate ion concentration was observed. After optimization the lowest detected concentration of nitrate ions in river water is 5 nM similar to that demonstrated in deionized water, highlighting the detection potential of our method in real samples. Benefiting from this effect of modified GO, complex pretreatment of the river water is no longer necessary, making our method simpler and appropriate for environment monitoring.

4. Conclusions

Herein we report on a simple and ultra-sensitive nitrate ion detection strategy with amino group modified GO. The proposed method exhibited remarkable sensitivity, yielding a very low limit of detection of 5 nM, much lower than the past reported methods based on absorption spectroscopy and required only 20 μl of nitrate solution to complete the detection within 75 min. The concentration of modified GO, the amount of GNPs and the incubation time are optimized to achieve the best detection sensitivity. The selectivity was assessed using common anions as control. Detection of nitrate ions at this concentration is possible because of the enrichment due to the interaction between nitrate ions and the amino group modified GO. The method developed can be used for routine environmental and agricultural water quality monitoring *in situ* with a portable spectrometer.

Acknowledgments

Funding for this this research was provided by NIH-NIEHS R01 ES017066-03 award and the USDA-ARS Food Safety Engineering grant. We are thankful to Pushpak Bhandari, Jing Liu, and Zhongwu Zhou for their assistance.

References

1. Johnson CJ, Kross BC. Continuing importance of nitrate contamination of groundwater and wells in rural-areas. *Am J Ind Med*. 1990; 18:449–456. [PubMed: 2248249]
2. Wakida FT, Lerner DN. Non-agricultural sources of groundwater nitrate: a review and case study. *Water Res*. 2005; 39:3–16. [PubMed: 15607159]
3. Brender JD, Olive JM, Felkner M, Suarez L, Marckwardt W, Hendricks KA. Dietary nitrites and nitrates, nitrosatable drugs, and neural tube defects. *Epidemiology*. 2004; 15:330–336. [PubMed: 15097014]
4. Daniel WL, Han MS, Lee JS, Mirkin CA. Colorimetric nitrite and nitrate detection with gold nanoparticle probes and kinetic end points. *J Am Chem Soc*. 2009; 131:6362–6363. [PubMed: 19368386]
5. Greer FR, Shannon M, N. Comm, H. Comm Environm. Infant methemoglobinemia: The role of dietary nitrate in food and water. *Pediatrics*. 2005; 116:784–786. [PubMed: 16140723]
6. Squillace PJ, Scott JC, Moran MJ, Nolan BT, Kolpin DW. VOCs, pesticides, nitrate, and their mixtures in groundwater used for drinking water in the United States. *Environ Sci Technol*. 2002; 36:1923–1930. [PubMed: 12026972]
7. Focazio MJ, Tipton D, Shapiro SD, Geiger LH. The chemical quality of self-supplied domestic well water in the United States. *Ground Water Monit Rem*. 2006; 26:92–104.
8. Chon K, Lee Y, Traber J, von Gunten U. Quantification and characterization of dissolved organic nitrogen in wastewater effluents by electrodialysis treatment followed by size-exclusion chromatography with nitrogen detection. *Water Res*. 2013; 47:5381–5391. [PubMed: 23916154]
9. Kodamatani H, Yamazaki S, Saito K, Tomiyasu T, Komatsu Y. Selective determination method for measurement of nitrite and nitrate in water samples using high-performance liquid chromatography with post-column photochemical reaction and chemiluminescence detection. *J Chromatogr A*. 2009; 1216:3163–3167. [PubMed: 19233366]
10. Afkhami A, Madrakian Ta, Ghaedi H, Khanmohammadi H. Construction of a chemically modified electrode for the selective determination of nitrite and nitrate ions based on a new nanocomposite. *Electrochim Acta*. 2012; 66:255–264.
11. Sachdeva V, Hooda V. A new immobilization and sensing platform for nitrate quantification. *Talanta*. 2014; 124:52–59. [PubMed: 24767445]
12. Kim K, Kim KL, Shin KS. Selective detection of aqueous nitrite ions by surface-enhanced Raman scattering of 4-aminobenzenethiol on Au. *Analyst*. 2012; 137:3836–3840. [PubMed: 22741157]
13. Ianoul A, Coleman T, Asher SA. UV resonance Raman spectroscopic detection of nitrate and nitrite in wastewater treatment processes. *Anal Chem*. 2002; 74:1458–1461. [PubMed: 11922318]
14. Akyuz M, Ata S. Determination of low level nitrite and nitrate in biological, food and environmental samples by gas chromatography-mass spectrometry and liquid chromatography with fluorescence detection. *Talanta*. 2009; 79:900–904. [PubMed: 19576462]
15. Marom H, Popowski Y, Antonov S, Gozin M. Toward the Development of the Direct and Selective Detection of Nitrates by a Bioinspired Mo-Cu System. *Org Lett*. 2011; 13:5532–5535. [PubMed: 21958375]
16. Mura S, Greppi G, Roggero PP, Musu E, Pittalis D, Carletti A, et al. Functionalized gold nanoparticles for the detection of nitrates in water. *Int J Environ Sci Technol*. 2014:1–8.
17. Watanabe S, Sonobe M, Arai M, Tazume Y, Matsuo T, Nakamura T, et al. Enhanced optical sensing of anions with amide-functionalized gold nanoparticles. *Chem Commun*. 2002:2866–2867.
18. Novoselov KS, Geim AK, Morozov SV, Jiang D, Zhang Y, Dubonos SV, et al. Electric field effect in atomically thin carbon films. *Science*. 2004; 306:666–669. [PubMed: 15499015]

19. Guo C, Book-Newell B, Irudayaraj J. Protein-directed reduction of graphene oxide and intracellular imaging. *Chem Commun.* 2011; 47:12658–12660.
20. Sun X, Liu Z, Welsher K, Robinson JT, Goodwin A, Zaric S, et al. Nano-graphene oxide for cellular imaging and drug delivery. *Nano Res.* 2008; 1:203–212. [PubMed: 20216934]
21. Gulbakan B, Yasun E, Shukoor MI, Zhu Z, You M, Tan X, et al. A dual platform for selective analyte enrichment and ionization in mass spectrometry using aptamer-conjugated graphene oxide. *J Am Chem Soc.* 2010; 132:17408–17410. [PubMed: 21090719]
22. Tang LAL, Wang J, Loh KP. Graphene-based SELDI probe with ultrahigh extraction and sensitivity for DNA oligomer. *J Am Chem Soc.* 2010; 132:10976–10977. [PubMed: 20698647]
23. Ren W, Fang Y, Wang E. A binary functional substrate for enrichment and ultrasensitive SERS spectroscopic detection of folic acid using graphene oxide/Ag nanoparticle hybrids. *Acs Nano.* 2011; 5:6425–6433. [PubMed: 21721545]
24. Ganesan P, Kamaraj R, Vasudevan S. Application of isotherm, kinetic and thermodynamic models for the adsorption of nitrate ions on graphene from aqueous solution. *J Taiwan Inst Chem Eng.* 2013; 44:808–814.
25. Frens G. Controlled nucleation for regulation of particle-size in monodisperse gold suspensions. *Nature-Phys Sci.* 1973; 241:20–22.
26. Haiss W, Thanh NTK, Aveyard J, Fernig DG. Determination of size and concentration of gold nanoparticles from UV-Vis spectra. *Anal Chem.* 2007; 79:4215–4221. [PubMed: 17458937]
27. Kim NH, Kuila T, Lee JH. Simultaneous reduction, functionalization and stitching of graphene oxide with ethylenediamine for composites application. *J Mater Chem A.* 2013; 1:1349–1358.
28. Maheswari PU, Modec B, Pevec A, Kozlevcar B, Massera C, Gamez P, et al. Crystallographic evidence of nitrate- π interactions involving the electron-deficient 1,3,5-triazine ring. *Inorg Chem.* 2006; 45:6637–6645. [PubMed: 16903718]
29. Daniel MC, Astruc D. Gold nanoparticles: Assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology. *Chem Rev.* 2004; 104:293–346. [PubMed: 14719978]
30. Li Y, Wu P, Xu H, Zhang Z, Zhong X. Highly selective and sensitive visualizable detection of Hg^{2+} based on anti-aggregation of gold nanoparticles. *Talanta.* 2011; 84:508–512. [PubMed: 21376980]
31. Wei H, Li B, Li J, Dong S, Wang E. DNzyme-based colorimetric sensing of lead (Pb^{2+}) using unmodified gold nanoparticle probes. *Nanotechnology.* 2008; 19
32. Wei H, Li B, Li J, Wang E, Dong S. Simple and sensitive aptamer-based colorimetric sensing of protein using unmodified gold nanoparticle probes. *Chem Commun.* 2007:3735–3737.

Highlights

- A sensing strategy for NO_3^- is demonstrated based on GO and GNPs.
- Enrichment of NO_3^- on amino functionalized GO-GNP sensors improve sensitivity.
- Very low concentration of NO_3^- is detected compared to other sensor techniques.
- The technique proposed is rapid and simple requiring minimum pretreatment.
- Opportunity for multiplex detection of ions in water exists.

Fig. 1. Optimization of detection conditions. Influence of (A) amino group modified GO concentration, (B) GNPs concentration and (C) incubation time to detect nitrate ion from samples with 50 nM nitrate ions.

Fig. 2. Morphological characterization of GNPs aggregation at, (A) 0 nM and (B) 500 nM of nitrate by TEM and SEM (shown in inset).

Fig. 3. UV-vis spectra of samples with no and 500 nM nitrate ions (A) and detection results of nitrate ions in different concentration (B).

Fig. 4.

A_{620}/A_{520} results from the samples of nitrate ions, CO_3^{2-} , acetate, Cl^- , Br^- , I^- , S^{2-} and SO_4^{2-} in concentration of 50 nM.

Fig. 5. UV-vis spectra of samples with no and 500 nM nitrate ions (A) and detection results of nitrate ions at different concentration in river water.

Scheme 1.

Illustration of nitrate ion detection concept in water.

Table 1
Zeta potential measurement conducted at 25 °C

	GO	Blank	500 nM Nitrate
Zeta potential (mV)	-29.4	-18.9	-26.6

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript