DEVELOPMENT AND APPLICATIONS OF NOVEL SOLVENT-MINIMIZED TECHNIQUES IN THE DETERMINATION OF CHEMICAL WARFARE AGENTS AND THEIR DEGRADATION PRODUCTS

LEE HOI SIM NANCY

NATIONAL UNIVERSITY OF SINGAPORE

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SUMMARY

Two approaches towards the development of solvent-minimized microextraction techniques are presented in this report. The first approach involved an attempt to develop solid-phase microextraction (SPME) fibers based on molecularly imprinted polymers (MIP) synthesized via the sol-gel route for the extraction of degradation products of chemical warfare agents. In the second approach, hollow fiber-protected liquid-phase microextraction (HF-LPME) was utilized for the determination of various chemical warfare agents and their degradation products.

Prior to the development of sol-gel MIPs as SPME fiber coatings, sol-gel MIPs were first synthesized as powder and evaluated as sorbent packings in solid-phase extraction (SPE) cartridges. A series of MIPs was synthesized using pinacolyl methylphosphonic acid (PMPA), thiodiglycol (TDG), triethanolamine (TEA) and 3-quinuclidinol (3Q) as the templates. A non-imprinted polymer (NIP) was also synthesized, but in the absence of a template. The polymers were evaluated for their binding properties towards their respective target analytes in aqueous matrices using SPE. The elution solvent and volume of elution solvent were optimized for each MIP. The MIP-SPE procedure was compared with other sample preparation procedures, namely strong anion-exchange (SAX) SPE and strong cation-exchange (SCX) SPE as well as a direct rotary evaporation procedure for the analysis of a range of analytes in an aqueous sample containing polyethylene glycol (PEG).

Commercially-available SPME fibers in which the polymer coatings have been stripped-off or damaged but with an intact fused silica backbone were used for the preparation of sol-gel MIP SPME fibers. Several attempts to synthesize the sol-gel MIP SPME fibers did not proceed well as the fiber coatings cracked and flaked off
upon drying. Hence, efforts were focused on the evaluation of a novel SPME coating based on poly(1-hydroxy-4-dodecyloxy-p-phenylene) polymer (PhPPP).

PhPPP was investigated as a coating for the SPME of Lewisites from aqueous samples. Several extraction parameters, namely the choice of derivatizing agent, pH, salting, and extraction time were thoroughly optimized. Upon optimization of the extraction parameters, the performance of the novel coating was compared against that of commercially-available SPME coatings.

HF-LPME was investigated for the extraction of various chemical warfare agents and their degradation products from aqueous samples. Optimization of several extraction parameters was carried out where the effects of the extraction solvent, the derivatizing agent, derivatization procedure, the amount of derivatizing agent (for degradation products), salting, stirring speed and extraction time were thoroughly investigated. Upon optimization of the extraction parameters, the HF-LPME technique was compared against SPME. In addition, the applicability of the technique for a 20th Official OPCW (Organization for the Prohibition of Chemical Weapons) Proficiency Test sample was demonstrated.
**LIST OF ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>A</td>
<td>Absorptivity</td>
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<tr>
<td>ACN</td>
<td>Acetonitrile</td>
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<tr>
<td>APTEOS</td>
<td>3-Aminopropyltriethoxysilane</td>
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<tr>
<td>BA</td>
<td>Benzilic acid</td>
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<tr>
<td>BCVAA</td>
<td>Bis(2-chlorovinyl)arsenous acid</td>
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<td>BDT</td>
<td>1,4-Butanedithiol</td>
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<td>BMPA</td>
<td>Isobutyl methylphosphonic acid</td>
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<td>BSTFA</td>
<td>N,O-Bis(trimethylsilyl)trifluoroacetamide</td>
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<tr>
<td>BT</td>
<td>Butanethiol</td>
</tr>
<tr>
<td>BZ</td>
<td>3-Quinuclidinyl benzilate</td>
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<tr>
<td>CAR/PDMS</td>
<td>Carboxen/polydimethylsiloxane</td>
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<td>CAS</td>
<td>Chemical Abstracts Service</td>
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<tr>
<td>CEES</td>
<td>2-Chloroethyl ethyl sulfide</td>
</tr>
<tr>
<td>CEPF</td>
<td>O-Cyclohexyl ethylphosphonofluoridate</td>
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<tr>
<td>CEPS</td>
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<td>CVAA</td>
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<td>CWA</td>
<td>Chemical warfare agent</td>
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<tr>
<td>CWC</td>
<td>Chemical Weapons Convention</td>
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<tr>
<td>CW/DVB</td>
<td>Carbowax/divinylbenzene</td>
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<tr>
<td>DBPP</td>
<td>O,O-Dibutyl n-propylphosphonate</td>
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<tr>
<td>DCHMP or DCMP</td>
<td>O,O-Dicyclohexyl methylphosphonate</td>
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<tr>
<td>DCM</td>
<td>Dichloromethane</td>
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DEEP or DEP  
**O,O-Diethyl N,N-diethylphosphoramidate**

**DIMP**  
**O,O-Diisopropyl methylphosphonate**

**DIPAE**  
2-(N,N-Diisopropylamino)ethanol

**DMEP**  
**O,O-Dimethyl ethylphosphonate**

**DMMP**  
**O,O-Dimethyl methylphosphonate**

**DVB/CAR/PDMS**  
divinylbenzene/carboxen/polydimethylsiloxane

**ECPP**  
**O-Ethyl O-cyclohexyl n-propylphosphonate**

**EDEA**  
**N-Ethyl diethanolamine**

**EDEPC**  
**O-Ethyl N,N-diethylphosphoramidocyanidate**

**EDT**  
1,2-Ethanedithiol

**EGDMA**  
Ethylene glycol dimethacrylate

**EHES**  
Ethyl 2-hydroxyethyl sulfide

**EMPA**  
Ethyl methylphosphonic acid

**EPA**  
Ethylphosphonic acid

**ET**  
Ethanethiol

**EtOH**  
Ethanol

**GA**  
Tabun or ethyl N,N-dimethylphosphoramidocyanidate

**GB**  
Sarin or isopropyl methylphosphonofluoridate

**GC–MS**  
Gas chromatography-mass spectrometry

**GD**  
Soman or pinacolyl methylphosphonofluoridate

**GF**  
Cyclohexyl methylphosphonofluoridate

**h**  
hour(s)

**HD or SM**  
Sulfur mustard or bis(2-chloroethyl) sulfide

**HF-LPME**  
Hollow fiber-protected liquid-phase microextraction

**HMDS**  
Hexamethyldisilazane
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<th>Abbreviation</th>
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<tr>
<td>HN1</td>
<td>Bis(2-chloroethyl)ethylamine</td>
</tr>
<tr>
<td>HN2</td>
<td>Bis(2-chloroethyl)methylamine</td>
</tr>
<tr>
<td>HN3</td>
<td>Tris(2-chloroethyl)amine</td>
</tr>
<tr>
<td>HP</td>
<td>Hewlett Packard</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
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<tr>
<td>IMPA</td>
<td>Isopropyl methylphosphonic acid</td>
</tr>
<tr>
<td>ISTD</td>
<td>Internal standard</td>
</tr>
<tr>
<td>L</td>
<td>Leak</td>
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<tr>
<td>LC-MS</td>
<td>Liquid chromatography-mass spectrometry</td>
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<td>LLE</td>
<td>Liquid-liquid extraction</td>
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<tr>
<td>LOD</td>
<td>Limit of detection</td>
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<tr>
<td>Log $K_{ow}$</td>
<td>Logarithm of octanol-water partition coefficient</td>
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<td>L1</td>
<td>Lewisite 1 or 2-chlorovinylidichloroarsine</td>
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<tr>
<td>L2</td>
<td>Lewisite 2 or bis(2-chlorovinyl)chloroarsine</td>
</tr>
<tr>
<td>L3</td>
<td>Lewisite 3 or tris(2-chlorovinyl)arsine</td>
</tr>
<tr>
<td>MAA</td>
<td>Methacrylic acid</td>
</tr>
<tr>
<td>MDEA</td>
<td>$N$-Methyldiethanolamine</td>
</tr>
<tr>
<td>min</td>
<td>minute(s)</td>
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<tr>
<td>MIP</td>
<td>Molecularly imprinted polymer</td>
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<td>MPA</td>
<td>Methylphosphonic acid</td>
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<tr>
<td>MSD</td>
<td>Mass selective detector</td>
</tr>
<tr>
<td>MTBSTFA</td>
<td>$N$-(tert.-butyldimethylsilyl)-$N$-methyltrifluoroacetamide</td>
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<tr>
<td>MW</td>
<td>Molecular weight</td>
</tr>
<tr>
<td>$m/z$</td>
<td>mass-to-charge ratio</td>
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<td>N</td>
<td>Non-endcapped NIP</td>
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<td>Abbreviation</td>
<td>Full Form</td>
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<td>-----------</td>
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<tr>
<td>NE</td>
<td>Endcapped NIP</td>
</tr>
<tr>
<td>NIP</td>
<td>Non-imprinted polymer</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance spectrometry</td>
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<tr>
<td>nPPA</td>
<td>n-Propylphosphonic acid</td>
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<td>OPCW</td>
<td>Organization for the Prohibition of Chemical Weapons</td>
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<td>P</td>
<td>Non-endcapped PMPA-MIP</td>
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<tr>
<td>PA</td>
<td>polyacrylate</td>
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<td>PDMS</td>
<td>polydimethylsiloxane</td>
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<td>PDMS/DVB</td>
<td>polydimethylsiloxane/divinylbenzene</td>
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<td>PDT</td>
<td>1,3-Propanedithiol</td>
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<tr>
<td>PE</td>
<td>Endcapped PMPA-MIP</td>
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<tr>
<td>PEG</td>
<td>polyethylene glycol</td>
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<tr>
<td>PhPPP</td>
<td>Poly(1-hydroxy-4-dodecyloxy-p-phenylene) polymer</td>
</tr>
<tr>
<td>PIPA</td>
<td>Propyl isopropylphosphonic acid</td>
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<tr>
<td>pK_a</td>
<td>Negative logarithm of acid dissociation constant</td>
</tr>
<tr>
<td>PMPA</td>
<td>Pinacolyl methylphosphonic acid</td>
</tr>
<tr>
<td>PPA</td>
<td>Propylphosphonic acid</td>
</tr>
<tr>
<td>PT</td>
<td>Propanethiol</td>
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<tr>
<td>PTMOS</td>
<td>Phenyl trimethoxysilane</td>
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<tr>
<td>Q</td>
<td>Sesquimustard or 1,2-bis(2-chloroethylthio)ethane</td>
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<tr>
<td>QOH</td>
<td>1,2-Bis(2-hydroxyethylthio)ethane</td>
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<tr>
<td>R</td>
<td>Recovery</td>
</tr>
<tr>
<td>r^2</td>
<td>Squared regression coefficient</td>
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<tr>
<td>ROP</td>
<td>Recommended operating procedure</td>
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<td>rpm</td>
<td>Revolutions per minute</td>
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<td>Abbreviation</td>
<td>Full Form</td>
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<td>-----------</td>
</tr>
<tr>
<td>RSD</td>
<td>Relative standard deviation</td>
</tr>
<tr>
<td>SAX</td>
<td>Strong anion-exchange</td>
</tr>
<tr>
<td>SCX</td>
<td>Strong cation-exchange</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>S/N</td>
<td>Signal-to-noise ratio</td>
</tr>
<tr>
<td>SPE</td>
<td>Solid-phase extraction</td>
</tr>
<tr>
<td>SPME</td>
<td>Solid-phase microextraction</td>
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<tr>
<td>T</td>
<td>O-mustard or bis(2-chloroethylthioethyl)ether</td>
</tr>
<tr>
<td>TBDMS</td>
<td>tert.-butyldimethylsilyl derivative</td>
</tr>
<tr>
<td>TDG</td>
<td>Thiodiglycol</td>
</tr>
<tr>
<td>TDGS</td>
<td>Thiodiglycol sulfoxide</td>
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<td>TDGSO</td>
<td>Thiodiglycol sulfone</td>
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<td>Triethylamine</td>
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<tr>
<td>TEA</td>
<td>Triethanolamine</td>
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<td>TEOS</td>
<td>Tetraethoxysilane</td>
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<td>TFA</td>
<td>Trifluoroacetic acid</td>
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<tr>
<td>TMCS</td>
<td>Trimethylchlorosilane</td>
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<tr>
<td>TMS</td>
<td>Trimethylsilyl derivative</td>
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<tr>
<td>TOH</td>
<td>Bis(2-hydroxyethylthioethyl)ether</td>
</tr>
<tr>
<td>TPP</td>
<td>Tripropyl phosphate</td>
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<tr>
<td>VERIFIN</td>
<td>Finnish Institute for Verification of the Chemical Weapons Convention</td>
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<td>VX</td>
<td>O-Ethyl S-2-diisopropylaminoethyl methylphosphonothiolate</td>
</tr>
<tr>
<td>w/v</td>
<td>weight per volume</td>
</tr>
<tr>
<td>3Q</td>
<td>3-Quinuclidinol</td>
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</table>
1 INTRODUCTION

1.1 The Chemical Weapons Convention

The Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and on Their Destruction, also known as the Chemical Weapons Convention (CWC), was opened for signature in Paris, France on 13 January 1993. The Convention had been the subject of nearly twenty years of negotiation with the aim to finalize an international treaty banning chemical weapons, and designed to ensure their worldwide elimination.

The CWC entered into force on 29 April 1997. Today, there are 184 State Parties with an additional 4 Signatory States that have signed the CWC. A State Party is one that has signed and ratified or acceded to the CWC and for which the initial 30-day period has passed (the CWC enters into force for a State only 30 days after its ratification or accession to the treaty) whereas a Signatory State is one that signed the CWC prior to its entry into force in 1997 but has yet to deposit its instrument of ratification with the United Nations in New York. Only 7 Non-Signatory States world-wide have not taken any action on the Convention. They are Angola, Democratic People's Republic of Korea, Egypt, Iraq, Lebanon, Somalia and Syrian Arab Republic. Singapore signed on 14 January 1993 and ratified on 21 May 1997 [1-4].

The Convention is unique because it is the first multilateral treaty to ban an entire category of weapons of mass destruction and to provide for the international verification of the destruction of these weapon stockpiles within stipulated deadlines. The Convention was also negotiated with the active participation of the global chemical industry, thus ensuring industry's on-going cooperation with the CWC's industrial verification regime. The Convention mandates the inspection of industrial
facilities to ensure that toxic chemicals are used exclusively for purposes not prohibited by the Convention [2].

For the purpose of implementing the CWC, several terms have been defined as follows. ‘Chemical Weapons’ refers to (a) toxic chemicals and their precursors, except where intended for purposes not prohibited under this Convention, as long as the types and quantities are consistent with such purposes; (b) munitions and devices, specifically designed to cause death or other harm through the toxic properties of those toxic chemicals specified in subparagraph (a), which would be released as a result of the employment of such munitions and devices; (c) any equipment specifically designed for use in connection with the employment of munitions and devices specified in (b). ‘Toxic Chemical’ refers to any chemical, which through its chemical action on life processes can cause death, temporary incapacitation, or permanent harm to humans or animals. This includes all such chemicals, regardless of their origin or their method of production, and regardless of whether they are produced in facilities, in munitions or elsewhere. ‘Precursor’ refers to any chemical reactant that takes part at any stage in the production, by whatever method, of a toxic chemical [5].

1.2 Chemicals Related To The Chemicals Weapons Convention

Besides the definitions, toxic chemicals and precursors, which have been identified for the application of verification measures, are grouped into lists known as Schedule 1, 2 and 3. The list of chemicals is tabulated in Appendix 1. Schedule 1 chemicals include those that have been or can be easily used as chemical weapons and which have very limited, if any, uses for peaceful purposes. These chemicals are subject to very stringent restrictions, including a ceiling on production of one ton per
annum per State Party, a ceiling on total possession at any given time of one ton per State Party, licensing requirements, and restrictions on transfers. These restrictions apply to the relatively few industrial facilities that use Schedule 1 chemicals. Some Schedule 1 chemicals are used as ingredients in pharmaceutical preparations or as diagnostics. The Schedule 1 chemical, saxitoxin, is used as a calibration standard in monitoring programs for paralytic shellfish poisoning, and is also used in neurological research. Ricin, another Schedule 1 chemical, has been employed as a biomedical research tool. Some Schedule 1 chemicals and/or their salts are used in medicine as anti-neoplastic agents. Other Schedule 1 chemicals are usually produced and used for protective purposes, such as for testing chemical weapons protective equipment and chemical agent alarms. Schedule 2 chemicals include those that are precursors to, or that in some cases can themselves be used as, chemical weapons agents, but have a number of other commercial uses (such as ingredients in resins, flame-retardants, additives, inks and dyes, insecticides, herbicides, lubricants and some raw materials for pharmaceutical products). For example, BZ (3-quinuclidinyl benzilate) is a neurotoxic chemical listed under Schedule 2, which is also an industrial intermediate in the manufacture of pharmaceuticals such as clindinium bromide. Thiodiglycol is both a mustard gas precursor as well as an ingredient in water-based inks, dyes and some resins. Another example is dimethyl methylphosphonate, a chemical related to certain nerve agent precursors that is used as a flame retardant in textiles and foamed plastic products. Schedule 3 chemicals include those that can be used to produce, or can be used as chemical weapons, but which are widely used for peaceful purposes (including plastics, resins, mining chemicals, petroleum refining fumigants, paints, coatings, anti-static agents and lubricants). Among the toxic chemicals listed under Schedule 3 are phosgene and hydrogen cyanide, which have been used as chemical
weapons, but are also utilized in the manufacture of polycarbonate resins and polyurethane plastics as well as certain agricultural chemicals. Triethanolamine, a precursor chemical for nitrogen mustard, is found in a variety of detergents (including shampoos, bubble baths and household cleaners) as well as being used in the desulfurization of fuel gas streams [2].

Based on their mode of action, that is, the route of penetration and their effect on the human body, chemical agents are commonly divided into several categories: nerve, blister, blood and choking agents [6,7]. The nerve agents such as Tabun, Sarin, Soman, VX, chlorosarin and chlorosoman are listed in Schedule 1. The blister agents, namely sulfur mustards, nitrogen mustards and Lewisites, are also listed in Schedule 1. The blood agents, for example hydrogen cyanide and cyanogen chloride, are listed in Schedule 3. Phosgene, is an example of a choking agent and is listed in Schedule 3.

The nerve agents, known as cholinesterase inhibitors, interfere with the central nervous system by reacting with the enzyme acetylcholinesterase and creating an excess of acetylcholine which affects the transmission of nerve impulses [8]. The classical symptoms of nerve agent poisoning includes difficulty in breathing, drooling and excessive sweating, vomiting, cramps, involuntary defecation and urination, twitching, jerking and staggering, headache, confusion, drowsiness, convulsion, coma, dimness of vision and pinpointing of the pupils [9]. Nerve agent poisoning may be treated with timely administration of antidotes such as atropine and diazepam.

The blister agents cause blistering of the skin and extreme irritation of the eyes and lungs. They can be very persistent in the environment. These chemicals cause incapacitation rather than death but can kill in large doses [10]. Some blister agents like Lewisite and phosgene oxime are immediately painful while mustard agents may cause little or no pain for as long as several hours after exposure. No effective medical
care exists for the treatment of mustard exposure and care is directed towards relieving the symptoms and preventing infections [8].

The blood agents are substances that block oxygen utilization or uptake from the blood, causing rapid damage to body tissues [9,11]. Symptoms are irritation of the eyes and respiratory tract, nausea, vomiting and difficulty in breathing. Death from poisoning follows quickly after inhalation of a lethal dose. The victim may recover quickly from a smaller dose without assistance [12].

The choking agents cause physical injury to the lungs through inhalation. Membranes may swell and lungs become filled with liquid, and in serious cases, the lack of oxygen causes death [8]. Phosgene and chlorine are classified as choking agents but in fact have several industrial uses as well.

Besides the above-mentioned major classes of chemical agents, there exist incapacitating agents such as vomiting, tearing and riot control agents. These are generally non-lethal agents that cause temporary physical or mental incapacitation rather than death. BZ is a hallucinating agent that produces similar effects to atropine such as changes in heart rate, confusion, disorientation, delusions and slurred speech. Tearing agents cause irritation to the eyes and skin. Some examples are chloroacetophenone, o-chlorobenzylidene malononitrile and dibenz-(b,f)-1,4-oxazepine, which are used as riot control agents. Vomiting agents cause nausea and vomiting and can also induce cough, headache, and nose and throat irritation. The vomiting agents are typically solids which when heated, vaporize and condense to form aerosols. Adamsite, an arsenic-containing chemical, is an example of a vomiting agent [9,10].

The chemical agents are usually not stable and when subjected to natural degradation in the environment or decontamination, a myriad of degradation products
arise through chemical processes such as hydrolysis, oxidation and elimination [13-18]. In cases where the parent agent no longer exists, verification of the presence of CWAs would most likely be based on the detection of the corresponding degradation products. Hence, the analysis of degradation products of CWAs is equally if not more important than that of the original substances. Table 1-1 lists the chemical agents and their corresponding degradation products investigated in this study.

<table>
<thead>
<tr>
<th>Chemical Agent</th>
<th>Degradation Product(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tabun (GA)</td>
<td>Not investigated</td>
</tr>
<tr>
<td>Sarin (GB)</td>
<td>Isopropyl methylphosphonic acid (IMPA)</td>
</tr>
<tr>
<td>Soman (GD)</td>
<td>Pinacolyl methylphosphonic acid (PMPA)</td>
</tr>
<tr>
<td>Cyclohexyl methylphosphonofluoridate (GF)</td>
<td>Cyclohexyl methylphosphonic acid (CMPA)</td>
</tr>
<tr>
<td>O-Ethyl S-2-diisopropylaminoethyl methyl phosphonothiolate (VX)</td>
<td>Ethyl methylphosphonic acid (EMPA)</td>
</tr>
<tr>
<td>2-(N,N-Diisopropylamino)ethanol (DIPAE)</td>
<td></td>
</tr>
<tr>
<td>Chemical Agent</td>
<td>Degradation Product(s)</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>---------------------------------------------</td>
</tr>
<tr>
<td>Sulfur mustard (HD)</td>
<td>Thiodiglycol (TDG)</td>
</tr>
<tr>
<td>Sesquimustard (Q)</td>
<td>1,2-Bis(2-hydroxyethylthio)ethane (QOH)</td>
</tr>
<tr>
<td>O-mustard (T)</td>
<td>Bis(2-hydroxyethylthioethyl)ether (TOH)</td>
</tr>
<tr>
<td>Bis(2-chloroethyl)ethylamine (HN1)</td>
<td>N-ethyldiethanolamine (EDEA)</td>
</tr>
<tr>
<td>Bis(2-chloroethyl)methylamine (HN2)</td>
<td>N-methyldiethanolamine (MDEA)</td>
</tr>
<tr>
<td>Tris(2-chloroethyl)amine (HN3)</td>
<td>Triethanolamine (TEA)</td>
</tr>
<tr>
<td>2-Chlorovinylidichloroarsine (L1)</td>
<td>2-Chlorovinylarsonous acid (CVAA)</td>
</tr>
<tr>
<td>Bis(2-chlorovinyl)chloroarsine (L2)</td>
<td>Bis(2-chlorovinyl)arsonous acid (BCVAA)</td>
</tr>
<tr>
<td>Tris(2-chlorovinyl)arsine (L3)</td>
<td>Not investigated</td>
</tr>
</tbody>
</table>
1.3 The Organization for the Prohibition of Chemical Weapons (OPCW)

The Chemical Weapons Convention mandated the Organization for the Prohibition of Chemical Weapons (OPCW), an independent, international organization based in The Hague, The Netherlands, to achieve the object and purpose of the Convention, to ensure the implementation of its provisions, including those for international verification of compliance with it, and to form a forum for consultation and cooperation among State Parties. Among the numerous roles of the OPCW, a complex verification regime is in place in order to ensure steps are taken towards meeting the objectives of the Convention. On-site inspections and data monitoring are conducted to ensure that activities within State Parties are consistent with the objectives of the Convention and the contents of declarations submitted to the OPCW. There are three types of inspections: routine inspections of chemical weapons-related facilities and chemical industry facilities using certain ‘dual-use’ chemicals; short-notice challenge inspections which can be conducted at any location in any State Party about which another State Party has concerns regarding non-compliance and finally investigations of alleged use of chemical weapons [19].

During these inspections, sampling and on-site analysis may be undertaken to check for the absence of undeclared scheduled chemicals. In cases of unresolved
ambiguities, samples may be sent to an off-site laboratory, subject to the inspected State Party's agreement [5]. This off-site laboratory will be selected among several OPCW designated laboratories. The designation of laboratories is determined through their performance in the Official OPCW Proficiency Tests.

1.4 The Official OPCW Proficiency Tests

The OPCW proficiency testing scheme was set up with the objective to simulate sample analysis in order to select laboratories that are capable of performing trace analysis (at parts per million levels) of chemicals scheduled under the CWC and/or their degradation products in a wide variety of matrices and of providing the OPCW with a detailed report on the analysis results that contains analytical proof of the presence of chemicals reported and provides high certainty of the absence of other chemicals relevant for the implementation of the CWC and does not contain information on chemicals not relevant to the CWC. Prior to the Official OPCW Proficiency Tests, there were four international inter-laboratory comparison tests, also known as round-robin tests, for laboratories to test the effectiveness of their procedures for the recovery of CWC-related chemicals and their precursors and degradation products from various sample matrices [20-23]. Thereafter, an additional inter-laboratory comparison test [24] was conducted to further test the recommended operating procedures [25] developed at the Finnish Institute for Verification of the Chemical Weapons Convention (VERIFIN). Before the 1st Official OPCW Proficiency Test in May 1996, two trial proficiency tests were held to train laboratories and to establish procedures for the conduct of this first official test [26].

A laboratory may participate in the official proficiency tests as a regular participant, whereby the laboratory is given fifteen calendar days to analyze the
samples and submit an analysis report to the OPCW [27]. Alternatively, a laboratory may assist in one of two roles, that of the sample preparation laboratory or the evaluating laboratory.

The sample preparation laboratory is tasked with formulating the composition of test samples according to a test scenario, performing stability studies to ensure the stability of spiking chemicals in the matrices, preparing the test samples as well as dispatching a set to each of the participating laboratories in addition to two sets each to the evaluating laboratory and the OPCW Laboratory. Thereafter, the sample preparation laboratory proceeds to perform stability studies starting on the dispatch date until the test period for all participants have expired. A sample preparation report is submitted to the OPCW Laboratory within two weeks after the stability studies have been completed. In addition, the sample preparation laboratory assists in the categorization of the test chemicals and participates in the meeting held at the OPCW Headquarters in The Hague to discuss the preliminary evaluation results with test participants [28].

On the other hand, the evaluating laboratory is tasked with analyzing the samples using at least two different analytical techniques, at least one of which must be a spectrometric technique, to identify the test chemicals. Thereafter, the evaluating laboratory submits a sample analysis report to the OPCW Laboratory within twenty eight days upon receipt of the samples. Upon receipt of copies of the test reports from participating laboratories (whereby pages identifying respective laboratories have been removed by the OPCW Laboratory), the evaluating laboratory performs a detailed evaluation of the reports and also assists in the categorization of the test chemicals. A draft preliminary evaluation report will be sent to the OPCW Laboratory within twenty eight days upon receipt of the complete set of copies of all participants’
reports. After discussion with the OPCW test coordinator on the draft preliminary evaluation report, a preliminary evaluation report would be submitted to the OPCW Laboratory within a week. The evaluating laboratory participates in the meeting held at the OPCW Headquarters in The Hague to discuss the preliminary evaluation results with test participants. Laboratories are allowed to submit comments on the preliminary evaluation results in writing to the OPCW Laboratory within fourteen calendar days following the preliminary evaluation meeting. If there are comments to the preliminary evaluation results, the evaluating laboratory will conduct a re-evaluation of the results affected by the comments, make corrections to the report and submit the final evaluation report to the OPCW Laboratory within one week following the receipt of the comments [29].

All participating laboratories that take part in an OPCW proficiency test will be awarded a performance rating according to the extent to which the laboratory fulfils the performance criteria as shown in Table 1-2. The sample preparation and evaluating laboratories will be awarded the maximum performance rating of A provided the test samples meet the required criteria and the evaluation of results is performed satisfactorily and within set time lines.

**Table 1-2.** Method of evaluating performance rating [27].

<table>
<thead>
<tr>
<th>Performance criteria fulfilled</th>
<th>Identification of chemicals</th>
<th>Performance scoring</th>
<th>Performance rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>Laboratory identifies all chemicals</td>
<td>Maximum possible score</td>
<td>A</td>
</tr>
<tr>
<td>Yes</td>
<td>Laboratory identifies all chemicals except one</td>
<td>Maximum possible score minus two</td>
<td>B</td>
</tr>
<tr>
<td>Yes</td>
<td>Laboratory identifies more than half of the chemicals</td>
<td>Score between zero and (maximum possible minus two)</td>
<td>C</td>
</tr>
<tr>
<td>Yes</td>
<td>Laboratory misses more chemicals than it identifies</td>
<td>Negative score</td>
<td>D</td>
</tr>
<tr>
<td>No</td>
<td>-</td>
<td>No score</td>
<td>Failure</td>
</tr>
</tbody>
</table>
The proficiency tests are a means for OPCW to assess laboratories in their technical competence and for certifying laboratories that are seeking designation or retention of designation status. To obtain designation, a laboratory should have established a quality system and have a valid accreditation by an internationally recognized accreditation body for the analysis of chemical warfare agents and related compounds in various types of samples. In addition, a laboratory must obtain a rating of three As or two As and a B in three consecutive proficiency tests in order for designation. To retain the designation status, a laboratory must participate in the proficiency tests at least once per calendar year and maintain the rating of three As or two As and a B. Otherwise, it may be temporarily suspended or withdrawn in cases where there is a substantial change in its accreditation status or deterioration in performance in any proficiency test.

Since 1996 to 2007, a total of twenty two official OPCW proficiency tests have been conducted. As of the 23rd Official OPCW Proficiency Test, there are twenty designated laboratories worldwide, namely Belgium, China (two laboratories), Czech Republic, Finland, France, Germany, India (two laboratories), The Netherlands, Poland, Republic of Korea, the Russian Federation, Singapore, Spain, Sweden, Switzerland, the United Kingdom and the United States of America (two laboratories) [30]. Singapore's participation in the official proficiency tests is undertaken by the Verification Laboratory of DSO National Laboratories. The laboratory has been actively taking part since the 2nd Official Proficiency Test and obtained the designation status in 2003 after the 10th, 11th and 12th Official OPCW Proficiency Tests. The laboratory has assisted as a sample preparation laboratory in the 14th Official OPCW Proficiency Test and as an evaluating laboratory in the 20th Official OPCW Proficiency Test.
1.5 **Recommended Operating Procedures**

The Recommended Operating Procedures (ROPs) for Sampling and Analysis in the Verification of Chemical Disarmament were proposed by VERIFIN and were subsequently tested and improved upon through the round-robin tests. In all these tests and in later proficiency tests, these ROPs have been widely and successfully applied [5].

The ROPs provide instructions on sampling, sample collection, packing and handling of samples as well as sample preparation, that is, sample treatment of various matrices, including air samples, soil, wipe (swab of solid surfaces using cotton buds, filter paper or glass filter beds), active charcoal, aqueous liquid, concrete, paint, rubber and other polymeric samples followed by analysis using various instrumental techniques such as gas chromatography (GC), gas chromatography-mass spectrometry (GC–MS), liquid chromatography-mass spectrometry (LC–MS) and nuclear magnetic resonance (NMR) spectrometry. The ROPs on sample treatment are essential for the determination of analytes of interest in samples since very often some form of sample treatment is required in order to extract and/or pre-concentrate the analytes of interest prior to instrumental analysis. The ROPs are largely based on solvent extraction and solid-phase extraction (SPE).

1.6 **Solvent Extraction**

Solvent extraction, in this context of sample treatment, is taken to include both liquid-liquid extraction (LLE) as well as extraction from solid matrices. Solvent extraction is often used interchangeably with LLE or liquid-liquid distribution, the term recommended by The International Union of Pure and Applied Chemistry (IUPAC). LLE involves the distribution of a solute between two immiscible liquid
phases in contact with each other. The solute, initially dissolved in only one of the two liquids, eventually distributes between the two phases when equilibrium is reached. LLE commonly takes place with an aqueous solution as one phase and an organic solvent as the other. Solvent extraction can facilitate the isolation of analyte(s) from the major component (matrix) and/or the separation of the particular analyte from concomitant trace or minor elements [31]. LLE has been proven to be an attractive method of concentrating various organic compounds from aqueous matrices [32]. On the other hand, solvent extraction can also be applied to the extraction of solutes from solid materials such as soils, sludges and sediments [33].

With regards to the ROPs for sample treatment of matrices for the analysis of CWC-related chemicals, LLE of aqueous liquid samples is performed using dichloromethane as the organic solvent in the first and second extractions for neutral and basic analytes respectively while a strong cation-exchange cartridge is used to recover polar acidic analytes in the third extraction. For the solvent extraction of soil samples, dichloromethane is used in the first extraction in order to extract any non-polar CWC-related chemicals while distilled, deionized water is used in the second extraction for polar analytes followed by the use of 1% triethylamine in methanol in the third extraction to recover basic CWC-related chemicals. Acetone or dichloromethane can be used in the first extraction of concrete and polymeric samples, followed by distilled, deionized water in the second extraction. For the solvent extraction of wipe samples, several non-polar organic solvents such as acetone, ethyl acetate, dichloromethane or deuterated chloroform (for subsequent NMR analysis) are recommended for the first extraction while polar solvents such as acetonitrile, methanol or water can be used in the second extraction. Suitable solvents
for the solvent extraction of active charcoal samples are acetone, dichloromethane, carbon disulfide and deuterated chloroform (for subsequent NMR analysis) [5,25].

1.7 Solid-Phase Extraction

SPE is a form of step-wise chromatography designed to extract, partition, and/or adsorb one or more components from a liquid phase (sample) onto a stationary phase (sorbent or resin) [34]. Prior to the loading of the sample, the SPE sorbent is first wetted and conditioned with solvents. The sorbent can be in the form of pre-packed cartridges, columns or disks onto which analytes of interest are trapped. A washing step is performed to remove contaminants trapped on the sorbent without influencing the elution of the analytes of interest. Finally, elution with a suitable solvent is carried out to recover the analytes of interest [35]. In this way, SPE may serve to achieve concentration of the analytes, sample clean-up by removal of interferences as well as sample matrix removal or solvent exchange into a form compatible with instrumental analysis [36]. With SPE, many of the problems associated with LLE, such as incomplete phase separations, less-than-quantitative recoveries, use of expensive, breakable specialty glassware, and disposal of large quantities of organic solvents, can be prevented. SPE is more efficient than LLE, yields quantitative extractions that are easy to perform, is rapid, and can be automated. Solvent use and laboratory time are reduced [37].

SPE is indeed an established sample treatment method [38] and has been widely utilized in a variety of applications [36,37,39-49]. Besides, SPE has been shown to be useful in the analysis of CWC-related chemicals in organic [26,50], aqueous liquid [51-53], soil [54-56] and biological [57-61] matrices. The SPE cartridges investigated included silica, C18, strong cation-exchange, strong anion-
exchange, quaternary amine and hydrophilic-lipophilic balance (HLB) cartridges. In fact, SPE can be used in place of LLE in the ROPs for sample treatment of aqueous samples for the analysis of CWC-related chemicals [62].

1.8 Motivation of the Project

The objective of the project is to improve on current extraction techniques for the analysis of CWC-related chemicals through the development of solvent-minimized extraction techniques. The current ROPs mainly utilize solvent extraction which requires large volumes of organic solvents in addition to substantial amounts of samples, typically 5 ml of aqueous samples or 5 g of soil samples or more. Furthermore, the entire procedure is time-consuming and labor-intensive. Besides, solvent extraction may not be specific such that analytes of interest are extracted together with contaminants and interfering chemicals. To address these issues, two approaches were undertaken.

The first approach involved an attempt to develop solid-phase microextraction (SPME) fibers coated with molecularly-imprinted polymers (MIPs) synthesized via the sol-gel route to address the issues of selectivity and analysis time. To date, there have been hardly any reports on sol-gel MIP SPME fibers. At the same time, another novel SPME coating based on a poly(paraphenylene) polymer was investigated. The second approach utilized hollow fiber-protected liquid-phase microextraction (HF-LPME) for the determination of various chemical warfare agents and their degradation products. This approach allows the miniaturization of the extraction procedure in terms of reducing the amount of sample, extracting solvents and glassware required during sample treatment as well as shortening the entire sample treatment process.
2 DEVELOPMENT OF NOVEL SOLVENT-MINIMIZED EXTRACTION TECHNIQUES

2.1 Solid-Phase Microextraction

SPME is a solvent-free sample preparation technique [63]. Since its introduction [64], SPME has been extensively investigated for a wide range of applications [65-81]. In SPME, a 1 cm length of fused silica fiber, coated with a polymer, is installed in a holder that looks like a modified microliter syringe. There is also a stainless steel needle that the fiber can be withdrawn into to protect it. The plunger moves the fused silica fiber into and out of the hollow needle. To use the unit, the analyst draws the fiber into the needle, passes the needle through the septum that seals the sample vial, and depresses the plunger, exposing the fiber to the sample or the headspace above the sample. Organic analytes absorb into/adsorb onto the coating on the fiber. After adsorption equilibrium is attained, usually in 2 to 30 minutes, the fiber is drawn into the needle, and the needle is withdrawn from the sample vial. Finally, the needle is introduced into the GC injector, where the adsorbed analytes are thermally desorbed and delivered to the GC column, or into the SPME/HPLC interface [82]. The SPME process is represented in Figure 2-1.

Figure 2-1. The extraction and desorption procedures in SPME [82].
SPME fibers are commercially available in various polymeric coatings and thickness for both GC and LC applications. Commercially-available coatings include polydimethylsiloxane (PDMS), carboxen/polydimethylsiloxane (CAR/PDMS), polydimethylsiloxane/divinylbenzene (PDMS/DVB), polyacrylate (PA), carbowax/divinylbenzene (CW/DVB), polyethylene glycol (PEG) and divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) [83].

PDMS and PA fibers are absorptive fibers whereby analytes are extracted by partitioning onto the liquid phase and are retained by the thickness of the coating. PDMS/DVB, CW/DVB, CAR/PDMS and DVB/CAR/PDMS fibers are adsorptive fibers which physically trap or chemically react and bond with analytes owing to their porous nature and high surface area. The relatively new PEG fibers do not contain an adsorbent polymer and are meant to replace the CW/DVB fibers [84]. In order to select the best fiber for the target analyte, several parameters such as the molecular weight and polarity of the analyte, the polarity and extraction mechanism of the fiber, minimum detection limit and the linear range requirements have to be considered [85,86].

A variety of commercial SPME coatings have been evaluated for the analysis of chemical warfare agents and degradation compounds [87-96]. The coatings that have been evaluated are 100 µm PDMS, 85 µm PA, 65 µm PDMS/DVB, 65 µm CW/DVB and 75 µm CAR/PDMS. Besides, a novel phenol-based polymer coating, consisting of hydrogen bond acidic hexafluorobisphenol groups alternating with oligo(dimethylsiloxane) segments, was designed for headspace SPME of Sarin [97].
2.1.1 Sol-gel SPME Fibers

Besides using commercial SPME fibers, SPME coatings made of silica-based polymers can also be fabricated through a simple procedure known as the sol-gel process. The sol-gel process refers to the preparation of ceramic materials by preparation of a sol, gelation of the sol and removal of the solvent [98]. A sol is a fluid, colloidal dispersion of solid particles in a liquid phase where the particles are sufficiently small to remain suspended by Brownian motion. A gel is a solid consisting of at least two phases wherein a solid phase forms a network that entraps and immobilizes a liquid phase [99,100]. The sol-gel process involves mild reaction conditions such that molecules, particularly those which are water soluble, may be readily introduced within a highly crosslinked porous host without problems of thermal or chemical decomposition. Materials in various configurations (for example, films, fibers, monoliths and powders) can be prepared easily. Specific organic functional groups can be combined with the inorganic precursor to introduce specific chemical functionalities into the framework and improve molecular selectivity and specificity. Furthermore, the materials are stable and the sol-gel processing conditions can be varied to control the porosity and surface area of the resultant material [101].

The starting materials in the preparation of sol-gel materials are typically inorganic metal salts or metal alkoxides (M(OR)_n) such as tetramethoxysilane (TMOS) or tetraethoxysilane (TEOS). A general sol-gel route is as follows:

Hydrolysis:  \[ \text{M(OR)}_n + x\text{H}_2\text{O} \rightarrow \text{M(OR)}_{n-x}(\text{OH})_x + x\text{ROH} \]

Condensation: \[ \text{M(OR)}_3(\text{OH}) + \text{M(OR)}_3(\text{OH}) \rightarrow (\text{RO})_3\text{M-O-M(OR)}_3 + \text{H}_2\text{O} \]

or \[ \text{M(OR)}_3(\text{OH}) + \text{M(OR)}_3(\text{OH}) \rightarrow (\text{RO})_3\text{M-O-M(OR)}_2(\text{OH}) + \text{ROH} \]
At the end, every oxygen is bridging and hence a pure and highly homogeneous oxide network is obtained [102]. Through the sol-gel process, materials can be fabricated in many forms, such as thin films, membranes, powders, dense ceramics and fibers. Useful applications of sol-gel materials include optical materials, chemical sensors, catalysts, coatings, membranes, electronic materials and chromatographic supports [103-109].

Several research groups are actively working on the area of sol-gel SPME fibers because of the advantages of sol-gel SPME fibers over commercially-available fibers. These advantages include enhanced thermal stability, solvent stability and the ease with which inorganic or organic components can be incorporated into the polymer framework. The enhanced thermal and solvent stability arises from the fact that sol-gel coatings are chemically bonded to the fused silica backbone. In contrast, most of the coatings of commercial fibers are physically deposited onto the fused silica surface [110,111]. Another significant advantage is the fact that sol-gel coatings contain polar moieties like silanol groups, resulting in the ability of seemingly non-polar coatings such as PDMS or C_{11}-PDMS to extract both polar and non-polar analytes [112].

The pioneering work of Malik and co-workers demonstrated that sol-gel PDMS coatings were capable of extracting both polar analytes such as dimethylphenols, long chain alcohols and anilines as well as nonpolar analytes such as polycyclic aromatic hydrocarbons (PAHs) and alkanes [113]. In contrast, conventionally-coated PDMS fibers do not show sufficient selectivity for polar compounds. The sol-gel PDMS fibers exhibited higher thermal stability compared to conventionally-coated PDMS fibers. The sol-gel fibers can be routinely used at 320°C and higher without any signs of bleeding. Enhanced thermal stability of sol-gel-coated
fibers allowed the sample carryover problem, often encountered in SPME of polar solutes with conventional PDMS fibers, to be overcome. Sol-gel coatings possess a porous structure and reduced coating thickness that provide enhanced extraction and mass transfer rates in SPME. High-temperature conditioning of sol-gel-coated PDMS fibers led to consistent improvement in peak area repeatability for SPME-GC analysis. Peak area relative standard deviation values of <1% was obtained for PAHs and dimethylphenols on sol-gel PDMS fibers conditioned at 320°C.

Besides conventional PDMS [114-116], PDMS/DVB [117] and PEG [111, 118-119] coatings, novel sol-gel coatings have been developed and evaluated. Oligomers [120], novel polymers [121-131], fullerol [132], acrylates [133-136], crown ethers [137-144], calix[4]arenes [145-151], cyclodextrins [152-154], hybrid organic-inorganic materials [155,156], silica particles [157] and carbon [158,159] have been incorporated as fiber coatings on SPME fibers. The fibers showed improved thermal and chemical stability as well as good extraction efficiency of target analytes.

Zeng and co-workers have published numerous papers of their work on sol-gel SPME fibers. Of special interest in the analysis of chemical warfare agents and related compounds is the development and evaluation of sol-gel PDMS/DVB for the extraction of trimethylphosphate, tributylphosphate and dimethyl methylphosphonate (a simulant of Sarin) [117] as well as PDMS coatings with acrylate components for the extraction of 2-chloroethyl ethyl sulfide (CEES), a simulant of HD [134]. The authors showed that the performance of the sol-gel PDMS/DVB fiber surpassed that of commercially-available fibers such as 100 μm PDMS, PA and PDMS/DVB for the extraction of the phosphates and phosphonates from air and water samples. In the other study, a comparison of acrylate components added to the sol mixture was made
and it was found that butyl methacrylate (BMA) gave the best results as compared to methyl acrylate or methyl methacrylate. The sol-gel PDMS/BMA fiber surpassed that of commercial PA for the extraction of CEES from soil.

### 2.1.2 Molecularly Imprinted Polymers for SPME Fibers

One drawback of current SPME coatings is that, other than the target analytes, the fibers may absorb/adsorb other compounds present in the matrix, which would possibly interfere with the analysis. One way of introducing selectivity to SPME fibers is through the use of MIPs as fiber coatings.

#### 2.1.2.1 Molecular Imprinting

Molecular imprinting is a technique used for preparing polymers with synthetic recognition sites having a predetermined selectivity for the analyte(s) of interest. The imprinted polymer is obtained by arranging polymerizable functional monomers around a template (target analyte). Complexes are then formed through molecular interactions between the analyte and the monomer precursors. These interactions can either be non-covalent bonds, for example, ionic bonds and hydrogen bonds, or reversible covalent bonds, for example, through boronic esters. Figure 2-2 depicts examples of the various interactions which may be employed in molecular imprinting [160]. The complexes are assembled in the liquid phase and fixed by cross-linking polymerization. Removal of the template from the resulting polymer matrix creates vacant recognition sites that exhibit affinity for the analyte [161]. The concept of molecular imprinting is illustrated in Figure 2-3.
Figure 2-2. Types of binding interactions that can be exploited during templating: (a) $\pi-\pi$ interaction; (b) hydrophobic or van der Waals interaction; (c) covalent bonds; (d) (transition) metal-ligand binding; (e) hydrogen binding; (f) “crown ether”-ion interaction; (g) ionic interaction [160].

Figure 2-3. Illustration of the concept of molecular imprinting [162].

Besides the specificity of MIPs, the other attractive features of MIPs are that they are easy to prepare in different configurations such as block polymers, particles, films or membranes and fibers, physically and chemically stable and reusable without loss of the imprinting effect [163-165]. Interest in molecular imprinting technology has grown at a phenomenal rate in recent years as seen from the number of original publications (Figure 2-4) and is being extensively investigated for applications in separations [167-196], sensors [198-202] and in synthesis and catalysis [203-208].
Several research groups have reported good results on the analysis of chemical warfare agents and their degradation products using MIPs. The techniques of molecular imprinting and sensitized lanthanide luminescence were combined to create the basis for sensors that can selectively measure pinacolyl methylphosphonic acid (PMPA), the hydrolysis product of the nerve agent, Soman, in water [209-213]. The sensor functions by selectively and reversibly binding PMPA to a functionality-imprinted copolymer possessing a coordinatively-bound luminescent lanthanide ion, Eu$^{3+}$. The MIP is formed by cross-linking styrene with divinylbenzene and templated for PMPA for the detection of PMPA and isopropyl methylphosphonic acid (IMPA), the hydrolysis products of Soman and Sarin respectively. This is feasible since the polymer-bound functional end of PMPA is the same for both PMPA and IMPA. The sensor is made from a fiber optic probe utilizing a luminescent europium complex. The use of lanthanide ions as spectroscopic probes of structure and content is an established technique. The narrow excitation and emission peaks of lanthanide spectra, typically in the order of 0.01-1 nm full width at half maximum, provide for the sensitive and selective analyses. Lanthanide complexes exhibit long luminescent
lifetimes and are intensely luminescent when complexed by appropriate ligands. Proper ligand choice, used both to immobilize the lanthanide probe and provide the enhancements needed for trace analysis, has been shown to provide limits of detection in parts per trillion or lower [214]. The device has been constructed using europium as the probe ion since its luminescence spectrum is least complex. Detection of the nerve agent is based upon changes that occur in the spectrum when the hydrolysis product is coordinated to Eu$^{3+}$. This is seen from the presence of a peak at 610 nm in the laser-excited luminescence spectrum of Eu(DMMB)$_3$(NO$_3$)$_2$(PMPA) as compared to that of Eu(DMMB)$_3$(NO$_3$)$_3$, where the peak is absent. DMMB refers to the ligand, methyl-3,5-dimethylbenzoate, which can be converted into polymerizable methyl-3,5-divinylbenzoate, providing an avenue for self-crosslinking. In addition, the sensor was evaluated for its physical properties such as luminescence properties, lifetime, response time and pH dependence. The effect of interferences was also investigated. The combination of molecular imprinting and luminescence detection provides multiple criteria of selectivity to virtually eliminate the possibilities of false positive readings. The sensor can be used in detecting the presence of chemical agents or pollutants near battlefields, in hospitals or military installations, or in community water supplies. Investigations were further extended to the imprinting of nerve agents in which the sensors were evaluated against the presence of nerve agents in various types of water such as tap, reverse osmosis, and deionized water [215].

In a study of MIP-SPE for the determination of degradation products of nerve agents (Figure 2-5) in human serum [216], the absorptivities of several degradation products of nerve agents, namely pinacolyl methylphosphonic acid (PMPA, degradation product of Soman), ethyl methylphosphonic acid (EMPA, degradation product of VX), isopropyl methylphosphonic acid (IMPA, degradation product of
Sarin), cyclohexyl methylphosphonic acid (CMPA, degradation product of GF), isobutyl methylphosphonic acid (BMPA, degradation product of Russian VX) and methylphosphonic acid (MPA, the final degradation product of all nerve agents) on MIPs imprinted with PMPA, EMPA and MPA were investigated. It was shown that the MIPs showed cross-selectivity as they could recognize not only the print molecule but also the degradation products of the other nerve agents because the degradation products of all nerve agents differ only in the alkyl chain of the phosphonate esters. On the other hand, the non-imprinted polymer (NIP) showed no absorptivity. The NIP is a polymer synthesized under the same conditions as the MIP but in the absence of the template. This is believed to have arisen from the imprinting effect. It was demonstrated that the cross-selectivity of the PMPA-MIP enabled the extraction of the possible degradation products of all the nerve agents from human serum with extraction recoveries of up to 90.5%. The interfering components for the capillary electrophoresis analysis were successfully removed. Detection limits of 0.1 μg ml\(^{-1}\) and relative standard deviations of <9% were obtained.

![Structures of the nerve agent degradation products](image.png)

**Figure 2-5.** The structures of the nerve agent degradation products investigated [216].

In a separate study [217], a PMPA-imprinted MIP (PMPA-MIP) was investigated for the analysis of EMPA using solid phase extraction. Two acrylate-based MIPs were prepared using methacrylic acid (MAA) as a monomer and ethylene
glycol dimethacrylate (EGDMA) or trimethylolpropane trimethacrylate (TRIM) as cross-linkers in dichloromethane and acetonitrile respectively. It was found that both the MIPs as well as the NIP showed absorptivity of the analyte. This was in contrast to the results of Meng and co-workers [216] where the NIP did not show any absorptivity of the analytes. In order to achieve a difference in the recovery between the MIP and the NIP, a washing step using 10 ml of acetonitrile:methanol (95:5) was performed. With this step, the MIP gave 87% recovery of EMPA whereas the NIP gave 34% upon elution with water. The MIP was next evaluated for the sample cleanup of soil spiked with IMPA and CMPA. An Oasis hydrophilic-lipophilic balance cartridge was selected to be used prior to the MIP cartridge, resulting in sample cleanup and 95% recovery of the analytes.

A plastic antibody, that is, an MIP for the specific recognition of sulfur mustard was fabricated using MAA and EGDMA [218]. The uptake of the plastic antibody was compared against that of the NIP and the imprinting efficiency was found to be 1.3. The imprinting efficiency is defined as the ratio of the binding ratio of the MIP to the NIP, where the binding ratio is the ratio of the adsorbed analyte to that remaining in solution. The plastic antibody did not experience interference from a stimulant, dichlorodiethyl ether. The same research group further designed an MIP-based potentiometric sensor for the specific recognition of MPA, the final degradation product of nerve agents [219]. The sensor was fabricated from MAA and EGDMA particles, dispersed in 2-nitrophenyloctyl ether followed by embedding in polyvinyl chloride matrix to form a polymer membrane. The polymer membrane sensor can be used for the analysis of MPA in natural waters in the presence of interfering compounds such as phosphoric acids.
In a different approach [220], covalent imprinting was carried out first by reacting MAA and EGDMA with 3,3-dimethylbutan-2-yl-4-vinylphenyl methylphosphonate, a vinylphenol template carrying functional groups of PMPA. This was followed by hydrolysis of the template using caesium fluoride. The binding affinity of the MIP was studied by colorimetric detection methods where the imprinting efficiency was determined to be 2.4.

Another interesting approach involved the introduction of a strong nucleophile (hydroxamic acid) into the MIP to efficiently attack the phosphorus-fluoride bond of organophosphonate nerve agents [221]. The proof-of-concept study showed that the MIPs synthesized to be specific for Sarin and Soman, led to accelerated hydrolysis of corresponding \( \rho \)-nitrophenyl substrates as compared to simultaneous hydrolysis in buffer. Further studies on actual nerve agents are currently underway.

2.1.2.2 Sol-Gel Molecularly Imprinted Polymers

Besides acrylate polymers, sol-gel MIP materials produced from silane monomers, have also been extensively investigated for applications mainly in separations and sensors [222-247]. Of particular interest is the work by Marx et al. since some of the analytes of interest studied were organophosphorus pesticides. In a study on the detection of organophosphate pesticides using thin film sol-gel MIPs [248], it was shown that the parathion-imprinted sol-gel films were highly selective for the template molecule and not for very closely similar analytes such as methyl-parathion, paraoxon, triethylphosphate and fenitrothion (Figure 2-6). In addition, the sol-gel MIP exhibited selective binding of the analyte even in an aqueous matrix. The sol-gel MIP films were further investigated for specific binding of analytes in the gas phase [249].
Direct comparisons between acrylic-based MIPs and sol-gel MIPs were made [231]. It was demonstrated that the thin film sol-gel MIPs, imprinted using propranolol as a template, possess superior properties over the acrylic MIPs in terms of selectivity for propanolol as compared to the corresponding NIPs. Even though the sol-gel system had a lower capacity for binding, the non-specific binding was lower than the acrylic system. In another study [250], acrylic and sol-gel MIPs imprinted for 2-aminopyridine were compared in terms of specificity and selectivity. Specificity was defined as the success of the imprinting process as seen from the difference in binding between the MIP and the NIP while selectivity was defined as the efficiency in binding structural analogues of the template. It was found that the sol-gel MIP showed a higher degree of specificity over the NIP in a polar solvent as compared to the acrylic polymers. In terms of selectivity, the selectivity of the polymers for the template over its structural analogues could be improved upon.
The use of sol-gel MIPs for the analysis of chemical warfare agents and degradation products is in fact fairly limited. The work by Markowitz et al. is of note where silica particles were surface-imprinted with PMPA, the degradation product of Soman [251]. The particle surfaces were imprinted during particle formation by adding PMPA to a microemulsion. The particles were functionalized with the addition of organotrialkoxysilanes such as N-trimethoxysilylpropyl-N,N,N-trimethyl ammonium chloride, 2-(trimethoxysilyl ethyl)pyridine or N-(3-triethoxysilyl)-4,5-dihydroimidazole and the particle size, surface area, adsorption properties and binding affinity of organophosphate compounds were studied. It was found that surface-imprinted quaternary amine, 2-ethylpyridine- and dihydroimidazole-functionalized silicates had a significantly higher degree of specificity for PMPA than for structurally similar organophosphates. The binding properties were conducted using 2-propanol as a solvent. Another study focused on the recognition of MPA, the final degradation product of nerve agents, using surface imprinting whereby the surface of an indium tin oxide electrode was modified with octadecylsiloxane [252]. High specificity, selectivity, stability and speed were demonstrated for this potentiometric chemosensor.

2.1.2.3 Current Status

Thus far, reports on the use of MIPs as SPME fiber coatings have been scarce. The first reports on MIPs as SPME adsorbents were published in 2001. The first study [253] involved the use of propranolol-MIP particles for use as a capillary column material for the determination of propranolol in serum samples by in-tube SPME. Another study [254], which is also the first report on MIPs as SPME fiber coatings, showed the feasibility of combining the selectivity of MIPs with the simplicity of
SPME. Silica fibers were coated with clenbuterol-imprinted polymers which were subsequently used for extraction of clenbuterol from biological samples. Bromobuterol (Figure 2-7), which is a structural analogue of the template, clenbuterol, and is baseline separated in the liquid chromatography system, was used to study the extraction and desorption characteristics of the MIP-coated fibers. Selectivity of the extraction with these fibers was evaluated by comparison with fibers coated with a NIP in order to investigate the non-selective interactions of the MIP. The NIP was prepared in the same way as the MIP but without the clenbuterol template. The selectivity of the MIP-coated fiber was shown by extraction yields of about 75 and <5% respectively, for bromobuterol from acetonitrile, using the imprinted and non-imprinted polymers. Application of the fiber to the extraction of bromobuterol from spiked human urine gave clean extracts and ~45% yield, demonstrating the suitability of the fibers for the analysis of biological samples.

Figure 2-7. The structures of clenbuterol and its structural analogue, bromobuterol [254].

After a lapse of several years, publications on MIPs as SPME fiber coatings started emerging in the recent couple of years. An interesting method of making MIP SPME fibers involved filling untreated fused silica capillaries with the MIP followed by etching of the fused silica with ammonium hydrogen difluoride [255,256]. Upon optimization of parameters such as fiber thickness, polymerization time, loading,
washing and elution solvents as well as time and temperature of the loading step, the fibers were used for the extraction of triazines from soil and vegetable samples.

In an alternative method, prometryn-imprinted polymers were coated onto silica fibers for SPME of triazines in soil and crop samples [257,258]. Further work by this research group involved the preparation and evaluation of MIP SPME fibers for the trace analysis of tetracycline antibiotics in chicken feed, chicken muscle and milk samples [259].

Using another straightforward preparation method, monolithic codeine-imprinted SPME fibers were fabricated through the use specially-prepared moulds. The resulting fibers were 2 cm in length and 0.3 mm in diameter [260]. The method was also used to fabricate diacetyl morphine-imprinted SPME fibers for the selective extraction of diacetylmorphine and its structural analogues followed by GC and/or GC–MS analysis [261].

2.1.3 Development of Novel SPME Coatings

The MIP SPME coatings mentioned in the previous section are solely acrylate-based coatings formed using methacrylic acid as the monomer and ethylene glycol dimethacrylate or trimethylolpropane trimethacrylate as the cross-linker. Hence, one of the aims of this project was to combine the fields of sol-gel chemistry, molecular imprinting and solid-phase microextraction in the development of novel SPME coatings in the form of sol-gel MIP SPME fibers for the selective extraction of degradation products of chemical warfare agents from water samples. To the best of our knowledge, there has only been one report on sol-gel MIP SPME fibers, for the determination of ascorbic acid [262].
At the same time, another novel SPME coating, based on an asymmetrically substituted polyhydroxylated poly(paraphenylene) polymer, poly(1-hydroxy-4-dodecyloxy-\(p\)-phenylene) (PhPPP) [263], was investigated. The chemical structure of the functionalized polymer used in the coating of the fiber is shown in Figure 2-8.

![Chemical structure of PhPPP](image)

**Figure 2-8.** The chemical structure of PhPPP.

The incorporation of a long alkoxy chain and hydroxyl groups on either side of the polymer backbone confers amphiphilic properties to the polymer backbone. The polymer has been characterized [263-266] and investigated as a coating for polymer-coated hollow fiber microextraction (PC-HFME) in which it functioned as the adsorbent for the enrichment of organochlorine pesticides in water [267].

In PC-HFME (Figure 2-9), a short length of the hollow fiber membrane is coated with a selected polymer. The extraction device is then placed into the sample solution and allowed to tumble freely and continuously throughout the extraction. Upon completion of extraction, the fiber is removed prior to desorption of the analytes in a suitable solvent [268-271].

![Experimental set-up for PC-HFME](image)

**Figure 2-9.** Experimental set-up for PC-HFME [267].
In our investigation of the PhPPP coating for extraction, the polymer was coated onto recycled commercial SPME fibers in which the polymeric coating had been removed, leaving the fused silica backbone intact. The performance of this novel fiber coating for the extraction of Lewisites from water samples was evaluated against that of commercially-available fibers.

2.2 Liquid-Phase Microextraction

Single-drop microextraction (SDME) and hollow-fiber protected liquid-phase microextraction (HF-LPME) are relatively new, solvent-minimized microextraction techniques. In SDME [80,272-275], also referred to as single drop extraction (SDE) [276-278], liquid-phase microextraction (LPME) [279-284], solvent microextraction (SME) [285-287], single-drop LPME (SD-LPME) [288] or liquid-liquid-liquid microextraction (LLLME) [289], a microdrop of solvent is suspended from the tip of a conventional microsyringe and then immersed in a sample solution in which it is immiscible or suspended in the headspace above the sample. After sampling, the microdrop is retracted into the syringe and transferred to the analytical instrument. The experimental set-up for SDME is depicted in Figure 2-10.

![Figure 2-10. Experimental set-up for SDME [80].](image-url)
An evolution from SDME involves the utilization of a polypropylene hollow fiber membrane to contain the acceptor phase either in a U-shaped or a rod-like configuration (Figures 2-11 and 2-12). The former technique was also known as LPME when it was first described [290-296] whereas the latter has been referred to as hollow fiber-protected LPME (HF-LPME) [297-307]. In the literature, the term, ‘LPME’, has been used to refer to different techniques and configurations and tends to lead to some confusion. Hence, the term, ‘HF-LPME’, will be used throughout the rest of this text to specifically refer to the use of the hollow fiber membrane in the rod-like (the so-called Lee-type) configuration. Alternative terminology for two-phase HF-LPME include liquid-liquid microextraction (LLME) and microporous membrane liquid-liquid extraction (MMLLE) [277] while three-phase HF-LPME has also been referred to as liquid-liquid-liquid microextraction (LLLME) [308-315] and supported liquid membrane (SLM) LLE [316].

**Figure 2-11.** Experimental set-up for HF-LPME in the U-shaped configuration [290].

**Figure 2-12.** Experimental set-up for HF-LPME in the rod-like configuration [297].
HF-LPME can be carried out as a two-phase or three-phase procedure. In two-phase HF-LPME, the hollow fiber is affixed to the tip of the syringe needle and contains an organic solvent for the extraction of analytes of interest from an aqueous sample. Upon completion of extraction, the organic solvent is withdrawn into the syringe and injected directly into a GC instrument for analysis [317]. The hollow fiber is then discarded and thus resolves any issues of carry-over. In contrast, three-phase HF-LPME involves extraction from an aqueous sample matrix, through an organic phase, that is immiscible with water, held in the pores of the hollow fiber, and back into a fresh aqueous phase inside the lumen (channel) of the hollow fiber [311].

Similar to SPME, besides direct immersion into an aqueous sample, headspace SDME [318-324] and HF-LPME [325-327] can be carried out over a sample. In addition, improvement to the technique is possible through dynamic HF-LPME, in which small volumes of the aqueous sample is a repeatedly pulled in and pushed out of the hollow fiber with the aid of a syringe pump. During withdrawal of the aqueous sample, a thin film of organic solvent builds up in the hollow fiber and vigorously extracts analyte from the sample segment, whereas, during sample expulsion, this thin film recombines with the bulk organic phase in the syringe. During this recombination, the portion of analyte extracted in the current cycle is trapped in the bulk organic solvent. After extraction, which includes many repeated cycles, a portion of the bulk organic solvent is subjected to further chromatographic analysis [275,328-333]. Further improvements to the techniques involve the use of ionic liquids [334-339] or binary solvents [340,341] as the extraction solvent.

Both SDME and HF-LPME techniques are simple and fast techniques to carry out with the use of inexpensive apparatus. In addition, minimal volumes of solvents, typically 5 to 50 µl, are consumed such that these techniques are practically solvent-
less. High pre-concentration of analytes and excellent clean-up can be achieved. However, SDME has its limitations: (a) the direct immersion mode requires careful and elaborate manual operation due to the problem of drop dislodgement and instability; (b) since more complex matrices will compromise the stability of the solvent drop during extraction, an extra filtration step of the sample is usually necessary; (c) the sensitivity and precision of SDME are not high due to relatively long extraction time and the slow stirring rates since fast stirring usually results in drop dissolution and/or dislodgement; and (d) SDME is not yet a routinely applicable on-line pre-concentration procedure. Even though HF-LPME is also limited by the unavailability of commercial equipment, with the protection of the hollow fiber membrane, the technique is far more robust where samples can be stirred or vibrated vigorously without any loss of the micro-extract. HF-LPME is expected to be an important sample preparation technique, complementing existing techniques like LLE, SPE and SPME [273,275,342,343].

Both SDME and HF-LPME have been investigated for the analysis of CWAs and their degradation products. In the first report on SDME of CWAs and related compounds [344], three toxic CWAs and six non-toxic markers of organophosphorus nerve agents were investigated. The structures of the compounds are shown in Figure 2-13. Extraction parameters were optimized, leading to extraction conditions requiring a combination of dichloromethane and carbon tetrachloride (3:1) as the extracting solvent, a stirring rate of 300 rpm, addition of 30% of sodium chloride and an extraction time of 30 min. The optimized SDME procedure was compared against SPME and LLE for the extraction of compounds E-I (Figure 2-13). SDME was shown to be superior over LLE and managed to extract all the compounds when SPME failed to extract Sarin.
In another study [345], HF-LPME was investigated for the extraction of CWAs from water. The structures of the analytes investigated are shown in Figure 2-14. The optimized extraction conditions were found to be the use of trichloroethylene as the extracting solvent, a stirring rate of 1000 rpm, an extraction time of 15 min and a salt concentration of 30% sodium chloride. It was found that HF-LPME is advantageous over SDME in terms of operation, sensitivity and reproducibility. The hollow fiber also provide a clean-up capability that does not apply to SDME whose drop is exposed directly to the sample.

In a subsequent study [346], the successful analysis of degradation products of CWAs using HF-LPME with in-situ derivatization was demonstrated. The structures
of the analytes investigated are shown in Figure 2-15. Prior to extraction, basification of the spiked water with potassium carbonate, addition of propyl bromide and stirring of the mixture at 100°C for 2 h were carried out. Upon completion of reaction, extractions were performed by HF-LPME prior to GC–MS analysis. The method was successfully used in the analysis of the 19th Official OPCW Proficiency Test water samples.

![Structures of alkylphosphonic acids](image)

**Figure 2-15.** Structures of alkylphosphonic acids investigated in HF-LPME [346].

This approach of developing solvent-minimized extraction techniques for the determination of various CWAs and related compounds by utilizing HF-LPME was carried out at approximately the same time as that reported by Gupta and co-workers described above [345,346]. Besides CWAs and acidic degradation products, basic degradation products were investigated as well. The results have been published as three separate articles [347-349] and are discussed in Sections 3.3 and 4.3 of this report.
3 EXPERIMENTAL

3.1 Sol-gel MIPs

Prior to the development of sol-gel MIPs as SPME fiber coatings, sol-gel MIPs were first synthesized as powder and evaluated as sorbent packings in SPE cartridges. A series of MIPs was synthesized using pinacolyl methylphosphonic acid (PMPA), thiodiglycol (TDG), triethanolamine (TEA) and 3-quinuclidinol (3Q) as the templates. A non-imprinted polymer was also synthesized, but in the absence of a template. Using the sol-gel process, the polymers formed were crushed up into powder and sieved. The templates were removed from the polymers by Soxhlet extraction in ethanol. The polymers were then dried under vacuum.

Endcapping of the polymers was carried out by stirring the polymers in an equimolar mixture of the endcapping reagents, trimethylchlorosilane (TMCS) and hexamethyldisilazane (HMDS). The effect of endcapping of the polymers was investigated by comparing the binding properties of the endcapped versus the non-endcapped NIP and the PMPA-imprinted MIP when they were used as the sorbent in SPE cartridges.

The various MIPs (PMPA-MIP, TDG-MIP, TEA-MIP and 3Q-MIP) and the NIP were evaluated for their binding properties towards their respective target analytes in aqueous matrices using SPE. The elution solvent and volume of elution solvent were optimized for each MIP. Subsequently, the MIPs and the NIP were challenged with water samples containing polyethylene glycol (PEG) to test their binding properties for the analytes of interest in complex environmental samples whereby the MIP-SPE procedure was compared with other sample preparation procedures, namely strong anion-exchange (SAX) SPE and strong cation-exchange (SCX) SPE as well as a direct rotary evaporation procedure for the analysis of a range
of analytes in an aqueous sample containing PEG. All experiments were carried out in triplicate.

3.1.1 Materials

The monomers (Figure 3-1), tetraethoxysilane (TEOS) (≥99%, Fluka, Buchs, Switzerland), phenyl trimethoxysilane (PTMOS) (98%, Lancaster, Lancashire, England, UK) and 3-aminopropyltriethoxysilane (APTEOS) (99%, Aldrich, Milwaukee, WI, USA), were used without further purification. Chemicals, methylphosphonic acid (MPA) (98%, Aldrich), ethyl methylphosphonate (EMPA) (98%, Aldrich), thiodiglycol (TDG) (99%, Merck, Darmstadt, Germany), thiodiglycol sulfoxide (TDGS) (Chem Service, West Chester, PA, USA), thiodiglycol sulfone (TDGSO) (65% weight % in aqueous solution, Lancaster), methyl diethanolamine (MDEA) (99+, Aldrich), ethyl diethanolamine (EDEA) (98%, Aldrich), triethanolamine (TEA) (98%, Aldrich), 3-quinuclidinol (3Q) (>98%, Fluka) and the internal standard, tripropyl phosphate (TPP) (99%, Aldrich), were commercially available while isopropyl methylphosphonate (IMPA), pinacolyl methylphosphonate (PMPA) and cyclohexyl methylphosphonate (CMPA) were synthesized by the Organic Synthesis Group of DSO National Laboratories. The endcapping reagents (Figure 3-2) used were trimethylchlorosilane (TMCS) (98%, Aldrich) and hexamethyldisilazane (HMDS) (99+, Lancaster). The solvents used included absolute ethanol (EtOH) (≥99.9%, Merck), tetrahydrofuran (GR, Merck), methanol (HPLC, J.T. Baker, Phillipsburg, NJ, USA), dichloromethane (DCM) (ultra resi, J.T. Baker) and acetonitrile (ACN) (HPLC, J.T. Baker). Concentrated hydrochloric acid (HCl) (min. 37% AR, Riedel-de Haën, Seelze, Germany) was used as a catalyst as well as for preparing 0.2 M HCl in methanol while trifluoroacetic acid (TFA) (99+,
Aldrich) and triethylamine (TE) (>99%, Merck) were used as solvent modifiers. Sodium hydroxide (NaOH) (Merck), of 1.0 M concentration, was used to clean the fused silica fibers. N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) (99% with 1% TMCS, Aldrich) was used as a derivatizing agent for GC analysis. Poly(ethylene glycol) (PEG) (Number average molecular weight, $M_n$, ca. 200, Aldrich) was used as a background contaminant for the water matrix. Deionized water was obtained from a Milli-Q system (Millipore, Bedford, MA, USA). Strong cation-exchange (SCX) and strong anion-exchange (SAX) cartridges were commercially available as Varian Bond Elute SCX (100 mg in 10 ml cartridges) and Supelco Supelclean LC-SAX (100 mg in 1 ml cartridges) respectively.

![TEOS](image1.png) ![PTMOS](image2.png) ![APTEOS](image3.png)

**Figure 3-1.** Structures of the monomers.

![TMCS](image4.png) ![HMDS](image5.png)

**Figure 3-2.** Structures of the endcapping reagents.

### 3.1.2 Synthesis of MIPs and NIPs

First, 120 ml of TEOS, 8 ml of PTMOS and 120 ml of ethanol were mixed. Next, 4 ml of concentrated HCl, 8 ml of APTEOS and 40 ml of deionized water were added dropwise in this order. The mixture was stirred at room temperature for 20 h.
For the PMPA-MIP, 100 ml portions of this sol were mixed with 10 ml of a 0.1 M solution of PMPA (180 mg in ethanol). The solution was stirred for an additional 4 h. The remaining mixture was used as the non-imprinted polymer [350,351]. The solutions were then left to stand at room temperature until all the solvent evaporated to yield hard and transparent polymers. The TDG-MIP, TEA-MIP and 3Q-MIP were similarly synthesized. A 100 ml portion of the sol solution was mixed with 10 ml of a 0.1 M solution of TDG (127 mg in ethanol), 10 ml of 0.1 M solution of TEA (123 mg in ethanol) and 10 ml of a 0.1 M solution of 3Q (149 mg in ethanol) respectively. The polymers were crushed up and ground into fine powder using a mortar and pestle and sieved through a 100 mesh (150 μm) Coulter Particle Sizer sifter screen. The polymers were repeatedly washed with ethanol using Soxhlet extraction. For the MIPs, washing was stopped when the template could no longer be detected by GC–MS in the wash. The polymers were then dried under vacuum to constant weight.

Endcapping is a procedure in which the surface silanol groups were reacted with an equimolar mixture of endcapping reagents. Here, TMCS and HMDS were used [230]. Endcapping has been shown to prevent the non-specific adsorption of analytes to surface silanol groups to a great extent and results in a higher imprint factor [236].

In order to compare the effect of endcapping on the properties of both the NIP and the MIPs, a portion of the various polymers were weighed out for the endcapping procedure while the rest remained non-endcapped. Typically, 5 g of polymer was stirred at room temperature for 24 h in an equimolar mixture of 2.5 g of TMCS and 3.75 g of HMDS. Next, the endcapped polymers were washed with anhydrous tetrahydrofuran, followed by ACN to remove excess reagents [236] and then dried under vacuum to constant weight.
3.1.3 Procedures

3.1.3.1 Evaluation of the effect of endcapping

First, 50 mg of PMPA-MIP or NIP was packed into recycled Varian 10 ml SCX cartridges which have been emptied of the contents. The polymer powder was packed between recycled frits in the cartridges. Next, 5 ml of a deionized water sample containing 10 μg ml\(^{-1}\) of MPA, EMPA, IMPA, PMPA and CMPA was applied to the cartridges. The eluents collected were analyzed for the percentage absorptivity, ie. the percentage of analyte that is retained on the polymer and is obtained by subtracting from 100%, the percentage recovered in the eluent upon loading of the sample. Elution with 5 × 2 ml of 1% TE in water, rotary evaporation to dryness of the eluents and reconstitution in ACN, followed by derivatization using BSTFA, gave the percentage recovery of the analytes from the polymers. TPP was used as an internal standard (ISTD). The MIP-SPE procedure is shown in Figure 3-3.

Figure 3-3. Procedure for MIP-SPE.
3.1.3.2 Evaluation of the effect of elution solvents and volume

The procedure for the packing of the SPE cartridges is as described in the previous section. In the experiments, 5 ml of a deionized water sample containing 10 μg ml\(^{-1}\) of PMPA was applied to the cartridges. For TDG-MIP SPE, 500 mg of TDG-MIP or NIP were used. In the experiments, 0.5 ml of a deionized water sample containing 10 μg ml\(^{-1}\) of TDG was applied to the cartridges. For TEA-MIP SPE, 500 mg of TEA-MIP or NIP were used. In the experiments, 0.5 ml of a deionized water sample containing 10 μg ml\(^{-1}\) of TEA was applied to the cartridges. For 3Q-MIP SPE, 200 mg of 3Q-MIP or NIP were used. In the experiments, 0.5 ml of a deionized water sample containing 100 μg ml\(^{-1}\) of 3Q was applied to the cartridges.

In order to select the elution solvent that gives the highest recovery of the analyte from the MIPs, 5 × 2 ml of ethanol, 1% TFA in water and 1% TE in water were used separately as the elution solvent. In addition, the volume of elution solvent required was investigated. Volumes of 1 × 1 ml, 1 × 2 ml, 3 × 2 ml, 4 × 2 ml, 5 × 2 ml and 10 × 2 ml of elution solvent (giving a total volume of 1, 2, 6, 8, 10 and 20 ml respectively) were applied to the cartridges.

3.1.3.3 Evaluation of binding properties

In order to evaluate the binding properties of the PMPA-MIP, 5 ml of a deionized water sample containing 10 μg ml\(^{-1}\) of MPA, EMPA, IMPA, PMPA and CMPA was applied to the cartridges. For the TDG-MIP, 0.5 ml of a deionized water sample containing 10 μg ml\(^{-1}\) of TDG, TDGS and TDGSO was applied to the cartridges. For the TEA-MIP, 0.5 ml of a deionized water sample containing 10 μg ml\(^{-1}\) of MDEA, EDEA and TEA was applied to the cartridges. For the 3Q-MIP, 0.5
ml of a deionized water sample containing 100 µg ml$^{-1}$ of 3Q was applied to the cartridges. In these experiments, the NIPs were evaluated alongside the MIPs.

The eluents collected were analyzed for the absorptivity. Elution with the optimal volume of the selected elution solvent, rotary evaporation to dryness of the eluents and reconstitution in ACN, followed by derivatization using BSTFA, gave the recovery of the analytes from the polymers. In addition, the polymers were further challenged with a tap water sample containing the analytes as well as 500 µg ml$^{-1}$ of PEG as a background contaminant. For such samples, the SPE procedure included a washing step of 5 ml of water (Figure 3-4).

**Figure 3-4.** Procedure for MIP-SPE for samples with PEG.

### 3.1.3.4 Comparison with other sample preparation techniques

The MIP-SPE procedures were compared against other sample preparation techniques using commercially-available SPE cartridges as well as with a procedure
involving no sample clean-up. To evaluate the PMPA-MIP, the sample consisted of 10 $\mu$g ml$^{-1}$ EMPA, IMPA, MPA, PMPA and CMPA with 500 $\mu$g ml$^{-1}$ PEG in tap water. For the TDG-MIP, 0.5 ml of 10 $\mu$g ml$^{-1}$ TDG, TDGS and TDGSO with 500 $\mu$g ml$^{-1}$ PEG in tap water was loaded onto the cartridges. For the TEA-MIP, 0.5 ml of 10 $\mu$g ml$^{-1}$ MDEA, EDEA and TEA with 500 $\mu$g ml$^{-1}$ PEG in tap water was loaded onto the cartridges. For the 3Q-MIP, 0.5 ml of 100 $\mu$g ml$^{-1}$ 3Q with 500 $\mu$g ml$^{-1}$ PEG in tap water was loaded onto the cartridges. Figures 3-5 and 3-6 illustrate the SCX and SAX SPE procedures respectively. For the procedure without sample clean-up, the water sample was directly rotary evaporated to dryness, followed by reconstitution with ACN and derivatization with BSTFA.

![Diagram of SCX SPE procedure](image1)

**Figure 3-5.** Procedure for SCX SPE.

![Diagram of SAX SPE procedure](image2)

**Figure 3-6.** Procedure for SAX SPE.

Lastly, the respective MIPs (PMPA-MIP, TDG-MIP, TEA-MIP and 3Q-MIP) were mixed in the ratio of 1:10:10:4 respectively and used for the extraction of the
four main template molecules from an aqueous sample. A 500 mg mixture of the MIPs and corresponding NIP were used for the evaluation of the three elution solvents, namely ethanol, 1% TFA in water and 1% TE in water. Next, 0.5 ml of a tap water sample containing 10 μg ml⁻¹ PMPA, TDG and TEA and 100 μg ml⁻¹ 3Q with 500 μg ml⁻¹ PEG as a matrix interference was loaded onto the MIP-SPE cartridges. Elution with 5 × 2 ml of solvents was carried out. This procedure was similarly compared against the SCX, SAX SPE procedures and the direct rotary evaporation procedure.

3.1.4 Instrumental Analysis

GC–MS analyses were performed on a HP6890 GC/5973 MSD system (Agilent Technologies, San Jose, CA, USA). Separation was carried out on a HP-5MS column, 30 m × 0.25 mm internal diameter, 0.25 μm film thickness, together with a 3 m precolumn. Splitless injections were performed. The temperature program used was: 60°C, held for 2 min, ramped at 10°C min⁻¹ to 220°C, then 20°C min⁻¹ to 280°C, held for 4 min. The carrier gas was helium at 35 cm s⁻¹. The split/splitless injector was maintained at 200°C while the transfer line was maintained at 280°C. The MS source and quadrupole were maintained at 230 and 150°C, respectively. All analyses were performed in GC–MS full scan mode over the range of m/z 40–550 at a scan rate of 1.49 scans s⁻¹.

3.1.5 Synthesis of sol-gel MIP SPME fibers

Commercially-available SPME fibers (Supelco, Bellefonte, PA, USA) in which the polymer coatings have been stripped-off or damaged but with an intact fused silica backbone were used for the preparation of sol-gel fibers. Any remaining
coating on the fibers was removed by dipping the fibers in DCM, after which the coating could be stripped off easily. Prior to coating, the fused silica fibers were dipped in 1 M NaOH for 1 h to expose the maximum number of silanol groups on the surface, rinsed with deionized water, dipped in 0.1 M HCl for 30 min to neutralize excess NaOH, rinsed with deionized water and dried at room temperature in a desiccator.

To prepare the sol solution, 750 µl of TEOS, 50 µl of PTMOS and 750 µl of ethanol were mixed. Then, 25 µl of concentrated HCl, 50 µl of APTEOS and 250 µl of deionized water were added dropwise in this order. The mixture was stirred at room temperature for 2 h. After this, 1 ml of this sol was mixed with 100 µl of a 0.1 M solution of PMPA. The remaining mixture was used as the NIP coating. The solutions were stirred for 24 h. A fused silica fiber was repeatedly dipped into the sol solution such that a coating was formed on the fiber. The sol-gel fibers were dried at room temperature in a desiccator.

3.2 SPME using PhPPP-coated fibers

PhPPP was investigated as a novel coating for the SPME of Lewisites from aqueous samples. Several extraction parameters, namely the choice of derivatizing agent, pH, salting, and extraction time were thoroughly optimized. Upon optimization of the extraction parameters, the performance of the novel coating was compared against that of commercially-available SPME coatings. All experiments were carried out in triplicate, unless stated otherwise.
3.2.1 Chemicals and Reagents

The analytes, 2-chlorovinylidichloroarsine (L1), bis(2-chlorovinyl)chloroarsine (L2) and tris(2-chlorovinyl)arsine (L3), were synthesized by the Organic Synthesis Group of DSO National Laboratories and shown to be 95%, 88% and 92% pure by NMR analysis respectively.

The solvents used included hexane, dichloromethane and acetonitrile (99.8%, J.T. Baker). Sodium chloride (NaCl) (Merck) and sodium sulfate (Na₂SO₄) (J.T. Baker) were used to investigate the effect of ionic strength of the sample during extraction. Hydrochloric acid (0.1 M, Merck) and ammonium hydroxide (28-30 wt % solution of NH₃ in water, Acros Organics, Geel, Belgium) were used for adjustment of sample pH. The derivatizing agents (Figure 3-7) used were ethanethiol (ET), propanethiol (PT), butanethiol (BT) (97%, Fluka), 1,2-ethanediithiol (EDT) (98%, Fluka), 1,3-propanediithiol (PDT) (99%, Aldrich) and 1,4-butanediithiol (BDT) (90%, Fluka). Deionized water was obtained from a Milli-Q system.

![Chemical structures](image)

**Figure 3-7.** Structures of the mono- and dithiol derivatizing agents.

3.2.2 Preparation of PhPPP as a coating for SPME

PhPPP was synthesized as summarized in the following scheme [263]:

(i) Br₂ in glacial acetic acid, 80%; (ii) NaOH, CH₃(CH₂)₁₁Br, 45-50°C, 10 h, 65%;
(iii) K₂CO₃, C₆H₅CH₂Br, 50°C, 10 h, 90%; (iv) n-butyllithium, tetrahydrofuran, –
78°C, triisopropyl borate, room temperature, 10 h, 70%; (v) 2 M K₂CO₃, toluene, 3.0 mol % Pd(PPh₃)₄, reflux, 3 days, (vi) H₂, 10% Pd/C, chloroform/ethanol/tetrahydrofuran.

Figure 3-8. Synthetic scheme of PhPPP [263].

Commercially-available SPME fibers with polymer coatings that were stripped off but with the fused silica backbone intact were used for coating with PhPPP. Thin films of the polymers on bare fused silica fibers were prepared by drop-casting from 0.5 mg ml⁻¹ polymer in chloroform solution under ambient conditions, without any air flow or temperature control aids. SEM images were taken with a JEOL JSM 6700 scanning electron microscopy (SEM) and the thickness of the fiber was measured to be approximately 7 µm.
3.2.3 Preparation of Stock Solutions and Samples

A stock solution of a mixture of the analytes at a concentration of 1 mg ml\(^{-1}\) was prepared in acetonitrile and stored at \(-20^\circ\text{C}\). Aqueous samples (3 ml) were freshly prepared by spiking deionized water with the analytes at a concentration of 0.5 \(\mu\text{g ml}^{-1}\). Quantitation of the analytes was done by external calibration where a series of standard solutions was obtained by dilution of the stock solution with dichloromethane, addition of the respective derivatizing agents and analysis by GC to obtain linear calibration plots for each analyte based on the total ion chromatographic peak area.

3.2.4 SPME Procedure

Prior to use, the network fiber was conditioned at 220\(^\circ\text{C}\) for 30 min in the GC injection port while the commercially-available fibers from Supelco, 30 \(\mu\text{m}\) and 100 \(\mu\text{m}\) PDMS, 65 \(\mu\text{m}\) PDMS/DVB, 75 \(\mu\text{m}\) CAR/PDMS, 85 \(\mu\text{m}\) PA, and 65 \(\mu\text{m}\) CW/DVB, were conditioned according to the manufacturer's recommendations [83]. SPME analyses were performed by direct immersion of the fiber to a water sample spiked with the analytes. Prior to extraction, 1 \(\mu\text{l}\) of derivatizing agent was added. The sample was stirred at the maximum rate during the extraction. After extraction, the fiber was desorbed in the heated injection port of the GC for 5 min.

3.2.5 Instrumental Analysis

Optimization experiments were performed with a HP6890 GC, equipped with a flame ionization detector (FID) (Agilent Technologies). Separation was carried out on a HP-5 column, 30 m \(\times\) 0.25 mm internal diameter, 0.25 \(\mu\text{m}\) film thickness. Splitless injections were performed. The temperature program used was: 60\(^\circ\text{C}\), held
for 2 min, ramped at 10°C min⁻¹ to 205°C, then 30°C min⁻¹ to 280°C and held for 4 min. The carrier gas was helium at 35 cm s⁻¹. The split/splitless injector was maintained at 250°C while the detector was maintained at 280°C.

The limits of detection (LODs) for the analytes were estimated at S/N = 3 under GC–MS full scan conditions. GC–MS analyses were performed in electron ionization mode (70 eV) on a HP6890 GC/5973 MSD system (Agilent Technologies). Separation was carried out on a HP-5MS column, 30 m × 0.25 mm internal diameter, 0.25 μm film thickness, together with a 2 m precolumn. The split/splitless injector was maintained at 250°C while the transfer line was maintained at 280°C. The temperature program was identical to that used for the GC–FID. The MS source and quadrupole were maintained at 230°C and 150°C respectively. Analyses were performed in GC–MS full scan mode over the range of m/z 40-400 at a scan rate of 1.36 scans s⁻¹.

3.3 HF-LPME

HF-LPME was investigated for the extraction of various chemical warfare agents and degradation products from aqueous samples. Optimization of several extraction parameters was carried out where the effects of the extraction solvent, the derivatizing agent and derivatization procedure and the amount of derivatizing agent (for degradation products), salting, stirring speed and extraction time were thoroughly optimized. Upon optimization of the extraction parameters, the HF-LPME technique was compared against SPME. In addition, the applicability of the technique for a 20th Official OPCW Proficiency Test sample was demonstrated. All experiments were carried out in triplicate, unless stated otherwise.
3.3.1 Chemicals and Reagents

The chemical warfare agents, isopropyl methylphosphonofluoridate (Sarin, GB), pinacolyl methylphosphonofluoridate (Soman, GD), ethyl \( N,N \)-dimethylphosphoramidocyanidate (Tabun, GA), bis(2-chloroethyl)sulfide (Sulfur mustard, HD) and \( O \)-ethyl-S-[(2-(diisopropylamino)ethyl] methylphosphonothiolate (VX), were synthesized by the Organic Synthesis Group of DSO National Laboratories. Ethyl methylphosphonic acid (EMPA) (98%), ethyl 2-hydroxyethyl sulfide (EHES) (97%), methylphosphonic acid (MPA) (98%) and \( n \)-propylphosphonic acid (nPPA) (95%) were purchased from Aldrich, thiodiglycol (TDG) (99%) and benzilic acid (BA) (98%) were bought from Merck and 1,2-bis(2-hydroxyethylthio)ethane (QOH) (98%) was supplied by Acros. Isopropyl methylphosphonic acid (IMPA), pinacolyl methylphosphonic acid (PMPA) and bis(2-hydroxyethylthio)ether (TOH) were synthesized by the Organic Synthesis Group of DSO National Laboratories. The basic degradation products, 2-(\( N,N \)-diisopropylamino)ethanol (DIPAE) (98%) and 3-quinuclidinol (3Q) (98%) were bought from Fluka while \( N \)-methylidethanolamine (MDEA) (99%), \( N \)-ethyldiethanolamine (EDEA) (98%) and triethanolamine (TEA) (98%), were from Aldrich.

The solvents used included chloroform (CHCl\(_3\)) (99.9%) and trichloroethylene (C\(_2\)HCl\(_3\)) (99%) from Aldrich, carbon tetrachloride (CCl\(_4\)) (99.9%) and toluene (99.9%) from Merck, tetrachloroethylene (C\(_2\)Cl\(_4\)) (99.7%), dichloromethane (CH\(_2\)Cl\(_2\), DCM) (99.8%) and acetonitrile (ACN) (99.8%) from J.T. Baker. Deionized water was obtained from a Milli-Q system.

Sodium chloride (NaCl) from Merck and sodium sulfate (Na\(_2\)SO\(_4\)) from J.T. Baker were used to investigate the effect of ionic strength of the sample on the
extraction. Solutions of hydrochloric acid (Aldrich) and sodium hydroxide (Acros Organics) were used to adjust the pH of the samples. \(N,O\)-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) (99% with 1% TMCS) and \(N\)-(tert.-butyldimethylsilyl)-\(N\)-methyltrifluoroacetamide (MTBSTFA) (97% with 1% tert.-butyldimethylchlorosilane) purchased from Aldrich were used as derivatizing agents.

\[
\begin{align*}
\text{BSTFA} & \quad \text{MTBSTFA}
\end{align*}
\]

**Figure 3-9.** Structures of the silylating agents used in this work.

### 3.3.2 Preparation of Stock Solutions and Samples

A stock solution of a mixture of the CWAs at a concentration of 1 mg ml\(^{-1}\) was prepared in acetonitrile and stored at \(-20^\circ\text{C}\). Aqueous samples (3 ml) were freshly prepared by spiking deionized water with CWAs at a concentration of 0.5 \(\mu\)g ml\(^{-1}\) of each while slurry samples were prepared by mixing 60 mg of soil (total organic content 30 g kg\(^{-1}\)) with 3 ml of deionized water prior to spiking CWAs at a concentration of 0.5 \(\mu\)g ml\(^{-1}\) (sample pH 6.5). Extraction of the samples was performed after \(-20\) min upon preparation. Quantitation of the analytes was done by external calibration where a series of standard solutions was obtained by dilution of the stock solution with dichloromethane and analysis by GC–MS to obtain linear calibration plots for each analyte based on the total ion chromatographic peak area.

A stock solution of a mixture of the analytes at a concentration of 2 mg ml\(^{-1}\) for EMPA, IMPA, BA, QOH and TOH, 1 mg ml\(^{-1}\) for EHES and PMPA and 20 mg
for MPA, nPPA and TDG was prepared in acetonitrile and stored at ~20°C. Additionally, a stock solution of a mixture of the analytes at a concentration of 10 mg ml⁻¹ for 3Q, MDEA, EDEA and TEA and 1 mg ml⁻¹ for DIPAE was prepared in acetonitrile and stored at ~20°C. Based on the results of preliminary experiments the analytes were prepared at varying concentrations as the extraction efficiency varies significantly among the analytes. If all the analytes were spiked at a particular concentration, analytes that were not easily extracted would not be detected while those that were easily extracted would give saturated signals. Aqueous samples (3 ml) were freshly prepared by spiking deionized water with the stock solution of the mixture of analytes. Quantitation of the analytes was done by external calibration where a series of standard solutions was obtained by dilution of the stock solution with dichloromethane, addition of BSTFA or MTBSTFA respectively, heating at 60°C for 30 min and analysis by GC–MS to obtain linear calibration plots for each analyte based on the total ion chromatographic peak area.

3.3.3 Typical HF-LPME Procedures

The HF-LPME device consisted of a 10 μl microsyringe with 0.63 mm outer diameter cone-tip needle (SGE, Sydney, Australia) and a 1.2 cm Accurel Q3/2 polypropylene hollow fiber membrane (Membrana, Wuppertal, Germany) with the dimensions, 600 μm inner diameter, 200 μm wall thickness and 0.2 μm pore size, attached to the needle tip.

For the extraction of the chemical agents, 5 μl of solvent was drawn into the syringe before a hollow fiber was affixed onto the tip of the syringe needle. The fiber was immersed in solvent for three seconds to immobilize the solvent in the pores of the hollow fiber prior to extraction. Upon immersion of the hollow fiber into the
sample solution, the syringe plunger was depressed to fill the hollow fiber with solvent. The sample was stirred during the extraction with a Heidolph MR 3001K (Kelheim, Germany) magnetic stirrer. After extraction, the analyte-enriched solvent was drawn back into the syringe and the hollow fiber was discarded. A volume of 1 μl of solvent was injected into the GC–MS. In a typical extraction of the degradation products, a solvent-derivatizing agent mixture was first prepared by mixing chloroform and MTBSTFA in equal amounts. The procedure is similar to that described previously. The extraction of the basic degradation products follows that for the degradation products except that 1 μl of extract was injected into the GC–MS together with another microliter of derivatizing agent.

3.3.4 Typical SPME Procedures

The optimized extraction conditions for direct immersion SPME of the chemical warfare agents were based on a previously-reported procedure [88]. Extractions were performed using a 65 μm PDMS/DVB fiber. Prior to use, the fiber was conditioned at 250°C for 30 min in the GC injection port. The fiber was directly exposed to a sample spiked with the analytes with 40% (w/v) NaCl. The sample was stirred at the maximum rate during the 30 min extraction. After extraction, the fiber was desorbed in the heated injection port of the GC–MS for 5 min. Direct immersion SPME analysis of the water and slurry samples was performed using the same extraction conditions.

The optimized extraction conditions for SPME of the degradation products were based on a previously-reported procedure [90]. Extractions of the acidic degradations were performed using a variety of fibers, namely 100 μm PDMS, 65 μm PDMS/DVB, 75 μm CAR/PDMS, 65 μm CW/DVB and 85 μm PA (Supelco) while
for SPME of the basic degradation products, the 30 μm PDMS was used in place of the 100 μm PDMS fiber and in addition, the newly-introduced 60 μm PEG fiber was evaluated as well. Prior to use, the fibers were conditioned in the GC injection port according to the manufacturer’s recommendations [83]. The extraction procedure involves first exposing a fiber to the MTBSTFA headspace for 5 min before directly inserting into a sample spiked with the analytes. The sample was saturated with salt and stirred during the entire extraction process. After extraction, the fiber was exposed to the MTBSTFA headspace again for 15 min prior to desorption in the GC injection port for 5 min.

3.3.5 Instrumental Analysis

GC–MS analyses were performed in electron ionization mode (70 eV) with a HP6890 GC/5973 MSD system. Separation was carried out on a HP-5MS column, 30 m×0.25 mm internal diameter, 0.25 μm film thickness, together with a 2 m precolumn. Splitless injections were performed. The carrier gas was helium at 35 cm s⁻¹. The split/splitless injector was maintained at 250°C while the transfer line was maintained at 280°C. The MS source and quadrupole were maintained at 230 and 150°C, respectively. For the analysis of the chemical agents, the oven temperature program used was: 40°C, held for 2 min, ramped at 10°C min⁻¹ to 205°C, then 30°C min⁻¹ to 280°C and held for 4 min. All analyses were performed in GC–MS full scan mode over the range of m/z 40–400 at a scan rate of 1.36 scans s⁻¹. The oven temperature program used with BSTFA derivatization was: 60°C, held for 2 min, ramped at 10°C min⁻¹ to 205°C, 25°C min⁻¹ to 280°C and held for 4 min while with MTBSTFA derivatization it was: 60°C, held for 2 min, ramped at 10°C min⁻¹ to
280°C and held for 4 min. All analyses were performed in GC–MS full scan mode over the range of \( m/z \) 40–550 at a scan rate of 1.49 scans s\(^{-1}\).

### 3.4 Health and Safety Aspects

Caution: extreme care was taken for synthesis of these compounds, as they are highly toxic. Trained professionals should prepare, handle and use these compounds in a fume hood equipped with an alkaline scrubber system and adopt proper protective measures; and international treaties and legal issues when synthesizing or working with these agents should be adhered to.
Results and Discussion

4.1 Sol-gel MIPs

4.1.1 Effect of endcapping on PMPA-MIP-SPE

Preliminary experiments on the binding properties of the PMPA-MIP and the NIP showed that the NIP did not demonstrate zero absorptivity of the analytes. It was reported in the study on MIP SPE of nerve agent degradation products using acrylate-based MIPs that the NIP could not absorb any of the analytes [216]. Hence, an attempt was made to endcap the surface silanol groups of the sol-gel MIPs and NIPs synthesized in order to reduce the non-specific adsorption of analytes to the surface silanol groups to a certain extent and achieve a higher imprint factor [236].

Figure 4-1 shows the structures of the analytes of interest. These analytes are of particular interest as they are the degradation products of a group of chemical warfare agents known as the nerve agents. The phosphonic acids, EMPA, IMPA, PMPA and CMPA, are the degradation products of VX, GA, GD and GF respectively while MPA is the final degradation product of the phosphonic acids. The difference in binding properties between the non-endcapped NIP (N) and the endcapped NIP (NE) as well as that between the non-endcapped PMPA-MIP (P) and endcapped PMPA-MIP (PE) for selected phosphonic acids, is shown in Figures 4-2 and 4-3 respectively.

![Figure 4-1. Structures of the phosphonic acids investigated.](image)
Figure 4-2. Comparison of % absorptivity and recovery between non-endcapped and endcapped NIPs.

The percentage absorptivity (\%A) is defined as the percentage of analyte that is retained on the polymer and is obtained by subtracting from 100\%, the percentage recovered in the eluent upon loading of the sample. The percentage recovery (\%R) is defined as the percentage recovered in the eluent upon elution with elution solvent.

It is expected that the endcapping procedure would reduce non-specific interactions between the analytes and the polymer. The % absorptivities of the analytes were observed to be reduced by more than half except for MPA. MPA is the most polar analyte and shows strong adsorption to the polymer. With endcapping, recovery of the analytes from the NIP was negligible.

Ideally, the NIP should not show absorptivity of the analytes. The results imply that the endcapping procedure may be insufficient to completely endcap all the silanol groups on the polymer and hence eliminate all the non-specific interactions between the analytes and the polymer such that the NIP shows zero absorptivity. The endcapping procedure can either be repeated several times or the amounts of endcapping reagents can be increased. However, as discussed below, endcapping of the PMPA-MIP adversely affected the binding properties of the MIP.
The non-endcapped PMPA-MIP shows 100% absorptivity for the analytes in water as none of the analytes was detected in the eluent upon loading of the sample. On the other hand, the endcapped PMPA-MIP does not show 100% absorptivity. This observation can be attributed to two factors. Firstly, the process of endcapping has reduced the non-specific interactions between the PMPA-MIP and the analytes to a certain extent, hence the endcapped PMPA-MIP is expected to show lower absorptivity of the analytes. Secondly, there exists competition between the analyte and the water molecules in the aqueous matrix for binding sites on the polymer. This would further reduce the absorptivity of the polymer for the analytes.

The recoveries of the analytes with 1% TE in water from the non-endcapped and endcapped PMPA-MIPs were not quantitative. This indicates relatively strong binding of the analytes with the polymer matrix such that even a strongly basic eluent like 1% TE in water was unable to disrupt most of the analyte-polymer interactions. It was observed that endcapping adversely affected the recovery of the analytes from the endcapped MIP. Since the non-endcapped MIP showed better binding properties as compared to the endcapped MIP, the non-endcapped PMPA-MIP and the other non-endcapped MIPs were used, together with non-endcapped NIPs, for further experiments.

**Figure 4-3.** Comparison of % absorptivity and recovery between non-endcapped and endcapped PMPA-MIPs.
4.1.2 Evaluation of elution solvents and volume for PMPA-MIP-SPE

Three elution solvents were evaluated for their ability to recover the template, PMPA, from the PMPA-MIP. Ethanol [249] was chosen as it successfully removed the templates from the MIPs during MIP synthesis. The two other elution solvents, 1% TFA in water and 1% TE in water, were evaluated since TFA and TE are common modifiers added to elution solvents used in molecular imprinting studies [352,353].

Although ethanol was effective in the removal of template from the polymer by Soxhlet extraction, it could not be used as an elution solvent as it was not able to elute PMPA from the PMPA-MIP. The elution solvents, 1% TFA in water (pH 0) and 1% TE in water (pH 11) were able to elute PMPA from the MIP, however, 1% TE in water was more effective as it recovered a higher percentage of the analyte loaded. Hence, 1% TE in water was chosen as the elution solvent in subsequent experiments.

![Figure 4-4](image.png)

**Figure 4-4.** Comparison of % recovery of PMPA with the various elution solvents.

Next, the volume of elution solvent required was investigated. Volumes of 1 × 1 ml, 1 × 2 ml, 3 × 2 ml, 4 × 2 ml, 5 × 2 ml and 10 × 2 ml of 1% TE in water (giving a total volume of 1, 2, 6, 8, 10 and 20 ml respectively) were applied to the cartridges. A comparison of percentage recovery using the various volumes of elution solvent is presented in Figure 4-5. As seen from the graph, the optimum volume of elution solvent was 10 ml. Increasing the volume of elution solvent to 20 ml did not increase
the % recovery of the analyte significantly. Hence $5 \times 2$ ml of 1% TE in water was used in subsequent experiments.

![Graph](image-url)

**Figure 4-5.** Comparison of % recovery of PMPA with the various elution volumes.

**4.1.3 Evaluation of binding properties by PMPA-MIP-SPE**

In order to study the binding properties of the PMPA-MIP as well as the NIP, the polymers were challenged with a water matrix spiked with the phosphonic acids, a range of analytes of similar structure to the template.

![Graph](image-url)

**Figure 4-6.** Comparison of % absorptivity and recovery of PMPA-MIP and NIP.

The % absorptivity and recovery of the PMPA-MIP and the NIP for the analytes from deionized water are compared in Figure 4-6. It can be seen that the PMPA-MIP shows 100% absorptivity for all the analytes while the NIP shows 100% absorptivity for only MPA and particularly low absorptivity for PMPA.
The study by Marx et. al. [249] showed that there was low cross-selectivity of the parathion-imprinted films for other similar organophosphate pesticides and that the non-imprinted films showed relatively lower binding. In this case, the PMPA-MIP was able to quantitatively bind with all the phosphonates. This may not be a disadvantage when it is necessary for the extraction of a range of unknown analytes of interest from an environmental sample. Ideally, the NIP should not show binding of the analytes. However, due to non-specific interactions of the analytes with the silanol groups on the polymer, the NIP was able to bind to the analytes to a certain extent. This problem was also encountered by other workers investigating the same polymer system but with lisinopril dihydrate as the template [238]. The PMPA-MIP showed similar recoveries of the analytes as compared to the NIP, except for the template molecule PMPA, where the ratio in % recoveries of the PMPA-MIP to that of the NIP was 5:1, the highest imprinting efficiency obtained among all the analytes.

Next, the polymers were challenged with a typical water matrix, one of most common sample types prepared for analysis during the proficiency tests organized by the OPCW. The analytes were spiked at a concentration of 10 µg ml⁻¹, together with 500 µg ml⁻¹ of PEG as a background contaminant, into tap water from the laboratory. The % absorptivity, leak and recovery of the PMPA-MIP and the NIP of the analytes from this water matrix are compared in Figure 4-7. The % leak (%L) is defined as the % of analytes found in the wash during the washing step.

It was found that the presence of PEG and the use of tap water as the matrix did not adversely affect the binding properties of the MIP and NIP as compared to when deionized water was used as the matrix. The MIP showed a slight decrease in % recovery for IMPA, PMPA and CMPA and an increase in % recovery for MPA. The NIP showed a slight decrease in % absorptivity for EMPA, IMPA and PMPA and a
decrease in % recovery for all the analytes except for an MPA, for which there was an increase. On comparison of the MIP and the NIP, it is observed that the ratio of the % recovery of the MIP to that of the NIP for PMPA was 8:1, the highest among the range of analytes.

The polymers did not retain much of the PEG present in the sample as PEG was found in the eluent upon loading of the sample. The purpose of the additional washing step was to remove more PEG from the polymers. The analytes did not elute from the MIP during the washing step, whereas for the NIP, the analytes, except for MPA, were detected in the wash eluent. The final eluent contained very insignificant amounts of PEG, showing that the MIP-SPE procedure is effective in the sample clean-up of a water matrix containing PEG as a background contaminant.

**Figure 4-7.** Comparison of % absorptivity, leak and recovery of PMPA-MIP and NIP for PEG samples.

### 4.1.4 Comparison of PMPA-MIP-SPE with other sample preparation techniques

The PMPA-MIP-SPE procedure was compared against other SPE techniques, namely SCX and SAX as well as a procedure without sample preparation. SCX and SAX SPE cartridges are used for the removal of cations and PEG in water samples respectively. These are usually present as background contaminants which may
interfere with the analysis. Figure 4-8 shows the % recovery of the analytes using the various procedures.

![Bar chart showing % recovery of various analytes using different procedures.]

**Figure 4-8.** Comparison of % recovery of the phosphonic acids using the various procedures.

Of the four procedures, only the MIP-SPE and SAX SPE procedures were capable of sample clean-up such that PEG was detected in insignificant amounts in the final eluent. PEG was still present in the SCX SPE eluent as well as the sample which was directly evaporated to dryness. Even though the % recovery of the analytes from the MIP-SPE procedure cannot surpass that of the SAX SPE procedure, it has been shown to be effective in the sample clean-up of aqueous samples containing PEG for the analysis of phosphonates. The total ion chromatograms of the various procedures are compiled in Appendix 2. The chromatograms were obtained by reconstitution with DCM instead of ACN as ACN gives split peaks with the HP-5MS column whereas DCM does not. This is due to the use of ACN, a more polar solvent as compared to DCM, on the relatively non-polar HP-5MS column.

### 4.1.5 Evaluation of elution solvents and volume for TDG-MIP-SPE

Preliminary experiments with TDG-MIP showed that the polymer does not give 100% absorptivity of TDG as TDG was detected in the eluent upon loading the
sample. Hence, to improve on the % absorptivity, a larger amount of polymer was used (500 mg) together with a smaller volume of sample (0.5 ml) as compared to those used for PMPA-MIP-SPE. Figure 4-9 shows the % recovery of TDG from the three elution solvents evaluated. EtOH and 1% TE in water gave comparable recoveries while 1% TFA in water was unable to recover TDG from the MIP. The highly acidic elution solvent promoted the interaction between the silanol groups and TDG such that TDG could not be eluted from the MIP. Since 1% TE gave the highest % recovery, it was used in subsequent experiments.

**Figure 4-9.** Comparison of % recovery of TDG with the various elution solvents.

**Figure 4-10.** Comparison of % recovery of TDG with the various elution volumes.
From the graph, the optimum volume of elution solvent was 10 ml. A decrease in % recovery was observed upon increasing the volume of elution solvent to 20 ml. Hence 5 × 2 ml of 1% TE in water was used for subsequent experiments.

### 4.1.6 Evaluation of binding properties by TDG-MIP-SPE

![Structures of the degradation products of HD.](image)

**Figure 4-11.** Structures of the degradation products of HD.

Figure 4-11 shows the structures of the range of analytes used in the evaluation of the binding properties of the TDG-MIP and the NIP. TDG is the degradation product of the blister agent, HD, while TDGS and TDGSO are oxidation products of TDG.

The % absorptivity and recovery of the TDG-MIP and the NIP for the analytes from deionized water are compared in Figure 4-12. The TDG-MIP shows 100% absorptivity only for TDGS while the NIP shows no recovery for TDGS. The % absorptivity and recovery of the NIP are generally lower than that of the MIP.

![Comparison of % absorptivity and recovery of TDG-MIP and NIP.](image)

**Figure 4-12.** Comparison of % absorptivity and recovery of TDG-MIP and NIP.
Next, the polymers were challenged with tap water samples containing the range of analytes with 500 μg ml\(^{-1}\) of PEG. An attempt to include a washing step to remove PEG in the TDG-MIP-SPE procedure resulted in the loss of the analytes as the analytes were eluted during the washing step. Hence, the TDG-MIP-SPE procedure was carried out according to Figure 3-3, that is, without a washing step. This ensured reasonable recovery while compromising sample clean-up.

![Figure 4-13](image.png)

**Figure 4-13.** Comparison of % absorptivity and recovery of TDG-MIP and NIP for PEG samples.

The % absorptivity and recovery of the TDG-MIP of the analytes from PEG samples was comparable with that from deionized water sample. The % absorptivity of the analytes of the NIP showed a decrease in the presence of PEG. The % recovery, however, remained unaffected. Again, it was observed that the % absorptivity and recovery of the NIP are generally lower than that of the MIP.

### 4.1.7 Comparison of TDG-MIP-SPE with other sample preparation techniques

The TDG-MIP-SPE procedure was compared against other SPE techniques, namely SCX and SAX as well as a procedure without sample preparation. Figure 4-14 shows the % recovery of the analytes using the various procedures.
Of the four sample preparation techniques, only SAX SPE was able to completely remove PEG from the sample such that PEG could not be observed in the GC chromatogram. However, none of the compounds were recovered. It was observed that the compounds were not retained on the cartridge and were eluted upon loading of the sample onto the cartridge. This implies that TDG, TDGS and TDGSO were not anionic at the sample pH (pH 6) and were thus not retained on the SAX cartridge. Even though the TDG-MIP-SPE, SCX and direct rotary evaporation methods could recover the analytes of interest, PEG could not be totally removed from the aqueous sample. However, on comparison of the residual amount of PEG, it is observed that the TDG-MIP-SPE procedure has considerably reduced the amount of PEG. The total ion chromatograms of the various procedures are compiled in Appendix 2.

### 4.1.8 Evaluation of elution solvents and volume for TEA-MIP-SPE

Like the TDG-MIP, the TEA-MIP does not give 100% absorptivity of TEA. Hence, 500 mg of polymer was used together with 0.5 ml of sample. Figure 4-15 show the % recovery of TEA from the three elution solvents evaluated. All three
elution solvents gave comparable recoveries with 1% TE giving the highest % recovery. Hence, the latter was used in subsequent experiments.

**Figure 4-15.** Comparison of % recovery of TEA with the various elution solvents.

The volume of elution solvent did not significantly affect the % recovery of TEA. A volume of 1 ml of 1% TE in water was sufficient for a reasonable recovery of TEA from the MIP. The % recovery did not improve with the use of 10 or 20 ml of elution solvent. From the graph, 4 × 2 ml of 1% TE was determined to be the optimum volume of elution solvent required.

**Figure 4-16.** Comparison of % recovery of TEA with the various elution volumes.

### 4.1.9 Evaluation of binding properties by TEA-MIP-SPE

Figure 4-17 shows the structures of the analytes used in the evaluation of the binding properties of the TEA-MIP and the NIP. The ethanolamines, EDEA, MDEA
and TEA, are the degradation products of the nitrogen mustards, HN1, HN2 and HN3 respectively.

Figure 4-17. Structures of the ethanolamines.

The % absorptivity and recovery of the TEA-MIP and the NIP for the analytes from deionized water are compared in Figure 4-18. Both the TEA-MIP and NIP show similar % absorptivity and recovery for each of the analytes. The NIP shows slightly higher % absorptivity of the analytes but lower % recovery as compared to the TEA-MIP.

Figure 4-19 shows the % absorptivity and recovery of TEA-MIP for PEG samples. The presence of PEG in the tap water resulted in a slight decrease in the % absorptivity and recovery as compared to the deionized water sample for both the TEA-MIP and the NIP. The % absorptivity and recovery of the NIP is generally lower as compared to that of the TEA-MIP.

Figure 4-18. Comparison of % absorptivity and recovery of TEA-MIP and NIP.
4.1.10 Comparison of TEA-MIP-SPE with other sample preparation techniques

The TEA-MIP-SPE procedure was compared against other SPE techniques, namely SCX and SAX as well as a procedure without sample preparation. Figure 4-20 shows the % recovery of the analytes using the various procedures.

Only the TEA-MIP-SPE and the direct rotary evaporation procedures were able to recover the ethanolamines from the PEG sample. The SCX and SAX SPE procedures could not recover the ethanolamines, implying that the ethanolamines are cationic at the sample pH (pH 6). The cationic ethanolamines were retained on the SCX cartridge while they eluted upon loading onto the SAX cartridge. Only the SAX SPE procedure was able to remove PEG from the sample. The total ion chromatograms are compiled in Appendix 2.

The recovery of the ethanolamines using the TEA-MIP-SPE procedure was less than half that of the direct rotary evaporation procedure. However, in terms of sample cleanup, the amount of PEG introduced into the GC system was considerably less as compared to the direct rotary evaporation procedure.
4.1.11 Evaluation of elution solvents and volume for 3Q-MIP-SPE

Here, 200 mg of polymer was used together with 0.5 ml of sample. Figure 4-21 shows the % recovery of 3Q from the three elution solvents evaluated. As 1% TFA gave the highest % recovery of 3Q, it was used in subsequent experiments.
From the graph, the optimum volume of elution solvent was determined to be 10 ml. Increasing the volume to 20 ml led to a decrease in the % recovery. Hence, 5 × 2 ml of 1% TFA in water was used in subsequent experiments.

4.1.12 Evaluation of binding properties by 3Q-MIP-SPE

Figure 4-23 shows the structure of 3Q which is the degradation product of the psychogenic agent, BZ. The % absorptivity and recovery of the 3Q-MIP and the NIP for 3Q from deionized water are compared in Figure 4-24. Both the 3Q-MIP and the NIP do not show 100% absorptivity of the analyte. The % absorptivity and the recovery of the NIP are lower as compared to that of the 3Q-MIP.

![3Q](attachment:image1)

**Figure 4-23.** The structure of 3Q.

![Graph](attachment:image2)

**Figure 4-24.** Comparison of the % absorptivity and recovery of 3Q-MIP and NIP.

![Graph](attachment:image3)

**Figure 4-25.** Comparison of the % absorptivity and recovery of 3Q-MIP and NIP for PEG samples.
The polymers were further challenged with a tap water sample containing 3Q with 500 μg ml\(^{-1}\) of PEG. Both the 3Q-MIP and NIP show a decrease in % absorptivity with the tap water sample containing PEG. On the other hand, the % recovery of the analyte is slightly higher as compared to the deionized water sample. Here, the ratio of % recovery improved slightly over the deionized water sample.

### 4.1.13 Comparison of 3Q-MIP-SPE with other sample preparation techniques

The 3Q-MIP-SPE procedure was compared against other SPE techniques, namely SCX and SAX as well as a procedure without sample preparation. Figure 4-26 shows the % recovery of the analytes using the various procedures.

![Figure 4-26. Comparison of % recovery of 3Q using the various procedures.](image)

Only the 3Q-MIP-SPE and the direct rotary evaporation procedures were able to recover 3Q from the PEG sample. Similar to the experiments with TEA, the SCX and SAX SPE procedures could not recover 3Q, implying that 3Q is also cationic at the sample pH (pH 8). The cationic 3Q was retained on the SCX cartridge [23] while it eluted upon loading onto the SAX cartridge. Only the SAX SPE procedure was able to remove PEG from the sample (Appendix 2).

The recovery of 3Q using the 3Q-MIP-SPE procedure marginally surpasses that of the direct rotary evaporation procedure. In addition, in terms of sample
cleanup, the amount of PEG introduced into the GC system was considerably less as compared to the direct rotary evaporation procedure.

4.1.14 Evaluation of elution solvents for MIP-SPE using a mixture of MIPs

Next, MIP-SPE using a mixture of the various MIPs was investigated for the extraction of the various template molecules from aqueous matrices. From the evaluation studies, 50 mg of PMPA-MIP, 500 mg of TDG-MIP and TEA-MIP and 200 mg of 3Q were used. Hence the MIPs, PMPA-MIP, TDG-MIP, TEA-MIP and 3Q-MIP, were mixed in the ratio of 1:10:10:4 respectively. The % absorptivity and recovery using the elution solvents, ethanol, 1% TFA in water and 1% TE in water, are compared in Figures 4-27 to 4-29.

![Figure 4-27. Comparison of % absorptivity and % recovery of the mixture of MIPs and NIP with EtOH.](image1)

![Figure 4-28. Comparison of % absorptivity and % recovery of the mixture of MIPs and NIP with 1% TFA in water.](image2)
Figure 4-29. Comparison of % absorptivity and % recovery of the mixture of MIPs and NIP with 1% TE in water.

Except for 1% TFA for the recovery of TDG where no recovery was obtained, all the solvents were able to recover the analytes to varying extents. The highest % recovery of 3Q and PMPA was obtained with 1% TFA while ethanol gave the highest % recovery of TDG and TEA. This is in contrast with the results from the evaluation of elution solvents for each MIP where deionized water samples without PEG were used. Those results showed that the highest % recovery for 3Q was obtained with 1% TFA while 1% TE gave the highest recovery for PMPA, TDG and TEA. The presence of the other analytes, presence of PEG and the use of tap water may have contributed to this observation. In terms of the biggest ratio of % recovery between the MIP and the NIP, 1% TFA was the solvent of choice for 3Q, 1% TE for PMPA and ethanol for TDG and TEA. The % absorptivity and recovery were generally lower for the NIP as compared to that of the mixture of MIPs.

4.1.15 Comparison of MIP-SPE using a mixture of MIPs with other sample preparation techniques

The % recovery of the analytes using the mixture of MIPs with the different elution solvents as well as the different sample preparation procedures are compared
in Figure 4-30. SAX SPE is the only method capable of removing PEG from the aqueous samples. However, it has its limitations. It is unable to recover the entire range of analytes as only PMPA was detected. SCX SPE was unable to remove PEG from the aqueous samples and in addition; it was only able to recover PMPA and TDG. Except for TDG using TFA as the elution solvent, the MIP-SPE procedure was able to recover the entire range of analytes. As expected, all the analytes were detected using the direct rotary evaporation procedure. The total ion chromatograms of the various procedures are compiled in Appendix 2. The chromatograms were obtained by reconstitution with DCM instead of ACN, as ACN gave split peaks with the HP-5MS column whereas DCM did not.

![Figure 4-30. Comparison of % recovery using the various procedures.](image)

Both the MIP-SPE and direct rotary evaporation procedures have their advantages and disadvantages. The direct rotary evaporation procedure is fast and gives a higher recovery of analytes. However, the procedure was found to introduce more PEG into the GC column as seen from the great difference in areas under the PEG peaks when the chromatograms are overlaid. This is important as it is not
advisable to introduce high concentrations of matrix interference into the GC–MS system as this may not only result in contamination of the column and MS detector but also in shortening the lifespan of the MSD filament. On the other hand, the MIP-SPE procedure gives lower recovery, and introduces less PEG into the GC column.

4.1.16 Preparation of sol-gel MIP fibers

Next, the development of sol-gel MIP fibers was attempted. Upon coating the sol solution onto the fused silica fiber, the coating was dried at room temperature in a desiccator. The coating solidified to give a shiny crystalline layer, however, it was of unequal thickness along the length of the fiber. Prior to use, the fiber was conditioned at 250°C for 1 h in the GC inlet. The heat treatment caused some of the coating to flake off. Preliminary experiments with the fiber for the extraction of analytes of interest from a water matrix and subsequent desorption in the heated inlet of the GC caused further flaking off of the coating. As such, further investigation of sol-gel MIP SPME was not carried out and efforts were instead focused on the evaluation of the novel PhPPP coating.

4.1.17 CONCLUSION

The MIPs as well as the NIP were evaluated for their binding properties for a range of analytes in deionized water samples as well as tap water samples containing PEG as a background contaminant. The MIPs were not only able to bind to their respective template but also to structurally similar compounds. This is an advantage for the analysis of a wide range of similar analytes. The NIPs used in this study showed some adsorption of the analytes. Ideally, they should not show adsorption of analytes. However, the absorptivity and recovery were generally lower than that of the MIPs. Only the PMPA-MIP showed 100% absorptivity of the analytes.
Each MIP-SPE procedure was compared with other sample preparation procedures, namely SAX SPE and SCX SPE as well as a direct rotary evaporation procedure for the analysis of a range of analytes in an aqueous sample containing PEG. All the procedures were able to recover the phosphonates. However, only the PMPA-MIP-SPE and SAX SPE procedures were capable of removing PEG from the sample. Except for PMPA-MIP-SPE, a problem encountered for the other MIP-SPE procedures was that analytes were eluted during the washing step. Hence to ensure recovery of the analytes, the washing step was omitted and this compromised the capability for sample clean-up. All the procedures except for SAX SPE were able to recover TDG while only the MIP-SPE and the direct rotary evaporation procedures were able to recover TEA and 3Q.

The various MIPs were mixed together for the analysis of a range of analytes, namely the templates used during the polymer synthesis. The MIP-SPE procedures using ethanol or 1% TE in water as the elution solvent, as well as the direct rotary evaporation procedure, were able to recover the entire range of analytes. This is in contrast to the procedures using commercially available SPE cartridges which did not work for all of the analytes investigated. Although the direct rotary evaporation procedure gave higher recovery, it was observed that the MIP-SPE procedure introduces a lower amount of PEG into the GC column.

The development of sol-gel MIP SPME fibers posed a problem as the sol-gel coatings synthesized using alkoxyisilane monomers cracked and flaked off upon drying at room temperature. Flaking off of the SPME coating was also observed upon exposure to the heated GC inlet.
4.2 SPME using PhPPP-coated fibers

L1 is an organoarsenic agent listed under Schedule 1 of the Chemicals Weapons Convention. The synthesized agent is actually composed of cis and trans isomers and contains impurities such as L2 and L3. The structures of the compounds are shown in Figure 4-31. Similar to sulfur mustard, L1 possesses vesicant properties. However, in contrast to sulfur mustard, it is known to produce an immediate burning sensation upon contact with the skin. Several arsenic-containing compounds were introduced as chemical warfare agents during World War I. Among arsine, diphenylchboroarsine, phenyldichloroarsine, diphenylaminechboroarsine, diphenylcyanoarsine, methyl dichloroarsine, ethyldichloroarsine and ethyl dibromoarsine, Lewisite was considered as the best arsenical war gas [354]. Unlike other chemical warfare agents, L1 requires derivatization in order to be amenable to GC–MS analysis as direct analysis leads to deterioration of the column performance and corrosion of the detector [58].

Upon contact with water, L1 is rapidly hydrolyzed to 2-chlorovinylarsonous acid (CVAA) which is as toxic. Subsequently, 2-chlorovinylarsenous oxide (CVAO) and polymerized chlorovinylarsenous oxide is formed. The hydrolysis pathway is shown in Figure 4-32. Similarly, L2 is expected to undergo similar hydrolysis to the corresponding bis(2-chlorovinyl)arsonous acid (BCVAA) [356]. Except in old abandoned chemical bombs where intact Lewisites 1 and 2 could still be found [357],
the existence of Lewisites in the environment would most likely be indicated by the presence of CVAA, BCVAA, L3 or other degradation products. The SPME of CVAA from aqueous and soil samples [89] as well as in urine [92] has previously been investigated. In these studies, dithiols such as 1,2-ethanedithiol and 1,3-propanedithiol were used as the derivatizing agents. In this study, we explored the use of the novel PhPPP fiber for the extraction of the arsenic compounds as well as using monothiols as derivatizing agents in SPME.

\[
\begin{align*}
\text{Cl} & \quad \text{As} & \quad \text{Cl} \\
\text{Cl} & \quad \text{As} & \quad \text{Cl} \\
+ & \quad \text{2 H}_2\text{O} & \quad \begin{array}{c}
\text{Cl} \\
\text{As} \\
\text{OH}
\end{array} & \quad + & \quad \text{2 HCl} \\
\text{L1} & \quad & \text{CVAA} & \quad & \text{CVAA}
\end{align*}
\]

\[
\begin{align*}
\text{Cl} & \quad \text{As} & \quad \text{O} \\
+ & \quad \text{H}_2\text{O} & \quad \begin{array}{c}
\text{Cl} \\
\text{As} \\
\text{O}
\end{array} & \quad + & \quad \text{H}_2\text{O} & \quad \text{CVAO}
\end{align*}
\]

\[
\begin{align*}
\text{Cl} & \quad \text{As} & \quad \text{O}_n
\end{align*}
\]

**Figure 4-32.** Hydrolysis pathway of L1 [355].

In the evaluation of the PhPPP-coated SPME fiber for the analysis of Lewisites, extraction conditions such as sample pH, ionic strength, derivatizing agent and extraction time were first optimized. Using the optimal extraction conditions, the precision, linearity and limit of detection of the method were investigated using spiked deionized water samples. The performance of the network fiber was compared
against that of a series of commercially available fibers, namely 30 µm and 100 µm PDMS, 85 µm PA, 65 µm PDMS/DVB, 75 µm CAR/PDMS and 65 µm CW/DVB. All experiments were performed in triplicate, unless stated otherwise.

4.2.1 Optimization of SPME conditions

Recommended operating procedures for the extraction of Lewisite in aqueous samples involve the adjustment of the pH of the sample to 2 [62]. Hence, the effect of sample pH on uptake by the fiber was investigated. The water sample was adjusted to an acidic pH of 0 and 2, left unadjusted at pH 6 and adjusted to a basic pH of 8.

Figure 4-33 shows the uptake of the analytes with respect to sample pH. It was found that adjustment of pH to 2 or analysis without pH adjustment did not significantly affect the uptake. On the other hand, an extremely acidic pH of 0 and a moderately basic pH of 8 adversely affected the uptake of the analytes. The behavior of the analytes at extreme acidity is not well-understood and the lower uptake may be due to the degree of ionization of the analytes while the poor uptake at pH 8 is the result of decomposition in alkaline media [354]. Hence, in subsequent experiments, the pH of the sample was not adjusted.

![Figure 4-33. Effect of pH on uptake of the Lewisites. Spiking concentration: 0.5 µg ml⁻¹; derivatizing agent: PT; salt concentration: 40% (w/v) NaCl; extraction time: 15 min.](image_url)
Increasing the ionic strength of the aqueous sample by the addition of salt has been shown to improve the SPME uptake of nerve agents [88]. However, the reverse is observed for the Lewisites. As seen from Figure 4-34, saturating the sample with the addition of 40% (w/v) NaCl or Na$_2$SO$_4$ led to a decrease in uptake of the analytes. Since the derivatized analytes were relatively non-polar as compared to the nerve agents, salting was not required for uptake onto the fiber. Hence, further experiments were conducted without the addition of salt. Omitting the need for adjustment of sample pH and addition of salt resulted in a simple and straightforward extraction procedure.

![Figure 4-34. Effect of salting on uptake of the Lewisites. Spiking concentration: 0.5 µg ml$^{-1}$; pH: 6; derivatizing agent: PT; extraction time: 15 min.](image)

L1 can be chromatographed on a new column but leads to rapid deterioration of the column. L2 can be chromatographed but is better derivatized. L3, on the other hand, does not require derivatization [358]. The dithiols form cyclic derivatives with L1 while for L2, a free thiol group remains. In other studies on CVAA, 1,2-ethanedithiol and 1,3-propanedithiol were commonly used as derivatizing agents [89,91,92,359]. An interesting fact to note is that derivatization of L1 or CVAA with any particular thiol leads to the same derivative. The same is true for L2 and BCVAA.
A series of monothiols and dithiols as derivatizing agents for the analytes was investigated. The monothiols investigated included ethanethiol, propanethiol and butanethiol while the dithiols were 1,2-ethanedithiol and 1,3-propanedithiol. The structures of the derivatives are presented in Figure 4-35. The results are shown in Figure 4-36. As 1,4-Butanedithiol was not fully soluble in water, it was excluded from further investigations.

Figure 4-35. Structures of the various derivatives of L1 and L2.

Figure 4-36. Effect of derivatizing agents on uptake of the Lewisites. Spiking concentration: 0.5 µg ml⁻¹; pH: 6; salt concentration: 0%; extraction time: 15 min.
Since L3 does not require derivatization, its uptake did not vary significantly with the derivatizing agent used while that of L2 and L1 showed significant variation. It was obvious that the monothiols were superior over the dithiols as derivatizing agents for L2 and L1. As our findings indicated that propanethiol gave the highest uptake of L2 and L1, propanethiol was thus selected as the derivatizing agent for the analytes in water samples in subsequent experiments.

Extraction time profiles (Figure 4.37) were obtained at extraction times ranging from 5 min to 60 min. It was observed that equilibrium was achieved for the analytes at 30 min. Hence, subsequent experiments were conducted using an extraction time of 30 min.

![Figure 4.37](image-url)

**Figure 4.37.** Effect of extraction time on uptake of the analytes. Spiking concentration: 0.5 µg ml\(^{-1}\); pH: 6; derivatizing agent: PT; salt concentration: 0%; extraction time: 15 min.

### 4.2.2 Method validation

Using the optimal extraction conditions, the precision, linearity and limit of detection of the method were investigated using spiked deionized water samples. The results are compiled in Table 4-1.
Table 4-1. Quantitative results of SPME of the Lewisites.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Equation&lt;sup&gt;a&lt;/sup&gt;</th>
<th>&lt;sup&gt;r&lt;/sup&gt;&lt;sup&gt;2&lt;/sup&gt;</th>
<th>% RSD&lt;sup&gt;b&lt;/sup&gt;</th>
<th>LOD&lt;sup&gt;b&lt;/sup&gt; (µg l&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>LOD&lt;sup&gt;c&lt;/sup&gt; (µg l&lt;sup&gt;-1&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L3</td>
<td>y = 0.2799x + 2.1886</td>
<td>0.9975</td>
<td>8</td>
<td>0.31</td>
<td>0.17</td>
</tr>
<tr>
<td>L2-PT</td>
<td>y = 0.4502x – 0.5715</td>
<td>0.9997</td>
<td>6</td>
<td>0.19</td>
<td>0.24</td>
</tr>
<tr>
<td>L1-PT</td>
<td>y = 0.5856x + 3.8997</td>
<td>0.9901</td>
<td>8</td>
<td>0.08</td>
<td>0.10</td>
</tr>
</tbody>
</table>

<sup>a</sup> linear range 0.001-0.5 µg ml<sup>-1</sup>
<sup>b</sup> LODs of SPME using PhPPP fiber at S/N = 3.
<sup>c</sup> LODs of SPME using 100 µm PDMS fiber at S/N = 3.

The linearity of SPME calibration plots was investigated over a concentration range of 0.001-0.5 µg ml<sup>-1</sup>. The analytes exhibited good linearity with good squared regression coefficients of >0.990. This allowed the quantification of the compounds by the method of external calibration. The relative standard deviations (RSDs) for six extractions were below 8% for all the analytes. The limits of detection (LODs) for the analytes were estimated at S/N = 3 under GC–MS full scan conditions. The LODs ranged between 0.08-0.31 µg l<sup>-1</sup>.

4.2.3 Comparison with commercial SPME fibers

![Figure 4-38](image)

**Figure 4-38.** Comparison of uptake using the various fibers. Spiking concentration: 10 µg l<sup>-1</sup>; pH: 6; derivatizing agent: PT; salt concentration: 0%; extraction time: 30 min.
The performance of the novel PhPPP fiber in the uptake of the analytes in aqueous samples was compared against that of several commercially available fibers from Supelco. As seen in Figure 4-38, the CAR/PDMS fiber gave the lowest uptake of the analytes since it is more suitable for gases and low molecular weight compounds up to a molecular weight of 225. Furthermore, it is not surprising that the CW/DVB and PA fibers, suitable for polar compounds, showed poor uptake of the analytes since they are considered to be relatively non-polar. Our results showed that the PhPPP fiber performed better than all the commercial fibers in the uptake of derivatives of CVAA and BCVAA but marginally poorer than the 100 μm PDMS and PDMS/DVB fibers in the uptake of L3. The LODs for the analytes using the PhPPP fiber and the best-performing fiber, the 100 μm PDMS fiber, are compared in Table 4-1. The use of propanethiol instead of propanedithiol as derivatizing agent for CVAA lowered the LOD of 1 μg L⁻¹ obtained in a previous study [89] by an order of magnitude. The lowest LOD reported for the analysis of CVAA is 7 pg ml⁻¹ in urine using an automated SPME–GC–MS system [92].

4.2.4 Conclusion

The novel PhPPP fiber shows great promise as an alternative to commercially available fibers for the SPME of Lewisites in aqueous samples. PhPPP fibers are easy to prepare and are significantly less costly than commercial fibers.
4.3 HF-LPME

4.3.1 Optimization of parameters for HF-LPME of chemical agents

The chemical warfare agents investigated included the nerve agents, GB, GD, GA and VX as well as the blister agent, HD. The structures of the analytes are presented in Figure 4-39. Factors affecting the extraction efficiency such as stirring speed, sample ionic strength, and extraction time were optimized. All experiments were performed in triplicate.

Several commonly-used organic solvents in microextraction such as toluene, cyclohexane, isoctane and their combinations were initially investigated. However, higher extraction efficiency was observed with chlorinated solvents and their combinations [344,345]. Hence, dichloromethane, chloroform, carbon tetrachloride, trichloroethylene and their combinations were studied. For each solvent, extractions were performed using deionized water spiked with CWAs, 30% (w/v) NaCl and with stirring at 700 rpm for 15 min. Figure 4-40 shows the effect of the different solvents on extraction efficiency.
Dichloromethane, when used as a single solvent, was not suitable as it evaporated too easily during the course of the extraction. As seen from Figure 4-40, chloroform gave the highest uptake for GB and VX and relatively similar uptake for GD, GA and HD as compared to the other solvents. In other preliminary studies, the uptake was found to decrease when chloroform was used in combination with other solvents. There is no observed relationship between the uptake of the CWAs as related to the polarity indices [360] or octanol/water partition coefficients (log $K_{ow}$) [361] of the solvents and the log $K_{ow}$ of the CWAs [15]. The polarity indices of the solvents as well as the log $K_{ow}$ of the CWAs are compiled in Tables 4-2 and 4-3. Chloroform, the most polar solvent among the chlorinated solvents investigated, was able to extract the two analytes with extreme log $K_{ow}$ values, GB and VX. On the other hand, trichloroethylene, the least polar solvent, showed poor uptake for most of the analytes. The results are in contrast to those reported previously [345] where chloroform, among other solvents, was discarded due to problems with non-
compatibility with fiber pores, instability and miscibility with water. Our results show that the extraction efficiency of the CWAs with chloroform is significantly better than that with toluene and with trichloroethylene. Several of the solvents were unable to extract VX. Hence, chloroform was selected as the extraction solvent.

Table 4-2. Data of the solvents.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Polarity Index [360]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform</td>
<td>4.1</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>3.1</td>
</tr>
<tr>
<td>Toluene</td>
<td>2.4</td>
</tr>
<tr>
<td>Carbon tetrachloride</td>
<td>1.6</td>
</tr>
<tr>
<td>Trichloroethylene</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Table 4-3. Data of the CWAs.

<table>
<thead>
<tr>
<th>CWA</th>
<th>Log $K_{ow}$ [361]</th>
</tr>
</thead>
<tbody>
<tr>
<td>GB</td>
<td>0.299</td>
</tr>
<tr>
<td>GD</td>
<td>1.824</td>
</tr>
<tr>
<td>GA</td>
<td>0.384</td>
</tr>
<tr>
<td>HD</td>
<td>1.37</td>
</tr>
<tr>
<td>VX</td>
<td>2.09</td>
</tr>
</tbody>
</table>

Extractions without the addition of salt were performed. However, uptake of the analytes was poor, particularly for VX which was not recovered. Hence experiments were performed with addition of salt. Increasing the ionic strength of the water sample has been shown to decrease the affinities of the chemical agents for the aqueous matrix and hence enhancing their uptake [88]. The effect of two different salts, NaCl and Na$_2$SO$_4$, was compared in Figure 4-41. At the same salinity level of 30% (w/v), neither NaCl nor Na$_2$SO$_4$ was the obvious choice. The uptake of VX was better whereas the uptake of HD was adversely affected with Na$_2$SO$_4$. However, since NaCl gave higher uptake of most the analytes, further experiments were conducted with NaCl. Salt concentrations were varied from a low salinity level of 10% (w/v) to a saturation level of 40% (w/v).
Figure 4-41. Effect of salt on uptake of the CWAs. Extraction solvent: chloroform; stirring speed: 700 rpm; extraction time: 15 min.

Figure 4-42. Effect of salt concentration on uptake of the CWAs. Extraction solvent: chloroform; stirring speed: 700 rpm; extraction time: 15 min.

Figure 4-42 shows the effect of salt concentration on extraction efficiency. It was observed that the optimum salt concentration for all the analytes was 30% (w/v). At higher salinity levels of 33% or higher, the sample solution was saturated such that the presence of suspended salt particles interfered with the uptake and led to the
decrease in recoveries observed. The salt concentration was thus maintained at 30\% (w/v) NaCl for subsequent experiments.

Although agitation of the sample can enhance the extraction and reduce the time to thermodynamic equilibrium, higher agitation speed may result in drop dislodgement and solvent loss particularly for SDME, over long extraction times. As mentioned above, an obvious advantage of HF-LPME over SDME is the fact that higher stirring speeds can be employed without affecting the solvent drop. The effect of stirring speed on the extraction efficiency was investigated over a range of 300 rpm to the maximum of 1250 rpm. Figure 4-43 shows the effect of stirring speed on extraction efficiency. The uptake of VX was relatively unaffected by stirring speed or extraction time due to its slow rate of diffusion [88], the extraction efficiencies of the other analytes increased with increasing stirring speeds. Hence the maximum allowable stirring speed on the magnetic stirrer, i.e. 1250 rpm, was used in subsequent experiments.

![Figure 4-43](image_url)

**Figure 4-43.** Effect of stirring speed on uptake of the CWAs. Extraction solvent: chloroform; salt concentration: 30\% (w/v); extraction time: 15 min.
HF-LPME is an equilibrium-based rather than exhaustive extraction process. Generally, the equilibrium time is selected as extraction time. However, it is usually not practical to prolong an extraction unnecessarily for equilibrium to be established. This is because the longer the extraction, the greater the potential of solvent loss due to dissolution in the sample solution. Additionally, there are obvious benefits in conducting more time-efficient extraction. Thus, for quantitative analysis, achieving equilibrium is not necessary as long as extraction conditions are kept rigorously constant. Figure 4-44 shows the effect of extraction time on extraction efficiency. Except for VX which was relatively unaffected by extraction time, the other analytes showed increasing uptake with increasing extraction times. The extraction time of 30 min was selected since reasonable uptake was achieved in this shorter analysis time.

![Figure 4-44](image)

**Figure 4-44.** Effect of extraction time on uptake of the CWAs. Extraction solvent: chloroform; salt concentration: 30% (w/v); stirring speed: 1250 rpm.

### 4.3.2 Method validation for HF-LPME of chemical agents

Using the optimal extraction conditions, the precision, linearity and limit of detection of the method were investigated using spiked deionized water samples. The results are shown in Table 4-4. External calibration was performed for all
experiments. To evaluate the linearity of the calibration plots, samples were spiked with CWA over a concentration range of 0.01–1 µg ml\(^{-1}\) and then extracted. The GC peak area counts were plotted against the respective analyte concentrations to generate calibration curves. The CWAs exhibited good linearity with very good squared regression coefficients of >0.9950. This allowed the quantification of the compounds by the method of external calibration. The limits of detection for the CWAs were estimated at S/N = 3 under GC–MS full scan conditions. The LODs ranged between 0.02 and 0.09 µg l\(^{-1}\) and were better than those reported for SDME and HF-LPME using trichloroethylene [344,345]. Also, these values better the requirement for the safe level (50-200 µg l\(^{-1}\) in 2-3 L) in drinking water contaminated with CWAs [88]. The RSDs for six extractions were below 10% for all the analytes.

### Table 4-4. Quantitative results of HF-LPME of the CWAs.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Equation(^a)</th>
<th>(r^2)</th>
<th>% RSD</th>
<th>LOD(^b) (n = 6)</th>
<th>LOD(^c)</th>
<th>LOD(^d)</th>
<th>LOD(^e)</th>
<th>LOD(^f)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GB</td>
<td>(y = 0.1164x + 1.0640)</td>
<td>0.9950</td>
<td>5</td>
<td>0.03</td>
<td>0.03</td>
<td>10</td>
<td>75</td>
<td>0.05</td>
</tr>
<tr>
<td>GD</td>
<td>(y = 0.1840x + 0.0447)</td>
<td>0.9999</td>
<td>7</td>
<td>0.09</td>
<td>0.10</td>
<td>-</td>
<td>-</td>
<td>0.05</td>
</tr>
<tr>
<td>GA</td>
<td>(y = 0.1802x + 0.2885)</td>
<td>1.0000</td>
<td>6</td>
<td>0.02</td>
<td>0.02</td>
<td>-</td>
<td>-</td>
<td>0.05</td>
</tr>
<tr>
<td>HD</td>
<td>(y = 0.1698x + 0.3817)</td>
<td>0.9999</td>
<td>9</td>
<td>0.09</td>
<td>0.10</td>
<td>1.0</td>
<td>30</td>
<td>-</td>
</tr>
<tr>
<td>VX</td>
<td>(y = 0.0375x + 1.9906)</td>
<td>0.9977</td>
<td>7</td>
<td>0.09</td>
<td>0.20</td>
<td>-</td>
<td>-</td>
<td>0.5</td>
</tr>
</tbody>
</table>

\(^a\) linear range 0.01-1 µg ml\(^{-1}\)

\(^b\) LODs (in µg l\(^{-1}\)) of HF-LPME for water samples at S/N = 3.

\(^c\) LODs (in µg l\(^{-1}\)) of HF-LPME for slurry samples at S/N = 3.

\(^d\) LODs (in µg l\(^{-1}\)) of HF-LPME using trichloroethylene as a solvent at S/N = 10 [345].

\(^e\) LODs (in µg l\(^{-1}\)) of SDME using dichloromethane:carbon tetrachloride as a solvent at S/N = 10 [344].

\(^f\) LODs (in µg l\(^{-1}\)) of SPME using 65 µm PDMS/DVB fiber at S/N =3 [88].

### 4.3.3 Comparison of HF-LPME of chemical agents with SPME

In order to demonstrate the capability of HF-LPME in the extraction of the CWAs, a relatively simple matrix such as deionized water samples as well as the much more complex slurry samples (20 mg of soil in 1 ml of water) were employed.
Figure 4-45 shows typical total ion chromatograms after HF-LPME extraction of the CWAs. As compared to the deionized water samples, the slurry samples were meant to pose a greater challenge to the extraction procedure due to the presence of soil particles as well as extraneous materials.

**Figure 4-45.** Total ion chromatograms after HF-LPME extraction of spiked deionized water sample (a) and spiked slurry sample (b) at a concentration of 0.5 µg ml\(^{-1}\). Extraction solvent: chloroform; salt concentration: 30% (w/v); stirring speed: 1250 rpm; extraction time: 30 min.

The extraction efficiency of HF-LPME was also compared with that of direct immersion-SPME. Previously-reported optimum conditions for direct immersion SPME were utilized [88]. As seen from Figure 4-46, neither technique demonstrated
superiority over the other. The HF-LPME technique gave higher uptake for GB, GA and VX while direct immersion SPME showed higher uptake of GD and HD. The PDMS/DVB fiber showed higher uptake of GD and HD, over the other CWAs, owing to their relative hydrophobicity. Except for VX, the uptake of the analytes using HF-LPME was not affected by the more complex slurry matrix. On the other hand, a difference between the uptake of the analytes by direct immersion SPME from water and slurry samples is observed. In fact, VX was not recovered at all from slurry samples by direct immersion-SPME. This experiment demonstrated an obvious advantage of HF-LPME over direct immersion SPME where the hollow fiber served as a filter that prevented the soil particles in the complex slurry matrix from interfering with the analysis. After each extraction, the hollow fiber can be discarded and a fresh one used for the next extraction. In contrast, the fiber was observed to deteriorate with repeated direct immersion SPME from the slurry samples.

![Figure 4-46. Comparison of HF-LPME and SPME extraction efficiency for the CWAs. HF-LPME extraction solvent: chloroform; salt concentration: 30% (w/v); stirring speed: 1250 rpm; extraction time: 30 min. SPME salt concentration: 40% (w/v); stirring speed: 1250 rpm; extraction time: 30 min.](image-url)
4.3.4 Optimization of parameters for HF-LPME of CWA degradation products

Several main degradation products of CWAs were selected, ranging from those of nerve agents such as GB, GD, VX to blister agents, including HD, 1,2-bis(2-chloroethylthio)ethane (sesquimustard, Q), bis(2-chloroethylthioethyl)ether (OMustard, T), and a psychotomimetic agent, BZ. The actual analytes of interest were EMPA (from VX), IMPA (from GB), PMPA (from GD), MPA which is the final degradation product of alkyl methylphosphonic acids, n-propylphosphonic acid (nPPA), ethyl 2-hydroxyethyl sulfide (EHES), thiodiglycol (TDG) (from HD), 1,2-bis(2-hydroxyethylthio)ethane (QOH) (from Q), bis(2-hydroxyethylthioethyl)ether (TOH) (from T), and benzilic acid (BA) (from BZ). The structures of the analytes are shown in Figure 4-47 while the $pK_a$ and log $K_{ow}$ data of the analytes are compiled in Table 4-5. Factors affecting the extraction efficiency such as extraction solvent, pH, sample ionic strength, stirring speed, and extraction time were optimized. All experiments were performed in triplicate.

![Chemical structures](image1)

Figure 4-47. CWA degradation products investigated in this study.
Table 4-5. $pK_a$ and $log K_{ow}$ data of the CWA degradation products.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>$pK_a$ [362,363]</th>
<th>$log K_{ow}$ [362]</th>
</tr>
</thead>
<tbody>
<tr>
<td>EMPA</td>
<td>2.32</td>
<td>-0.15</td>
</tr>
<tr>
<td>EHES</td>
<td>14.73</td>
<td>0.44</td>
</tr>
<tr>
<td>IMPA</td>
<td>2.32</td>
<td>0.27</td>
</tr>
<tr>
<td>PMPA</td>
<td>2.30</td>
<td>Not available</td>
</tr>
<tr>
<td>MPA</td>
<td>2.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-0.70</td>
</tr>
<tr>
<td>nPPA</td>
<td>3.13</td>
<td>0.28</td>
</tr>
<tr>
<td>TDG</td>
<td>14.68</td>
<td>-0.63</td>
</tr>
<tr>
<td>BA</td>
<td>3.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.30</td>
</tr>
<tr>
<td>QOH</td>
<td>14.64</td>
<td>Not available</td>
</tr>
<tr>
<td>TOH</td>
<td>Not available</td>
<td>Not available</td>
</tr>
</tbody>
</table>

<sup>a</sup> Obtained from the Interactive PhysProp Database [362].

HF-LPME for analytes requiring derivatization prior to GC–MS analysis has been carried out by extracting the analytes of interest first using pure solvent before drawing up derivatizing agent into the hollow fiber prior to injection [364], or introducing derivatizing agent into the GC injection port immediately after injection of the extract [365]. Alternatively, the derivatizing agent is added to the sample for simultaneous derivatization and extraction of analytes [366,367]. Coupled two-step derivatizations in which hydroxycarbonyls were first extracted and derivatized with $O$-(2,3,4,5-pentafluorobenzyl)-hydroxylamine by HF-LPME followed by single drop microextraction over the headspace of bis(trimethylsilyl)trifluoroacetamide (BSTFA) have been reported [368]. Another successful approach for derivatization was to use a mixture of solvent and derivatizing agent as the extraction medium held within a HF for the simultaneous clean-up, extraction and derivatization of 11-nor-$\Delta^9$-tetrahydrocannabinol-9-carboxylic acid from urine [369]. The solvent mixture consisted of BSTFA and octane in the ratio of 5:1. After an 8-min extraction, 6 µl of the mixture was analyzed by GC–MS. Similarly, a HF-LPME technique involving simultaneous in-fiber silylation for unconjugated anabolic steroids in urine has been developed [370]. The hollow fiber was first pre-conditioned with dihexyl ether and
then filled with a mixture of $N$-methyl-$N$-(trimethylsilyl)trifluoroacetamide (MSTFA), ammonium iodide and dithioerythritol as the acceptor phase. The extraction was performed at 45°C for 30 min. A 2-μl portion of the acceptor phase was then drawn from the fiber and injected directly into the GC–MS instrument.

In-situ derivatization HF-LPME of alkylphosphonic acids has been carried out by basification of the spiked water with potassium carbonate, addition of propyl bromide and stirring of the mixture at 100°C for 2 h. Upon completion of reaction, extractions were performed by HF-LPME prior to GC–MS analysis [346]. Here, HF-LPME with in-situ derivatization was investigated for the extraction of degradation products of CWAs from aqueous samples in which extractions were performed using a mixture of solvent and derivatizing agent held in the hollow fiber. Although the derivatizing agent used, $N$-(tert.-butyldimethylsilyl)-$N$-methyltrifluoroacetamide (MTBSTFA), is moisture-sensitive, it is protected within the hollow fiber and allowed the successful extraction of the analytes of interest prior to GC–MS analysis. In addition, tert.-butyldimethylsilyl (TBDMS) derivatives are known to be $10^4$ times more stable to hydrolysis than trimethylsilyl (TMS) derivatives [371].

In order to determine an optimum derivatization method, three derivatization procedures were compared. In the first method, pure solvent (chloroform) was held in the hollow fiber for the extraction of the analytes. Extractions were performed using deionized water spiked with the analytes, 30% (w/v) NaCl and with stirring at 700 rpm. After 15 min of extraction, 1 μl of MTBSTFA was combined with 1 μl of extract in the syringe for injection. In the second method, a mixture of chloroform and MTBSTFA in a 1:1 ratio was held in the hollow fiber for extraction, followed by injection of 1 μl of the extract. The third method was essentially identical to the second except that the 1 μl of extract was combined with another 1 μl of MTBSTFA.
prior to injection. It was concluded from our experiments that the second method using the chloroform/MTBSTFA solvent-derivatizing agent mixture (1:1) without the additional microliter of derivatizing agent was optimum for extractions. The effect of the derivatization procedure on the uptake of the analytes is shown in Figure 4-48.

![Figure 4-48. Effect of derivatization method on uptake of the degradation products.](image)

The experiments on HF-LPME of CWAs, as discussed in Section 4.3.1, demonstrated that chlorinated solvents were ideal for this class of compounds. Hence, several chlorinated solvents, namely chloroform, carbon tetrachloride, trichloroethylene and tetrachloroethylene, were evaluated together with a commonly-used non-chlorinated solvent, toluene. Each of the solvents was mixed in a 1:1 ratio with MTBSTFA and 5 µl of the solvent-derivatizing agent mixture was held in the hollow fiber for extraction. From Figure 4-49, it can be seen that none of the solvents gave the highest extraction for all the analytes. Chloroform was selected for further experiments as it gave the optimum extraction for most of the analytes.
Next, two commonly-used silylating derivatizing agents, BSTFA and MTBSTFA, were compared in order to determine the derivatizing agent of choice. The results are presented in Figure 4-50. Except for BA where BSTFA gave higher uptake than MTBSTFA, MTBSTFA is the derivatizing agent of choice for the rest of the analytes. Subsequent experiments were carried out using MTBSTFA as the derivatizing agent.
The amount of MTBSTFA required for derivatization was investigated (Figure 4-51). The use of 100% MTBSTFA was not feasible as the liquid rapidly solidified upon exposure to water. Solidification was also observed with an MTBSTFA content of 65% and above. Hence, experiments were conducted using chloroform with an MTBSTFA content of 10%, 40%, 50% and a maximum of 60% as extracting solvent. It was found that 50% of MTBSTFA in chloroform, that is, chloroform/MTBSTFA (1:1), gave optimal results and was hence used in subsequent experiments.

As the analytes possess acidic protons, pH has a significant impact on the degree of ionization and hence extraction. A comparison of the extraction of the analytes at an extreme pH of 0, pH 1.5 and pH 4, where the sample pH was unadjusted, was made. Figure 4-52 clearly shows that pH 0 was optimum for the extraction of the analytes since they are expected to be fully unionized at this pH. Hence the pH of the samples was adjusted to 0 in subsequent experiments.
Figure 4-52. Effect of pH on uptake of the degradation products. Spiking concentration: 2 µg ml\(^{-1}\) for EMPA, IMPA, BA, QOH and TOH, 1 µg ml\(^{-1}\) for EHES and PMPA and 20 µg ml\(^{-1}\) for MPA, nPPA and TDG; extraction solvent: chloroform/MTBSTFA (1:1); salt concentration: 30% (w/v); stirring speed: 700 rpm; extraction time: 15 min.

Increasing the ionic strength of the water sample has been shown to decrease the affinities of the CWAs for the aqueous matrix, thus enhancing their extraction by the extractant solvent [88]. The effect of two different salts, NaCl and Na\(_2\)SO\(_4\), was compared. At the same salinity level of 30% (w/v), NaCl gave higher uptake of most of the analytes as compared to Na\(_2\)SO\(_4\) (Figure 4-53).

Figure 4-53. Effect of salt on the uptake of the degradation products. Spiking concentration: 0.7 µg ml\(^{-1}\) for EMPA, IMPA, BA, QOH and TOH, 0.3 µg ml\(^{-1}\) for EHES and PMPA and 7 µg ml\(^{-1}\) for MPA, nPPA and TDG; extraction solvent: chloroform/derivatizing agent (1:1); pH: 1.5; salt concentration: 30% (w/v); stirring speed: 700 rpm; extraction time: 15 min.
NaCl concentrations were then varied from a salinity level of 20% (w/v) to a highly saturated level of 40% (w/v). PMPA and nPPA showed vastly opposite trends with increasing ionic strength. This can be explained by the fact that nPPA is relatively more polar than PMPA and its uptake is enhanced by increasing salt concentration. From Figure 4-54, the optimum salt concentration was determined to be 33% (w/v). The salt concentration was adjusted to 33% (w/v) NaCl for subsequent experiments.

![Figure 4-54. Effect of salt concentration on the uptake of the degradation products. Spiking concentration: 0.7 µg ml\(^{-1}\) for EMPA, IMPA, BA, QOH and TOH, 0.3 µg ml\(^{-1}\) for EHES and PMPA and 7 µg ml\(^{-1}\) for MPA, nPPA and TDG; extraction solvent: chloroform/MTBSTFA (1:1); pH: 0; stirring speed: 700 rpm; extraction time: 15 min.](image)

The effect of stirring speed on the extraction efficiency was investigated over a range of 300 rpm to the maximum of 1250 rpm. As seen from Figure 4-55 extraction of several of the analytes were relatively unaffected by the stirring speed. However, it was observed that the extraction of PMPA, IMPA and TOH increased with increasing stirring speed up to 1000 rpm. Increasing the stirring speed led to increased diffusion of these analytes, which possess branched or long alkyl chains.
Hence the stirring speed was set at 1000 rpm in subsequent experiments, since at this value, most of the analytes were optimally extracted.

Figure 4-55. Effect of stirring on uptake of the degradation products. Spiking concentration: 0.7 µg ml\(^{-1}\) for EMPA, IMPA, BA, QOH and TOH, 0.3 µg ml\(^{-1}\) for EHES and PMPA and 7 µg ml\(^{-1}\) for MPA, nPPA and TDG; extraction solvent: chloroform/MTBSTFA (1:1); pH: 0; salt concentration: 33% (w/v); extraction time: 15 min.

Figure 4-56. Effect of extraction time on uptake of the degradation products. Spiking concentration: 0.7 µg ml\(^{-1}\) for EMPA, IMPA, BA, QOH and TOH, 0.3 µg ml\(^{-1}\) for EHES and PMPA and 7 µg ml\(^{-1}\) for MPA, nPPA and TDG; extraction solvent: chloroform/MTBSTFA (1:1); pH: 0; salt concentration: 33% (w/v); stirring speed: 1000 rpm.
Figure 4-56 shows the time profile of extraction up to 60 min. Except for EHES and BA which were relatively unaffected by extraction time, the uptake of the other analytes increased with increasing extraction times up to 45 min. The extraction time of 45 min was selected since it was the optimum extraction time for most of the analytes.

### 4.3.5 Method validation for HF-LPME of CWA degradation products

Using the optimum extraction conditions, the precision, linearity and LODs of the method were investigated using spiked water samples. The results are shown in Table 4-6.

**Table 4-6. Quantitative results of HF-LPME of the degradation products.**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Equation</th>
<th>$r^2$</th>
<th>% RSD (n = 6)</th>
<th>LOD$^a$ (µg l$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EMPA</td>
<td>$y = 0.1108x + 0.4105$</td>
<td>0.9997</td>
<td>17</td>
<td>0.16</td>
</tr>
<tr>
<td>EHES</td>
<td>$y = 0.0436x + 5.4783$</td>
<td>1.0000</td>
<td>9</td>
<td>0.08</td>
</tr>
<tr>
<td>IMPA</td>
<td>$y = 0.1751x + 1.7730$</td>
<td>0.9977</td>
<td>12</td>
<td>0.03</td>
</tr>
<tr>
<td>PMPA</td>
<td>$y = 0.4045x + 2.7154$</td>
<td>0.9989</td>
<td>13</td>
<td>0.05</td>
</tr>
<tr>
<td>MPA</td>
<td>$y = 0.0022x + 2.4999$</td>
<td>0.9940</td>
<td>14</td>
<td>0.02</td>
</tr>
<tr>
<td>nPPA</td>
<td>$y = 0.0187x + 3.5046$</td>
<td>0.9995</td>
<td>13</td>
<td>0.01</td>
</tr>
<tr>
<td>TDG</td>
<td>$y = 0.0079x - 0.8692$</td>
<td>0.9996</td>
<td>11</td>
<td>0.10</td>
</tr>
<tr>
<td>BA</td>
<td>$y = 0.0073x + 0.7016$</td>
<td>0.9987</td>
<td>22</td>
<td>0.54</td>
</tr>
<tr>
<td>QOH</td>
<td>$y = 0.1434x - 5.6745$</td>
<td>0.9934</td>
<td>22</td>
<td>0.46</td>
</tr>
<tr>
<td>TOH</td>
<td>$y = 0.2744x - 4.9917$</td>
<td>0.9929</td>
<td>20</td>
<td>0.39</td>
</tr>
</tbody>
</table>

$^a$ at S/N = 5.

The linearity of HF-LPME calibration plots was investigated over a concentration range of 0.005–5 µg ml$^{-1}$. The analytes exhibited good linearity with squared regression coefficients of >0.993. This allowed the quantification of the compounds by the method of external calibration. The LODs for the analytes were estimated at S/N = 5 under GC–MS full scan conditions by considering the lowest concentration of each of the analytes that could be detected. The LODs ranged
between 0.01 and 0.54 µg l⁻¹. The relative standard deviations for six extractions were between 9 and 22% for the analytes. Due to the need for derivatization, the % RSD values tend to be larger in general than that of extractions without the need for this additional step.

4.3.6 Comparison of HF-LPME of CWA degradation products with SPME

In order to compare the performance of HF-LPME with in-situ derivatization with that of SPME, extractions of the degradation products from deionized water samples were conducted under the optimized HF-LPME conditions and previously-reported optimum conditions for SPME [90]. Several commercially available SPME fibers were used, ranging from non-polar PDMS to polar PA fibers. Figure 4-57 shows the results obtained. It was obvious that HF-LPME with in-situ derivatization surpassed that of SPME for all of the analytes except for BA. The CW/DVB fiber gave the highest extraction for BA owing to the polarity as well as porosity of the CW/DVB coating. Due to its hydrophilic nature as compared to the other analytes, MPA posed a problem for both extraction techniques as seen from the low recoveries of both procedures. Besides the simplicity and low cost, an added advantage of HF-LPME over SPME is that after each extraction, the hollow fiber can be discarded and a fresh one used for the next extraction. In contrast, the SPME fiber required additional conditioning steps prior to and after extractions in order to prevent carryover problems. The limited lifetime of an SPME fiber subjected to direct immersion extraction is also an unfavorable consideration.
Figure 4-57. Comparison of HF-LPME and SPME of the degradation products. Spiking concentration: 1 µg ml$^{-1}$ for EMPA, IMPA, BA, QOH and TOH, 0.5 µg ml$^{-1}$ for EHES and PMPA and 10 µg ml$^{-1}$ for MPA, nPPA and TDG; HF-LPME extraction solvent: chloroform/MTBSTFA (1:1); pH: 0; salt concentration: 33% (w/v); stirring speed: 1000 rpm; extraction time: 45 min. SPME pH: 1.5; salt concentration: 40% (w/v); stirring speed: 1000 rpm; extraction time: 30 min.

4.3.7 Optimization of parameters for HF-LPME of basic degradation products

Several basic degradation products of CWAs were selected, namely those of a nerve agent (VX), the nitrogen mustards blister agents (HN1, HN2 and HN3) and a psychotomimetic agent (BZ). The actual analytes of interest were 2-(N,N-diisopropylamino)ethanol (DIPAE) (from VX), N-methyldiethanolamine (MDEA) (from HN2), N-ethylidethanolamine (EDEA) (from HN1), triethanolamine (TEA) (from HN3) [15] and 3-quinuclidinol (3Q) (from BZ) [372]. The structures of the analytes are shown in Figure 4-58. The negative logarithm of the acid dissociation constants ($pK_a$) and logarithms of the octanol-water coefficients ($\log K_{ow}$) of the analytes are tabulated in Table 4-7.

Figure 4-58. Structures of the basic analytes investigated in this study.
<table>
<thead>
<tr>
<th>Analyte</th>
<th>$pK_a$ [362,363]</th>
<th>Log $K_{ow}$ [362]</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIPAE</td>
<td>10.1</td>
<td>0.88</td>
</tr>
<tr>
<td>3Q</td>
<td>9.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.17</td>
</tr>
<tr>
<td>MDEA</td>
<td>8.52</td>
<td>-1.50</td>
</tr>
<tr>
<td>EDEA</td>
<td>8.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-1.01</td>
</tr>
<tr>
<td>TEA</td>
<td>7.76</td>
<td>-1.00</td>
</tr>
</tbody>
</table>

<sup>a</sup> Based on the SPARC On-Line Calculator [363]

Factors affecting the extraction efficiency such as choice of derivatizing agent, extraction solvent for HF-LPME, pH, sample ionic strength, stirring speed, and extraction time were optimized. All experiments were performed in triplicate.

In order to determine an optimum derivatization method for HF-LPME, three derivatization procedures were compared together with the evaluation of MTBSTFA and BSTFA as the more suitable derivatizing agent. In the first method, pure solvent (chloroform) was held in the hollow fiber for the extraction of the analytes. Extractions were performed using deionized water spiked with the analytes, 30% (w/v) NaCl and with stirring at 1000 rpm. After 15 min of extraction, 1 μl of derivatizing agent was combined with 1 μl of extract in the syringe for injection. In the second method, a mixture of chloroform and derivatizing agent in a 1:1 ratio was held in the hollow fiber for extraction, followed by injection of 1 μl of the extract. The third method was essentially identical to the second except that the 1 μl of extract was combined with another 1 μl of derivatizing agent prior to injection. The results are shown in Figure 4-59.
It is interesting to note that in the case of derivatization with MTBSTFA, 3Q was largely underivatized, and only a small amount of the MTBSTFA derivative was observed. This problem did not occur with BSTFA derivatization. This observation can conceivably be attributed to the steric bulk of both 3Q and MTBSTFA that prevents effective derivatization. Furthermore, the secondary alcohol group of 3Q could have affected its reactivity towards MTBSTFA. Hence, quantification was based on the underivatized 3Q instead of the MTBSTFA derivative for all subsequent experiments that involved MTBSTFA.

The extraction with the chloroform:MTBSTFA mixture followed by another 1 μl of MTBSTFA gave the highest uptake of 3Q even though, as mentioned above, it remained largely underivatized in the presence of MTBSTFA. Separate experiments were performed to investigate the recovery of 3Q using HF-LPME without MTBSTFA which resulted in negligible recovery of 3Q. Hence it is speculated that MTBSTFA probably aided in the uptake of 3Q similar to an ion-pairing mechanism.

Figure 4-59. Effect of derivatization method on uptake of the basic analytes. Spiking concentration: 1 μg ml⁻¹ for DIPAE and 10 μg ml⁻¹ for 3Q, MDEA, EDEA and TEA; extraction solvent: chloroform/derivatizing agent (1:1); pH: 10; salt concentration: 30% (w/v) NaCl; stirring speed: 1000 rpm; extraction time: 15 min.
but eventually failed to fully derivatize the compound. This phenomenon has not been thoroughly investigated and hence is not yet fully understood. This could be the subject of future studies. It was concluded from our experiments that the third method using the chloroform/BSTFA solvent-derivatizing agent mixture (1:1) together with the additional microliter of derivatizing agent was optimum for HF-LPME as it gave the highest uptake for most of the analytes, especially TEA which could not be extracted using the other methods. On the other hand, SPME using derivatization with BSTFA gave negligible recoveries of the analytes. Hence, SPME experiments were conducted using MTBSTFA as the derivatizing agent.

The experiments on HF-LPME of CWAs and degradation products, as discussed in Sections 4.3.1 and 4.3.4, demonstrated that chlorinated solvents were ideal for this class of compounds. Hence, several chlorinated solvents, namely chloroform, carbon tetrachloride, trichloroethylene and tetrachloroethylene, were evaluated together with a commonly-used non-chlorinated solvent, toluene. Each of the solvents was mixed in a 1:1 ratio with BSTFA and 5 μl of the solvent-derivatizing agent mixture was held in the hollow fiber for extraction. As seen from Figure 4-60(a), chloroform was the best extraction solvent and was selected for further experiments. On the other hand, the uptake of the analytes using various fibers was evaluated. Several fibers gave negligible uptake of the analytes. As seen from Figure 4-60(b), even though the PDMS fiber gave the highest uptake of DIPAE, the most non-polar analyte, the CAR/PDMS gave the highest uptake for the other analytes and was thus used in subsequent experiments.
Figure 4-60. (a) Effect of extraction solvent on uptake of the basic analytes. Spiking concentration: 1 µg ml\(^{-1}\) for DIPAE and 10 µg ml\(^{-1}\) for 3Q, MDEA, EDEA and TEA; extraction solvent: chloroform/BSTFA (1:1); pH: 10; salt concentration: 30% (w/v) NaCl; stirring speed: 1000 rpm; extraction time: 15 min. (b) Effect of SPME fiber on uptake of the basic analytes. Spiking concentration: 5 µg ml\(^{-1}\) for DIPAE and 50 µg ml\(^{-1}\) for 3Q, MDEA, EDEA and TEA; pH: 10; salt concentration: 30% (w/v) Na\(_2\)SO\(_4\); stirring speed: 1000 rpm; extraction time: 15 min.

The amount of BSTFA required for derivatization in HF-LPME was investigated through experiments using chloroform with a BSTFA content of 25%, 50% and 75% and pure BSTFA as extracting solvent. It was found that 50% of BSTFA in chloroform, that is, chloroform/BSTFA (1:1), gave optimal results (Figure
4-61) and was hence used in subsequent experiments. The reason for the decreasing recoveries at higher amounts of BSTFA could be attributed to the decreasing solubility of the analytes with decreasing amounts of CHCl₃ solvent. The use of BSTFA means that the poor derivatization of 3Q by MTBSTFA as observed earlier was no longer a concern in HF-LPME.

Figure 4-61. Effect of amount of derivatizing agent on uptake of the basic analytes. Spiking concentration: 1 µg ml⁻¹ for DIPAE and 10 µg ml⁻¹ for 3Q, MDEA, EDEA and TEA; extraction solvent: chloroform/BSTFA; pH: 10; salt concentration: 30% (w/v) NaCl; stirring speed: 1000 rpm; extraction time: 15 min.

As they are basic compounds (Table 4-7), pH has a significant impact on the degree of ionization and hence extraction of the analytes. A comparison of HF-LPME of the analytes at an extreme pH of 14, pH 12, pH 10 and pH 7, where the sample pH was unadjusted, was made. On the other hand, the extraction pH using SPME was evaluated at pH 8, pH 9, pH 10 and pH 11, which was the maximum acceptable working pH of the Carboxen/PDMS fiber. Figure 4-62 shows that pH 12 and pH 10
was optimum for HF-LPME and SPME respectively. Hence, the pH of the samples was adjusted accordingly in subsequent experiments.

**Figure 4-62.** (a) Effect of pH on HF-LPME of the basic analytes. Spiking concentration: 1 µg ml⁻¹ for DIPAE and 10 µg ml⁻¹ for 3Q, MDEA, EDEA and TEA; extraction solvent: chloroform/BSTFA (1:1); salt concentration: 30% (w/v) NaCl; stirring speed: 1000 rpm; extraction time: 15 min. (b) Effect of pH on SPME of the basic analytes. Spiking concentration: 5 µg ml⁻¹ for DIPAE and 50 µg ml⁻¹ for 3Q, MDEA, EDEA and TEA; salt concentration: 30% (w/v) Na₂SO₄; stirring speed: 1000 rpm; extraction time: 15 min.
Increasing the ionic strength of the water sample has been shown to decrease the affinities of analytes for the aqueous matrix and hence enhancing their extraction by the extractant solvent [90]. The effect of two different salts, NaCl and Na$_2$SO$_4$, was compared. At the same salinity level of 30% (w/v), Na$_2$SO$_4$ gave higher uptake of the analytes as compared to NaCl for both HF-LPME and SPME (Figure 4-63).

**Figure 4-63.** (a) Effect of salt on HF-LPME of the basic analytes. Spiking concentration: 1 µg ml$^{-1}$ for DIPAE and 10 µg ml$^{-1}$ for 3Q, MDEA, EDEA and TEA; extraction solvent: chloroform/BSTFA (1:1); pH 12; salt concentration: 30% (w/v); stirring speed: 1000 rpm; extraction time: 15 min. (b) Effect of salt on SPME of the basic analytes. Spiking concentration: 5 µg ml$^{-1}$ for DIPAE and 50 µg ml$^{-1}$ for 3Q, MDEA, EDEA and TEA; pH 10; salt concentration: 30% (w/v); stirring speed: 1000 rpm; extraction time: 15 min.
The concentration of Na$_2$SO$_4$ was then varied from a salinity level of 20% (w/v) to a highly saturated level of 40% (w/v) for HF-LPME while that of SPME was varied from 15% (w/v) to 35% (w/v). Extractions without the addition of salt are expected to yield negligible recoveries of the analytes. Figure 4-64 shows the effect of salt concentration on extraction efficiency where the optimum salt concentration was determined to be 30% (w/v). In both cases, increasing salt concentration aided in the uptake of the polar analytes except for HF-LPME of DIPAE, where increasing salt concentration led to a decrease in the uptake of the least polar analyte. The salt concentration was adjusted to 30% (w/v) Na$_2$SO$_4$ for subsequent experiments.

**Figure 4-64.** (a) Effect of salt concentration on HF-LPME of the basic analytes. Spiking concentration: 0.5 µg ml$^{-1}$ for DIPAE and 5 µg ml$^{-1}$ for 3Q, MDEA, EDEA and TEA; extraction solvent: chloroform/BSTFA (1:1); pH 12; stirring speed: 1000 rpm; extraction time: 15 min. (b) Effect of salt concentration on SPME of the basic analytes. Spiking concentration: 5 µg ml$^{-1}$ for DIPAE and 50 µg ml$^{-1}$ for 3Q, MDEA, EDEA and TEA; pH 10; stirring speed: 1000 rpm; extraction time: 15 min.
The effect of stirring speed on the extraction efficiency was investigated over a range from 300 rpm to the maximum of 1250 rpm. As seen from Figure 4-65, the optimum stirring speed was achieved at 1000 rpm for HF-LPME while the uptake of DIPAE and 3Q by SPME showed opposite trends with increasing stirring speed. This observation may be attributed to competition between DIPAE and 3Q, both relatively bulky molecules, for uptake onto the fiber. Hence the stirring speed was set at 1000 rpm for HF-LPME and at 700 rpm for SPME in subsequent experiments.

**Figure 4-65.** (a) Effect of stirring on HF-LPME of the basic analytes. Spiking concentration: 0.5 µg ml\(^{-1}\) for DIPAE and 5 µg ml\(^{-1}\) for 3Q, MDEA, EDEA and TEA; extraction solvent: chloroform/BSTFA (1:1); pH 12; salt concentration: 30% (w/v) Na\(_2\)SO\(_4\); extraction time: 15 min. (b) Effect of stirring on SPME of the basic analytes. Spiking concentration: 5 µg ml\(^{-1}\) for DIPAE and 50 µg ml\(^{-1}\) for 3Q, MDEA, EDEA and TEA; pH 10; salt concentration: 30% (w/v) Na\(_2\)SO\(_4\); extraction time: 15 min.
Figure 4-66 shows the time profile from 10 min to 25 min for HF-LPME and from 15 min to 60 min for SPME respectively. Extraction times of 20 min for HF-LPME and 30 min for SPME were selected since these were the optimum extraction times for virtually all of the analytes.

**Figure 4-66.** (a) Effect of extraction time on HF-LPME of the basic analytes. Spiking concentration: 0.5 µg ml\(^{-1}\) for DIPAE and 5 µg ml\(^{-1}\) for 3Q, MDEA, EDEA and TEA; extraction solvent: chloroform/BSTFA (1:1); pH 12; salt concentration: 30% (w/v) Na\(_2\)SO\(_4\); stirring speed: 1000 rpm. (b) Effect of extraction time on SPME of the basic analytes. Spiking concentration: 2.5 µg ml\(^{-1}\) for DIPAE and 25 µg ml\(^{-1}\) for 3Q, MDEA, EDEA and TEA; pH 10; salt concentration: 30% (w/v) Na\(_2\)SO\(_4\); stirring speed: 700 rpm.
4.3.8 Method validation for HF-LPME of basic degradation products

Using the optimum extraction conditions, the precision, linearity and limit of detection of both methods were investigated using spiked deionized water samples. The results are shown in Tables 4-8 and 4-9.

Table 4-8. Quantitative results of HF-LPME of the basic analytes.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Equation</th>
<th>$r^2$</th>
<th>% RSD (n = 6)</th>
<th>LOD (S/N = 5) (µg l$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIPAE</td>
<td>$y = 0.0556x + 2.2385$</td>
<td>0.9966</td>
<td>9</td>
<td>0.04</td>
</tr>
<tr>
<td>3Q</td>
<td>$y = 0.0016x - 0.1049$</td>
<td>0.9996</td>
<td>6</td>
<td>0.22</td>
</tr>
<tr>
<td>MDEA</td>
<td>$y = 0.0009x - 1.7186$</td>
<td>0.9975</td>
<td>10</td>
<td>0.09</td>
</tr>
<tr>
<td>EDEA</td>
<td>$y = 0.0072x - 8.1536$</td>
<td>0.9959</td>
<td>9</td>
<td>0.08</td>
</tr>
<tr>
<td>TEA</td>
<td>$y = 0.0001x - 0.0649$</td>
<td>0.9982</td>
<td>8</td>
<td>0.36</td>
</tr>
</tbody>
</table>

Table 4-9. Quantitative results of SPME of the basic analytes.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Equation</th>
<th>$r^2$</th>
<th>% RSD (n = 6)</th>
<th>LOD (S/N = 5) (µg l$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIPAE</td>
<td>$y = 0.0434x + 45.988$</td>
<td>0.9997</td>
<td>14</td>
<td>0.06</td>
</tr>
<tr>
<td>3Q</td>
<td>$y = 0.0022x - 1.6675$</td>
<td>0.9998</td>
<td>5</td>
<td>0.34</td>
</tr>
<tr>
<td>MDEA</td>
<td>$y = 0.0001x - 0.0444$</td>
<td>0.9985</td>
<td>22</td>
<td>0.11</td>
</tr>
<tr>
<td>EDEA</td>
<td>$y = 0.0005x - 0.7572$</td>
<td>0.9946</td>
<td>21</td>
<td>0.19</td>
</tr>
<tr>
<td>TEA</td>
<td>$y = 0.0001x - 0.8472$</td>
<td>0.9965</td>
<td>6</td>
<td>0.77</td>
</tr>
</tbody>
</table>

The linearity of calibration plots was investigated over a concentration range of 0.05-25 µg ml$^{-1}$ for HF-LPME and 0.5-25 µg ml$^{-1}$ for SPME. The analytes exhibited good linearity with squared regression coefficients of >0.994 for both techniques. This allowed the quantification of the compounds by the method of external calibration. The limits of detection for the analytes were estimated at a S/N ratio of 5 under GC–MS full scan conditions. The LODs ranged between 0.04 and 0.36 µg l$^{-1}$ for HF-LPME and 0.06 and 0.77 µg l$^{-1}$ for SPME. The relative standard deviations for six extractions were between 6 and 10% for LPME and 5 and 22% for SPME.
4.3.9 Comparison of HF-LPME of basic degradation products with SPME

In order to compare the performance of HF-LPME with in-situ derivatization with that of SPME, extractions of the degradation products from deionized water samples were conducted under the respective optimized HF-LPME and SPME conditions. Figure 4-67 shows the results obtained.

![Figure 4-67](image)

**Figure 4-67.** Comparison of HF-LPME and SPME of the basic analytes. Spiking concentration: 1 µg ml\(^{-1}\) for DIPAE and 10 µg ml\(^{-1}\) for 3Q, MDEA, EDEA and TEA; HF-LPME extraction solvent: chloroform/BSTFA (1:1); pH 12; salt concentration: 30% (w/v) Na\(_2\)SO\(_4\); stirring speed: 1000 rpm; extraction time: 20 min. SPME pH 10; salt concentration: 30% (w/v) Na\(_2\)SO\(_4\); stirring speed: 700 rpm; extraction time: 30 min.

It was obvious that HF-LPME with in-situ derivatization surpassed that of SPME for all of the analytes. Besides the simplicity and low cost, an added advantage of HF-LPME over SPME is that after each extraction, the hollow fiber can be discarded and a fresh one used for the next extraction. In contrast, the SPME fiber required additional conditioning steps prior to and after extractions in order to prevent carryover problems. Furthermore, unlike SPME where the fibers are limited to a
working pH range, HF-LPME does not have this constraint. In addition, the limited lifetime of an SPME fiber subjected to direct immersion extraction is an unfavorable consideration.

4.3.10 Analysis of a 20th Official OPCW Proficiency Test water sample

The OPCW conducts a biannual proficiency test for chemical verification laboratories around the world to benchmark their capability. In the 20th Official OPCW Proficiency Test held in October 2006, two chemicals, namely EMPA and 2-(N,N-diisopropylamino)ethanol (DIPAE), were spiked into an aqueous waste sample. The samples were prepared by Centre d’Etudes du Bouchet (CEB), France. Using only 0.3 ml of sample adjusted to pH 0 and topped up to 3 ml with deionized water, EMPA was successfully detected by HF-LPME with in-situ derivatization using the optimized extraction conditions discussed in Section 4.3.4. Similarly, after adjusting the pH to 12 of only 0.3 ml of sample topped up to 3 ml with deionized water, DIPAE was successfully detected by the same technique using the optimized extraction conditions described in Section 4.3.7. Figures 4-68 and 4-69 show the respective chromatograms obtained. The large signal at 11.39 min in Figure 4-68 was confirmed to be ethyl tert.-butyldimethylsilylmethyl phosphonate (the TBDMS derivative of EMPA) while this analyte was absent in the blank, as expected. The large signal at 9.38 min in Figure 4-69 was confirmed to be 2-(N,N-diisopropylamino)ethyl trimethylsilyl ether (the TMS derivative of DIPAE) which was not present in the blank.
Figure 4-68. Total ion chromatograms of HF-LPME under acidic conditions with in-situ derivatization of a 20th Official OPCW Proficiency Test aqueous sample (top) and blank (bottom). HF-LPME extraction solvent: chloroform/MTBSTFA (1:1); pH 0; salt concentration: 33% (w/v); stirring speed: 1000 rpm; extraction time: 45 min.
Figure 4-69. Total ion chromatograms of HF-LPME under basic conditions with in-situ derivatization of a 20th Official OPCW Proficiency Test aqueous sample (top) and blank (bottom). HF-LPME extraction solvent: chloroform/BSTFA (1:1); pH 12; salt concentration: 30% (w/v) Na₂SO₄; stirring speed: 1000 rpm; extraction time: 20 min.
These experiments show that the technique can be applied to a wide range of analytes of interest, whether they are acidic or basic in nature and only a very small amount of sample and extraction solvent are required. The technique will continue to be further tested and evaluated in future OPCW proficiency tests in order to demonstrate its applicability to the entire range of analytes of interest. It is hoped that the technique will complement and even substitute liquid-liquid extraction in the recommended operating procedures for the analysis of CWAs and related compounds.

4.3.11 Conclusion

HF-LPME has been successfully utilized for the analysis of the several CWAs and degradation products in aqueous samples. For analytes that required derivatization, even though the derivatizing agents used were moisture-sensitive, protection afforded by the hollow fiber allowed simultaneous extraction and derivatization to be carried out followed by direct injection into the GC-MS, without the need for an additional off-line or separate derivatization step which requires heating of the samples prior to HF-LPME. The procedure is simple, convenient to perform and only a few microliters of organic solvent and derivatizing agents are required. In addition, HF-LPME significantly improved the LODs over that of SPME. The significantly lower cost of the hollow fiber membrane (one bundle of 2600 pieces of 53.5 cm length costs only ~$200 USD) [297] as compared to commercially-available SPME fibers (~$120 USD) further enhances its advantages over SPME. The successful analysis of an OPCW Proficiency Test sample further affirms the capability of the procedure.
5 CONCLUDING REMARKS

Thus far, there have been many reports on sol-gel solid-phase microextraction (SPME) fibers, sol-gel molecularly imprinted polymers (MIPs), MIP SPME fibers but very few reports on sol-gel MIP SPME fibers. In an attempt to develop sol-gel MIP SPME fibers, the synthesized MIPs were first evaluated as sorbent material for solid-phase extraction (SPE). It was found that the non-imprinted polymers (NIPs) did not show zero absorptivity of the analytes. Hence, endcapping of the polymers was carried out to reduce the non-specific adsorption of analytes on the surface silanol groups of the polymers in order to achieve a high imprint factor. With endcapping, the non-specific interaction of analytes with the NIPs was reduced but not totally eliminated. Moreover, endcapping resulted in lower absorptivities and recoveries of the analytes with the MIPs. Hence, non-endcapped MIPs and NIPs were used in subsequent experiments. The various MIPs were evaluated for their binding properties towards their respective templates as well as similar analytes. In addition, the MIP-SPE procedure was compared against several sample preparation techniques for the extraction of analytes of interest in samples with polyethylene glycol (PEG) as matrix interference. Next, the development of sol-gel MIP SPME fibers was attempted. However, the sol-gel coatings were prone to cracking, and flaked off upon drying at room temperature. To successfully develop sol-gel MIP SPME fibers, it is expected that a lot more effort has to be invested in optimizing the composition of the sol-gel solution used for coating of the fibers. This can be achieved by using computer modeling for the selection of the best monomers for molecular imprinting [373] prior to the synthesis of the actual polymer systems.

On the other hand, a simple method of fabricating SPME fibers was achieved by coating a novel poly(1-hydroxy-4-dodecyloxy-p-phenylene) polymer (PhPPP) as
the SPME coating. The novel PhPPP-coated fibers were evaluated for the analysis of Lewisites after derivatization with thiols. The in-house prepared PhPPP fibers gave comparable performance as compared to commercially-available SPME fibers. In addition, they were easy to prepare and were significantly less costly in contrast to the fairly expensive commercially-available fibers.

The extraction and determination of selected CWAs and degradation products has been successfully demonstrated using hollow fiber-protected liquid-phase microextraction (HF-LPME) and gas chromatography-mass spectrometry (GC–MS). In-situ derivatization of degradation products with silylating agents such as BSTFA and MTBSTFA was possible as a result of the protection of the moisture-sensitive derivatizing agents afforded by the hollow fiber. In order for HF-LPME to fully substitute liquid-liquid extraction as a recommended operating procedure in the analysis of chemical warfare agents and their degradation products, it is essential to show that HF-LPME is applicable to all the various classes of chemicals. Future work on HF-LPME will include the investigation of HF-LPME for the determination of extremely volatile chemicals such as pinacolyl alcohol and chloropicrin as well as for the determination of Lewisites with in-situ derivatization using mono- or dithiols as derivatizing agents.

**Future Work**

A recently-introduced sample preparation technique, dispersive liquid-liquid microextraction (DLLME), is being extensively investigated for various applications [374-377] but has yet to be explored for the analysis of chemical warfare agents and their degradation products. DLLME is a very simple and rapid method for extraction and pre-concentration of organic compounds from water samples. In this method
an appropriate mixture of extraction solvent (8.0 µl of C₂Cl₄) and disperser solvent (1.0 ml of acetone) is rapidly injected into the aqueous sample (5.0 ml) using a syringe. As a result, a cloudy solution is formed consisting of fine droplets of extraction solvent dispersed entirely into the aqueous phase. Through centrifugation, the extraction solvent is collected at the bottom of a conical sample vial, withdrawn with a microsyringe and injected into a GC. DLLME is characterized by very short extraction times, mainly due to the large surface area between the solvent and the aqueous phase. Other advantages are the simplicity of operation, low cost, high recoveries (82-111% at a spiking level of 5 µg l⁻¹) and enrichment factors (603-1113), offering potential for ultra trace analysis (LODs between 0.007 and 0.030 µg l⁻¹, with RSD below 10% at 2 µg l⁻¹) [374]. It will be highly worthwhile to investigate this microextraction technique in comparison with SPME and HF-LPME. It is proposed that this be explored in a future study.

Figure 5-1. Photography of different steps in DLLME: (a) before injection of mixture of disperser solvent (acetone) and extraction solvent (C₂Cl₄) into sample solution, (b) starting of injection, (c) end of injection, (d) optical microscopic photography, magnitude 1000 (that shows fine particles of C₂Cl₄ in cloudy state), (e) after centrifuging and (f) enlarged view of sedimted phase (5.0±0.2 µl) [374].
6 REFERENCES


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[160] Figure 2-2 reprinted from J. Steinke, D.C. Sherrington, I.R. Dunkin, Adv. Polym. Sci. 123 (1995) 81 Figure 9, with kind permission of Springer Science + Business Media.


Figure 3-8 reprinted from reference with permission from Reference Citation. Copyright 2001 American Chemical Society.


[297] G. Shen, H.K. Lee, Anal. Chem. 74 (2002) 648. Figure 2-12 reprinted from reference with permission from Reference Citation. Copyright 2002 American Chemical Society.


<table>
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<th>Schedule number</th>
<th>Chemical name</th>
<th>CAS number</th>
<th>Chemical Structure</th>
<th>Molecular formula (Molecular Weight)</th>
</tr>
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<td>I.A.01</td>
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<td>107-44-8</td>
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<td></td>
<td><img src="image2" alt="Chemical Structure" /></td>
<td>C₄H₇F₂O₂P (182)</td>
</tr>
<tr>
<td></td>
<td>e.g. Soman: O-Pinacolyl methylphosphonofluoridate (GD)</td>
<td></td>
<td><img src="image3" alt="Chemical Structure" /></td>
<td></td>
</tr>
<tr>
<td>I.A.02</td>
<td>O-Alkyl (&lt;C10, incl. cycloalkyl) N,N-dialkyl (Me, Et, n-Pr or i-Pr) phosphoramidocyanidates</td>
<td>77-81-6</td>
<td><img src="image4" alt="Chemical Structure" /></td>
<td>C₇H₁₄N₂O₂P (162)</td>
</tr>
<tr>
<td></td>
<td>e.g. Tabun: O-Ethyl N,N-dimethyl phosphoramidocyanidate (GA)</td>
<td></td>
<td><img src="image5" alt="Chemical Structure" /></td>
<td></td>
</tr>
<tr>
<td>I.A.03</td>
<td>O-Alkyl (H or &lt;C10, incl. cycloalkyl) S-2-dialkyl(Me, Et, n-Pr or i-Pr)-aminoethyl alkyl(Me, Et, n-Pr or i-Pr) phosphonothiolates and corresponding alkylated or protonated salts</td>
<td>50782-69-9</td>
<td><img src="image6" alt="Chemical Structure" /></td>
<td>C₁₁H₂₆NO₂PS (267)</td>
</tr>
<tr>
<td></td>
<td>e.g. VX: O-Ethyl S-2-dissopropylaminoethyl methyl phosphonothiolate</td>
<td></td>
<td><img src="image7" alt="Chemical Structure" /></td>
<td></td>
</tr>
<tr>
<td>I.A.04</td>
<td>Sulfur mustards: 2-Chloroethylchloromethylsulfide</td>
<td>2625-76-5</td>
<td><img src="image8" alt="Chemical Structure" /></td>
<td>C₅H₃ClS (144)</td>
</tr>
<tr>
<td></td>
<td>Mustard gas: Bis(2-chloroethyl)sulfide (HD)</td>
<td>505-60-2</td>
<td><img src="image9" alt="Chemical Structure" /></td>
<td>C₅H₅ClS (158)</td>
</tr>
<tr>
<td></td>
<td>Bis(2-chloroethylthio)methane</td>
<td>63896-13-6</td>
<td><img src="image10" alt="Chemical Structure" /></td>
<td>C₆H₆Cl₂S (204)</td>
</tr>
<tr>
<td></td>
<td>Sesquimustard: 1,2-Bis(2-chloroethylthio)ethane (Q)</td>
<td>3563-36-8</td>
<td><img src="image11" alt="Chemical Structure" /></td>
<td>C₇H₁₀Cl₂S (218)</td>
</tr>
<tr>
<td></td>
<td>1,3-Bis(2-chloroethylthio)-n-propane</td>
<td>63905-10-2</td>
<td><img src="image12" alt="Chemical Structure" /></td>
<td>C₇H₁₀Cl₂S (232)</td>
</tr>
<tr>
<td></td>
<td>1,4-Bis(2-chloroethylthio)-n-butane</td>
<td>142868-93-7</td>
<td><img src="image13" alt="Chemical Structure" /></td>
<td>C₈H₁₂Cl₂S₂ (246)</td>
</tr>
<tr>
<td></td>
<td>1,5-Bis(2-chloroethylthio)-n-pentane</td>
<td>142868-94-8</td>
<td><img src="image14" alt="Chemical Structure" /></td>
<td>C₉H₁₄Cl₂S₂ (260)</td>
</tr>
<tr>
<td></td>
<td>Bis(2-chloroethylthiomethyl)ether</td>
<td>63918-90-1</td>
<td><img src="image15" alt="Chemical Structure" /></td>
<td>C₇H₁₂Cl₂S₂ (234)</td>
</tr>
<tr>
<td></td>
<td>O-Mustard: Bis(2-chloroethylthioethyl)ether (T)</td>
<td>63918-89-8</td>
<td><img src="image16" alt="Chemical Structure" /></td>
<td>C₈H₁₄Cl₂OS₂ (262)</td>
</tr>
<tr>
<td>I.A.05</td>
<td>Lewisites: Lewisite 1: 2-Chlorovinylchloroarsine (L1)</td>
<td>541-25-3</td>
<td><img src="image17" alt="Chemical Structure" /></td>
<td>C₃H₃AsCl (206)</td>
</tr>
<tr>
<td></td>
<td>Lewisite 2: Bis(2-chlorovinyl)chloroarsine (L2)</td>
<td>40334-69-8</td>
<td><img src="image18" alt="Chemical Structure" /></td>
<td>C₅H₆AsCl (232)</td>
</tr>
<tr>
<td></td>
<td>Lewisite 3: Tris(2-chlorovinyl)arsine (L3)</td>
<td>40334-70-1</td>
<td><img src="image19" alt="Chemical Structure" /></td>
<td>C₆H₇AsCl (258)</td>
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<tr>
<td>Schedule number</td>
<td>Chemical name</td>
<td>CAS number</td>
<td>Chemical Structure</td>
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<tr>
<td>1.A.06</td>
<td>Nitrogen mustards: HN1: Bis(2-chloroethyl)ethylamine</td>
<td>538.07-8</td>
<td></td>
<td>C₁₀H₁₉Cl₂N (169)</td>
</tr>
<tr>
<td></td>
<td>HN2: Bis(2-chloroethyl)methylamine</td>
<td>51-75-2</td>
<td></td>
<td>C₅H₁₁Cl₂N (155)</td>
</tr>
<tr>
<td></td>
<td>HN3: Tris(2-chloroethyl)amine</td>
<td>555-77-1</td>
<td></td>
<td>C₆H₁₂Cl₃N (203)</td>
</tr>
<tr>
<td>1.A.07</td>
<td>Saxitoxin</td>
<td>35523-89-8</td>
<td></td>
<td>C₁₀H₁₇N₇O₄ (299)</td>
</tr>
<tr>
<td>1.A.08</td>
<td>Ricin</td>
<td>9009-86-3</td>
<td>Structure undefined</td>
<td>60 kDa</td>
</tr>
<tr>
<td>1.B.09</td>
<td>Alkyl (Me, Et, n-Pr or i-Pr) phosphonyldifluorides e.g. DF: Methylphosphonyldifluoride</td>
<td>676-99-3</td>
<td></td>
<td>CH₃F₂OP (100)</td>
</tr>
<tr>
<td>1.B.10</td>
<td>O-Alkyl (H or &lt;C₁₀, incl. cycloalkyl) O-2-dialkyl(Me, Et, n-Pr or i-Pr)-aminoethyl alkyl(Me, Et, n-Pr or i-Pr) phosphonites and corresponding alkylated or protonated salts e.g. QL: O-Ethyl O-2-diisopropylaminoethyl methylphosphonite</td>
<td>57856-11-8</td>
<td></td>
<td>C₁₃H₂₀NO₂P (235)</td>
</tr>
<tr>
<td>1.B.11</td>
<td>Chlorosarin: O-Isopropyl methylphosphonochloridate</td>
<td>1445-76-7</td>
<td></td>
<td>C₃H₆ClO₂P (156)</td>
</tr>
<tr>
<td>1.B.12</td>
<td>Chlorosoman: O-Pinacolyl methylphosphonochloridate</td>
<td>7040-57-5</td>
<td></td>
<td>C₅H₈ClO₂P (198)</td>
</tr>
<tr>
<td>2.A.01</td>
<td>Amiton: O,O-Diethyl S-[2-(diethylamino)ethyl] phosphorothioate and corresponding alkylated or protonated salts</td>
<td>78-53-5</td>
<td></td>
<td>C₁₆H₂₆NO₃S (269)</td>
</tr>
<tr>
<td>2.A.02</td>
<td>PFIB: 1,1,3,3,3-Pentafluoro-2-(trifluoromethyl)-1-propene</td>
<td>382-21-8</td>
<td></td>
<td>C₆F₃ (200)</td>
</tr>
<tr>
<td>2.A.03</td>
<td>BZ: 3-Quinuclidinyl benzilate</td>
<td>6581-06-2</td>
<td></td>
<td>C₁₅H₂₀NO₃ (337)</td>
</tr>
<tr>
<td>Schedule number</td>
<td>Chemical name</td>
<td>CAS number</td>
<td>Chemical Structure</td>
<td>Molecular formula (Molecular Weight)</td>
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</tr>
<tr>
<td>2.B.04</td>
<td>Chemicals, except for those listed in Schedule 1, containing a phosphorus atom to which is bonded one methyl, ethyl or propyl (normal or iso) group but not further carbon atoms e.g. Methyl(phosphonyl) dichloride</td>
<td>676-97-1</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>CH$_3$ClOP (132)</td>
</tr>
<tr>
<td></td>
<td>Dimethyl methylphosphonate</td>
<td>756-79-6</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>C$_3$H$_9$O$_2$P (124)</td>
</tr>
<tr>
<td></td>
<td>Exemption: Fonofos: O-Ethyl S-phenyl ethylphosphonothiolothionate</td>
<td>944-22-9</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>C$<em>{10}$H$</em>{15}$OPS$_2$ (246)</td>
</tr>
<tr>
<td>2.B.05</td>
<td>N,N-Dialkyl (Me, Et, n-Pr or i-Pr) phosphoramidic dihalides</td>
<td></td>
<td><img src="image" alt="Chemical Structure" /></td>
<td></td>
</tr>
<tr>
<td>2.B.06</td>
<td>Dialkyl (Me, Et, n-Pr or i-Pr) N,N-dialkyl(Me, Et, n-Pr or i-Pr)-phosphoramidates</td>
<td></td>
<td><img src="image" alt="Chemical Structure" /></td>
<td></td>
</tr>
<tr>
<td>2.B.07</td>
<td>Arsenic trichloride</td>
<td>7784-34-1</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>AsCl$_3$ (180)</td>
</tr>
<tr>
<td>2.B.08</td>
<td>Benzilic acid: 2,2-Diphenyl-2-hydroxyacetic acid</td>
<td>76-93-7</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>C$<em>{14}$H$</em>{12}$O$_3$ (228)</td>
</tr>
<tr>
<td>2.B.09</td>
<td>Quinuclidin-3-ol</td>
<td>1619-34-7</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>C$<em>7$H$</em>{13}$NO (127)</td>
</tr>
<tr>
<td>2.B.10</td>
<td>N,N-Dialkyl (Me, Et, n-Pr or i-Pr) aminoethyl-2-chlorides and corresponding protonated salts</td>
<td></td>
<td><img src="image" alt="Chemical Structure" /></td>
<td></td>
</tr>
<tr>
<td>2.B.11</td>
<td>N,N-Dialkyl (Me, Et, n-Pr or i-Pr) aminoethane-2-ols and corresponding protonated salts</td>
<td></td>
<td><img src="image" alt="Chemical Structure" /></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Exemptions: N,N-Dimethylaminoethanol and corresponding protonated salts</td>
<td>108-01-0</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>C$<em>4$H$</em>{11}$NO (89)</td>
</tr>
<tr>
<td></td>
<td>Exemptions: N,N-Diethylaminoethanol and corresponding protonated salts</td>
<td>100-37-8</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>C$<em>4$H$</em>{11}$NO (117)</td>
</tr>
<tr>
<td>2.B.12</td>
<td>N,N-Dialkyl (Me, Et, n-Pr or i-Pr) aminoethane-2-thiols and corresponding protonated salts</td>
<td></td>
<td><img src="image" alt="Chemical Structure" /></td>
<td></td>
</tr>
<tr>
<td>2.B.13</td>
<td>Thiodiglycol: Bis(2-hydroxyethyl)sulfide</td>
<td>111-48-8</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>C$<em>4$H$</em>{11}$O$_2$S (122)</td>
</tr>
<tr>
<td>Schedule number</td>
<td>Chemical name</td>
<td>CAS number</td>
<td>Chemical Structure</td>
<td>Molecular formula (Molecular Weight)</td>
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<tr>
<td>2.B.14</td>
<td>Pinacolyl alcohol: 3,3-Dimethylbutan-2-ol</td>
<td>464-07-3</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>C₆H₁₄O (102)</td>
</tr>
<tr>
<td>3.A.01</td>
<td>Phosgene: Carbonyl dichloride</td>
<td>75-44-5</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>CCl₂O (98)</td>
</tr>
<tr>
<td>3.A.02</td>
<td>Cyanogen chloride</td>
<td>506-77-4</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>CCIN (61)</td>
</tr>
<tr>
<td>3.A.03</td>
<td>Hydrogen cyanide</td>
<td>74-90-8</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>CHN (27)</td>
</tr>
<tr>
<td>3.A.04</td>
<td>Chloropicrin: Trichloronitromethane</td>
<td>76-06-2</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>CCl₃NO₂ (163)</td>
</tr>
<tr>
<td>3.B.05</td>
<td>Phosphorus oxychloride</td>
<td>10025-87-3</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>POCl₃ (152)</td>
</tr>
<tr>
<td>3.B.06</td>
<td>Phosphorus trichloride</td>
<td>7719-12-2</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>PCl₃ (136)</td>
</tr>
<tr>
<td>3.B.07</td>
<td>Phosphorus pentachloride</td>
<td>10026-13-8</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>PCl₅ (206)</td>
</tr>
<tr>
<td>3.B.08</td>
<td>Trimethyl phosphite</td>
<td>121-45-9</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>C₃H₆O₃P (124)</td>
</tr>
<tr>
<td>3.B.09</td>
<td>Triethyl phosphite</td>
<td>122-52-1</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>C₆H₁₄O₃P (166)</td>
</tr>
<tr>
<td>3.B.10</td>
<td>Dimethyl phosphite</td>
<td>868-85-9</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>C₂H₅O₃P (110)</td>
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<tr>
<td>3.B.11</td>
<td>Diethyl phosphite</td>
<td>762-04-9</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>C₆H₁₂O₃P (138)</td>
</tr>
<tr>
<td>3.B.12</td>
<td>Sulfur monochloride</td>
<td>10025-67-9</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>SCl₂ (134)</td>
</tr>
<tr>
<td>3.B.13</td>
<td>Sulfur dichloride</td>
<td>10545-99-0</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>SCl₂ (102)</td>
</tr>
<tr>
<td>3.B.14</td>
<td>Thionyl chloride</td>
<td>7719-09-7</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>SOCl₂ (118)</td>
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<tr>
<td>3.B.15</td>
<td>Ethyldiethanolamine</td>
<td>139-87-7</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>C₆H₁₄NO₂ (133)</td>
</tr>
<tr>
<td>3.B.16</td>
<td>Methylidethanolamine</td>
<td>105-59-9</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>C₆H₁₄NO₂ (117)</td>
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<tr>
<td>3.B.17</td>
<td>Triethanolamine</td>
<td>102-71-6</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>C₆H₁₂NO₂ (149)</td>
</tr>
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</table>
Figure A2-1. Total ion chromatograms of samples upon (a) direct rotary evaporation; (b) SCX; (c) SAX; (d) loading onto PMPA-MIP-SPE; (e) washing of PMPA-MIP-SPE; (f) elution of PMPA-MIP-SPE cartridges.
Figure A2-2. Total ion chromatograms of samples upon (a) direct rotary evaporation; (b) SCX; (c) SAX; (d) loading onto TDG-MIP-SPE; (e) elution of TDG-MIP-SPE cartridges.
Figure A2.3. Total ion chromatograms of samples upon (a) direct rotary evaporation; (b) SCX; (c) SAX; (d) loading onto TEA-MIP-SPE; (e) elution of TEA-MIP-SPE cartridges.
Figure A2.4. Total ion chromatograms of samples upon (a) direct rotary evaporation; (b) SCX; (c) SAX; (d) loading onto 3Q-MIP-SPE; (e) elution of 3Q-MIP-SPE cartridges.
(a) Direct

(b) SCX

(c) SAX

(d) Absorptivity of MIP-SPE

(e) Recovery of MIP-SPE with EtOH
Figure A2-5. Total ion chromatograms of samples upon (a) direct rotary evaporation; (b) SCX; (c) SAX; (d) loading onto MIP-SPE; (e) elution of MIP-SPE cartridges with EtOH; (f) loading onto MIP-SPE; (g) elution of MIP-SPE cartridges with 1% TFA in water; (h) loading onto MIP-SPE; (i) elution of MIP-SPE cartridges with 1% TE in water.
**APPENDIX 3**

**MASS SPECTRA**

Figure A3-1. Mass spectrum of GB (MW: 140)

Figure A3-2. Mass spectrum of GD (MW: 182)

Figure A3-3. Mass spectrum of GA (MW: 162)

Figure A3-4. Mass spectrum of HD (MW: 158)

Figure A3-5. Mass spectrum of VX (MW: 267)

Figure A3-6. Mass spectrum of L3 (MW: 258)

Figure A3-7. Mass spectrum of L2-ET (MW: 258)

Figure A3-8. Mass spectrum of L1-ET (MW: 258)
Figure A3-25. Mass spectrum of PMPA-TMS (MW: 252)

Figure A3-26. Mass spectrum of PMPA-TBDMs (MW: 294)

Figure A3-27. Mass spectrum of CMPA-TMS (MW: 250)

Figure A3-28. Mass spectrum of CMPA-TBDMs (MW: 292)

Figure A3-29. Mass spectrum of BA-TMS (MW: 372)

Figure A3-30. Mass spectrum of BA-TBDMs (MW: 456)

Figure A3-31. Mass spectrum of EHES-TMS (MW: 178)

Figure A3-32. Mass spectrum of EHES-TBDMs (MW: 220)
Figure A3-33. Mass spectrum of TDG-TMS (MW: 266)

Figure A3-34. Mass spectrum of TDG-TBDMS (MW: 350)

Figure A3-35. Mass spectrum of TDGS-TMS (MW: 282)

Figure A3-36. Mass spectrum of TDGSO-TMS (MW: 298)

Figure A3-37. Mass spectrum of QOH-TMS (MW: 326)

Figure A3-38. Mass spectrum of QOH-TBDMS (MW: 410)

Figure A3-39. Mass spectrum of TOH-TMS (MW: 370)

Figure A3-40. Mass spectrum of TOH-TBDMS (MW: 454)
Figure A3-49. Mass spectrum of 3Q (MW: 127)

Figure A3-50. Mass spectrum of 3Q-TMS (MW: 199)

Figure A3-51. Mass spectrum of 3Q-TBDMS (MW: 241)
APPENDIX 4 LIST OF POSTER PRESENTATIONS AND PUBLICATIONS

Poster Presentations


Publications


