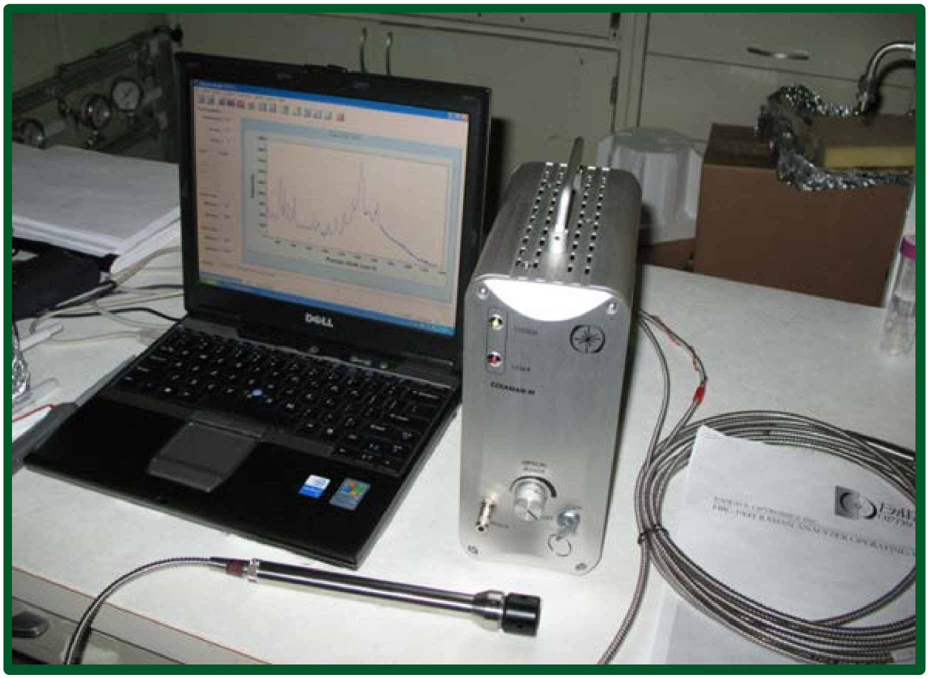
ESTCP
Cost and Performance Report

(ER-201327)



Demonstration and Validation of a Portable Raman Sensor for in-situ Detection and Monitoring of Perchlorate (ClO4-)

July 2017

This document has been cleared for public release;
Distribution Statement A

ENVIRONMENTAL SECURITY
TECHNOLOGY CERTIFICATION PROGRAM

U.S. Department of Defense

Page Intentionally Left Blank

Page Intentionally Left Blank

REPORT DOCUMENTATION PAGE	*Form Approved* *OMB No. 0704-0188*

1. REPORT DATE *(DD-MM-YYYY)* 07/30/2017	**2. REPORT TYPE** ESTCP Cost & Performance Report	**3. DATES COVERED** *(From - To)*

4. TITLE AND SUBTITLE Demonstration and Validation of a Portable Raman Sensor for in-situ Detection and Monitoring of Perchlorate (ClO4-)	**5a. CONTRACT NUMBER**
	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S) Baohua Gu	**5d. PROJECT NUMBER** ER-201327
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Oak Ridge National Laboratory 1 Bethel Valley Road, Bldg. 1505, Rm 218 Oak Ridge, TN 37831	**8. PERFORMING ORGANIZATION REPORT NUMBER** ER-201327
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Environmental Security Technology Certification Program 4800 Mark Center Drive, Suite 17D03 Alexandria, VA 22350-3605	**10. SPONSOR/MONITOR'S ACRONYM(S)** ESTCP
	11. SPONSOR/MONITOR'S REPORT NUMBER(S) ER-201327

12. DISTRIBUTION/AVAILABILITY STATEMENT
Distribution A; unlimited public release

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The overall goal of the project was to field-demonstrate, validate, and optimize the performance and sensitivity of a portable Raman sensor for rapid detection and analysis of the energetic compound perchlorate (ClO_4^-) in contaminated groundwater at multiple U.S. Department of Defense (DoD) military installations. Specific objectives were to: (1) construct and validate the performance of a portable Raman sensor for ClO4- detection in groundwater with varying characteristics, (2) optimize the performance and sensitivity of the sensor through nano-fabrication of surface-enhanced Raman scattering (SERS) substrates and the fiber-optic sensor probe, (3) optimize field-testing methodologies and establish testing protocols, (4) partner with a commercial vendor for large-scale production of SERS substrates via nanoimprinting, and (5) evaluate and document the cost effectiveness of the new sensing technology by comparing with conventional laboratory-based analytical protocols.

15. SUBJECT TERMS
Demonstration, Validation, Portable Raman Sensor, for in-situ Detection and Monitoring of Perchlorate (ClO4-)

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON** Baohua Gu
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(Include area code)*
UNCLASS	UNCLASS	UNCLASS	UNCLASS	66	865-574-7286

Page Intentionally Left Blank

COST & PERFORMANCE REPORT
Project: ER-201327

TABLE OF CONTENTS

Page

TABLE OF CONTENTS (Continued)

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

ACRONYMS AND ABBREVIATIONS

σ	standard deviation
µg	microgram(s)
µL	microliter(s)
µM	micromolar
Ag	silver
Au	gold
cis-DCE	1,2-*cis* dichloroethene
Cl^-	chloride
ClO_4^-	perchlorate
cm^{-1}	wavenumber in reciprocal centimeters
CNMS	Center for Nanophase Materials Sciences (ORNL)
Cr	chromium
cVOC	chlorinated volatile organic compound
DMAET	2-(dimethylamino) ethane-thiol
DO	dissolved oxygen
DoD	U.S. Department of Defense
DOE	U.S. Department of Energy
ea	each
EBL	electron beam lithography
EF	enhancement factor
EPA	U.S. Environmental Protection Agency
ESI	electrospray ionization
ESTCP	Environmental Security Technology Certification Program
Fe	iron
ft	foot/feet
HPLC	high-performance liquid chromatography
hr	hour(s)
IC	ion chromatography
IHDIV	Indian Head Division, Naval Surface Warfare Center
in	inch(es)
L	liter
LC	liquid chromatography
LTM	long-term monitoring
M	molar
MCL	maximum contaminant level

min	minute
mg	milligram(s)
mL	milliliter(s)
Mn	manganese
MS	mass spectrometry
mV	millivolt(s)
mW	milliwatt(s)
MW	monitoring well
N	nitrogen
$NaClO_4$	sodium perchlorate
nm	nanometer
NO_3^-	nitrate
O	oxygen
OB/OD	open burn/open detonation
ORNL	Oak Ridge National Laboratory
ORP	oxidation reduction potential
*p*MA	*p*-mercaptoaniline
PO_4^{3-}	phosphate
ppb	part(s) per billion
ppm	part(s) per million
SEM	scanning electron microscope
SERS	surface-enhanced Raman scattering
Si	silicon
SO_4^{2-}	sulfate
TCE	trichloroethene
TDS	total dissolved solid
U.S.	United States
VOC	volatile organic compound
wk	week

ACKNOWLEDGEMENTS

We wish to thank Ms. Xiangping Yin at Oak Ridge National Laboratory (ORNL) for technical support, Dr. Andrew Ritts and Xin Sun at Nanova Inc. for assistance in surface-enhanced Raman scattering (SERS) analysis, Dr. Randi Rothmel and Mr. Paul Hedman from CB&I Federal Services for sampling and analytical assistance, and Dr. Andrea Leeson of the Environmental Security Technology Certification Program (ESTCP) for her support and oversight during this project. We also wish to acknowledge personnel at both the Indian Head Division, Naval Surface Warfare Center (IHDIV) and Redstone Arsenal (Redstone) for providing site access and supporting this research effort. The project was funded by the ESTCP Program (Project ER-201327) to ORNL, which is managed by UT-Battelle, LLC, for U.S. Department of Energy (DOE) under contract DE-AC05-00OR22725. The fabrication of elevated gold (Au) ellipse dimers by electron beam lithography (EBL) was conducted at the ORNL Center for Nanophase Materials Sciences (CNMS), which is sponsored by the DOE Scientific User Facilities Division.

Page Intentionally Left Blank

EXECUTIVE SUMMARY

OBJECTIVES OF THE DEMONSTRATION

The overall goal of the project was to field-demonstrate, validate, and optimize the performance and sensitivity of a portable Raman sensor for rapid detection and analysis of the energetic compound perchlorate (ClO_4^-) in contaminated groundwater at multiple U.S. Department of Defense (DoD) military installations. Specific objectives were to: (1) construct and validate the performance of a portable Raman sensor for ClO_4^- detection in groundwater with varying characteristics, (2) optimize the performance and sensitivity of the sensor through nano-fabrication of surface-enhanced Raman scattering (SERS) substrates and the fiber-optic sensor probe, (3) optimize field-testing methodologies and establish testing protocols, (4) partner with a commercial vendor for large-scale production of SERS substrates via nanoimprinting, and (5) evaluate and document the cost effectiveness of the new sensing technology by comparing with conventional laboratory-based analytical protocols.

TECHNOLOGY DESCRIPTION

The technology used herein is SERS—million-fold or greater enhancements of the Raman signal of target analyte molecules adsorbed at or near nanostructured noble metal surfaces (SERS substrates) and thus allows the detection of analytes or pollutants at ultra-low concentrations in water. SERS also provides molecular signatures via vibrational frequency shifts of specific chemical bonds (e.g., ClO_4^- at ~935 reciprocal centimeters [cm^{-1}], and nitrate [NO_3^-] at 1,050 cm^{-1}) so that the technique is selective to specific analyte molecules, potentially enabling simultaneous, multi-species detection and analysis. The key to successful application of SERS for chemical and environmental analyses is the fabrication of nanostructured substrates with small gap sizes that are reproducible, sensitive, and selective to target analyte molecules. This project developed and utilized a new type of ordered and elevated gold (Au) bowtie and ellipses nano-arrays with controllable gap sizes to <10 nanometers (nm). The substrate showed superior reproducibility and sensitivity with an enhancement factor (EF) on the order of 10^9–10^{11}(Hatab et al., 2010).

DEMONSTRATION RESULTS

Field demonstration of the Raman sensor technology was conducted multiple times at two DoD sites: the Indian Head Division, Naval Surface Warfare Center (IHDIV) in Maryland, and Redstone Arsenal (Redstone) in Alabama, with varying groundwater geochemical characteristics. Major findings include the following:

1. A SERS sensor based on elevated Au ellipse dimer architectures was designed and developed for ClO_4^- with a detection limit of ~10^{-6} molarity (M) (or 100 micrograms per liter [µg/L]). The performance of these sensors was evaluated and optimized through variation of their geometric characteristics (i.e., dimer aspect ratio, dimer separation, etc.).
2. Large-scale commercial production of SERS substrates via nanoimprinting technology was successfully demonstrated. This is a substantial step toward the commercialization of the SERS sensors and may potentially lead to significantly reduced fabrication costs of SERS substrates.

3. Commercially-produced SERS sensors were demonstrated to detect ClO_4^- at levels $<10^{-6}$ M using a portable Raman analyzer. The performance of the commercial SERS sensors for ClO_4^- detection in the presence and absence of interferences was determined for a series of standard solutions.
4. Field demonstration of the portable Raman sensor with commercially-produced SERS substrates was completed twice at IHDIV and once at Redstone. Multiple wells were sampled at both sites, where a standard addition method was employed using the sensor to determine the ClO_4^- for each groundwater sample. Groundwater samples were also collected for method intercomparison with the standard ion chromatography (IC) approach. Results were generally comparable, although significant variations were observed in a small set of samples due to the presence of interference ions in the groundwater.

To our knowledge, this is the first demonstration of a field portable SERS Raman sensor that combines a portable Raman analyzer with novel elevated Au ellipse nanostructural arrays. The technology shows the potential to provide a tool for rapid, *in-situ* screening and analysis of ClO_4^- and possibly other energetics that are both important for environmental monitoring and of interest for national security.

IMPLEMENTATION ISSUES

As commonly observed with other analytical techniques, SERS technology is also prone to interferences due to its sensitivity and responses to other ionic species, such as NO_3^-, sulfate (SO_4^{2-}), and dissolved organics present in water, which could potentially mask the SERS signal of the target analyte (ClO_4^-). As such, SERS analysis could be subject to significant variations (e.g., ±20%). The biggest challenge is thus to reduce its variability due to the presence of various groundwater interferences so as to increase its detection limit. The reported ClO_4^- detection limit (~100 µg/L) and variability may not be suitable for routine quantitative analysis, particularly at low ClO_4^- concentrations. However, the portable Raman sensor developed in this project could be used as a rapid screening tool for ClO_4^- at concentrations $>10^{-6}$ M during site assessment work to aid more effective and timely decision-making during remediation projects. Future studies are warranted to further develop the technology and to optimize its performance, and eventually to bring the technology to market. With additional development and demonstration, the technology has the potential to reduce analytical costs by eliminating shipping and typical costs associated with laboratory analysis. A cost saving of 30–45% is estimated during a typical sampling event. The technology also allows rapid turnaround of information to decision makers for site characterization and remediation.

1.0 INTRODUCTION

1.1 BACKGROUND

Costs for environmental analysis and monitoring represent a significant percentage of the total and future remedial expenses at many of the U.S. Department of Defense (DoD) contaminated sites. It has been reported that about 30–40% of the remediation budget is usually spent on long-term monitoring (LTM), of which a large percentage represents laboratory analytical costs. Perchlorate (ClO_4^-) is one of the prevalent contaminants in groundwater and surface water at DoD military testing and training ranges such as the Indian Head Division, Naval Surface Warfare Center (IHDIV) at Indian Head, Maryland, and Dahlgren, Virginia; and Redstone Arsenal (Redstone) in Alabama (Clausen et al., 2009; Gu and Coates, 2006; Sass, 2004; Suidan et al., 2008). Perchlorate in these contaminated sites ranges from sub-parts per billion (ppb) (micrograms per liter [µg/L]) up to hundreds or thousands of parts per million (ppm) (milligrams per liter [mg/L]) concentrations (e.g., IHDIV) and often occurs with many other common ionic species in groundwater such as nitrate (NO_3^-), chloride (Cl^-), and sulfate (SO_4^{2-}) with wide variations in total dissolved solids (TDS). ClO_4^- is a key component of explosives and solid rocket fuels, although it is also present in a number of commercial products such as fireworks, road flares, and Chilean NO_3^- fertilizers (Sass, 2004; Gu and Coates, 2006). Currently, the established detection and analysis methods for ClO_4^- in water are ion chromatography (IC) (U.S. Environmental Protection Agency [EPA] Method 314.0) and IC with electrospray ionization (ESI) and mass spectrometry (MS) (IC/ESI/MS; EPA Method 6860). Each of these analytical techniques requires sample collection and shipping in addition to the use of expensive analytical equipment, and significant time for sample preparation and analysis. Furthermore, typical turnaround time for these analyses by commercial laboratories is generally two weeks.

Therefore, rapid, cost-effective field detection and screening technologies are highly desirable for site assessments and both short- and long-term monitoring. In this work, we demonstrate a portable Raman sensor based on surface-enhanced Raman scattering (SERS), allowing real-time analysis of ClO_4^- in contaminated groundwater or surface water. A field-ready portable instrument with the capability of multiple species detection would thus be of significant benefit for site assessment work at many DoD training and testing ranges and may significantly reduce the costs associated with LTM at these sites.

1.2 PROJECT OBJECTIVES

The overall goal of the project was to field-demonstrate, validate, and optimize the performance and sensitivity of a portable Raman sensor for rapid detection and analysis of the energetic compound ClO_4^- in groundwater.

Specific objectives were to:

1. Construct and validate the performance of a portable Raman sensor for the detection of ClO_4^- as a target analyte in groundwater with varying characteristics,
2. Optimize the performance and sensitivity of the sensor through nano-fabrication of SERS substrates and the fiber-optic sensor probe,

3. Optimize field-testing methodologies and establish testing protocols,
4. Partner with a commercial vendor for large-scale production of SERS substrates via nanoimprinting, and
5. Evaluate and document the cost-effectiveness of the new sensing technology by comparing with conventional laboratory-based analytical protocols.

1.3 REGULATORY DRIVERS

There is currently no Federal drinking water standard (maximum contaminant level [MCL]) for ClO_4^-. However, the U.S. Environmental Protection Agency (USEPA) has previously listed ClO_4^- on the Draft Drinking Water Candidate Contaminant List[1] and the Unregulated Contaminant Monitoring Regulation List,[2] and has previously announced their intention to establish a Federal MCL for ClO_4^- under the Safe Drinking Water Act.[3] The states of Massachusetts and California currently have drinking water MCL values for ClO_4^- of 1 µg/L and 6 µg/L, respectively. Although there is no Federal MCL, many DoD sites are actively involved in the sampling and analysis of ClO_4^-. The objective of this project is to demonstrate a field-based method for rapid ClO_4^- analysis.

---

[1] http://water.epa.gov/scitech /drinkingwater/dws/ccl/ccl3.cfm
[2] http://water.epa.gov/lawsregs/rulesregs/sdwa/ucmr/factsheet.cfm
[3] http://water.epa.gov/drink/contaminants/unregulated/upload/FactSheet_PerchlorateDetermination.pdf

2.0 TECHNOLOGY

2.1 TECHNOLOGY DESCRIPTION

Raman spectroscopy is a technique that gives information about the vibrational frequency shifts or modes of chemical bonds in certain molecules such as ClO_4^- (Gu et al., 2009; Hatab et al., 2010; Kneipp et al., 1997; Nie and Emory, 1997; Ruan et al., 2006b; Ruan et al., 2007). The chloride-oxygen (Cl-O) bond in ClO_4^- gives a characteristic Raman frequency shift at ~935 reciprocal centimeters [cm^{-1}] (the strongest Raman band for ClO_4^-). Since each molecule (or chemical bond) has its own vibrational frequency, the technique is specific and can in principle provide unique fingerprinting for various organic and inorganic compounds that are Raman active. However, one of the major limitations of conventional Raman spectroscopy is its relatively weak signal when compared to other optical techniques such as fluorescence spectroscopy. This shortcoming has greatly limited the use of conventional Raman spectroscopy as an analytical tool in environmental analysis.

SERS overcomes these limitations by providing orders of magnitude higher (10^4–10^{12}) enhanced Raman signal from Raman-active molecules that are either adsorbed onto or at the close proximity of specially prepared noble metal surfaces (Gu et al., 2009; Hatab et al., 2010; Kneipp et al., 1997; Nie and Emory, 1997; Ruan et al., 2006b; Ruan et al., 2007). Such surfaces are usually made with nanostructured gold (Au) or silver (Ag) arrays with nanometer (nm) gap sizes between nanostructured particles or dimers. First demonstrated in 1974 (Kneipp et al., 1997; Nie and Emory, 1997), SERS has developed into an ultra-sensitive technique for detecting and analyzing a variety of chemical and biological agents. Target analyte molecules near or adsorbed at nanostructured Au or Ag surfaces give rise to a million-fold or greater enhancement of the Raman signals due to interactions between target molecules and the SERS-active surface. As a result, the SERS technique allows the detection of analyte molecules at ultra-trace to single molecular concentration levels (Hatab et al., 2010; Ruan et al., 2007). Thus, in comparison with fluorescence and optical absorption spectroscopic techniques, SERS is much more sensitive with greater molecular selectivity due to the molecular vibrational information provided by the Raman methodology. This is a significant advantage of SERS since the surface selectivity and sensitivity extends the utility of Raman spectroscopy to a wide variety of applications previously impossible to achieve with conventional Raman techniques. Furthermore, SERS is nondestructive and can be performed under ambient conditions in water, thereby allowing direct analysis of environmental samples such as groundwater and surface water.

2.2 ADVANTAGES AND LIMITATIONS OF THE TECHNOLOGY

The new SERS technology is designed to provide rapid detection and screening of ClO_4^- in environmental samples such as contaminated groundwater and surface water. It is useful for field sensing applications that require rapid identification outside the typical laboratory environment. The technology also has the potential to detect other energetic compounds or pollutants such as 2,4,6-trinitrotoluene (TNT) in groundwater although additional development and optimization are needed since SERS substrate design is affected by specific contaminant molecules. As stated earlier, in comparison with fluorescence and optical absorption spectroscopic techniques, SERS is more sensitive with greater molecular selectivity (or fingerprinting) due to the molecular vibrational information provided by the Raman methodology.

This is a significant advantage of using SERS since the surface selectivity and sensitivity extends Raman spectroscopy utility to a wide variety of applications previously impossible to achieve with conventional techniques. Furthermore, SERS is nondestructive and can be performed under ambient conditions in water, thereby allowing direct analysis of environmental samples such as groundwater and surface water.

Similar to other techniques, however, analysis of realistic environmental samples by SERS presents a challenge because of the complex geochemistry and mixed contaminants resulting in interference with the analysis or false-positive responses. Analysis is also complicated by the fact that concentrations of the analytes of interest (ClO_4^- in this case) are usually orders of magnitude lower than interfering ions such as total organic carbon (TOC), NO_3^-, and SO_4^{2-}, which also give strong Raman signals and can thus mask the detection of the analyte itself or cause significant variations in analysis. The standard addition method is sometimes used to minimize potential matrix interferences due to unknown background organic or inorganic materials in the groundwater. As part of this Environmental Security Technology Certification Program (ESTCP) demonstration, the factors that cause interference issues with the Raman technique were evaluated, the lower limits of instrument accuracy were better defined, and the overall utility of the instrument for site assessment applications and potentially LTM were confirmed.

3.0 PERFORMANCE OBJECTIVES

Table 3-1 provides a list of performance objectives, data requirements, and success criteria for the project. Since the overall objective of the project is to develop, demonstrate, and validate a new instrument (field-ready Raman sensor) for ClO_4^- detection and analysis in groundwater, the main performance objectives are to determine the selectivity and sensitivity of the instrument to quantify ClO_4^- in groundwater under differing conditions (such as differing natural anion concentrations, pH, oxidation reduction potential (ORP), and co-contaminants, such as trichloroethene [TCE]) and varying ClO_4^- concentration ranges at multiple DoD sites. Results between SERS measurements and EPA-approved IC methodologies are compared to determine if the two methods are comparable at the confidence level of 80%. This confidence level is used primarily because SERS is a spectroscopy-based technique, in which spectral peak position and peak height may vary due to substrate variations, laser focusing, and environmental geochemical conditions or interferences. Nonetheless, our goal was to establish a new SERS methodology that could be used for rapid screening and/or *in-situ* field monitoring of ClO_4^- in contaminated groundwater or surface water.

Table 3-1. Performance Objectives, Data Requirements, Success Criteria.

Performance Objective	Data Requirements	Success Criteria	Result
Quantitative Performance Objectives			
Development of a portable Raman sensor; SERS substrate optimization and commercial production	NA	• Field-ready meter and optimized substrates • Commercially-produced SERS substrates	• A field-ready instrument was developed and commercial SERS substrates produced.
Selective identification and detection of ClO_4^-	Raman spectra; peak intensities; common anions, cations, and co-contaminants	• Identification of ClO_4^- in the presence of common anions and cations at <10 mM. • ClO_4^- detection in the presence of co-contaminants such as VOCs at <1 ppm.	• ClO_4^- was readily identified in the presence of common anions, cations, and co-contaminants. • However, the presence of interfering ions (>0.2 mM) made SERS detection less sensitive.
Sensitive detection and quantification of ClO_4^-	Raman data; peak intensities; standard addition and calibration; IC data by EPA methods	• Quantitative analysis of ClO_4^- at LOD better than 3 ppb. • Analysis of ClO_4^- at wide concentration ranges from ~3 to 100,000 μg/L. • Measurement accuracy and precision at 20% or better	• The anticipated LOD for ClO_4^- was not achieved in site groundwater. The LOD was ~100 ppb for the method. • Detection of ClO_4^- at >100 μg/L, and the precision may vary >20%, depending on interferences.
Qualitative Performance Objectives			
Sensor methodology developed	Method development based on above data and analysis	• Method established for field monitoring and quantification • System robustness and ease of use	• Method established and easy to use

LOD = limit of detection; mM = millimolar; NA = not applicable; VOC = volatile organic compound

Page Intentionally Left Blank

4.0 SITE DESCRIPTION

Field testing of the Raman sensor technology was conducted at two different sites: the Indian Head Division, Naval Surface Warfare Center (IHDIV), in Indian Head, Maryland, and Redstone Arsenal (Redstone) outside of Huntsville, Alabama. Two field demonstrations were performed at IHDIV between October 2015 and October 2016, and one at Redstone in November 2016.

4.1 FIELD DEMONSTRATION AT INDIAN HEAD DIVISION, NAVAL SURFACE WARFARE CENTER

4.1.1 Site Background

The IHDIV is located near Indian Head, Maryland, in Charles County. This facility, located approximately 50 kilometers (km) from Washington, D.C., manufactures explosives and tests energetic compounds for the U.S. Navy. Figure 4-1 shows the field site location where the portable Raman sensor was tested. The site is located on the southeast side of IHDIV Building 1419, which was used to clean out or "hog out" solid propellant containing ammonium perchlorate from various devices, including rockets and ejection seat motors, that have exceeded their useful life span. The hog out process and former waste handling methods have impacted the groundwater near Building 1419. The presence of a former nitration plant in this area also has apparently resulted in high NO_3^- in some locations (as high as 14 mg/L as nitrogen [N]).

Figure 4-1. Location of the Raman Sensor Field Demonstration at the IHDIV Site.

4.1.2 Geochemistry and Contaminant Concentrations

Previous site assessment work performed at Building 1419 is described in Hatzinger et al. (2006). Historical groundwater characterization data (samples collected January 2002) are provided in Table 4-1 and Table 4-2. Groundwater samples were collected from 17 Geoprobe® borings to the southeast of Building 1419 and analyzed for ClO_4^-, NO_3^-, SO_4^-, pH, and dissolved oxygen (DO). Results of the chemical analyses from the Geoprobe borings are provided in Table 4-1. Groundwater samples collected from six monitoring wells (MWs) onsite were also analyzed for ClO_4^-, pH, and DO (Table 4-2). The field characterization data revealed a shallow, narrow plume of ClO_4^- contamination behind Building 1419 with levels ranging from below detection to approximately 430 mg/L. With a few exceptions, the pH of the site was <5, and the DO levels were <2 mg/L. In some instances, dissolved metal ions, such as iron (Fe) and manganese (Mn), were also elevated at this site.

Table 4-1. Historical Groundwater Properties at the IHDIV Site (Hatzinger et al., 2006).

Boring	ClO_4^- (mg/L)	Nitrate as N (mg/L)	SO_4^{2-} (mg/L)	pH	DO (mg/L)[1]
GP-1	120	0.6	66	4.67	NA[2]
GP-2	<2.5	3.0	220	8.08	NA
GP-3	8.2	1.9	280	5.23	NA
GP-4	57	0.3	110	4.54	NA
GP-5	65	0.1	130	4.21	1
GP-6	280	11	69	5.62	1
GP-7	35	1.5	66	4.21	0.1
GP-8	430	14	62	4.57	NA
GP-9	73	0.4	56	4.44	0.8
GP-10	300	12	70	4.31	1
GP-11	230	14	72	4.71	0.8
GP-12	55	2.0	110	6.46	NA
GP-13	230	3.8	64	4.61	1.5
GP-14	14	1.5	250	4.97	NA
GP-15	9.8	<0.2	160	5.34	0.2
GP-16	270	2.8	74	4.16	1
GP-17	<5	<0.2	140	4.83	0.2

[1] Analysis performed by colorimetric field method (CHEMetrics).

[2] NA: Not analyzed.

Table 4-2. Historical Groundwater Properties and ClO4- Concentrations in MW 1–6 at the IHDIV Site (Hatzinger et al., 2006).

Monitoring Well	ClO_4^- (mg/L)	pH	DO (mg/L)
MW-1	84.7	5.02	1.49
MW-2	1.9	6.75	NA
MW-3	1.6	4.13	NA
MW-4	181	5.00	1.64
MW-5	82.8	6.20	1.13
MW-6	142.4	5.03	1.33

NA = not applicable

Two previous demonstration plots, each consisting of two injection wells, two extraction wells, and nine groundwater MWs, were installed near Building 1419 in 2002, and a small field demonstration was conducted in which lactate was actively injected into one demonstration plot and another served as an unamended control plot. Full details of the demonstration are presented in Hatzinger et al. (2006). All of the groundwater wells for this demonstration and the wells installed for site assessment remain in place. A subset of these wells was selected for groundwater sampling during two field demonstrations of the Raman technology conducted at the IHDIV site.

4.2 REDSTONE ARSENAL

4.2.1 Site Background

Redstone is an active U.S. Army facility in northern Alabama, adjacent to Huntsville in Madison County. The current Redstone Arsenal originated as the Huntsville Arsenal in 1941, a facility built to produce chemical weapons for World War II, and the adjacent Redstone Ordnance Plant, used to manufacture grenades, bombs, and other ordnance. After World War II, the facility was reorganized to become a center for the U.S. Army's rocket and missile projects, and now includes several different commands and centers involved with the testing and development of missiles. Redstone is also the home of the National Aeronautics and Space Administration (NASA) Marshall Space Flight Center.

The demonstration was conducted using groundwater collected from the open burn/open detonation (OB/OD) area at Redstone. The OB/OD area is an active facility located within the RSA-151 groundwater unit in the southern portion of Redstone (Figure 4-2). The OB/OD area lies on topographically high ground surrounded by wetlands on three sides. The far northern boundary of the OB/OD area is adjacent to a creek and wetland area. The far western boundary of the OB/OD area is adjacent to wetlands and is approximately 250 feet (ft) from a lake located farther to the west. The OB/OD area is located approximately 1,000 ft east of the Tennessee River. Portions of RSA-013 (Unlined Open Burn Pads) and RSA-014 (Waste Burn Trenches)—currently inactive sites—are also located within the OB/OD area site boundary.

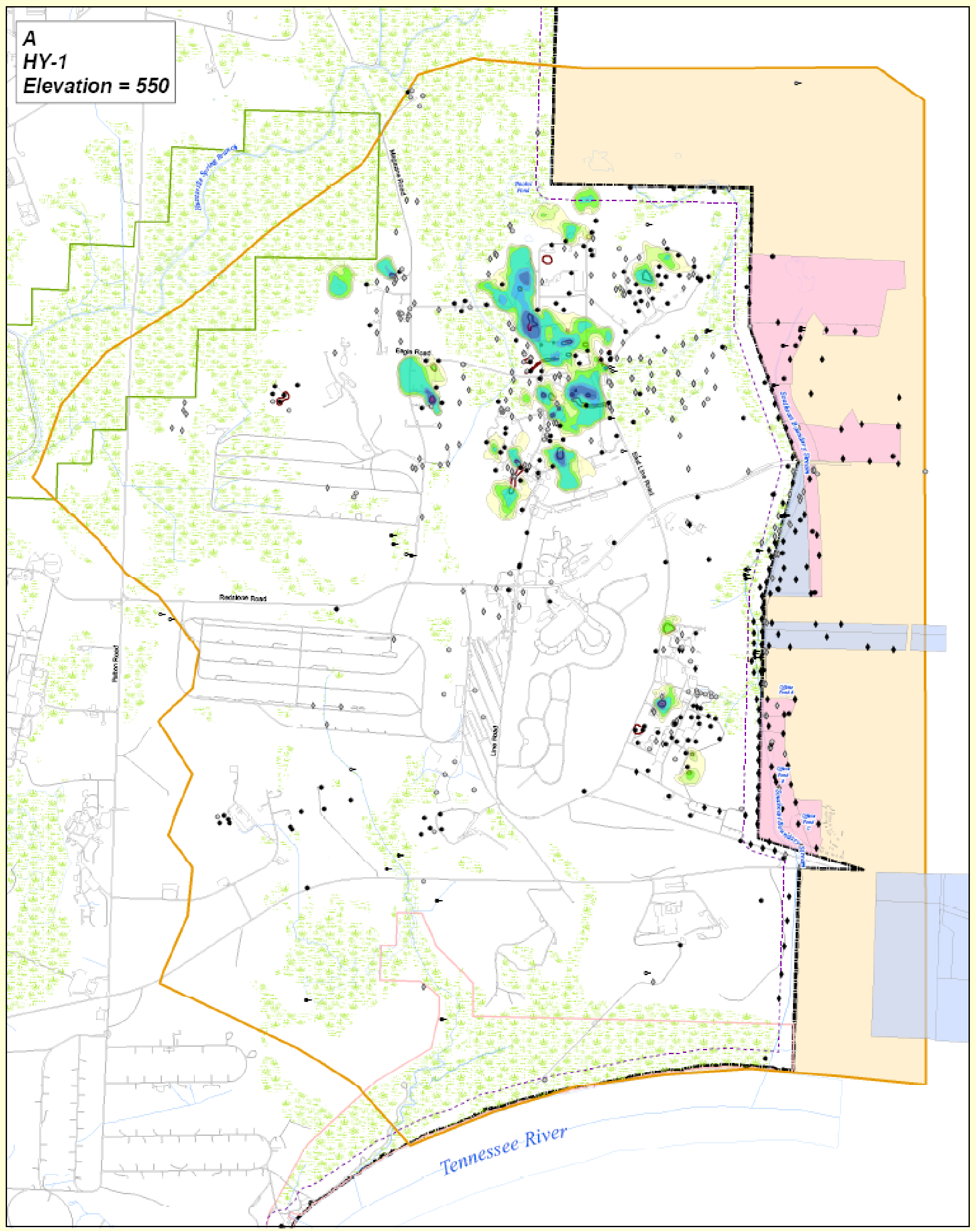


Figure 4-2. Location and ClO_4^- Concentration Contours in Hydrostratigraphic Zone A of the Groundwater Unit at Redstone.

4.2.2 Geochemistry and Contaminant Concentrations

The groundwater at Redstone has been previously characterized. In HY-zone 1, ClO_4^- concentrations in the site database range from ~0.05 µg/L to >200 mg/L. A number of the wells also have TCE as a co-contaminant, at concentrations ranging from <1 µg/L to >800 mg/L.

Tetrachloroethene (PCE) and lower-chlorinated degradation products of these two chlorinated ethenes, including 1,2-*cis* dichloroethene (*cis*-DCE) and vinyl chloride (VC), are also detected sporadically at the site. The groundwater across the large site is generally neutral in pH (~5.5–7.5), and ORP values are variable with location and time, ranging about -200 to +300 millivolts (mV), similar to DO, which varies from <1 to >10 mg/L. Alkalinity is generally >100 mg/L as calcium carbonate ($CaCO_3$). This site was selected as a test site for the Raman sensor primarily to evaluate the impact of volatile organic compounds (VOCs), such as TCE and possibly other chlorinated solvents, on ClO_4^- detection. The ORP, DO, alkalinity, pH, and other parameters across the site was also taken into consideration when selecting wells to sample in order to maximize the number of variables to be tested.

In the OB/OD area, eight wells are annually monitored. The pH in these wells is also variable, ranging from 4.1 to 6.9 in different wells during the 2015 sampling event. Wells across this pH range were sampled for the demonstration. The DO at the site ranged from 0 to 3.8 mg/L in 2015, and the ORP varied from -108 mV to +242 mV in different wells. ClO_4^- concentrations ranged from 3 to 7580 µg/L in 2015. There are also variable levels of TCE present in several of the groundwater wells with concentrations ranging from 3 to 541 µg/L in 2015. The highest concentration was present in well PS12RS240, which also contained 187 µg/L of cis-DCE in 2015. This well was selected for Raman testing based on the elevated levels of VOCs in addition to ClO_4^-. Other contaminants were present in select wells, including 1,4-dioxane and RDX, although the concentrations were generally <15 µg/L in 2015.

Page Intentionally Left Blank

5.0 TEST DESIGN

5.1 EXPERIMENTAL DESIGN

Unlike many ESTCP Environmental Restoration (ER) projects for which a single demonstration site is chosen to test a remediation approach, this project is being conducted to test the performance of the SERS sensor as an instrument for ClO_4^- detection. As such, the test was performed multiple times at two sites, covering a general range of geochemical and contaminant conditions. The field testing was conducted following initial characterization of the groundwater samples, and a significant amount of optimization and testing occurred for both the field Raman instrument and the SERS substrates that are required for contaminant analysis by the SERS technology. In addition, laboratory studies were completed in conjunction with the field demonstration(s) to better understand the potential interferences with the SERS technique that are likely to be encountered in the field. Additional field sites, including Naval Surface Warfare Center (NSWC), Dahlgren, Virginia; the Fort Wingate Depot Activity (FWDA) site in Gallup, New Mexico; and Kirtland Air Force Base in California, were considered but field trials were not conducted, partly due to the low or non-detect ClO_4^- concentrations found in groundwaters and the time and budget constraints.

5.2 GROUNDWATER TESTING

5.2.1 Sample Collection

Site groundwater samples were collected and analyzed for this project. In general, low-flow sampling procedures were followed (USEPA, 2010). Groundwater sampling was performed using pumps appropriate to the well depth and desired flow rate. For shallow groundwater (<10 ft), a peristaltic pump was used to collect samples. For deeper groundwater (>10 ft), a Grundfos submersible pump (or equivalent) was used to collect groundwater samples. After the appropriate pump was installed/set up, groundwater was passed through a field meter (e.g., YSI multi-parameter meter or equivalent) at a low flow rate, and geochemical parameters were measured with time, including DO, pH, conductivity, temperature, and oxidation-reduction potential. When these parameters stabilized according to the specifications in low-flow groundwater sampling guidance (USEPA, 2010), groundwater samples for ClO_4^- analysis were collected. Sampling for other desired chemical analyses were also performed at this time.

In addition to ClO_4^-, geochemical parameters including major anions (e.g., Cl^-, SO_4^-, NO_3^-), dissolved metal ions, pH, TDS, or alkalinity were measured for most of the samples collected for the demonstration. Other more specialized analyses for explosives (EPA Method 8330) or VOCs (EPA Method 8260) were performed on a site-by-site basis based upon the expected co-contaminants in groundwater.

5.2.2 Supporting Analytical Methods

5.2.2.1 Perchlorate analysis

Perchlorate concentrations measured by the portable Raman sensor were compared with those determined by EPA Method 314.0 (IC with conductivity detection) performed in the CB&I Federal Services (CB&I) Analytical Laboratory in Lawrenceville, New Jersey. IC analysis was also conducted at Oak Ridge National Laboratory (ORNL) for samples for comparison.

5.2.2.2 Field parameters

Typical geochemical parameters were collected at each well during sampling using a field meter (e.g., YSI multi-parameter meter). The parameters, which provide a basic geochemical baseline for each well, include temperature, DO, oxidation-reduction potential, conductivity, and pH. The stabilization of these parameters with time was also used to determine when to collect field samples, according to USEPA guidelines on low-flow sampling (USEPA, 2010).

5.2.2.3 Anions and cations

Common anions, including Cl^-, SO_4^-, NO_2^-, and NO_3^-, were measured by EPA Method 300.0 (IC with conductivity detection) in the CB&I Analytical Laboratory in Lawrenceville, New Jersey, as well as in laboratories at ORNL. Common metal cations, including calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), Fe, aluminum (Al), Mn, and copper (Cu) were measured at ORNL by EPA Method 6020A using inductively coupled plasma (ICP)-MS.

5.2.2.4 Co-contaminants

A variety of co-contaminants often co-exists in groundwater wells, particularly in the Redstone OB/OD area. These co-contaminants include explosives, pesticides, and chlorinated volatile organic compounds (cVOCs), among others. Because the SERS demonstration was coordinated with the annual OB/OD groundwater sampling event at this location, a variety of analyses were conducted on the groundwater samples. The analyses of these co-contaminants include the following: nitroaromatic compounds by SW-846 Method 8330A, cVOCs by SW-846 Method 8260B, semivolatile organic compounds (sVOCs) and polycyclic aromatic hydrocarbons (PAHs) by SW-846 Method 8270D, pesticides and polychlorinated biphenyls (PCBs) by SW-846 Methods 8081B and 8082A, herbicides by SW-846 Method 8151A, dioxin/furans by SW-846 Method 8280B, and metals and tin by SW-846 Methods 6010C and 7470A.

5.3 FIELD TESTING

5.3.1 Instrument and Operating Parameters

A portable Raman sensor (shown in Figure 5-1) was utilized throughout the field demonstration. The application of the technology requires the following: (1) preparation of nano-structured SERS substrates (pre-prepared in the laboratory); (2) interfacing the SERS substrate with the Raman probe through a SERS module (pre-fabricated); (3) collection of the groundwater sample; (4) pipette a small drop of the groundwater sample onto the SERS substrate; (5) spectral collection and analysis of ClO_4^- by the SERS Raman probe; (6) calculation of ClO_4^- concentrations against standard calibration curves; and (7) statistical analysis, interpretation, and correlation of data with those obtained by standard EPA methods. Multiple wells at each of the test sites were used for analysis, and multiple analyses (usually triplicate) were performed for select samples.

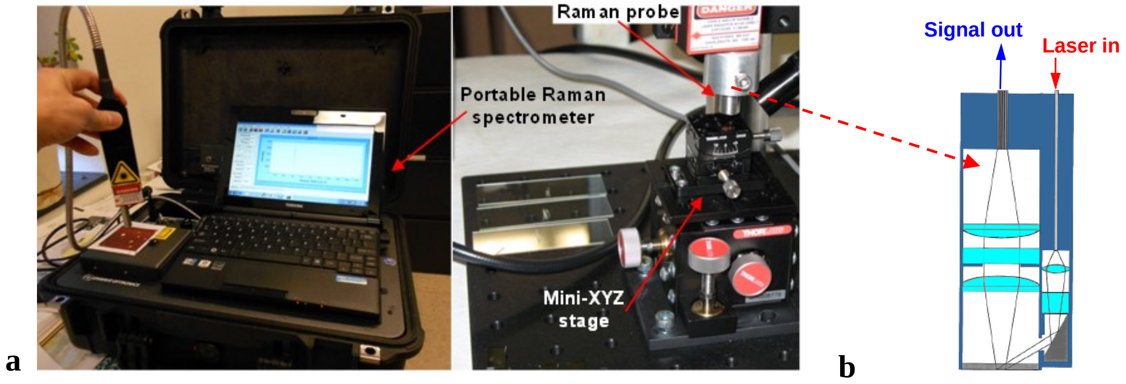


Figure 5-1. (a) A portable Raman Sensor Equipped with a Fiber-optic Raman Probe Was Used to Detect ClO_4^-. (b) A Schematic, Enlarged View of the Raman Probe with Optical Lens and Fibers for the Incident Laser and Signal Collection.

A standard addition method was employed for some samples due to matrix interferences with unknown background organic or inorganic ions in the groundwater. In brief, the method involves the addition of varying amounts of ClO_4^- (standard solutions in deionized water) to a fixed amount (e.g., 5 milliliters [mL]) of the same groundwater. The samples (with ClO_4^- standards) were made up to a fixed final volume (e.g., 10 mL) so that all samples contain the same matrix interferences, except the ClO_4^- concentration differs. Samples were then analyzed, and the characteristic Raman intensities for ClO_4^- (y-axis) were plotted against the final added ClO_4^- concentration (x-axis). A linear regression was then used to calculate the absolute value of the x-axis intercept, which corresponds to the true concentration of ClO_4^- in the unknown groundwater sample.

5.3.2 Sampling Time

Sampling and analysis at each site lasted from one to three days depending on the number of wells requiring sampling, site conditions, analytical parameters to be collected. At the IHDIV site, field demonstrations were performed in October 2015, and October 2016. For Redstone, the field demonstration was performed in coordination with the schedule of the field crew performing annual groundwater sampling in the OB/OD area in November 2016.

5.3.3 Residuals Handling

Site regulations or guidance concerning disposal of groundwater were followed. Since no chemicals or pre-treatments were necessary for the demonstration of the Raman sensor, there were no other residuals or chemicals requiring disposal.

5.3.4 Health and Safety

Site-specific Health and Safety Plans (HASPs) were followed during the demonstrations.

5.4 DATA ANALYSIS

5.4.1 Data Analysis, Interpretation, and Evaluation

Selective identification of ClO_4^- from complex groundwater matrices was a key measure of the success of the portable Raman sensor. This is accomplished by the collection of the Raman spectra, in which characteristic Raman peak at about 935 cm^{-1} is obtained and analyzed under varying environmental conditions or groundwater matrices. The peak position may shift slightly depending on the groundwater chemistry since the vibrational frequency of the Cl-O bond could be affected by its neighboring atoms or the bonding environment and the co-existing ions. The peak height or intensity can then be used for the quantification of ClO_4^- in unknown samples through calibration standards. The response of the sensor to ClO_4^- concentration defines the analytical detection limit, which is another key measure of the success of the project. In many cases, the standard addition technique was used so that the ClO_4^- concentration in the unknown samples could be better determined.

5.4.2 Statistical Analysis

An evaluation of the new Raman technique for ClO_4^- analysis versus the traditional EPA Method 314.0 was conducted using multiple techniques. A subset of split samples (at least two wells for each site) were analyzed multiple times by each technique giving a measure of method variability. The means for each sample set were then compared by a standard two-sample t-test. Raman peak position and peak intensity changes with groundwater characteristics (e.g., pH and anion concentrations) was used for assessing their influence on ClO_4^- detection. Analyzing a sample multiple times also shows the repeatability of the analysis.

The dataset was also evaluated according to the basic procedures described in Bland and Altman (1986) for comparing two different measurement techniques. The data from all wells for which samples were analyzed by both analytical methods were initially plotted against each other on a correlation plot (Raman on the y-axis and IC on the x-axis), and the data were compared. In instances where the sample was analyzed multiple times by both methods, error bars were displayed for the points. This analysis shows the correlation between the data or any bias. A correlation coefficient (R) for the data was calculated.

6.0 PERFORMANCE ASSESSMENT

6.1 CONSTRUCTION OF A PORTABLE RAMAN SENSOR

A SERS-based portable Raman sensor was constructed in the laboratory (Figure 5-1). The sensor consists of a Raman analyzer equipped with a 300-milliwatt (mW) near infrared laser and a fiber-optic probe for laser excitation and signal collection (Figure 5-1a). A key component of the sensor probe is the interface where the nanostructured SERS array substrate is attached, and the Raman signal becomes substantially enhanced (Hatab et al., 2010; Hatab et al., 2011). A diode laser operated at 785 nm was used as the excitation source with a high Rayleigh rejection fiber optic probe, which serves three purposes: (1) transmission of the incident laser to the SERS substrate or the sample, (2) collection of the scattered Raman signal to the spectrograph, and (3) removal of unwanted background signals through an optical filtering device. The incident laser is focused onto the SERS substrate, which is mounted on an XYZ stage to allow precise focusing to obtain maximal signal. The scattered SERS signals are then collected through a separate optical fiber to the spectrograph/detector system. A schematic drawing of the Raman probe is provided in Figure 5-1b.

To facilitate the field analysis, a focal-length-adjustable SERS probe module (Figure 6-1b) was designed and fabricated by Nanova Inc., which was intended to be used with the portable Raman sensor for field demonstration. The module allows the vertical adjustment of the focal length so that the excitation laser beam can be better focused on the SERS substrate for optimal detection of ClO_4^-. Additional adjustments can be made to further improve the ergonomics and repeatability for SERS detection. Figure 6-1 illustrates the use of an active SERS substrate mounted on a glass slide, which is then inserted into a SERS module, allowing collection of Raman signal via a fiber optic SERS probe to the Raman analyzer.

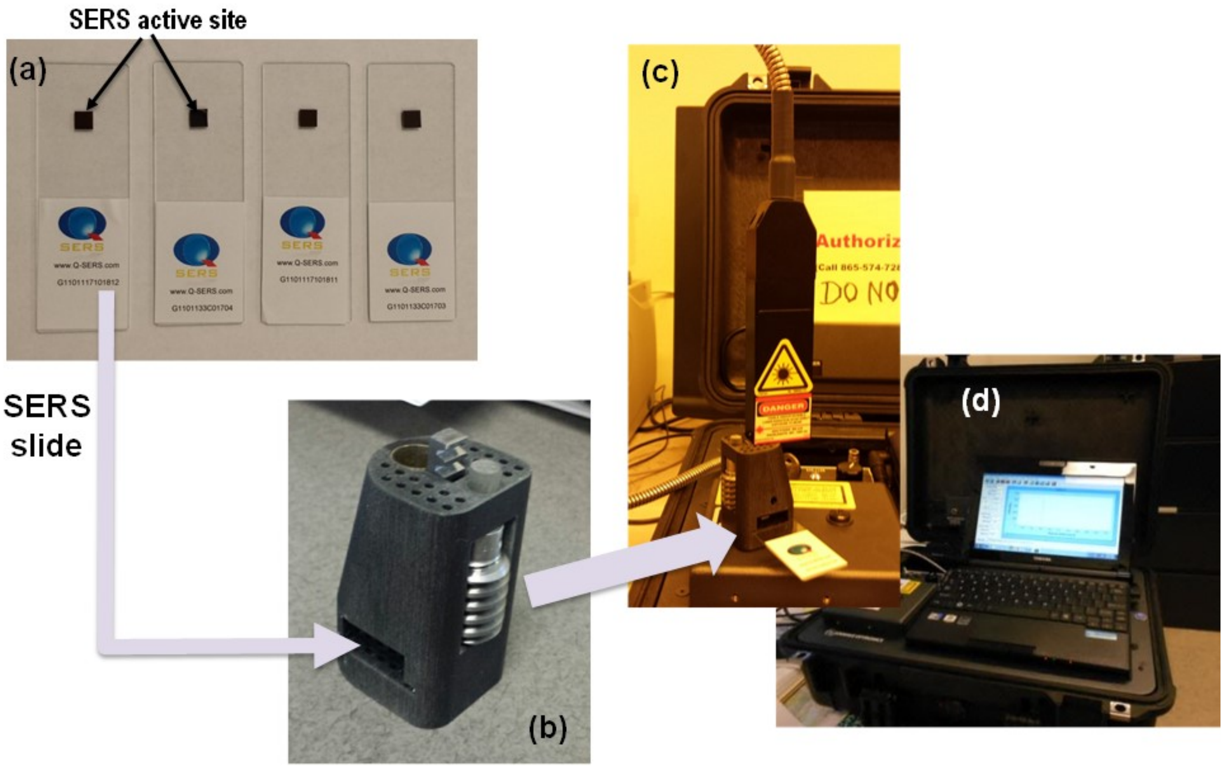


Figure 6-1. Illustration of SERS Detection Using a Portable Raman Analyzer.

(a) Glass slides mounted with active SERS substrates, (b) a SERS module for holding the glass slide and the fiber optic SERS probe (c), and (d) signal collected via fiber optics by a portable Raman analyzer.

6.2 SERS SUBSTRATE FABRICATION AND OPTIMIZATION

To optimize SERS detection sensitivity, a uniform and sensitive novel SERS template was designed based on elevated Au ellipse dimers with 10±2 nm gaps. Elevating the SERS active structures from the underlying substrate has been previously demonstrated to offer greater signal enhancements for both bowtie and mushroom geometries compared to SERS structures in direct contact with the substrate (Hatab et al., 2010; Hatab et al., 2011; Jubb et al., 2016; Polemi et al., 2011; Wells et al., 2011). The ellipse dimers with 10±2 nm gap size are fabricated by electron beam lithography (EBL) and subsequent Au deposition following lift-off.

The effect of tuning the ellipse aspect ratio on the plasmon resonance frequency is studied, and a shift in the localized surface plasmon (LSP) resonance frequency is observed, consistent with theoretical predictions (Hatab et al., 2010; Jackson and Halas, 2004; Jain et al., 2007; Zhao et al., 2006). This tunability enables a more flexible SERS substrate design and systematic control of the resulting SERS signal intensity compared to previously-reported elevated Au bowtie arrays (Hatab et al., 2010; Hatab et al., 2011). The SERS response following excitation by two common Raman excitation wavelengths—633 nm and 785 nm—are tested and compared. When optimized for 785 nm excitation, the elevated Au ellipse dimer substrates were found to have an enhancement factor (EF) up to 109 for adsorbed *p*-mercaptoaniline (*p*MA) molecules (Jubb et al., 2016).

The optimized SERS substrate conditions were found to be a gap of 10±2 nm, an aspect ratio close to 1:1, and the polarization of the 785 nm excitation beam aligned with the ellipse x-axis. Note that the tunability of the ellipse dimers make them an attractive practical choice for a broad range of SERS applications including chemical and biological sensing (Hatab et al., 2011; Jubb et al., 2017; Ruan et al., 2006a; Xu et al., 1999). The optimized SERS substrates are subsequently utilized for ClO_4^- detection in environmental samples.

6.3 COMMERCIAL FABRICATION OF SERS SUBSTRATES

6.3.1 Substrate Production by Nanoimprinting

While the EBL technique has been successfully used for the fabrication of desired SERS substrates, it requires the use of a high-resolution electron microscope and a nanometer pattern generator. The fabrication process also requires specialized skills and is a rather slow process. To reduce the cost of fabrication and move toward commercialization, the demonstration team joined Nanova Inc. and its partner NIL Technology for the commercial production of optimized ORNL SERS substrates based on the elevated Au ellipse dimer architectures. Here, nanoimprinting technology is utilized for large-scale production. Nanoimprinting lithography is based on pressure-induced transfer of a topographic pattern (e.g., ellipses in this case) from a rigid mold into a thin thermoplastic polymer film or resist heated above its glass transition temperature (Veres et al., 2010; Weiss et al., 2011). It is a physical process that does not use any energetic beams and is under rapid development to meet the needs of the new generation of applications extended into the nanoscale arena. Imprint molds can be fabricated with multiple layers of topography stacked vertically. Resulting imprints replicate both layers with a single imprint step, which reduces chip fabrication costs and improves product throughput. A broader range of materials with varying properties are also available for use with imprint lithography, thus increasing the potential range of SERS substrates for Raman SERS applications. Its high resolution, high throughput, and relatively low cost have made nanoimprint litholography one of the leading nanofabrication techniques. The simplified requirements of the technology lead to its easy production at relatively low costs.

A mold designed for high throughput, nanoimprint fabrication of SERS substrates was first made at ORNL Center for Nanophase Materials Science (CNMS). The mold was made with about 100 desired nanoparticle arrays on a 4-inch (in) silicon (Si) wafer (Figure 6-2). The mold was later used to transfer topographic patterns (e.g., ellipses) from a rigid mold into a thin thermoplastic polymer film or resist heated above its glass transition temperature (nanoimprinting). Once the imprinted materials are fabricated (or patterns transferred), they are processed through the usual lift-off and Au deposition to obtain the elevated Au ellipse nanoarrays for SERS detection. After numerous attempts, successful transfer of topographic patterns from the mold onto a thin thermoplastic polymer film or resist, and subsequently to the Si wafer (nanoimprinting), was performed. The transfer and integrity of the ellipse dimer architectures were verified by scanning electron microscope (SEM) analysis (e.g., Figure 6-3).

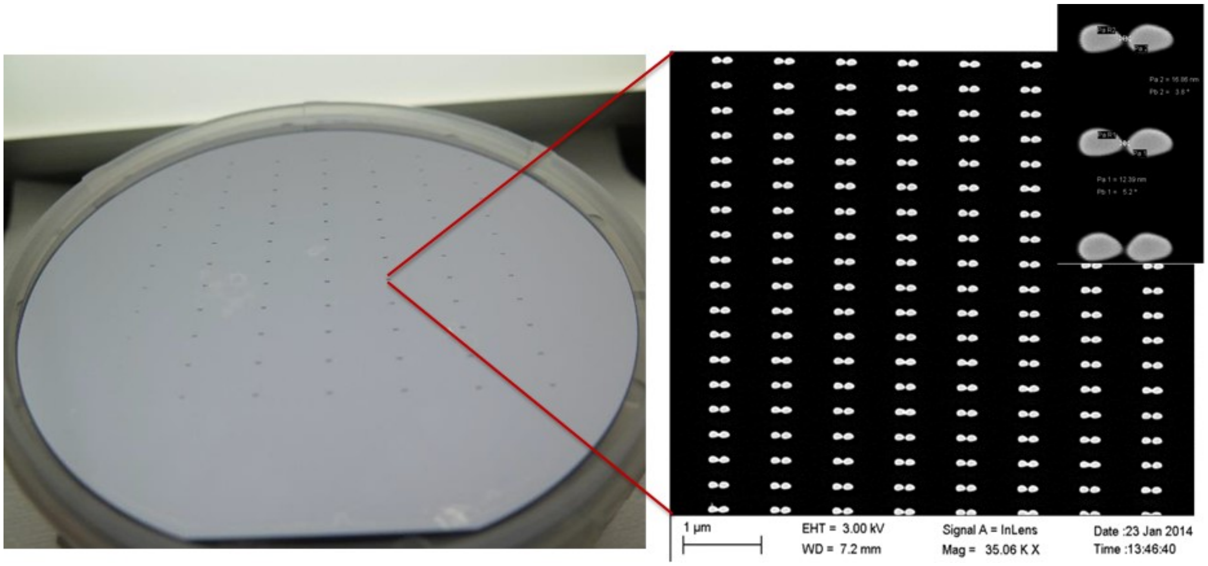


Figure 6-2. A Mold Designed for High Throughput, Nanoimprint Fabrication of SERS Substrates was Made at ORNL CNMS.

The mold was used to transfer topographic patterns (e.g., ellipses in this case) by a commercial company, resulting in substantially decreased SERS chip fabrication costs and improved product throughput.

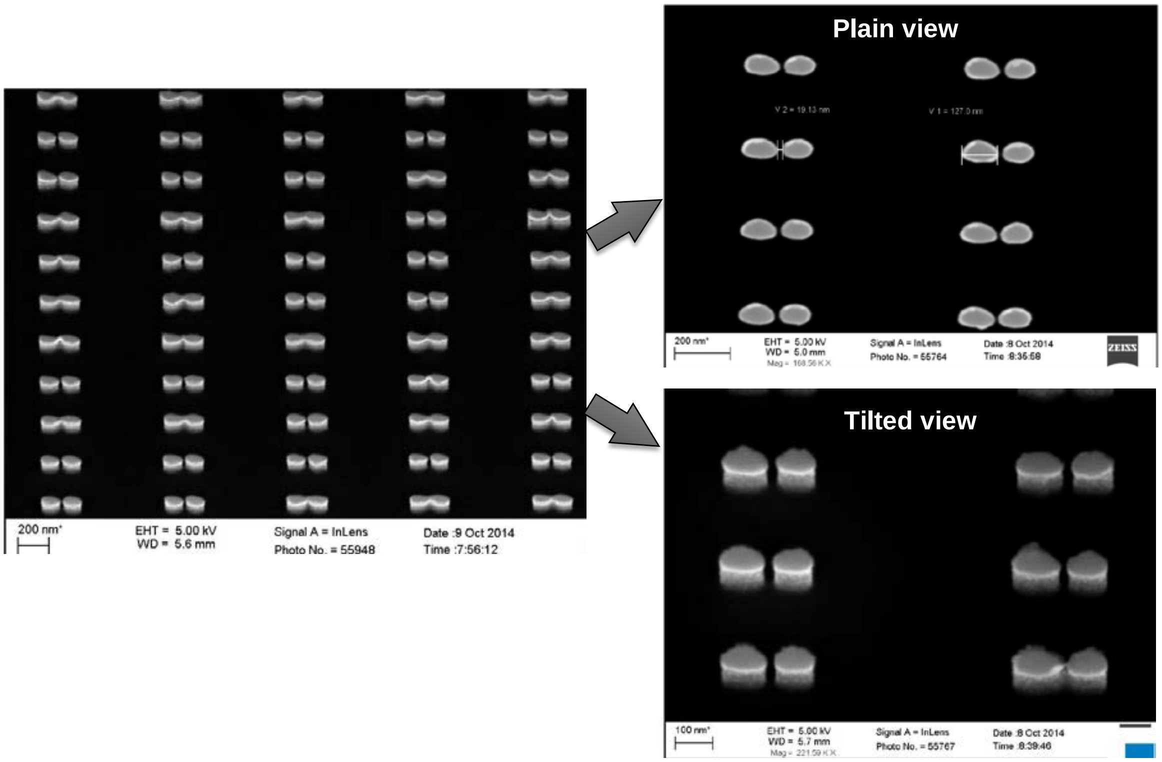


Figure 6-3. SEM Images of Commercially-fabricated SERS Substrates via High Throughput Nanoimprinting (without chromium [Cr] and Au coatings).

The topographic patterns (i.e., ellipses) were successfully transferred.

6.3.2 Substrate Optimization

Following successful transfer of the topographic features, the imprinted SERS substrates were coated with a thin layer of Cr (8–10 nm, as an adhesion layer) and subsequently coated with Au at various thicknesses for performance evaluations. Extensive studies were then carried out to evaluate the performance and sensitivity of the commercially-produced SERS substrates for ClO_4^- detection. For example, the Au coating thicknesses were varied—20, 30, 40, 60, 80, 110, and 140 nm—and the effect of Au thickness on ClO_4^- detection at various concentrations in water were studied. Results suggest that the optimal Au coating thickness is ~60 nm. Quality control testing of these commercial substrates with a model thiol compound, *p*MA (C6H4NH2SH), and with ClO_4^- standards in nanopure deionized water was also performed, and results show performance comparable, in most cases, to the SERS arrays fabricated at ORNL using EBL. This is a substantial step forward toward the commercialization of these sensors.

One potential source of variation in the detected ClO_4^- signal is the inhomogeneous distribution of ions across the active SERS substrate surface. This arises due to the inherently random nature of drying the sample droplet in which surface tension forces effectively pull ions toward the outer edge of the drop. One approach to lessen this effect is to functionalize the surface of the SERS arrays with an agent that attracts ClO_4^- ions. Toward this end, laboratory optimization was also performed with coating the elevated Au ellipse dimer SERS substrates with 2-(dimethylamino) ethane-thiol (DMAET), which can form a monolayer on the Au surface through the thiol moiety while leaving the positively charged dimethyl amino group pointed into the sample solution. One concern with this approach is the added baseline signal from SERS enhanced vibrational modes of the DMAET. Results show that this is not a significant issue, in line with previous literature reports that have taken this approach with colloidal Au spheres SERS substrates (Gu et al., 2009). Coating the SERS substrates with DMAET had the benefit of increasing the detection efficiency as it enhances the adsorption of ClO_4^- to the SERS active region (Figures 6-4 and 6-5).

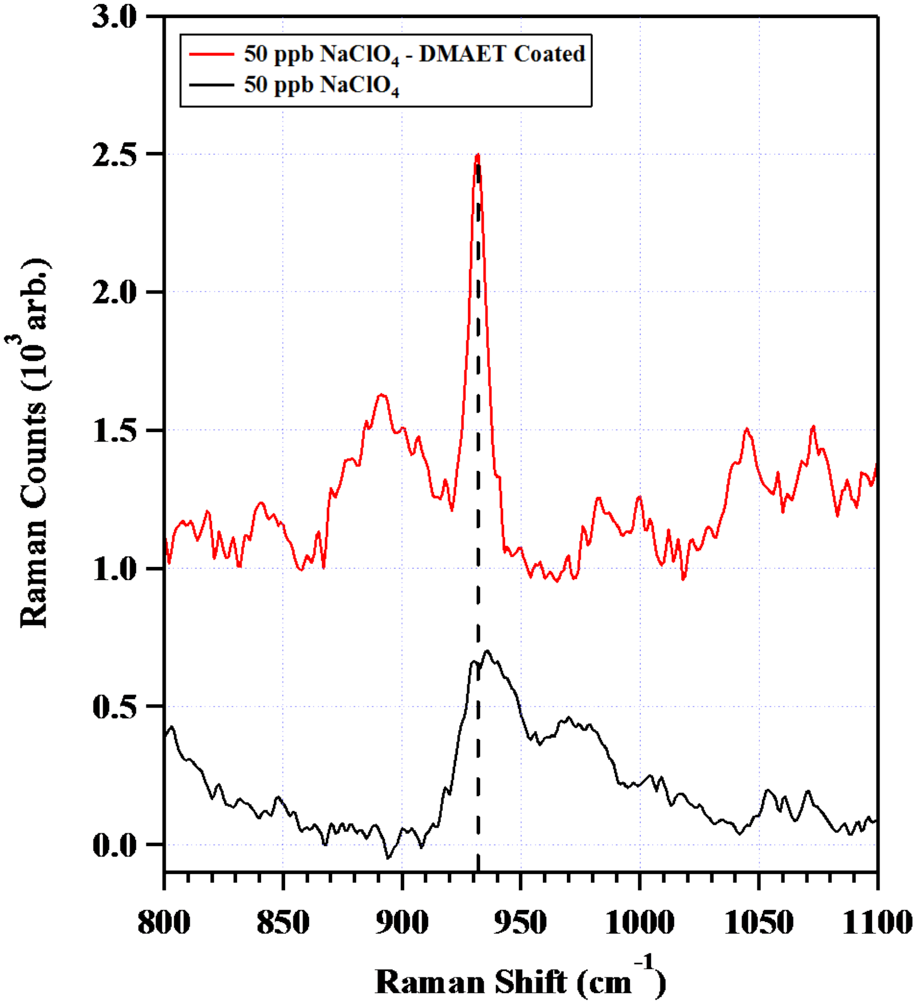


Figure 6-4. Comparison between DMAET-coated SERS Array (red trace) and Blank SERS Array (black trace) with 50 ppb Sodium Perchlorate (NaClO4) Standard.

Dashed vertical line represents ClO4- symmetric stretching peak. All spectra have been baseline-corrected and scaled for clarity. DMAET-coated SERS array has ~3× higher signal compared to uncoated array.

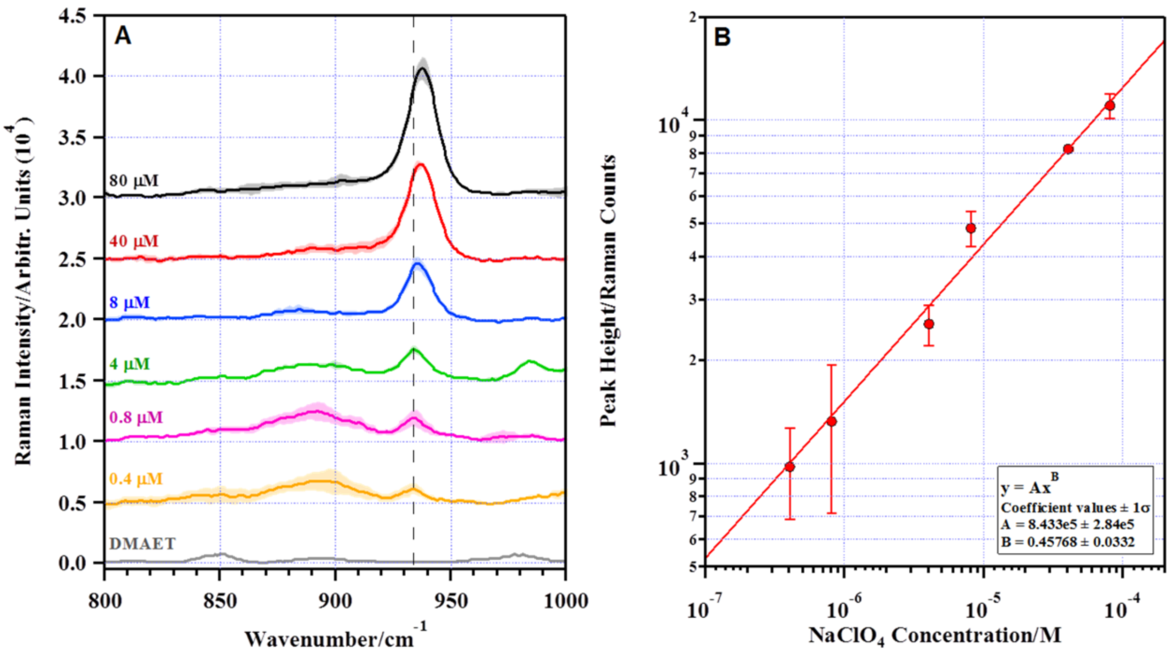


Figure 6-5. (A) Representative SERS Spectra of NaClO4 Solutions Varying in ClO4- Concentration from 0.4–80 Micromolars (μM). (B) Log-log Plot of the νSS-ClO4- Peak Height versus NaClO4 Concentration.

(A) A spectrum of the DMAET-coated SERS substrate is given (gray trace) showing minimal contributions to the SERS spectrum from DMAET vibrational modes within the ν_{SS}-ClO_4^- *region. Data are represented by solid traces while shaded regions represent ±1 standard deviation (σ). All spectra are the average of 3–5 spots and have been baseline corrected. Spectra are offset (y-axis) for clarity. The vertical dashed line represents the peak center of the* ν_{SS}-ClO_4^- *mode for the 0.4 μM spectrum (orange trace). Note shift of the* ν_{SS}-ClO_4^- *mode wavenumber with increasing concentration. (B) Data (red markers) and power law fit to the data (red trace) are provided. Error bars on data correspond to ±1σ between peak height values determined from the Lorentzian fitting analysis for the spectra acquired at each* ClO_4^- *concentration.*

The SERS response to trace levels of ClO_4^- can be seen in the spectra shown in Figures 6-4 and 6-5A. These spectra clearly exhibit the symmetric stretching peak of ClO_4^- (ν_{SS}-ClO_4^-) molecules at ~935 cm^{-1} for solution concentrations as low as 0.4 μM (~40 μg L^{-1}). With a concentration increase, the ClO_4^- signal increases correspondingly, and the relationship is well fit with a power law expression (Figure 6-5B). The clear ClO_4^- signal apparent for solution concentrations as low as 0.4 μM demonstrates that the elevated Au ellipse dimer SERS architecture is well-suited for the detection of trace quantities of ClO_4^-.

6.4 EVALUATION OF INTERFERENCES ON SERS PERFORMANCE

To determine potential interference effects present in natural groundwater or surface water, the sensitivity of commercially-produced SERS substrates based on elevated Au ellipse dimers for rapid detection of ClO_4^- were investigated. These dimers provided higher sensitivity than previously reported elevated Au bowtie geometries (Hatab et al., 2010; Hatab et al., 2011).

Additionally, the interference effects of other common ions (e.g., Cl^-, NO_3^-, and SO_4^{2-} typically found in environmental samples) on SERS detection of ClO_4^- were systematically explored. The systematic quantification of interferences in SERS is vital for analyzing complex samples as the SERS technique is non-specific and will report on any Raman active compounds within the working region of the sensor.

Interference effects of other ions and solutes on the ClO_4^- detection efficiency were examined for SERS sensors based on colloidal Au nanoparticles functionalized with DMAET by Gu et al. in simulated groundwater where ClO_4^- existed concomitantly with Cl-, NO_3^-, and SO_4^{2-} ions (Gu et al., 2009). For these solutions, NO_3^- was observed to interfere with ClO_4^- detection, based on detection of the NO_3^- symmetric stretch at ~1,045 cm^{-1}, while SO_4^{2-}, phosphate (PO_4^{3-}), and Cl^- ions were not observed to impact ClO_4^- detection. These observations were attributed to the high hydration energies of SO_4^{2-} and PO_4^{3-} compared to ClO_4^-, which may inhibit their adsorption/ion-pairing at the DMAET-modified SERS active site surface, while an explicit explanation on the effect of Cl- was not given.

By fitting the normalized peak heights versus the log of the interfering ion concentration with linear regressions, it is possible to compare the relative effects of the different interfering species on ClO_4^- detection (Hao et al., 2010). At low and intermediate concentrations of interfering ions (i.e., ≤200 μM), the interference strength is ion-specific with the effect decreasing as: $SO_4^{2-} > NO_3^- > Cl^-$, in agreement with the findings of Hao et al. (2010). However, at higher ion concentrations (>200 μM), the identity of the interfering ion seems to play less of a role in determining the overall interference effect. This is attributed to physical displacement of ClO_4^- ions from the SERS active region within the nanogap of the elevated Au ellipse dimers by the much more prevalent interfering ions. This behavior has not been observed previously in studies examining interference effects on the SERS detection of ClO_4^-, likely due to the major differences between typical nanoparticle SERS substrates with much larger surface areas able to accommodate higher ion loadings than those of the elevated Au ellipse dimer architectures used here. These finding would imply that efforts to use these SERS architectures for ClO_4^- analysis in highly saline environmental samples may be complicated by the ability to actually deposit ClO_4^- molecules within the active region of the sensor. Taken together, the results presented in this study make a case for the applicability of the SERS-based portable Raman sensor for rapid field measurements of trace levels of ClO_4^- in contaminated water.

6.5 FIELD DEMONSTRATION AND EVALUATION

6.5.1 Detection and Analysis of ClO_4^- at Field Sites

To evaluate the ability of the elevated Au ellipse dimer SERS substrates to detect ClO_4^- within impacted groundwater, where multiple interferences exist, groundwater samples were collected from multiple DoD sites. The ClO_4^- concentrations were determined with a portable SERS sensor in the field, and the samples were subsequently tested again for ClO_4^- by IC. Sections 6.5.2 and 6.5.3 detail two field demonstrations at the IHDIV site between October 2015, and October 2016. Section 6.5.4 provides details of another field demonstration at Redstone in November 2016.

6.5.2 IHDIV Demonstration 1

An initial field demonstration of the portable Raman sensor for ClO_4^- was conducted at IHDIV in October 2015. Site background was provided previously in Section 4. Groundwater samples were collected from six representative MWs behind the ClO_4^- hog-out facility (Figure 4-1) for onsite SERS analysis as well as further laboratory analysis by both IC and SERS at a later date. Of the six groundwater samples collected, four were analyzed onsite with the portable Raman system, including samples CPMW-2D, MW-4, CPMW-5, and MW-8. These four were selected since they were the first four wells sampled. MW-1 and MW-11 were not analyzed onsite with the portable SERS system due to time constraints at the IHDIV site but were analyzed later in the laboratory.

The experimental procedure used for the onsite SERS analysis is briefly described below. Following collection of the groundwater samples, an aliquot of sample—typically 1–5 microliter (µL)—was placed on a previously fabricated SERS sensor based an elevated Au ellipse dimer substrate template that had been taped onto a glass microscopy slide (Jubb et al., 2017; Jubb et al., 2016). The sample aliquot was subsequently dried onto the SERS array and placed on the portable Raman sample stage under the laser excitation beam spot. A SERS spectrum was then collected. SERS spectra were collected with six 10-second scans using either 75 mW or 300 mW of 785 nm laser light. At the lower laser power (75 mW), most spectra featured a broad strong background signal (Figure 6-6). This is attributed to fluorescence from potential naturally dissolved organic compounds within the groundwater samples as well as the non-specificity of the SERS response (to other interfering ions), which also “report” on chemical compounds with Raman active vibrational modes. In the frequency region relevant for ClO_4^- detection (850–1,100 cm^{-1}), three peaks are observed that can be assigned to ClO_4^-, SO_4^{2-}, and NO_3^-. The symmetric stretch of the ClO_4^- ion should occur at ~940 cm^{-1}; this can be seen clearly for CPMW-2D and to a lesser extent for the other three well waters tested (Figure 6-6).

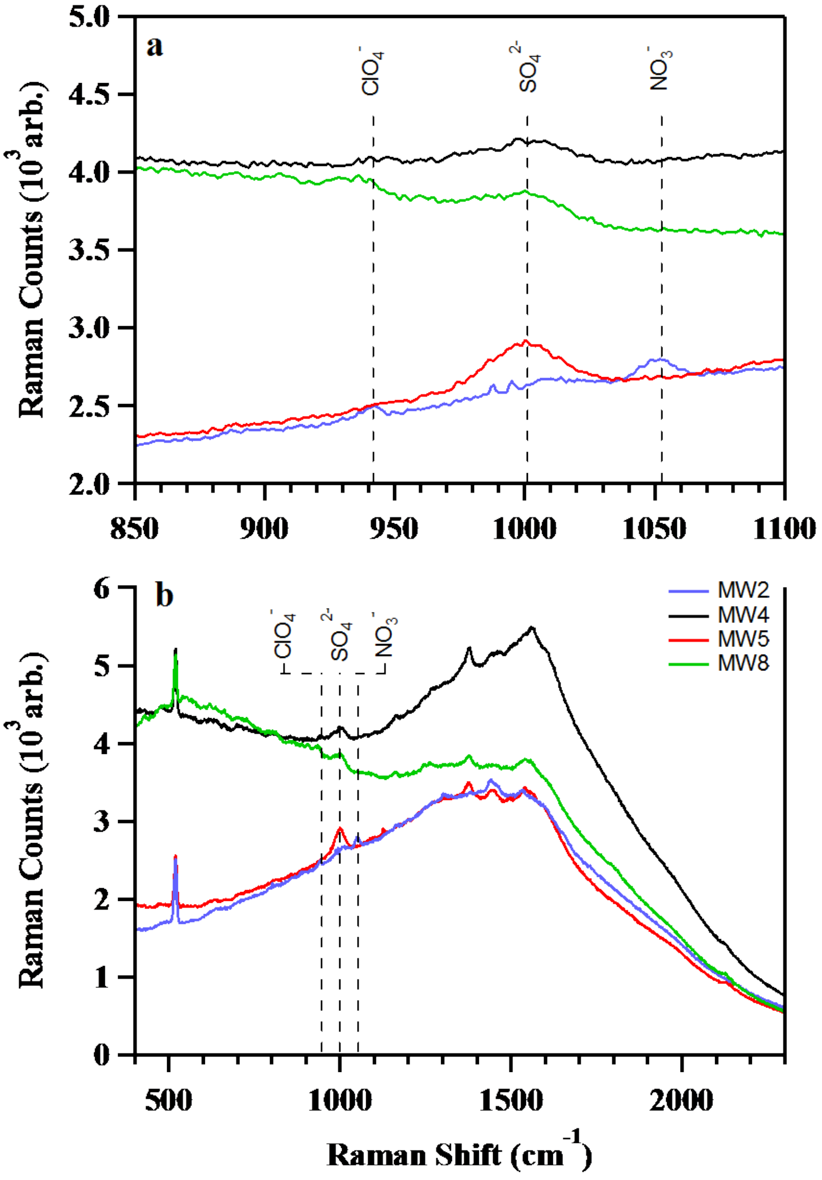


Figure 6-6. SERS Spectra of Four Groundwater Samples Collected at IHDIV: CPMW-2D (MW2) (blue trace), MW4 (black trace), CPMW-5 (MW5) (red trace), and MW8 (green trace).

(a) SERS spectra of the four groundwater samples in the ClO_4^- symmetric stretching region. (b) Full SERS spectra of the four groundwater samples. Vibrational signatures of ClO_4^-, SO_4^{2-}, and NO_3^- ions indicated by dashed lines and annotations.

The symmetric stretches of SO_4^{2-} and NO_3^-—likely interferences due to their environmental ubiquity—occur at ~990 cm^{-1} and 1,050 cm^{-1}, respectively. Peaks are observed in several of the groundwater spectra at these frequencies and are attributed to the presence of these chemical compounds. When the laser power of the SERS excitation beam is increased to ~300 mW, the presence of ClO_4^- within CPMW-2D is very clear with a signal-to-noise ratio >50 (Figure 6-7).

While increasing the power of the excitation beam increases the SERS response, and hence the detection limit, incident powers of ~300 mW are typically to be avoided as it is possible to damage the SERS array through heating effects, which may lead to non-reproducibility. These results indicate that the portable Raman sensor worked as expected. Onsite standard addition (described in Section 5.3.1) was also tested to construct calibration curves from previously generated standard solutions of ClO_4^- as well as standard solutions of ClO_4^- with known amounts of interfering compounds (e.g., SO_4^{2-}, NO_3^-).

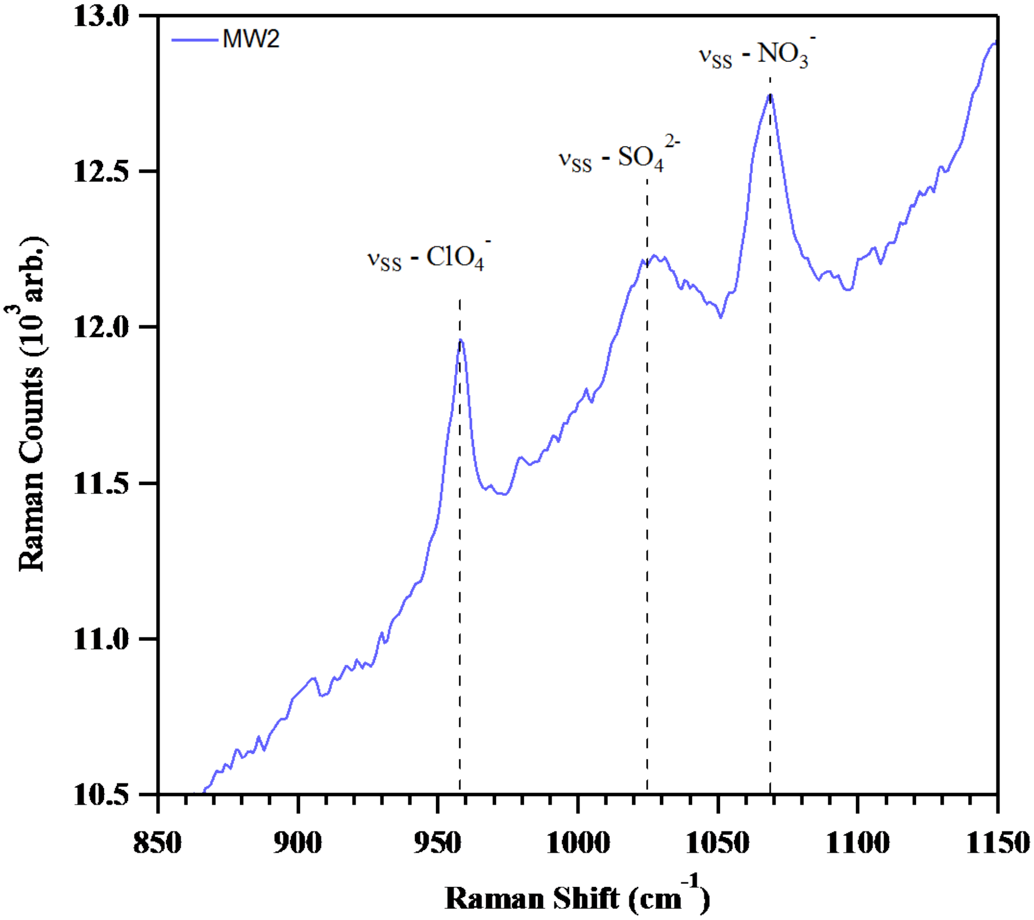


Figure 6-7. SERS Spectrum of CPMW-2D (MW2) Groundwater with ~300 mW Incident 785 nm Power.

ClO_4^-, SO_4^{2-}, and NO_3^- vibrational features are clearly present and indicated with dashed lines and annotations.

The six groundwater samples collected from IHDIV were also analyzed for ClO_4^- by IC following EPA Method 314.0 at ORNL and CB&I (Table 6-1). The agreement between the two laboratory IC analyses is within ±10% for five of the six groundwaters tested. For CPMW-2D, the deviation between the two labs is higher (~20%), likely caused by its relatively high ClO_4^- concentration in this sample exceeding the linear region of the calibration curve used. Each standard deviation is the result of triplicate analyses of split samples (i.e., from the same individual sample bottle).

Table 6-1. ClO_4^- Concentrations of the Six groundwater samples determined via IC by CB&I and ORNL in October 2015.

ClO_4^- (μg/L) determination with IC					
	CB&I		ORNL		
	Average	Standard deviation (σ)	Average	Standard deviation (σ)	% Deviation
MW-01	627	9	611.5	5	-2.5
CPMW-2D	2060	26	2468	18	19.8
MW-4	73.0	0.7	73.4	1	0.5
CPMW-5	261	6	269.0	2	3.1
MW-08	375	5	376.5	3	0.3
MW-11	98.9	0.3	91.9	1	-7.6

In addition to determining ClO_4^- concentration with IC, the concentration of a suite of other inorganic cations and anions as well as TDS was also determined. This is important in the context of SERS detection of ClO_4^-, as other anions, especially oxyanions, are vital to quantify as they can provide possible interferences due to their molecular vibrations. The major anions present in the six groundwater samples were Cl-, SO_4^{2-}, and NO_3^-. Both SO_4^{2-} and NO_3^- appeared to interfere with the SERS detection of ClO_4^-. Additionally, they are present at levels equal to or greatly exceeding the amount of ClO_4^- within the samples. These IC results also confirm the earlier assignments of peaks at ~990 cm^{-1} and ~1,050 cm^{-1} in the SERS spectra collected onsite for SO_4^{2-} and NO_3^-, respectively. The results for major anions are given in Table 6-2.

Table 6-2. Anion and TDS Results from October 16, 2015 Sampling at IHDIV.

All results presented in mg/L.

	F^-	Cl^-	NO_2^-	SO_4^{2-}	Br^-	NO_3^-	PO_4^{3-}	TDS
MW-01	0.2	61.9	0.2	28.4	0.2	2.52	0.2	227
CPMW-2D	0.2	30.3	0.2	64.3	0.2	1.07	0.2	189
MW-4	0.2	9.12	0.2	57.7	0.2	0.2	0.2	156
CPMW-5	0.2	6.96	0.2	71.9	0.2	0.2	0.2	214
MW-08	0.2	79.7	0.2	52.8	0.2	0.61	0.2	229
MW-11	0.2	32.0	0.2	55.4	0.2	0.23	0.2	177

Br^- = bromine; F^- = fluoride

The six groundwater samples previously collected from IHDIV on August 16, 2015, (MW-1, CPMW-2D, MW-4, CPMW-5, MW-08, and MW-11) were further analyzed with the portable SERS instrument in a laboratory setting following an identical procedure as was used in the field, i.e., an aliquot of sample (~1 μL) was placed on a Au ellipse dimer nanoantennae SERS sensor, dried, placed on the portable Raman sample stage, and a SERS spectrum collected. The SERS spectra of a series of ClO_4^- standard solutions made in 18.3 megaohms (MΩ) water were also collected in a similar manner. The SERS spectra collected from the ClO_4^- standard solutions indicate that the detection limit of the SERS technique is ~100 ppb.

The SERS spectra from two groundwaters, CPMW-2D and CPMW-5, collected from separate sampling wells are shown in Figure 6-8. While these spectra exhibit both a different number of peaks as well as relative intensity differences, the νSS-ClO_4^- is clearly visible at ~935 cm^{-1} for each groundwater spectrum. Ion chromatography was used to establish the concentration of ClO_4^- within each groundwater sample along with several common coexisting ions that potentially interfere with the SERS detection of ClO_4^- (see Tables 6-1 and 6-2). A standard addition approach was also used to validate the identification of ClO_4^- and to determine its concentration in the two groundwater samples. As the groundwaters had differing matrix compositions and concentrations of co-existing ions, it was necessary to use individual standard addition curves for each groundwater sample (Figure 6-9). The determined ClO_4^- concentrations from the SERS analysis for CPMW-2D and CPMW-5 are given in Table 6-3 and agree well with the IC ClO_4^- concentrations.

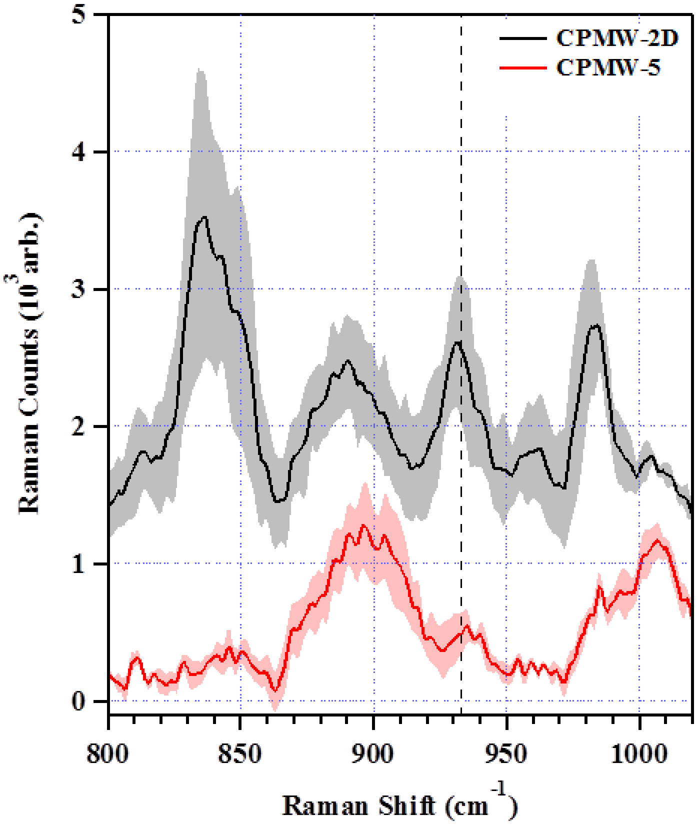


Figure 6-8. SERS Spectra of Two Groundwater Samples, CPMW-2D (black trace) and CPMW-5 (red trace), Collected from IHDIV.

Shaded regions represent ±1σ. The spectra are the average of 3–4 spots and have been baseline corrected. Spectra are scaled for clarity. Vertical dashed lines represent the peak center of the ν_{SS}-ClO_4^- *mode.*

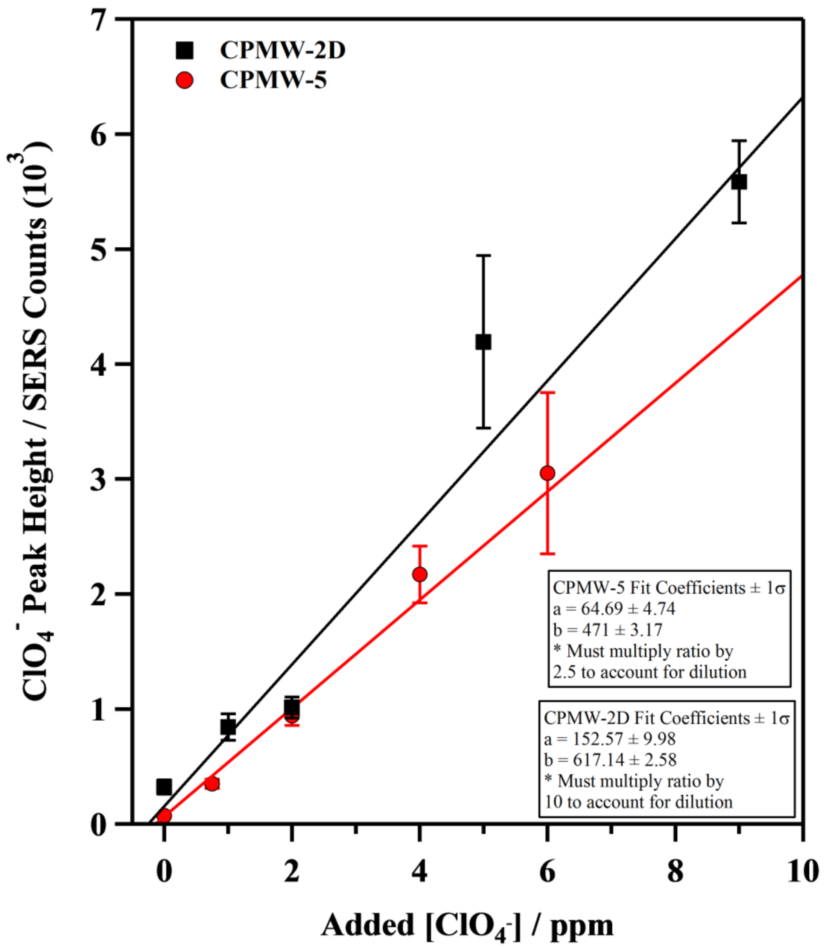


Figure 6-9. Standard addition curves for groundwaters CPMW-2D (black squares) and CPMW-5 (red circles).

Solid traces are linear regression fits to the data with the respective fit coefficients given in the boxes in lower right corner along with the dilution factor used for each standard addition. Data are average of four measurements where error bars represent ±1σ.

Table 6-3. Comparison between Groundwater ClO4- Concentration Determined with SERS versus EPA Method 314.0.

Groundwater ID	SERS ClO_4^- (a) (mg L^{-1})	IC ClO_4^- (b) (mg L^{-1})
CPMW-5	0.343 ± 0.025	0.261 ± 0.005
CPMW-2D	2.47 ± 0.16	2.19 ± 0.18

(a) Error corresponds to the ±1σ precision uncertainty of the linear regression curve used in the standard addition analysis.

(b) Error corresponds to the ±1σ level of uncertainty determined from three measurements.

6.5.3 IHDIV Demonstration 2

A second field demonstration of the portable Raman sensor was conducted in October 2016, at IHDIV using the improved SERS substrates. The same six groundwater wells were sampled from the ClO_4^- hog-out facility. Of the six groundwater samples collected, five were analyzed as is (undiluted) onsite with portable Raman system, including samples MW-1, CPMW-2D, MW-4, CPMW-5, and MW-11. MW-8 groundwater was not measured in the field with the SERS substrate due to time limitation. All groundwater samples were further analyzed at CB&I and ORNL. Additionally, the ClO_4^- concentrations in all six samples were analyzed with IC in the laboratory for comparisons and to track concentration changes with time (Table 6-4). From the ClO_4^- concentrations detailed in Table 6-4, it is apparent that the groundwater composition is quite dynamic where both increases and decreases in ClO_4^- concentrations are observed. Note that a relatively large standard deviation was observed for the October 2016 sample from well CPMW-2D, since one of the triplicate samples showed a much higher ClO_4^- concentration than the other two field samples.

Table 6-4. 2015 and 2016 ClO_4^- Concentrations from Six Groundwater Samples from IHDIV as Determined by IC.

Standard deviations are from triplicates.

Well	**October 2015**		**October 2016**	
	Average	**Standard Deviation (σ)**	**Average**	**Standard Deviation (σ)**
MW-01	627	9	423	13
MW-08	375	5	1640	26
CPMW-5	261	5	6.86	0.22
CPMW-2D	2060	18	665	91
MW-4	73.0	0.7	2.38	0.42
MW-11	98.9	0.3	26.1	0.8

ClO_4^- (µg/L)

The SERS sensor was demonstrated to detect a ClO_4^- signal in five of the undiluted groundwaters directly (MW-01, CPMW-2D, CPMW-5, MW-4, and MW-11) during the in-field demonstration (Figure 6-10), in contrast to earlier results from the October 2015 visit. This is attributed to the use of DMAET as a surface coating on the SERS substrates, which can enhance the affinity of ClO_4^- ions for the SERS sensor surface (Gu et al., 2009; Jubb et al., 2017). However, we point out that the measured SERS intensity is not always proportional to the actual ClO_4^- concentration in these undiluted environmental samples because groundwater matrix interferences were not corrected. This may explain why the MW-1 spectrum from the field showed a higher SERS intensity than other groundwater samples (Figure 6-10) such as CPMW-2D, which in fact has a higher ClO_4^- concentration than the MW-1 water (Table 6-4). Another important factor could be slight differences in the SERS EFs between different SERS substrates used in the field, which could result in large differences in measured SERS intensities. Therefore, standard addition techniques were used to determine the actual ClO_4^- concentrations, as previously described. Figure 6-11 presents a standard addition representation for MW-1. Using this technique, the SERS-determined ClO_4^- concentrations from the 2016 IHDIV groundwaters are given in Table 6-5.

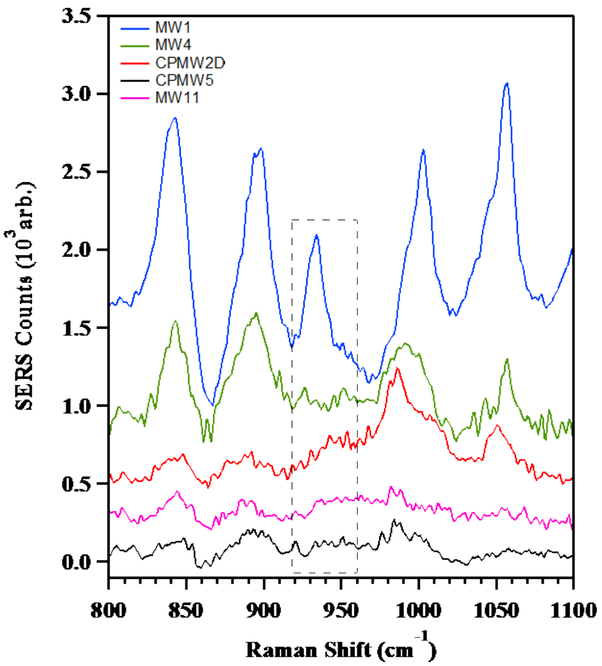


Figure 6-10. Field SERS Spectra of Undiluted Groundwater from IHDIV (October 2016).

Spectra represent the average of three individual spectra and have been baseline-corrected and scaled for clarity. The boxed region represents the ClO_4^- *symmetric stretching region.*

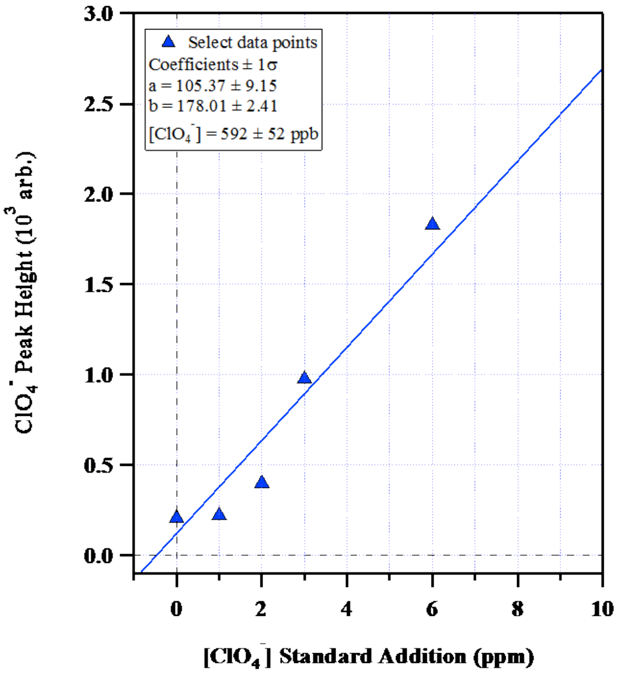


Figure 6-11. Field-collected SERS Standard Addition Determination of ClO_4^- Concentration in MW-1 Groundwater.

Data are markers. The solid trace is the linear regression fit to the data with coefficients and determined ClO_4^- *concentration given in the inset box.*

Table 6-5. Comparisons of ClO4- Concentrations Determined by IC and by Onsite Portable Raman SERS Sensor for IHDIV Groundwaters (October 2016).

Standard deviations are from triplicate analyses.

Groundwater ID	IC ClO_4^- (mg L^{-1})	SERS ClO_4^- (mg L^{-1})
MW-1	0.423 ± 0.013	0.592 ± 0.052
CPMW-2D	0.665 ± 0.091	1.390 ± 0.215
MW-4	0.002 ± 0.001	BD
CPMW-5	0.007 ± 0.001	BD
MW-8	1.640 ± 0.026	2.00 ± 0.193
MW-11	0.026 ± 0.001	BD

BD – Concentration below the SERS detection limit.

The SERS determined ClO_4^- concentrations were observed to vary with the IC results from ±18% to ~50%. Note that the SERS-determined ClO_4^- concentration trends in the groundwater samples between 2015 and 2016 track those determined by IC. ClO_4^- concentrations in MW-4, CPMW-5, and MW-11 were below the detection limit of the SERS approach.

6.5.4 Field Demonstration at Redstone

6.5.4.1 Field demonstration

The general site conditions at the Redstone OB/OD area are provided in Section 4.2. The field demonstration of the Raman sensor at Redstone occurred in November 2016 (Figure 4-2). This enabled a determination of the SERS substrate feasibility for use on samples with differing chemical composition as the groundwater chemistry and co-contaminants at Redstone are generally different than conditions at IHDIV. All Redstone SERS data were collected with commercially-produced SERS sensors.

The well water location and sample codes are given in Table 6-6 along with the ClO_4^- concentrations determined in 2015 and 2016 using EPA Method 314.0 versus the concentration determined with the SERS approach. As the ClO_4^- contaminant levels for the wells sampled at Redstone were generally quite high, it was possible to directly detect ClO_4^- in the undiluted well water for six of the eight wells sampled. Two of the wells sampled had ClO_4^- concentrations below the detection limit of the SERS instrument, ~100 µg L^{-1}. The ClO_4^- concentrations determined using SERS and a standard addition approach show that the groundwater concentration for this contaminant is quite variable with time. The standard addition curves used to determine the ClO_4^- concentrations are given in Figure 6-12. Standard deviation for the SERS measurements of the Redstone samples varied mostly within ±30%, similar to those observed at the IHDIV site.

Table 6-6. Comparisons of ClO4- Concentrations Determined by IC and by Onsite Portable Raman SERS Sensor from Redstone (November 2016).

IC data from 2016 field demonstration represent an average of all measurements performed at ORNL and CB&I.

Groundwater Location Code	Sample Code	$[ClO_4^-]$ – (mg L^{-1}) IC – 2015	$[ClO_4^-]$ – (mg L^{-1}) IC – 2016	$[ClO_4^-]$ – (mg L^{-1}) SERS – 2016
P133-RS107	3149	0.530	0.669 ± 0.134	4.530 ± 2.300
P12-RS187	3150	2.220	2.217 ± 0.207	1.700 ± 0.343
P13-RS476	3151	0.006	0.989 ± 0.162	0.669 ± 0.069
P131-RS210	3152	7.580	26.24 ± 5.151	18.00 ± 8.200
P14-RS253	3154	0.003	BD	BD
P12-RS240	3155	1.640	2.346 ± 0.334	1.250 ± 0.190
P12-RS241	3156	4.740	4.627 ± 0.717	5.300 ± 2.600
P131-RS337	3157	0.007	BD	BD

BD – Concentrations below SERS detection limit.

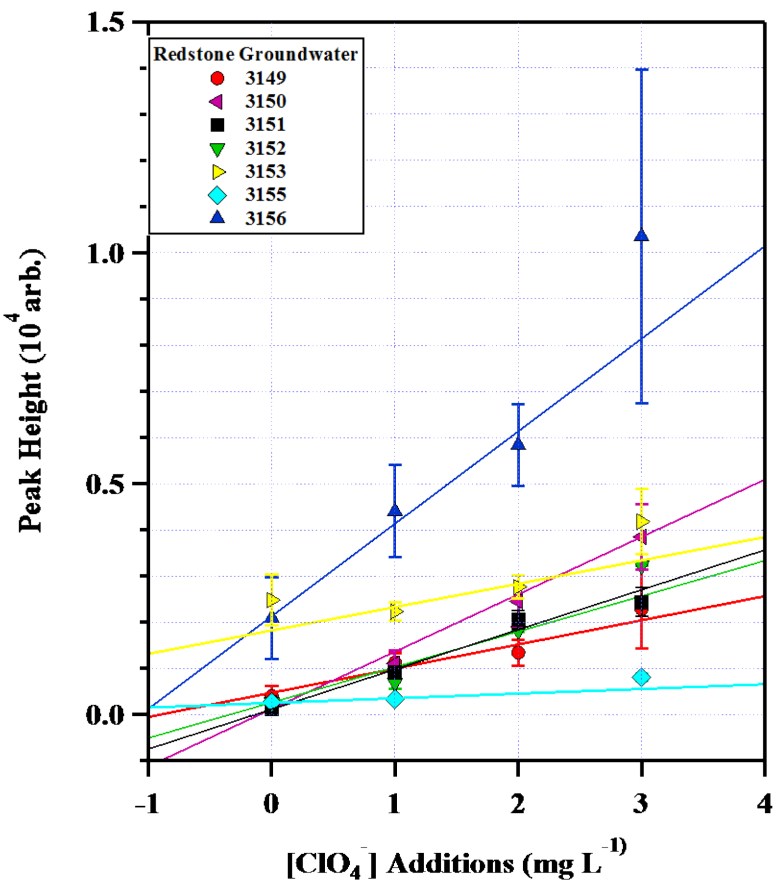


Figure 6-12. Standard Addition Data Representation for Six Groundwater Samples from Redstone.

Data are markers while solid traces represent linear regression fits to the data.

However, significant variations (>30%) were observed for two of the samples (RS 3149 and RS 3155). These large variations again may be attributed to the sensitivity of SERS to environmental variables, as discussed earlier, since SERS technology is prone to interferences (such as other organic and inorganic ionic species present in groundwater), which can mask the SERS signal of the target molecule. In particular, one data outlier (sample RS 3149) had an error >100% (see Figure 6-13 and Tables 6-6); SERS analysis substantially over-estimated its concentration. It is unknown the exact cause of this high variation since interfering anion concentrations (e.g., SO_4^{2-}: 7.7–22.0 mg/L, Cl^-: 1.3–33.0 mg/L, and NO_3^-: 0.7–2.0 mg/L) are within the normal range. However, it is speculated that the presence of high concentrations of several organic solvents in this groundwater may be responsible. These organic co-contaminants include: 1,4-benzenediamine at 1,900 µg/L, a,a-dimethylphenethyl-amine at 1,900 µg/L, hexachlorophene at 4,700 µg/L, and methanol at 40 mg/L. The presence of relatively high concentrations of these co-contaminants could result in background spectral peaks, which may overlap with the ClO_4^- peak, thereby leading to a substantially over-estimated ClO_4^- concentration. Additionally, both 1,4-benzenediamine and a,a-dimethylphenethyl-amine could strongly interact with Au surfaces via amine functional groups and therefore may also contribute to the observed large variation.

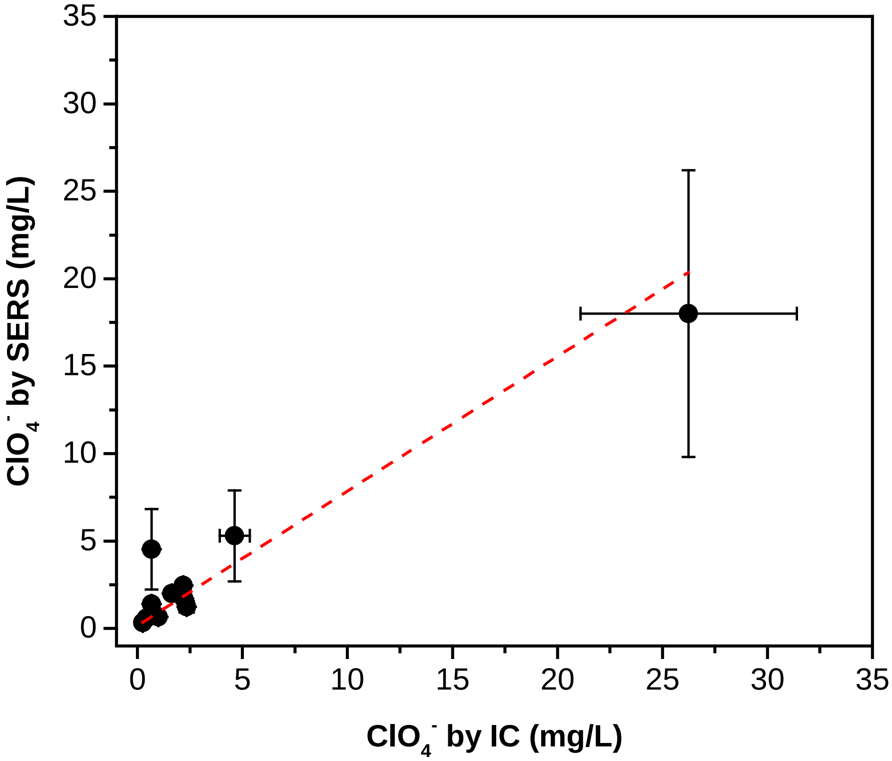


Figure 6-13. Comparisons between ClO_4^- Concentrations Measured by the SERS Sensor and Standard IC Methods (using data from Tables 6-3, 6-5, and 6-6).

Results show a general agreement between the SERS and IC methods for ClO_4^- determination, although significant variations were observed with a few selected groundwater samples.

6.5.5 Statistical Analyses and SERS/IC Data Comparisons

Based on three field demonstrations with the portable Raman sensor (using elevated Au ellipses as SERS substrates) carried out in 2015 and 2016 at the IHDIV and Redstone sites, it is concluded that these sensors were able to rapidly determine the ClO_4^- concentrations for groundwater samples with concentrations >~0.1 mg L^{-1}. Statistical analyses and comparisons of all measurable SERS data with those determined by IC (Table 6-7) (from Tables 6-3, 6-5, and 6-6) showed a general agreement between measured ClO_4^- concentrations by SERS and by IC (Figure 6-13). About half of the samples showed a measurement error within 20% (meeting our performance objective) between IC and SERS measurements. However, significant variations (>30%) were observed with three of the samples (RS 3149, RS 3155, and IH CPMW-2D, 2016). Many samples had an estimated error of ±30%, exceeding our performance objective of ±20%. This large variation is again attributed to the sensitivity of SERS to environmental variables since SERS technology is prone to interferences (such as other organic and inorganic ionic species present in groundwater), which can mask the SERS signal of the target molecule. Also, as stated earlier, one data outlier from Redstone (sample RS 3149) had an error >100% (see Figure 6-13 and Tables 6-6 and 6-7); SERS analysis substantially over-estimated its concentration. The exact cause of this high variation is unknown, but it is suspected that the presence of relatively high concentrations of several organic solvents in this groundwater may be responsible. These organic co-contaminants include: 1,4-benzenediamine at 1,900 µg/L, a,a-dimethylphenethyl-amine at 1,900 µg/L, hexachlorophene at 4,700 µg/L, and methanol at 40 mg/L. The presence of relatively high concentrations of these organic solvents likely caused high background spectral peaks, which may overlap with the ClO_4^- peak, thereby leading to a substantially over-estimated ClO_4^- concentration.

Table 6-7. Statistical Analyses and Comparisons of all Field SERS-sensor Measured ClO4- Concentrations with those Determined by IC (combined data from Tables 6-3, 6-5, and 6-6).

Data were obtained from 2016 field campaigns, except the first two samples.

Groundwater sample name	[ClO_4^-] (mg L^{-1}) IC	[ClO_4^-] (mg L^{-1}) SERS	[ClO_4^-] (mg L^{-1}) Average	Error (%)
IH-CPMW-5 (2015)	0.261±0.005	0.343±0.025	0.302±0.058	19.20
IH CPMW-2D (2015)	2.190±0.180	2.470±0.160	2.330±0.198	8.50
IH MW-01	0.423±0.013	0.592±0.052	0.508±0.120	23.55
IH CPMW-2D	0.665±0.091	1.390±0.215	1.028±0.513	49.89
IH MW-08	1.640±0.026	2.000±0.193	1.820±0.255	13.99
RS 3149	0.669±0.134	4.530±2.300	2.599±2.730	105.05
RS 3150	2.217±0.207	1.700±0.343	1.958±0.365	18.65
RS 3151	0.989±0.162	0.669±0.069	0.829±0.226	27.26
RS 3152	26.24±5.15	18.00±8.20	22.12±5.83	26.35
RS 3155	2.346±0.334	1.250±0.190	1.798±0.775	43.10
RS 3156	4.627±0.717	5.300±2.600	4.964±0.476	9.59

An additional factor influencing SERS measurements could be the SERS substrate itself; slight differences in the SERS EFs between different SERS substrates (e.g., nanostructural inhomogeneity, defects) could result in large differences in measured SERS intensities or measurement errors. The presence of relatively high interfering inorganic anions such as SO_4^{2-} and NO_3^- could also mask the ClO4- signal, as previously described. The threshold levels of these two interfering anions are ~200 µM, above which the SERS signal could be significantly suppressed (as described in Section 6.4). Nevertheless, results presented in Table 6-7 and Figure 6-13 demonstrate the feasibility of implementing SERS as a tool for rapid in-field detection of ClO_4^- within impacted waters. Additional studies and optimization are recommended to bring the technology to the market.

Page Intentionally Left Blank

7.0 COST ASSESSMENT

7.1 COST MODEL

A goal of the project was to evaluate the overall costs of onsite ClO_4^- analysis with the portable Raman sensor. The cost of Raman sensor analysis on a per sample event basis is thus estimated and compared with those using standard groundwater analyses such as EPA Methods 314.2 (IC), 331.0 (high-performance liquid chromatography [HPLC]-ESI-MS), and 6850 (liquid chromatography [LC]-ESI-MS).

7.2 COST DRIVER

Typical costs associated with Raman sensor analyses include: capital equipment, i.e., a typical portable Raman spectrometer ($15,000–$25,000) versus IC and IC-MS, LC-MS, or HPLC-MS used in conventional methods; labor and analytical time including sample preparation and pre-treatment both in the laboratory and in the field; materials and consumables; sampling equipment; vehicle and rental costs (e.g., pumps, generators, pump controllers); shipping; and data analysis and interpretation (Table 7-1). The capital cost of a portable Raman spectrometer is roughly equivalent to or slightly lower than that of a typical IC system, but should be much lower than an IC-MS, LC-MS, or HPLC-MS. For simplified calculations, it is assumed there are no capital differences based on analytical equipment. If this technique becomes standard, rental agencies would have incentive to purchase and supply the Raman field instruments on a time basis, much like many of the onsite sampling and analytical pieces of equipment. For a cost comparison, $400/week (wk) rental cost for the Raman spectrometer rather than a capital expenditure is included.

Table 7-1. Basic Cost Analysis of the Raman Sensor for in-field Analysis of ClO_4^- in Groundwater.

The cost analysis is framed around field sampling and field analytical costs.

Cost Element	Field Raman Elements ($)	Cost/ unit	Cost/24 wells sampled	Field Elements Offsite lab	Cost/ unit	Cost/24 wells sampled		
						EPA Method 314.2	EPA Method 331.0	EPA Method 6850
Materials, SERS substrates	SERS Chip	$20/ea	$480	Corning Collection tubes	$20/ea	$480	$480	$480
	Corning Collection tubes	$1/ea	$24		$1/ea	$24	$24	$24
Sampling	Field labor - sampling	$70/hr	$2,240	Field labor - sampling	$70/hr	$2,240	$2,240	$2,240
	Field labor - Raman	$70/hr	$840	Cooler packing, shipping	$70/hr	$420	$420	$420
	Sampling equipment rental	$400/wk	$400	Sampling equipment rental	$400/wk	$400	$400	$400
	Raman sensor rental	$400/wk	$400	Truck rental	$375/wk	$375	$375	$375
	Truck rental	$375/wk	$375	Room and per diem	$142/day	$568	$568	$568
	Room and per diem	$142/day	$568					
Analytical	Field technician, included in sampling labor	--	--	Per sample analytical cost	Variable (see following columns)	$2,040 ($85 per sample)	$3,960 ($165 per sample)	$2,160 ($90 per sample)
Shipping	None	--	--	Cooler	$30	$120	$120	$120
				Ice	$5	$20	$20	$20
				Cooler shipping to field	$25	$100	$100	$100
				Cooler shipping to lab	$75	$300	$300	$300
Total			**$ 5,327**			**$ 7,087**	**$ 9,007**	**$ 7,207**

7.3 COST ANALYSES

The overall costs are detailed below and potential savings to the DoD of the Raman sensor approach versus traditional offsite analytical techniques are documented. A basic cost comparison between Raman SERS technology and that currently being used for the analysis of ClO_4^- [e.g., EPA Methods 314.2 (IC), 331.0 (HPLC-ESI-MS), and 6850 (LC-ESI-MS)] (Table 7-1) was performed. For the test scenario, it was assumed that a total of 24 groundwater wells were to be sampled by a single field technician, and that the technician could sample 6 wells/day (80 minutes [min]/well) and conduct the Raman analysis on the samples during that same day (30 min/well). Packing and shipping of coolers for offsite analysis required 1.5 hours (hr)/day. The following additional assumptions were made for the cost comparison:

1. Rental of required sampling pumps and meters (other than Raman sensor) ($400/wk);
2. Rental of Raman sensor ($400/wk);
3. Commercial SERS substrates ($20/each [ea]);
4. Field labor ($70/hr);
5. Vehicle rental ($375/wk);
6. Hotel and per diem, 2016 standard rate ($142/day);
7. Coolers ($30/ea);
8. Shipping empty coolers to site ($25/ea);
9. Shipping samples to laboratory ($75/ea); and
10. Other miscellaneous (sample tubes at $1/ea and ice at $5/cooler)

Analytical costs were requested from commercial laboratories for EPA Methods 314.2 ($85/sample), 331.0 ($165/sample), and 6850 ($90/sample). Based on the assumptions provided, the estimated cost of a 4-day sampling event (24 wells) using the Raman sensor was $4,687 (Table 7-1). By comparison, the cost for offsite analysis by the different methods was $6,655 using EPA Method 314.2, $8,575 using EPA Method 331.0, and $6,775 by EPA Method 6850. The majority of the cost savings is realized by reduced analytical costs, with the assumption of a rental Raman sensor at $400 and total labor to analyze the samples at $840 (total of $1,240 for 24 wells). This compares to $2,040–$3,960 for offsite analysis by the different techniques, excluding shipping costs of ~$135/day, which would be added on. The savings for the sampling event using the Raman technique compared to traditional sampling and offsite analysis ranged from ~30% to 45%. Thus, assuming that commercial instruments are available for use (and realizing that this same instrument could potentially be utilized for a variety of other DoD contaminants, including various explosives and organics), the potential cost savings to DoD of this onsite technique is potentially significant. However, this also assumes that the precision of the technique can be improved with further work.

Finally, the ability to have real-time measurements when monitoring remediation systems is likely to result not only in cost savings, but also in more effective and timely decision making during remediation projects. In many instances, decisions made concerning the location and screening of wells during site assessment work could be vastly improved if real-time contaminant data are available. Moreover, the precision of these data is often less important than for regulatory field data.

8.0 IMPLEMENTATION ISSUES

In this project, a SERS-based portable Raman sensor to detect ClO_4^- in contaminated groundwater was developed and demonstrated. To our knowledge, this is the first demonstration of a field portable SERS/Raman sensor that combines a portable Raman analyzer with elevated Au ellipse nanostructural array substrates. However, similar to other new technologies, additional development and optimization are needed to bring the technology to commercialization. Below, several implementation issues for further technology development are identified.

8.1 INSTRUMENTATION AND SERS SUBSTRATES

Hand-held portable Raman analyzers are commercially available, but none of them currently interface with a SERS probe or with the SERS substrate, where enhanced Raman signal is obtained. However, this may be accomplished by modifications of existing Raman probes available on the market. The key is to ensure that the excitation laser focal point is on the active SERS substrate to maximize signal collection. A minor implementation issue is laser safety, since a near infrared laser (at 785 nm) is used as an excitation source for surface-enhanced Raman scattering. This issue may be resolved by using an enclosure for the laser or the SERS probe (in this case).

Large-scale fabrication of nanostructured SERS substrates is necessary to lower substrate costs. This was demonstrated using nanoimprint techniques for the fabrication of SERS substrates so that costs and time required for SERS fabrication by EBL could be substantially reduced. While nanoimprint techniques are widely used in semiconductor industries, it has not been used for SERS substrate fabrication, partly due to small market demands. Future application of the SERS technology thus requires working together with nanoimprinting companies for commercialization.

Future work is also needed to increase the sensitivity and selectivity of SERS substrates and to minimize the matrix interference effect, thereby increasing the reliability for detection and quantification, especially at low ClO_4^- concentrations. First, the controlled fabrication of reproducible nanostructured SERS dimer arrays with <10 nm gap sizes is highly desirable since SERS activity and/or enhancement increases exponentially with decreasing nanogap sizes. However, reducing the gap size below 10 nm is a formidable challenge due to EBL limits in fabrication, and difficulties in controlling Au deposits and homogeneity on nanostructured arrays. As described earlier, nanostructural inhomogeneity and defects on SERS substrates could result in large differences in SERS EFs and thus the measured SERS intensities or measurement errors. Additional studies and optimization are thus warranted in this regard. Second, while SERS is highly sensitive to the analyte, it is also sensitive to surface contamination and any adsorbed molecules complicating SERS signals. Even with the fabrication issues resolved, the stability and longevity of the fabricated SERS substrates require additional investigation because the nanostructured arrays may undergo surface and morphological changes over time due to processes such as surface oxidation and atomic rearrangements at the nanoscale. Furthermore, active SERS sites may be readily contaminated because of surface adsorption of many organic and inorganic molecules present in the air, thereby decreasing SERS activity and selectivity during storage. Thus, freshly prepared SERS substrates often show better sensitivity and performance and are desirable for targeted measurements.

8.2 METHOD INTERFERENCES

Because of its high sensitivity, SERS technology is also prone to interferences due to its responses to other ionic species, such as NO_3^-, SO_4^{2-}, and dissolved organics present in water, as commonly observed with other analytical techniques. The presence of these interfering ions or organics could potentially mask the SERS signal of the target analyte (i.e., ClO_4^-). Relatively high concentrations of salts or TDS (e.g., SO_4^{2-} and NO_3^- at >200 µM) in groundwater is a particular concern as they can smear the Au SERS surface upon drying and suppress the SERS signal or cause variability. Additionally, the presence of dissolved organics or relatively high concentrations of organic solvents, such as 1,4-benzenediamine at 1,900 µg/L, a,a-dimethylphenethyl-amine at 1,900 µg/L, and hexachlorophene at 4,700 µg/L found in some contaminated groundwaters (see Section 6.5.4), can result in high background spectral peaks. These background spectra may overlap with the ClO_4^- peak, thereby interfering with ClO_4^- measurements. Some of these organics (e.g., 1,4-benzenediamine and a,a-dimethylphenethyl-amine) could also strongly interact with Au surfaces via amine functional groups and thus decrease the selectivity and sensitivity of SERS substrates for ClO_4^- analysis. As such, SERS analysis could be subject to significant variations (e.g., ±20% or more), as described earlier.

Additional development and optimization are needed to reduce SERS measurement variability due to the presence of various groundwater interferences so as to increase its detection limit. The reported ClO_4^- detection limit (~100 µg/L) and variability (8–105%) in this project may not be suitable for routine quantitative analysis, particularly at low ClO_4^- concentrations. Future work should be directed to minimize the matrix interference effect, thereby increasing the sensitivity and reliability for ClO_4^- detection and quantification. For example, the Au SERS substrate surface may be further modified to increase its selective sorption and concentration of ClO_4^- so that the SERS substrate may be rinsed or washed with deionized water following its reaction with the sample before SERS analysis. This treatment could remove most of the salts or interfering ions (assuming that ClO_4^- is selectively sorbed), resulting in increased detection limit but decreased variability. Additionally, potential interferences resulting from dissolved organics or organic co-contaminants have not been studied (beyond the scope of this project). Systematic studies are recommended, for example, by determining the interferences and threshold levels using different types of organics and their concentrations.

8.3 END USER CONCERNS

The primary end-users of this technology are expected to be industrial or military clients that have a history of ClO_4^- usage or contamination at their facility. Additional users with interest in this sensor technology may include environmental companies and contractors that perform routine groundwater monitoring and remediation at ClO_4^- contamination sites. The potential concerns for all of the end users may include but are not limited to: (1) ease of operation and (2) technology robustness. As stated above, since this is a new technology with little or no current market demands, additional effort and improvement can be made for the sensor to be more user-friendly. For example, the sensor probe could potentially be made as a down-well probe (via fiber-optics) for direct analysis of ClO_4^- in groundwater. This would eliminate the need for pumping groundwater out for analysis, minimizing labor and analytical time. These issues could be addressed in future application and optimization processes.

Nevertheless, results presented in this work make a case for the applicability of the SERS-based portable Raman sensor for rapid field measurements of trace levels of ClO_4^- in contaminated water. Although the technology may not be currently used as a quantitative analytical tool, it could be used as a rapid screening tool for ClO_4^- at concentrations $>10^{-6}$ molarity (M) during site assessment to aid in more effective and timely decision-making during remediation projects. Future studies are warranted to further develop the technology and to optimize its performance, and eventually to bring the technology to market. With additional development and demonstration, the technology has the potential to reduce analytical costs by eliminating shipping and typical costs associated with laboratory analysis.

Page Intentionally Left Blank

9.0 REFERENCES

Bland, J. M.; Altman, D. G., 1986. Statistical Methods for Assessing Agreement between Two Methods of Clinical Measurement. *Lancet,* 1 (8476), 307-310.

Clausen, J., Cramer, R., Clough, S., Gray, M., Gwinn, P., 2009. Assessing the sensitivity of quantitative structural activity analysis models for evaluating new military compounds. Water Air Soil Poll. 202(1-4), 141-147.

Gu, B., Coates, J.D. (2006). *Perchlorate Environmental Occurrence, Interactions and Treatment*. New York: Springer.

Gu, B., Ruan, C., Wang, W., 2009. Perchlorate detection at nanomolar concentrations by surface-enhanced Raman scattering. Appl. Spectr. 63, 98-102.

Hao, J.M., Xu, Z.H., Han, M.J., Xu, S.Y., Meng, X.G., 2010. Surface-enhanced Raman scattering analysis of perchlorate using silver nanofilms deposited on copper foils. Colloids and Surfaces a-Physicochemical and Engineering Aspects 366(1-3), 163-169.

Hatab, N.A., Hsueh, C.H., Gaddis, A., Retterer, S.T., Li, J.H.; Eres, G., Zhang, Z., Gu, B.,, 2010. Free-standing optical gold bowtie nanoantenna with variable gap size for enhanced Raman spectroscopy. Nano Letters 10, 4952-4955.

Hatab, N.A., Rouleau, C., Retterer, S.T., Eres, G., Hatzinger, P.B., Gu, B.,, 2011. An integrated portable Raman sensor with nanofabricated gold bowtie array substrates for energetics detection. Analyst 136(8), 1697-1702.

Hatzinger, P.B., Diebold, J., Yates, C.A., Cramer, R.J. (2006). Field demonstration of in situ perchlorate bioremediation in groundwater. In B. Gu, J.D. Coates (Eds.), *Perchlorate Environmental Occurrences, Interactions, and Treatment* (pp. 311-341). New York: Springer.

Jackson, J.B., Halas, N.J., 2004. Surface-enhanced Raman scattering on tunable plasmonic nanoparticle substrates. PNAS USA 101(52), 17930-17935.

Jain, P.K., Huang, W., El-Sayed, M.A., 2007. On the universal scaling behavior of the distance decay of plasmon coupling in metal nanoparticle pairs: A plasmon ruler equation. Nano Lett. 7(7), 2080-2088.

Jubb, A.M., Hatzinger, P., GU, B., 2017. Trace-level perchlorate analysis of impacted groundwater by elevated gold ellipse dimer nanoantenna surface-enhanced Raman scattering. J. Raman Spect., DOI: 10.1002/jrs.5070.

Jubb, A.M., Jiao, Y., Eres, G., Retterer, S.T., Gu, B., 2016. Elevated gold ellipse nanoantenna dimers as sensitive and tunable surface enhanced Raman spectroscopy substrates. Nanoscale 8, 5641-5648.

Kneipp, K., Wang, Y., Kneipp, H., Perelman, L.T., Itzkan, I., Dasari, R., Feld, M.S., 1997. Single molecule detection using surface-enhanced Raman scattering (SERS). Phys. Rev. Lett. 78(9), 1667-1670.

Nie, S., Emory, S.R., 1997. Probing single molecules and single nanoparticles by surface-enhanced Raman scattering. Science 275, 1102-1106.

Polemi, A., Wells, S.M., Lavrik, N.V., Sepaniak, M.J., Shuford, K.L., 2011. Dispersion Characteristics in Disk-on-Pillar Array Nanostructures for Surface-Enhanced Raman Spectroscopy. Journal of Physical Chemistry C 115(28), 13624-13629.

Ruan, C., Gu, B., Wang, W., Eres, G., Zhang, Z., 2007. Controlled fabrication of nanopillar array substrates for surface-enhanced Raman spectroscopy. Langmuir 23, 5757-5760.

Ruan, C., Wang, W., Gu, B., 2006a. Detection of alkaline phosphatase using surface-enhanced Raman spectroscopy. Analytical Chemistry 78(10), 3379-3384.

Ruan, C., Wang, W., Gu, B., 2006b. Surface-enhanced Raman scattering for perchlorate detection using cystamine-modified gold nanoparticles. Anal Chim Acta 567(1), 114-120.

Sass, J., 2004. US Department of Defense and white house working together to avoid cleanup and liability for perchlorate pollution. International Journal of Occupational and Environmental Health 10(3), 330-334.

Suidan, M.T., Atikovic, E., Maloney, S.W., 2008. Anaerobic treatment of army ammunition production wastewater containing perchlorate and RDX. Chemosphere 72(11), 1643-1648.

USEPA, 2010. Low Stress (Low Flow) Purging and Sampling Procedure for the Collection of Groundwater Samples from Monitoring Wells. *EQASOP-GW 001. Rev. 3. January 2010.* https://www.epa.gov/sites/production/files/2015-06/documents/EQASOP-GW001.pdf.

Veres, T., Cui, B., Clime, L., 2010. Fabrication of nanostar arrays by nanoimprint lithography. J. Vac. Sci. Technol. B 28(6), C6o26-C26o29.

Weiss, S.M., Ryckman, J.D., Liscidini, M., Sipe, J.E., 2011. Direct imprinting of porous substrates: A rapid and low-cost approach for patterning porous nanomaterials. Nano Letters 11(5), 1857-1862.

Wells, S.M., Polemi, A., Lavrik, N.V., Shuford, K.L., Sepaniak, M.J., 2011. Efficient disc on pillar substrates for surface enhanced Raman spectroscopy. Chemical Communications 47(13), 3814-3816.

Xu, H., Bjerneld, E.J., Kall, M., Borjesson, L., 1999. Spectrscopy of single hemoglobin molecules by surface enhanced Raman scattering. Phys. Rev. Let. 83, 4357-4360.

Zhao, K., Xu, H., Gu, B., Zhang, Z., 2006. One-dimensional arrays of nanoshell dimers for single molecule spectroscopy via surface-enhanced raman scattering. J. Chem. Phys. 125(8).

APPENDIX A POINTS OF CONTACT

Point of Contact Name	Organization Name Address	Phone Fax Email
Dr. Baohua Gu	Environmental Sciences Division ORNL PO Box 2008, MS-6036 Oak Ridge, TN 37831	865-574-7286 (phone) 865-576-8543 (fax) gub1@ornl.gov
Dr. Paul B. Hatzinger	CB&I Federal Services, LLC 17 Princess Road Lawrenceville, NJ 08648	609-895-5356 (phone) 609-895-1858 (fax) Paul.hatzinger@cbifederalservices.com
Dr. Andrea Leeson	Strategic Environmental Research and Development Program (SERDP)/ESTCP 901 N Stuart Street, Suite 303 Arlington VA 22203	703-696-2118 (phone) 703-696-2114 (fax) Andrea.leeson@osd.mil

ESTCP Office
4800 Mark Center Drive
Suite 17D08
Alexandria, VA 22350-3605
(571) 372-6565 (Phone)
E-mail: estcp@estcp.org
www.serdp-estcp.org