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Detection and analysis of cyclotrimethylenetrinitramine (RDX) in environmental samples by surface-enhanced Raman spectroscopy

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Techniques for rapid and sensitive detection of energetics such as cyclotrimethylenetrinitramine (RDX) are needed both for environmental and security screening applications. Here we report the use of surface-enhanced Raman scattering (SERS) spectroscopy to detect traces of RDX with good sensitivity and reproducibility. Using gold (Au) nanoparticles (∼90–100 nm in diameter) as SERS substrates, RDX was detectable at concentrations as low as 0.15 mg/l in a contaminated groundwater sample. This detection limit is about two orders of magnitude lower than those reported previously using SERS techniques. A surface enhancement factor of ∼6 × 10^4 was obtained. This research further demonstrates the potential for using SERS as a rapid, *in situ* field screening tool for energetics detection when coupled with a portable Raman spectrometer. Copyright © 2010 John Wiley & Sons, Ltd.

Keywords: RDX; explosives; SERS detection; gold nanoparticles; groundwater

Introduction

The real-time detection of explosive compounds for environmental monitoring and security screening is a rapidly evolving field and is important for both environmental safety and national security. Cyclotrimethylenetrinitramine (RDX) is one of the most commonly used explosives, and it is frequently detected in soils and groundwater at or near military installations because of its long persistence and high mobility.[1–5] For example, the U.S. Army is reported to have 583 sites at 82 installations with explosives in groundwater and 87 additional locations with suspected groundwater contamination.[5] In addition, studies have shown that munition compounds on ranges and other military testing sites are often heterogeneously distributed in surface soils, with RDX concentrations ranging from less than 0.5 mg/kg up to 10 000 mg/kg for samples collected only a few feet from each other.[1,2] Currently, the detection and analysis of RDX is conducted primarily via high-performance liquid chromatography (HPLC) (USEPA Method 8330), which requires expensive capital equipment and significant costs for sample collection and shipping as well as for laboratory extraction and analysis. Moreover, typical turnaround times for explosives analysis by commercial laboratories are 2 weeks or longer. Rapid, *in situ* field detection and screening technologies are therefore desirable to perform cost-effective assessments within military testing and training ranges to identify potential areas of groundwater contamination with explosives such as RDX.

Surface-enhanced Raman scattering (SERS) spectroscopy has the potential to serve as a rapid screening tool for detecting many different chemical and biological agents. For example, SERS has been demonstrated to be a useful technique for the identification of explosives,[6–10] chemical warfare agents,[11,12] and bacteria.[13–15] The vibrational spectrum generated using this technique provides a 'fingerprint' of the chemical composition of each agent. In addition, SERS is often more sensitive than traditional Raman spectroscopy because the vibrational modes of the analyte can be easily enhanced 10^2–10^9 times when the analyte is adsorbed on noble-metal surfaces. This is due to the high effective SERS cross section per molecule, which allows for detection of up to only a few molecules. Two mechanisms have been known to account for the SERS effect.[16,17] The electromagnetic mechanism is believed to be responsible for the main enhancement that occurs as a result of the excitation of surface plasmons localized on roughened metal surfaces. The second mechanism is related to an increased polarizability of adsorbed molecules due to specific interactions or coupling effects between the metal surface and the adsorbed molecule, although this mechanism has a much smaller contribution to the overall enhancement. Also, the SERS technique requires little or no sample preparation or pretreatment and a small sample volume (usually tens of microliters). Moreover, it is rapid and virtually

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14. ABSTRACT
Techniques for rapid and sensitive detection of energetics such as cyclotrimethylenetrinitramine (RDX) are needed both for environmental and security screening applications. Here we report the use of surface-enhanced Raman scattering (SERS)spectroscopy to detect traces of RDX with good sensitivity and reproducibility. Using gold (Au) nanoparticles (∼90?100 nm in diameter) as SERS substrates, RDXwas detectable at concentrations as low as 0.15mg/l in a contaminated groundwater sample. This detection limit is about two orders of magnitude lower than those reported previously using SERS techniques. A surface enhancement factor of∼6?04 was obtained. This research further demonstrates the potential for using SERS as a rapid, in situ field screening tool for energetics detection when coupled with a portable Raman spectrometer.

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nondestructive, and most analytes can be analyzed directly in water. The availability of portable Raman spectrometers also makes it possible to interface it with an SERS probe, permitting rapid, *in situ* field detection and screening.[18] Therefore, the SERS-based methods have the potential to reduce time, operational costs, and waste generated during the analysis while providing discriminative quantification of analytes in a single measurement.

The potential of SERS for trace analysis of explosives has been explored in recent years.[7,8,11,19] In an early study, Kneipp *et al.*[7] reported a minimum detection limit of 10^{-7} M for trinitrotoluene (TNT) adsorbed on gold and silver nanoparticles in laboratory-prepared solutions. More recently, a detection limit of 10^{-12} M for TNT was achieved by using synthesized Ag nanoparticles as SERS substrates and by adjusting the pH of the TNT aqueous solution.[11] SERS was also found to be applicable for detecting the chemical vapor signature emanating from buried TNT-based landmines at a concentration level of 5 µg/l using a flow-through probe head design and electrochemically etched gold substrates.[8] Gold substrates produced by a nanosphere imprinting technique were recently shown to be applicable not only for SERS detection of TNT but also for detection of RDX and cyclotetra-methylene-tetranitramine (HMX) at 4.5×10^{-4} M for both nitramines.[19] While the SERS technique shows great promise for detection of explosives, most of these previous studies were performed in laboratory-prepared solutions rather than in environmental samples. Trace detection by SERS is known to face potential complications from environmental matrixes, which can compromise the performance and accuracy of the analysis. In addition, to the best of our knowledge, there have been no SERS studies to detect RDX in realistic environmental groundwater samples.

In this work, we report on the synthesis and use of Au nanoparticles as sensitive SERS substrates for detecting RDX explosive at concentrations as low as about 1×10^{-6} M in a contaminated groundwater. The substrates were evaluated in terms of both sensitivity and reproducibility for detecting RDX. An SERS enhancement factor of about 6×10^4 was obtained, and the technique was validated by detecting RDX in an environmental sample using the standard addition method.

Experimental

RDX reference standard (1 mg/ml in acetonitrile) was purchased from AccuStandard, Inc. Thionine, $HAuCl_4$, cetyltrimethylammonium bromide (CTAB), trisodium citrate hydrate, and sodium borohydride were obtained from Aldrich. Deionized (DI) water with resistivity higher than 18.2 MΩ·cm (Barnstead E-pure) was used when preparing aqueous solutions. The gold nanoparticles were prepared by a seed-mediated growth approach[20,21] involving the reduction of $HAuCl_4$ with borohydride in the presence of trisodium citrate. Au seeds were first prepared and used to grow larger nanoparticles in the presence of CTAB, additional $HAuCl_4$, and ascorbic acid as a reducing agent. Briefly, 2.0 ml of ice-cold 0.1 M $NaBH_4$ was added to a 40 ml aqueous solution containing 2.5×10^{-3} M $HAuCl_4$ and 2.5×10^{-4} M trisodium citrate with vigorous stirring. The solution immediately turned orange, indicating the formation of gold nanoparticles. Citrate serves as a capping agent in this solution, and the gold seeds were observed to be stable for at least 1 month. Larger gold nanoparticles were subsequently synthesized as follows. A 100-ml vial and four 45-ml vials were labeled '1' through '5'. To each of these vials, 8 ml of 2.5×10^{-3} M $HAuCl_4$ and 0.5 ml 1×10^{-3} M CTAB were added, followed by the addition of 70 µl of 0.10 M of freshly prepared ascorbic acid solution under gentle stirring. The seed solution (3 ml) was then added to sample 1 and, after 1 min, the sample 1 suspension (11.5 ml) was drawn and added to sample 2 with gentle stirring. This procedure was repeated from sample 2 to sample 5, and the ending Au nanoparticle suspension (sample 5) was stored in a refrigerator for 48 h before use. Excess CTAB was removed by centrifugation and washing with DI water three times.

The extinction spectra of as-synthesized Au nanoparticles were recorded using an HP UV-VIS 8453 spectrophotometer (Agilent Technologies) after diluting 5 µl of the Au suspension in 6 ml of DI water. Scanning electron microscopy (SEM) images were obtained using a Hitachi S4800 FEG-SEM scanning electron microscope operated at 4 kV. SERS spectra were obtained using a Renishaw micro-Raman system (Renishaw Inc.) equipped with a 300 mW near-infrared diode laser for excitation at a wavelength of 785 nm. The laser beam was set in position with a 50×, 0.5 NA (numerical aperture) Leica microscope objective at a lateral spatial resolution of ∼2 µm. A charge-coupled device array detector was used to achieve signal detection from a 1200 groove/mm grating light path controlled by Renishaw WiRE software and analyzed by ThermoGalactic GRAMS software.

RDX working standard solutions of 10^{-4} to 10^{-7} M were prepared from a stock solution of 8×10^{-4} M by diluting the reference standard in DI water. SERS samples were prepared by mixing 0.5 ml of the Au nanoparticle suspension with 250 µl of RDX standard solutions at different concentrations. This mixed RDX and Au nanoparticle suspension (50 µl) was then placed on a glass slide for SERS analysis after the droplet was air-dried. The calibration curves and the reproducibility studies were performed on the same day to minimize error associated with instrument variability.

To evaluate the applicability of SERS technique to detect RDX in the environment, a contaminated groundwater sample was obtained from a U.S. Navy facility in Virginia and used as received. This sample is characterized with a low pH and contained 18 mg/l of total organic carbon (TOC). Major cations and anions included sulfate (21.5 mg/l), chloride (18.0 mg/l), sodium (39.7 mg/l), magnesium (7.65 mg/l), calcium (19.7 mg/l), and potassium (7.5 mg/l). The analysis was performed by the standard addition method, in which 15 ml of the groundwater sample (with an unknown concentration of RDX) were spiked with 0, 50, 70, 175, or 300 µl of a stock solution of RDX (at 177.7 mg/l). The final volume was made up to 25 ml with DI water. The characteristic Raman band intensity at 874 cm^{-1} for RDX for each sample was plotted against the final added concentration of RDX, and a linear regression routine was used to calculate the absolute value of the X-intercept, which is equal to the concentration of RDX in groundwater.

Results and Discussion

The as-synthesized Au nanoparticles were first characterized for their absorption spectra by UV–vis spectroscopy and for their size and morphology by SEM (Fig. 1(A) and (B)). Both spectra of Au nanoparticles and Au seeds (Fig. 1(A)) showed a broad absorption band which has been referred to as the plasmon resonance absorption band and is ascribed to a collective oscillation of the conduction electrons in response to optical excitation.[22–24] Absorption bands are centered at 528 nm for Au seeds and 635 nm for Au nanoparticles; the position of the plasmon band

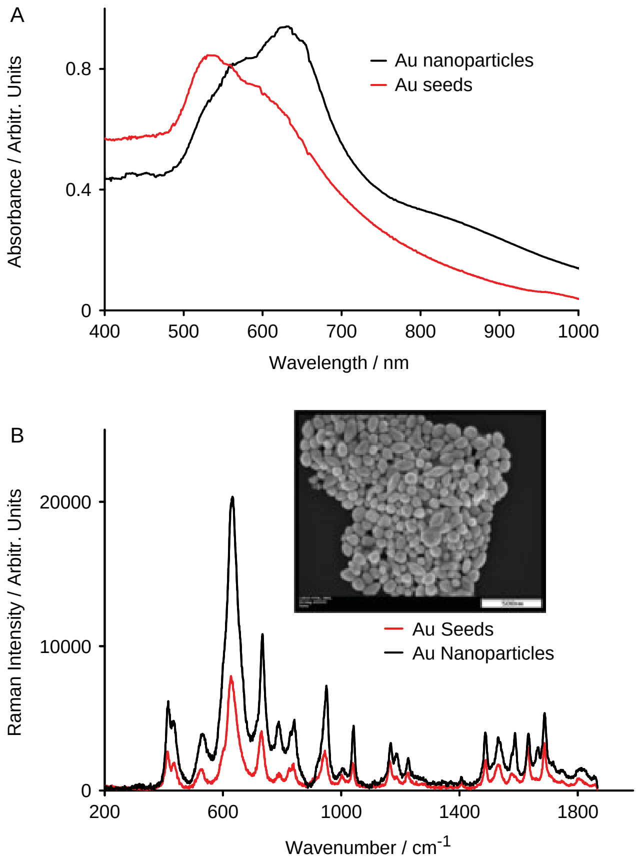

Figure 1. (A) UV-visible spectra of the Au seeds and Au nanoparticles in water. (B) SERS spectra of (1×10^{-6} M thionine) from Au seeds and Au nanoparticles. Inset is a scanning electron micrograph of synthesized Au nanoparticles with an average diameter of 90 nm.

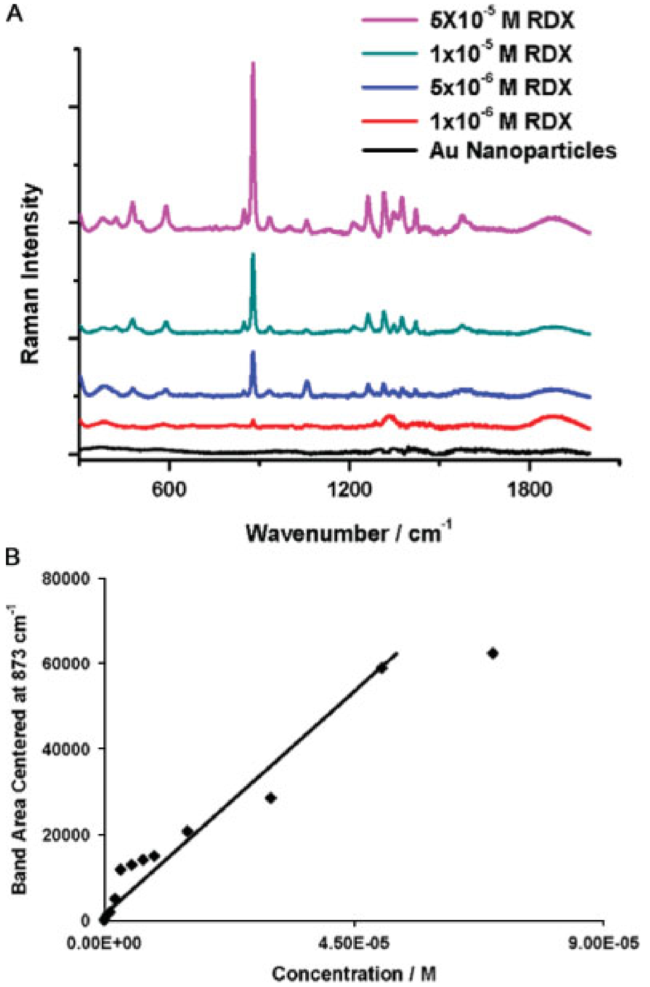

Figure 2. (A) SERS spectra of RDX at concentrations ranging from 1×10^{-6} M to 5×10^{-5} M. Laser power was ~1 mW at the exit of the microscope objective, and the scan time was ~10 s. (B) Peak area at 873 cm^{-1} as a function of RDX concentration.

thus red-shifted from Au seeds to Au nanoparticles due to increased particle size (confirmed by SEM measurements shown in Fig. 1(B)). Au nanoparticles had an average diameter of 90±10 nm, and exhibited spherical to diamond shaped morphologies when compared with spherical Au seeds (~3.5 nm).[20] These observations are consistent with previous studies, in which the absorption maximum of Au nanoparticles was found to depend on the size and shape of the particles.[25–27] For spherical Au nanoparticles, the plasmon band maximum typically falls between 520 and 530 nm.

The synthesized Au nanoparticles were observed to give a good SERS response when thionine was used as the probing molecule (Fig. 1(B)). At the concentration of 10^{-6} M, SERS spectral intensity using Au nanoparticles was found to be ~80% greater than that using the Au seeds. This result is consistent with previously reported data by Ruan *et al.*[28] It has been empirically established that strong SERS effects are obtained when the laser excitation is within the long-wavelength absorption band corresponding to the collective plasmon oscillations.[29,30] Because the absorption spectra of the Au nanoparticles show a single resonance at about 635 nm (Fig. 1(A)), either the red (633 nm) or near-IR (785 nm) laser excitation should be in resonance with surface plasmon of the Au surface and thus suitable for the analysis. For example, Kwon *et al.*[20] reported that gold nanoparticles with an average size of 60–70 nm showed the most intense SERS response when excited at 650 nm. Wei *et al.*[31] found an optimum Au particle size of roughly 90 nm for excitation at 785 nm. The 785-nm laser excitation was used in this study partly because the use of near-IR excitation appears to give higher SERS enhancement and produce the lowest fluorescence background from samples.[28,32–35] This property is particularly advantageous in detecting complex organic molecules such as pyridine,[34] adenine,[35] thionine,[28] and RDX, as shown in this study. For example, Chase and Parkinson[34] found that the SERS enhancement factor for pyridine on Au is higher by using the near-IR rather than visible laser excitation. Kneipp *et al.*[35] showed a large SERS enhancement factor for adenine when near-IR excitation was used.

The Au nanoparticles were subsequently used to investigate the detection of RDX explosive at varying concentrations ranging from 10^{-4} to 10^{-6} M, and the resulting SERS spectra are shown in Fig. 2(A). All spectra were collected in the range from 300 to 2000 cm^{-1} to cover most Raman bands of RDX along with the conventional Raman spectrum of pure RDX for comparison.[36] Since only the vibrational modes of analyte moieties adsorbed on the Au surface are enhanced, there are typically fewer peaks in the SERS spectrum than in the conventional Raman spectrum (without using gold as an SERS substrate). This observation is consistent with those reported in the literature.[19,37,38] The typical

Raman bands assignments of RDX are as follows: the band at 874 cm^{-1} is attributed to the symmetric ring-breathing mode; the band at 930 cm^{-1} is assigned to ring stretching and N–O deformation; the band at 1258 cm^{-1} is due to CH_2 scissoring and N–N stretch vibration;[37] the band at 1312 cm^{-1} results from CH_2 wagging; the band at 1370 cm^{-1} is υNO_2 symmetric stretching vibration and βCH_2 scissoring; finally, the band at 1560 cm^{-1} is attributed to the υNO_2 asymmetric stretch in nitro amines.[37,38]

The strongest SERS Raman band for RDX occurred at 874 cm^{-1} and its intensity increased with an increase of the RDX concentration in solution (Fig. 2(A)). SERS spectra of RDX at different concentrations showed that most of the bands could be identified at the lowest concentration of 1×10^{-6} M RDX with negligible background. On the basis of previously established techniques, the enhancement factor (EF) can be estimated using the following equation:[28,39]

$$EF = \left(\frac{N_{\text{bulk}} I_{\text{SERS}}}{N_{\text{surf}} I_{\text{norm}}}\right) \qquad (1)$$

where I_{SERS} and I_{norm} are the measured peak areas at 874 cm^{-1} from the SERS spectra and the normal Raman spectra of RDX, respectively. N_{bulk} and N_{surf} are the estimated number of RDX molecules in the reference sample (normal Raman without Au nanoparticles) and the SERS enhanced sample, respectively. These measurements were performed under the same experimental conditions for direct comparisons of data and calculations of EF. The maximum number of adsorbed RDX molecules was calculated following the method developed by Ding *et al.*[40] and found to be approximately 4.65×10^6 per Au particle.[28] The calculated EF for SERS detection of RDX explosive was therefore about 6×10^4, which is higher than the EF previously observed for thionine in our group.[28] This enhancement, which can be attributed to an enhanced electromagnetic field upon laser excitation, is consistent with many theoretical and experimental studies showing that a large SERS enhancement occurs when the analyte is situated at the junction or nanoneck of two adjacent Au nanoparticles.[41–44]

Quantitative SERS analysis of RDX at different concentrations was also evaluated (Fig. 2(B)). The characteristic peak area at 874 cm^{-1} was integrated through the instrument software and plotted as a function of the RDX concentration. The linear dynamic range for SERS analysis of RDX was found from the lower detection limit of 1×10^{-6} M to 5×10^{-5} M (Fig. 2(B)). The band area increased linearly with RDX concentration throughout this range, but linearity was lost when the RDX concentration exceeded 5×10^{-5} M. This detection limit (1×10^{-6} M or 0.22 mg/l) is more than two orders of magnitude lower than that previously reported in the literature (4.5×10^{-4} M or 100 mg/l) using SERS analysis.[19] However, we note that the previous study[19] used a gold-coated polystyrene nanosphere film as the SERS substrate and data were recorded using a portable Raman spectrometer. The reported differences in sensitivity are partially attributed to the use of different SERS substrates and instrumentation.

The reproducibility of SERS measurements of RDX from a given substrate is important especially for sensitive and trace level detections. We thus evaluated the sample-to-sample variations using different batches of Au nanoparticles at a fixed concentration of 5×10^{-5} M RDX (Fig. 3). The results indicate that all spectra overlap with a standard deviation of about 21% for the band area centered at 874 cm^{-1}. This standard deviation is considered reasonable for SERS measurements and suggests that our synthesis approach resulted in the production of Au nanoparticles of consistent size and shape among replicate batches. The data also suggest that the SERS technique presented herein holds promise as a quantitative and qualitative tool for rapid screening of RDX.

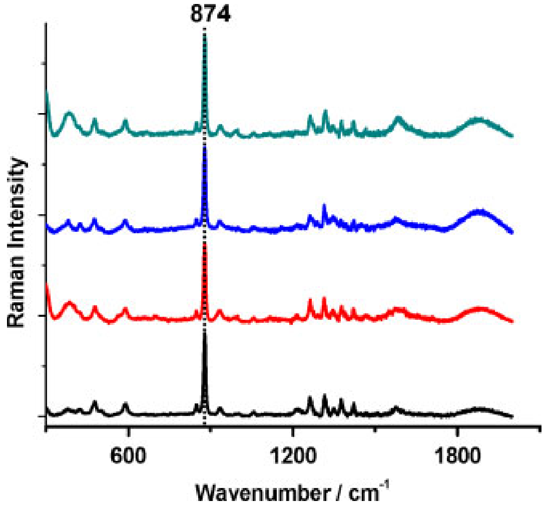

Figure 3. SERS spectra of 5×10^{-5} M RDX using different batches of Au nanoparticles as SERS substrates for reproducibility evaluation.

The SERS technique for detecting RDX was further validated using a contaminated groundwater sample obtained form a U.S. Navy site (Fig. 4(A) and (B)). Rapid detection and screening of RDX explosives in the environment represent a significant challenge because current analytical techniques involve lengthy laboratory preparations and the use of expensive instrumentation (e.g. EPA Method 8330).[45,46] Moreover, groundwater can contain organic impurities which can interfere with the detection and identification of explosives such as RDX, since the EPA method relies on HPLC with analyte detection via retention time only, which is potentially susceptible to false positives. The SERS Raman technique holds promise because the vibrational mode of the SERS spectrum of a given molecule is specific and can provide unique fingerprinting for various organic or inorganic molecules. Here we showed that SERS can be used for sensitive detection of RDX in environmental samples and could potentially offer a valuable tool for rapid screening and characterization of energetics in the environment (Fig. 4(A) and (B)). Using the standard addition technique, SERS analysis revealed that the site groundwater contains about 0.15 ± 0.12 mg/l RDX. The concentration determined by SERS compared favorably with that determined by HPLC via EPA Method 8330 (i.e. 0.12 ± 0.4 mg/l) for the same sample. The standard addition technique was used to correct for matrix interferences because of the presence of unknown constituents in the groundwater. Results showed that the peak intensity at about 874 cm^{-1} increased consistently by spiking the groundwater sample with increasing concentrations of RDX. The error reported in the RDX concentration represents the standard deviation determined using the method by Skoog *et al.*[47]

Our results represent the first use of SERS to detect RDX in a contaminated groundwater at an environmentally relevant concentration, although the SERS technique has been previously demonstrated to detect a range of pollutants such as TNT, perchlorate, pertechnetate, and uranium in groundwater at low

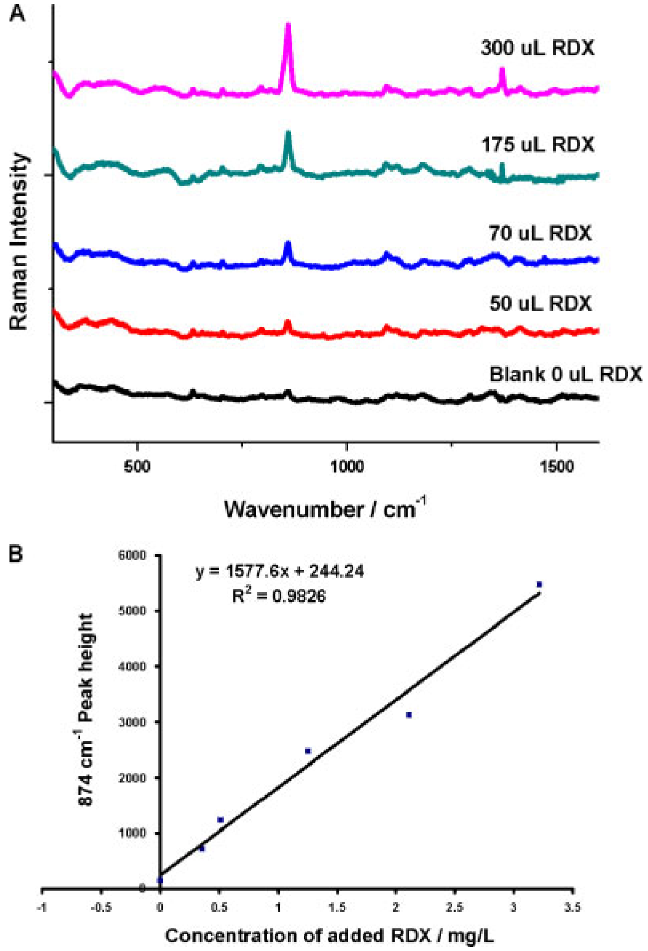

Figure 4. (A) SERS spectra of a contaminated groundwater sample using the standard addition method. (B) Standard addition curve for determining the RDX concentration in groundwater.

concentrations.[48–50] Our work further demonstrates the potential for developing a field SERS detector coupled with a portable Raman spectrometer. The tool would provide environmental and national security professionals with the ability to rapidly screen samples and potentially identify a range of compounds including pollutants, explosives, and other relevant chemical and biological materials.

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