Characterization of chemical warfare G-agent hydrolysis products by surface-enhanced Raman spectroscopy

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ABSTRACT

The United States and its allies have been increasingly challenged by terrorism, and since the September 11, 2001 attacks and the war in Afghanistan and Iraq, homeland security has become a national priority. The simplicity in manufacturing chemical warfare agents, the relatively low cost, and previous deployment raises public concern that they may also be used by terrorists or rogue nations. We have been investigating the ability of surface-enhanced Raman spectroscopy (SERS) to detect extremely low concentrations (e.g. part-per-billion) of chemical agents, as might be found in poisoned water. Since trace quantities of nerve agents can be hydrolyzed in the presence of water, we have expanded our studies to include such degradation products. Our SERS-active medium consists of silver nanoparticles incorporated into a sol-gel matrix, which is immobilized in a glass capillary. The choice of sol-gel precursor allows controlling hydrophobicity, while the porous silica network offers a unique environment for stabilizing the SERS-active silver particles. Here we present the use of these silver-doped sol-gels to selectively enhance the Raman signal of the hydrolyzed products of the G-series nerve agents.

Keywords: chemical warfare agent detection, CWA, hydrolysis, SERS, Raman spectroscopy

1. INTRODUCTION

The potential use of chemical and biological warfare agents by terrorist organizations directed against U.S. military and Coalition forces in the Middle East, and civilians at home, is an issue that has generated considerable concern in the post 9/11 era. The ability to counter such attacks, requires recognizing likely deployment scenarios, among which includes poisoning water supplies with chemical warfare agents (CWAs). The G-series nerve agents are a particular concern due to their extreme toxicity (LD_{50} man for GB = 25 mg/kg, GD = 5 mg/kg, GF = 5mg/kg),[1] persistence (hydrolysis half-life of 1-3 days),[2] relatively high solubility (5-25 g/L, see Table 1), and their previous use in Iraq[3] and Japan.[4] The nerve agents, isopropyl methylphosphonofluoridate (GB), pinacolyl methylphosphonofluoridate (GD), and cyclohexyl methylphosphonofluoridate (GF) initially hydrolyze to isopropyl methylphosphonic acid (IMPA), pinacolyl methylphosphonic acid (PMPA), and cyclohexyl methylphosphonic acid (CMPA), respectively, and subsequently, at a much slower rate, to a common final, stable product methylphosphonic acid (MPA, see Figure 1).[5,6] Clearly any analysis designed to detect nerve agents in poisoned water must not only be able to detect μg/L concentrations,[7] but also be able to detect and distinguish the resultant hydrolysis products. In addition, the ability to quantify the relative amounts of the initial agent and its hydrolysis products would provide a means to estimate when the water supply was poisoned. It is also worth noting that an analyzer capable of measuring these hydrolysis products at such low concentrations would also be valuable in establishing prior presence of nerve agents through soil and groundwater analysis,[8,9] verify successful destruction during decommissioning operations,[5,10,11] and establishing extent of exposure during an attack.[12]

Several technologies have recently been investigated as potential at-site analyzers for chemical agents, as well as their hydrolysis products.[6,13] This includes liquid chromatography combined with mass spectrometry (LC/MS),[9,14-17] infrared spectroscopy[18,19,20] and Raman spectroscopy (RS).[21] However, LC/MS remains a labor intensive technique, infrared is limited by the strong absorption of water which obscures much of the spectrum, while Raman spectroscopy does not have sufficient sensitivity.[21] In the past few years, we and others have explored the potential of surface-enhanced Raman spectroscopy (SERS) to detect CWAs,[22-28] and their degradation products.[29] The utility of SERS is based upon the extreme sensitivity of this technique and the ability to identify molecular structure through the abundant vibrational information provided by Raman spectroscopy. SERS employs the interaction of surface plasmon modes of metal particles with target analytes to increase scattering efficiency by as much as 1 million times.[30]

In our studies, we have employed metal-doped sol-gels to promote the SERS effect. The porous silica network of the alkoxide sol-gel matrix offers a unique environment for immobilizing and stabilizing SERS-active metal particles of both silver and gold.[31-34] The choice of metal and Si-alkoxide composition provides a means for chemically selecting the target analyte to be enhanced based on charge and polarity. Electropositive silver or electronegative gold particles can selectively enhance the Raman signals of negative or positive chemical species, respectively, while different alkoxides (or combinations of) can be used to select for polar or non-polar molecules. Previously, we used glass vials internally coated with the SERS-active sol-gel to measure cyanide, HD, VX, and MPA.[28] More recently, we have developed glass capillaries filled with the SERS-active sol-gel that can be attached to a syringe to perform simple and rapid sample extraction and SERS analysis.[35] This paper employs these extractive and SERS-active capillaries to examine the ability of SERS to measure and distinguish the hydrolysis products of GB, GD, and GF. Both Raman and surface-enhanced Raman spectra are presented along with preliminary vibrational mode assignments.

Table 1. Properties of chemical agents and their primary hydrolysis products investigated in the present study.[2]

Chemical Agent	**Hydrolysis ½ life**		**Water Solubility** at 25°C
Sarin (GB)	39 hr (pH 7)		completely miscible
IMPA	stable	(can hydrolyze to MPA)	4.8 g/L
MPA	very stable	(resistant to further degradation)	>1000 g/L
Soman (GD)	45 hr (pH 6.6)		21 g/L (@20°C)
PMPA	stable	(can hydrolyze to MPA)	no data
Cyclosarin (GF)	slower than GB		3.7 g/L
CMPA	no data	(can hydrolyze to MPA)	no data

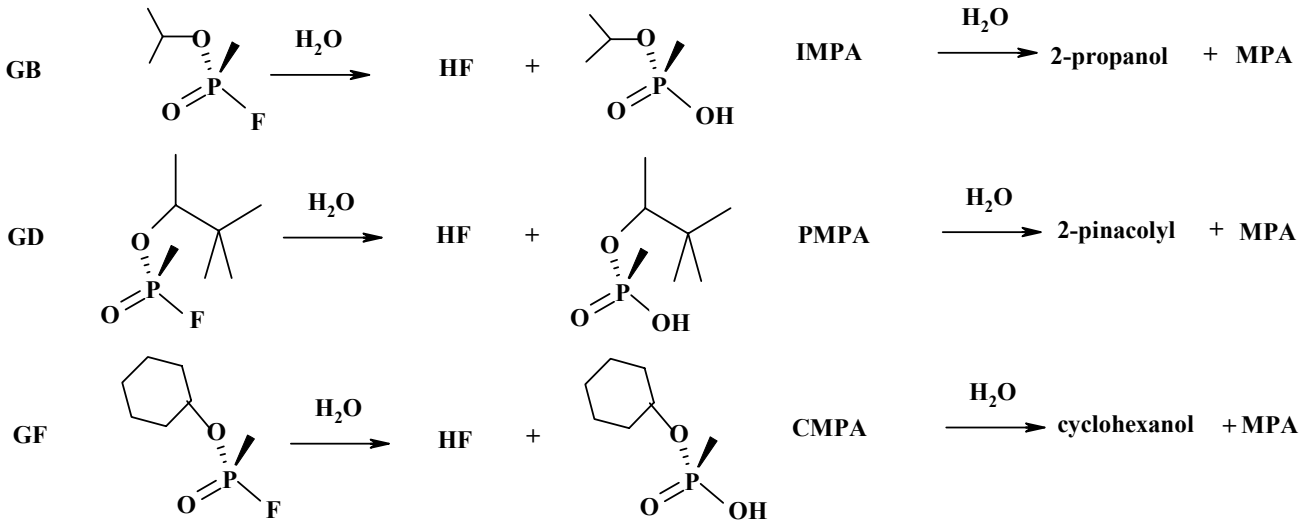

Figure 1. Hydrolysis pathways for G-Series nerve agents.

2. EXPERIMENTAL

The hydrolysis degradation chemicals measured in this study (IMPA, PMPA, CMPA) were obtained as analytical reference materials from Cerilliant (Round Rock, TX) and used without further purification. MPA and all chemicals used to prepare the silver-doped sol-gel coated capillaries were acquired from Sigma-Aldrich (St. Louis, MO) and used as received. For the purpose of safety, samples were prepared in a chemical hood, transferred to a sampling device and sealed prior to being measured. All samples were measured initially by Raman in their pure state at room temperature; MPA as a solid powder, with IMPA, and PMPA as neat liquids. CMPA was obtained in forensic quantities (1 mg/mL in MeOH), and was not amenable to RS studies at these concentration levels.

Methanol or water (HPLC grade) was used to prepare solutions of the target chemicals for SERS measurements at a

concentration of 1 mg/mL from solid powders or 0.1% v/v from neat liquids unless noted otherwise. Lower concentrations were prepared from these solutions by serial dilution, and all solutions were stored at 10°C until needed. The Raman and SERS spectra of the target chemicals presented here were all measured in capillaries.

SERS-active capillaries were prepared using the following general methodology. A silver-doped sol-gel solution, prepared according to previous published procedures from a mixture of two precursor solutions,[31] was drawn via a syringe into pre-cleaned 1-mm diameter capillaries. This procedure was modified for the SERS-active capillaries, in particular by replacing TMOS with an alkoxide mixture composed of tetramethyl orthosilicate (TMOS), octadecyltrimethoxysilane (ODS), and methyltrimethoxysilane (MTMS) at a v/v/v ratio of 1/1/5.

A 50 µL sample from each of the prepared analyte solutions was drawn into a SERS-active capillary for measurement. The capillaries were mounted horizontally on an XY positioning stage (Conix Research, Springfield, OR), such that the focal point of an f/0.7 aspheric lens was positioned just inside the glass wall. The probe optics and fiber optic interface have been described previously.[35] A Fourier transform Raman spectrometer (Real-Time Analyzers, model IRA-785, East Hartford, CT) equipped with a 785 nm diode laser (Process Instruments Inc. model 785-600, Salt Lake City, UT) and a silicon photo-avalanche detector (Perkin Elmer model C30902S, Stamford, CT) was used to deliver 100 mW of power to the SERS and RS samples and generate spectra with 8 cm^{-1} resolution.

3. RESULTS AND DISCUSSION

The SERS spectra of chemicals are often different than their Raman spectral counterparts due to the surface interactions that can enhance various vibrational modes to different extents. Therefore the Raman spectra were measured and included in this study to aid interpretation of the corresponding SERS spectra. The simplest chemical specific to the G series nerve agents is methylphosphonic acid, which has been well characterized by IR and Raman spectroscopy,[36,37] and subsequent normal coordinate analysis for assigning the vibrational modes.[38] The Raman spectrum of MPA contains 10 discernable peaks between 350 and 1650 cm^{-1} (Figure 2B). Four PO_3 bending modes are observed at 408, 462, 491 (shoulder) and 504 cm^{-1}. The PC symmetric stretch is the most intense peak observed at 774 cm^{-1}. A CH_3 rocking mode occurs at 892 cm^{-1} with little intensity, while the PO_3 stretching mode produces a peak to 956 cm^{-1}. Two additional CH_3 and PO_3 modes produce peaks at 1004 and 1054 cm^{-1}, also with moderate intensity. The 10th mode in this region is a CH_3 bending mode which occurs at 1424 cm^{-1}.

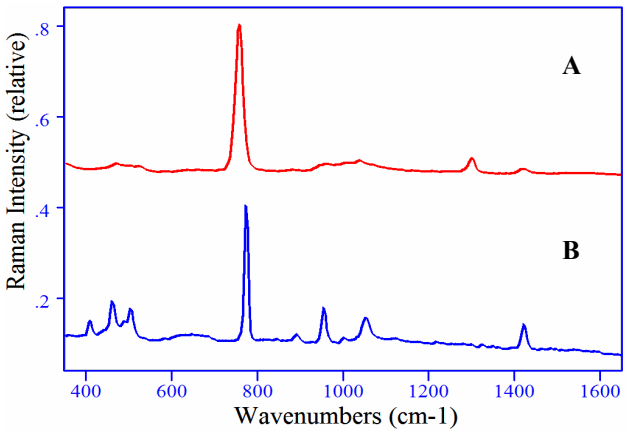

Figure 2. A) SERS and B) Raman spectra of MPA. Conditions: A) 0.1 mg/ml in water, TMOS/ODS/MTMS sol-gel in capillary, 1-min acquisition time. B) solid, 5-min acquisition time.

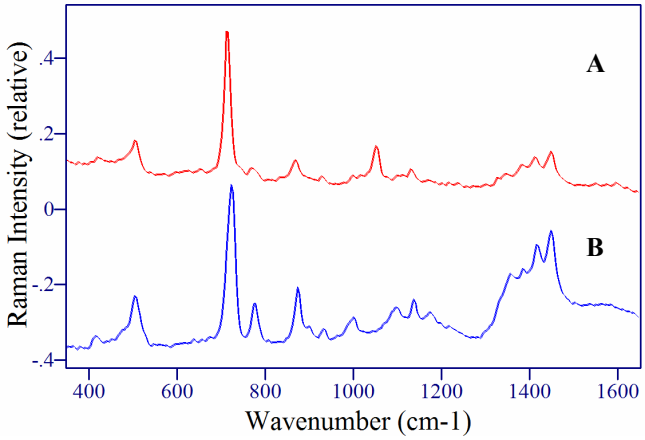

Figure 3. A) SERS and B) Raman spectra of IMPA. Conditions as in Fig. 2, but: A) 0.1 % v/v in MeOH, B) neat liquid.

The SERS spectrum of MPA (Figure 2A) is considerably simpler than that of the solid powder Raman spectrum, with weak peaks observed at 469, 521, 958, 1003, 1038, and 1420 cm^{-1}. These SERS spectral peaks can all be assigned to the modes observed at similar frequencies in the Raman spectrum, albeit the 521 and 1038 cm^{-1} peaks have shifted significantly from the 504 and 1054 cm^{-1} Raman spectral peaks. The most characteristic SERS spectral peaks are the

intense 756 cm^{-1} peak and the unique peak at 1300 cm^{-1}. The former peak clearly corresponds to a nearly pure PC symmetric stretch, while the latter is likely a CH_3 twist.

The next hydrolysis product studied was isopropyl methylphosphonic acid. Like MPA, both the Raman and SERS spectra of IMPA are dominated by a peak in the 700 cm^{-1} region, specifically at 728 and 716 cm^{-1}, respectively (Figure 3). However, these peaks are not simply a PC stretch, but include a considerable amount of the backbone CPOCC mode created by the addition of the isopropyl group. Both spectra also contain moderate peaks at 782 and 772 cm^{-1} that may also be PC containing backbone modes, as has been suggested by a theoretical treatment for sarin.[39] It is also worth noting that the Raman spectrum of IMPA is very similar to that of a published spectrum of sarin.[21] A number of the peaks assigned to PO_3 modes for MPA have shifted moderately from the Raman to the SERS spectra for IMPA, and includes the following respective peaks; 510 and 508 cm^{-1}, 938 and 931 cm^{-1}, and 1006 and 1004 cm^{-1}. The latter peak likely contains significant methyl character. Similarly, the methyl rocking and bending modes observed for MPA are now at 880 and 874 cm^{-1}, and 1420 and 1416 cm^{-1} in the respective Raman and SERS spectra of IMPA. Not surprisingly, the isopropyl group not only increased the intensity of these bands, but also gives rise to a CH deformation, and additional CH_3 and CH_2 wagging modes, at 1359 and 1349 cm^{-1}, 1390 and 1388 cm^{-1} and 1453 and 1451 cm^{-1}, in the respective Raman and SERS spectra. The isopropyl group also gives rise to a CC bend at 421 and 424 cm^{-1}, and a CC stretch at 1179 and 1173 cm^{-1} in the respective Raman and SERS spectra. In the Raman spectrum of IMPA a peak also appears at 1104 cm^{-1} that is characteristic of CO or CC stretches, while in the SERS spectrum a peak appears at 1055 cm^{-1} and is assigned to a PO_3 stretch, as was the 1038 cm^{-1} peak in the MPA SERS spectrum.

The Raman spectrum of pinacolyl methylphosphonic acid, like IMPA, contains an increasing amount of CC and CH_n character (Figure 4B). This includes new peaks at 541, 934, 977, 1212 and 1264 cm^{-1} that are assigned to a CC_3 wag, a CC_3 bend, a CCC bend, and two CC stretching modes based on a theoretical treatment for soman.[39] The 1300 to 1500 cm^{-1} region again contains a number of CH_n bending modes, and the peaks are assigned accordingly. The most obvious change in the spectrum is that the PC plus backbone mode in the IMPA spectrum has split into two distinct peaks at 732 and 761 cm^{-1}. The SERS spectrum for PMPA is dominated by these latter peaks, except that they overlap considerably producing a peak centered at 750 cm^{-1} with a shoulder at 729 cm^{-1} (Figure 4A). The remaining SERS peaks are evident, but have little intensity, except for the CC_3 wag at 543 cm^{-1}, the PO_3 stretch at 1037 cm^{-1}, and the CH_2 bend at 1444 cm^{-1}.

Cyclohexyl methylphosphonic acid was only available as 1 mg/mL in methanol and a Raman spectrum at this concentration could not be obtained. The SERS spectrum in many ways is like IMPA with the addition of cyclohexane modes (Figure 5). This includes peaks at 622, 1023, and 1262 cm^{-1}, that are attributed to ring CC stretching modes, and a peak at 811 cm^{-1} that is assigned to a ring CH_2 bending mode. The most intense peak observed at 747 cm^{-1} is again assigned to a PC stretch plus backbone mode.

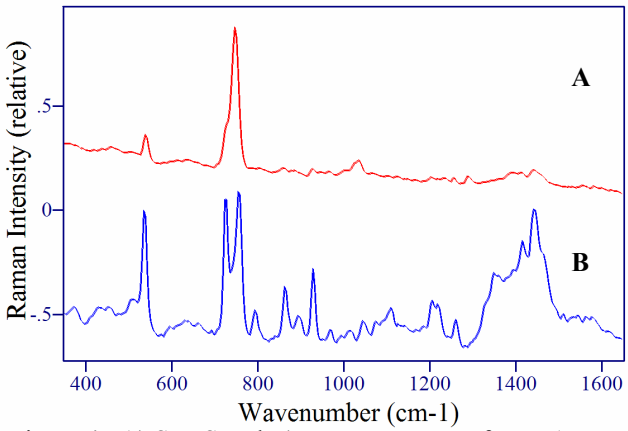

Figure 4. A) SERS and B) Raman spectra of PMPA. Conditions as in Fig. 3.

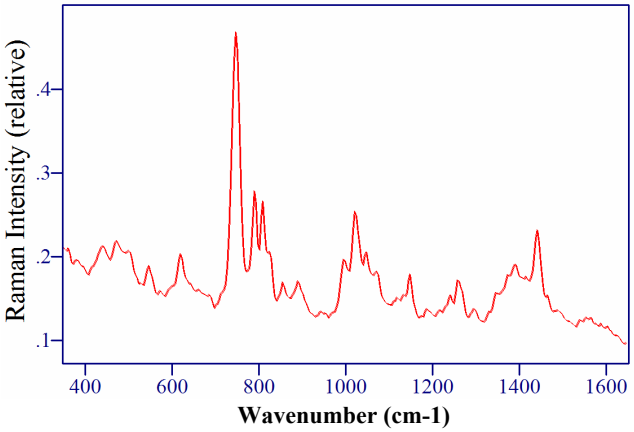

Figure 5. SERS spectrum of CMPA. Conditions as in Fig. 3, but A) 1 mg/mL in MeOH.

In general, the SERS spectra for these alkyl methylphosphonic acids have two common features, the PC stretch produces the most intense peak, more so than the Raman spectra when compared to the intensity of the other peaks, and the most

substantial shift in peak frequencies occurs for PO_3 modes when compared to the Raman spectra. The increased intensity of the PC mode suggests that it is perpendicular to the surface, based on previous research that has shown that modes couple to the plasmon field more effectively in this orientation.[40] The shift in the PO_3 frequencies suggests strong surface interactions through this group. Taken together, the SERS data suggests that these molecules are oriented with the PO_3 group interacting with the silver surface and the methyl group away from the surface. In the case of MPA, especially for the doubly deprotonated anion, the three oxygens could form the base of a tripod on the surface. This orientation may become less likely for the other molecules as the alkoxide groups replace the hydroxide group with surface interaction through the other two oxygens. This change in orientation along with increasing amounts of backbone character to the PC stretch could explain the shift and splitting of this mode.

Table 2. Tentative vibrational mode assignments for Raman and SERS peaks for VX and its hydrolysis products.

MPA		**IMPA**		**PMPA**		**CMPA**	Tentative Assignments[a]
RS	SERS	RS	SERS	RS	SERS	SERS[b]	
408		421	424				PO_3 bend
462[c,d]	469			441	442	441	PO_3 bend
491[c]						475	PO_3 bend
504[c]	521	510	508	514		495	C-PO_3 bend
				541[e]	543	549	C-C_3 bend
						622	Ring breathing
		728	716	732	729sh		PC stretch and backbone
774	756	782	772	761	750	747	PC stretch and backbone
				799		792	CH bend
						811	Ring CH_2
		880[e]	874	869[e]	863	857	CCC bend
892[c,d]				902	888	896	CH_3 rock
				934[e]	929		C-C_3 bend
956[c,d]	958	938	931				PO_3 stretch
				977[e]			CCC stretch
1004	1003	1006	1004	1015		1000	PO_3 or CH_3 bend
						1023	Ring breathing sym
1054	1038[d]		1055	1052	1037	1050	PO_3 stretch
				1079		1073	CCC bend
		1104		1116			OC or CC stretch
		1143	1132			1150	CC stretch
		1179	1173	1212[e]	1206		CC stretch
				1224	1236	1243	CH_2 bend or above
				1264[e]	1257	1262	CC stretch
	1300				1291		CH_3 bend
		1359	1349	1355		1347	CH deformation
						1374	CH_n bend
		1390	1388	1390	1394	1393	CH_3 rock
1424[c,d]	1420	1420	1416	1420	1415	1416	CH_3 bend (bound to P)
		1453	1451	1447	1444	1443	CH_2 rock

a - Assignment terminology is simplified since assignments refer to multiple molecules. b - no Raman spectrum measured, c = Ref. 36, d = Ref. 37, e = Ref. 39.

4. CONCLUSION

The ability to measure and identify the various hydrolysis degradation products with our SERS-active silver-doped sol-gel coated capillaries has been demonstrated. The SERS spectra of these chemicals were somewhat different than their Raman spectral counterparts, which is attributed to the interaction of these chemicals with the silver. In general, the Raman and SERS spectra for the alkyl methylphosphonic acid hydrolysis products were dominated by one or two peaks between 715 and 765 cm^{-1}, which have been assigned to PC stretching modes with varying amounts of backbone mode

contributions. The spectral intensity of this mode and the shift in frequency of the PO_3 modes in the SERS spectra suggest a strong surface interaction for these molecules. It is clear from the present study that the hydrolysis products can easily be identified as a class by these 700 cm^{-1} peaks, but quantifying each in a mixture is likely to require chemical separations or chemometric approaches. These approaches, as well as measurements to determine the detection limits and pH dependence of these hydrolysis products are in progress.

5. ACKNOWLEDGMENTS

The authors are grateful for the support of the U.S. Army (DAAD13-02-C-0015, Joint Service Agent Water Monitor program), and the National Science Foundation (DMI-0215819), and would like to thank Dr. Steve Christesen for helpful discussions, and Mr. Chetan Shende for sol-gel chemistry development.

6. REFERENCES

1. Committee on Toxicology. Review of Acute Human-Toxicity Estimates for Selected Chemical-Warfare Agents, Nat. Acad. Press (Washington, D.C.) 1997.
2. Munro, N.B., Talmage, S.S., Griffin, G.D., Waters, L.C., Watson, A.P., King, J.F., and Hauschild V. "The Sources, Fate, and Toxicity of Chemical Warfare Agent Degradation Products", *Environ. Health Perspect.*, 107, 933-974 (1999).
3. Hoenig, S.L. Handbook of Chemical Warfare and Terrorism, Greenwood Press (Westport, CT) 2002.
4. Nozaki, H. and Aikawa, N. "Sarin poisoning in Tokyo subway", *Lancet*, 345 1446-1447 (1995).
5. Wagner, G. and Yang, Y. "Rapid nucleophilic/oxidative decontamination of chemical warfare agents", *Ind. Eng. Chem. Res.,* 41, 1925-1928, (2002).
6. Creasy, W., Brickhouse, M., Morrissey, K., Stuff, J., Cheicante, R., Ruth, J., Mays, J., Williams, B., O'Connor, R., and Durst, H. "Analysis of chemical weapons decontamination waste from old ton containers from Johnston atoll using multiple analytical methods", *Environ. Sci. Technol.*, 33, 2157-2162, (1999).
7. McKone, T.E., Huey, B.M., Downing, E., and Duffy, L.M., Editors. Strategies to Protect the Health of Deployed U.S. Forces: Detecting, Characterizing, and Documenting Exposures, Nat. Acad. Press (Washington, D.C.) p.207, 1999.
8. Johnston, R.L., Hoefler, C.M., Fargo, J.C., and Moberley, B. "The Defense Nuclear Agency's Chemical/ Biochemical Weapons Agreements Verification Technology Research, Development, Test and Evaluation Program and its Requirements for On-Site Analysis", *AT-ONSITE*, 5-8 (1994).
9. D'Agustino, P.A, Hancock, J.R., and Provost, L.R. "Determination of sarin, soman and their hydrolysis products in soil by packed capillary liquid chromatography-electrospray mass spectrometry", *J. Chromatography A*, 912, 291-299 (2001).
10. Yang, Y., Baker, J., and Ward, J. "Decontamination of chemical warfare agents", *Chem. Rev.*, 92, 1729-1743 (1992).
11. Christesen, S., MacIver, B., Procell, L., Sorrick, D., Carrabba, M., and Bello, J. "Nonintrusive analysis of chemical agent identification sets using a portable fiber-optic Raman spectrometer", *Appl. Spec.*, 53, 850-855 (1999).
12. Hui, D.-M. and Minami, M. "Monitoring of fluorine in urine samples of patients involved in the Tokyo sarin disaster, in connection with the detection of other decomposition products of sarin and the by-products generated during sarin synthesis", *Clin. Chim. Acta*, 302, 171-188 (2000).
13. "The Chemical Weapons Convention Redefines Analytical Challenge", *Analytical Chemistry News & Features*, June 1, 397A (1998).
14. Sega, G.A., Tomkins, B.A., and Griest, W.H. "Analysis of methylphosphonic acid, ethyl methylphosphonic acid and isopropyl methylphosphonic acid at low microgram per liter levels in groundwater" *J. Chromatography A*, 790, 143-152 (1997).
15. Creasy, W.R. "Postcolumn Derivatization Liquid Chromatography/Mass Spectrometry for Detection of Chemical-Weapons-Related Compounds" *Am. Soc. Mass Spectrom.*, 10, 440-447 (1999).
16. Katagi…, *J. Chromatography A*, 833, 169-179 (1999).
17. Liu, Q., Hu, X., and Xie, J. "Determination of nerve agent degradation products in environmental samples by liquid chromatography–time-of-flight mass spectrometry with electrospray ionization", *Analytica Chimica Acta,* 512, 93-101 (2004).

18. Hoffland, L.D., Piffath, R.J., and Bouck, J.B. "Spectral signatures of chemical agents and simulants", *Optical Engineering*, 24, 982-984, (1985).
19. Braue, E.H.J., and Pannella, M.G. "CIRCLE CELL FT-IR Analysis of Chemical Warfare Agents in Aqueous Solutions", *Applied Spectroscopy*, 44, 1513-1520, (1990).
20. Kanan, S. and Tripp, C. "An infrared study of adsorbed organophosphonates on silica: a prefiltering strategy for the detection of nerve agents on metal oxide sensors", *Langmuir*, 17, 2213-2218, (2001).
21. Christesen, S.D. "Raman cross sections of chemical agents and simulants", *Appl. Spec.*, 42, 318-321 (1988).
22. Lee, Y. and Farquharson, S. "Rapid chemical agent identification by SERS", *SPIE*, 4378, 21-26 (2001).
23. Farquharson, S., Maksymiuk, P., Ong, K., and Christesen, S. "Chemical agent identification by surface-enhanced Raman spectroscopy", *SPIE*, 4577, 166-173 (2001).
24. Spencer, K.M., Sylvia, J., Clauson, S. and Janni, J. "Surface Enhanced Raman as a Water Monitor for Warfare Agents in Water", *SPIE*, 4577, 158-165 (2001).
25. Premasiri, W., Clarke, R., Londhe, S., and Womble, M. "Determination of cyanide in waste water by low-resolution surface enhanced Raman spectroscopy on sol-gel substrates", *J. Ram. Spec.*, 32, 919-922 (2001).
26. Tessier, P., Christesen, S., Ong, K., Clemente, E., Lenhoff, A., Kaler, E., and Velev, O. "On-line spectroscopic characterization of sodium cyanide with nanostructured Gold surface-enhanced Raman spectroscopy substrates", *App. Spectrosc.*, 56, 1524-1530 (2002).
27. Christesen, S.D., Lochner, M.J., Ellzy, M., Spencer, K.M., Sylvia, J., and Clauson, S. "Surface Enhanced Raman Detection and Identification of Chemical Agents in Water", *23rd Army Science Conf.*, Orlando, 2002.
28. Farquharson, S., Gift, A., Maksymiuk, P., Inscore, F., Smith, W., Morrisey, K., and Christesen, S. "Chemical agent detection by surface-enhanced Raman spectroscopy", *SPIE*, 5269, 16-22 (2004).
29. Farquharson, S., Gift, A., Maksymiuk, P., Inscore, F., and Smith, W. "pH dependence of methyl phosphonic acid, dipicolinic acid, and cyanide by surface-enhanced Raman spectroscopy", *SPIE*, 5269, 117-125 (2004).
30. Weaver, M.J., Farquharson, S., and Tadayyoni, M.A. "Surface-enhancement factors for Raman scattering at silver electrodes", *J. Chem. Phys.*, 82, 4867-4874 (1985).
31. Lee, Y. and Farquharson, S. "SERS Sample Vials Based on Sol-Gel Process for Trace Pesticide Analysis", *SPIE*, 4206, 140-146 (2000).
32. Farquharson, S. and Lee, Y. "Trace Drug Analysis by Surface-Enhanced Raman Spectroscopy", *SPIE*, 4200-16 (2000).
33. Lee, Y., Farquharson, S., and Rainey, P.M. "Surface-Enhanced Raman Sensor for Trace Chemical Detection in Water", *SPIE,* 3857, 76-84 (1999).
34. Lee, Y, Farquharson, S., Kwong, H., and Shahriari, M. "Surface-Enhanced Raman Sensor for Surface-Enhanced Raman Spectroscopy", *SPIE,* 3537, 252-260 (1998).
35. Farquharson, S., Gift, A., Maksymiuk, P., and Inscore, F. "Rapid dipicolinic acid extraction from Bacillus spores detected by surface-enhanced Raman spectroscopy", *Appl. Spec.* 58, 351-354 (2004).
36. Nyquist, R. "Vibrational spectroscopic study of $(R\text{-}PO_3)^{2-}$", *J. Mol. Struct.*, 2, 123-135, (1968).
37. Van der Veken, B.J. and Herman, M.A. "Vibrational analysis of methylphosphonic acid and its anions: I. Vibrational spectra", *J. Molec. Struct.*, 15, 225-236 (1973).
38. Van der Veken, B.J. and Herman, M.A. "Vibrational analysis of methylphosphonic acid and its anions: II. Normal coordinate analysis", *J. Molec. Struct.*, 15, 237-248 (1973).
39. Hameka, H. and Jensen, J. "Theoretical prediction of the infrared spectra of nerve agents", CRDEC-TR-326, 1992.
40. Suh, J.S. and Moskovitz, M. "SERS of amino acids and nucleotide bases adsorbed on silver" *J. Am. Chem. Soc.* 108, 4711-4718 (1986).