

# Analytical Methods for CWA

## 1. Surface acoustic wave (SAW) sensors

- One of the most popular approaches for selective sensing of CW agents is the SAW sensor. First reported by Wohltjen and Dessy in 1979 and based on the GC piezoelectric response demonstrated by King in 1964, this sensor detects adsorption of an analyte on the surface of a piezoelectric crystal.
- When a time-varying electric field is applied to one side of a piezoelectric material, it sets up an acoustic wave that is propagated along the surface of the piezoelectric material and detected by electrodes located at the other end of the material. Changes in amplitude or phase of this wave occur when an analyte adsorbs onto the surface of the piezoelectric material. When the surface is coated with a thin film, which adsorbs chemicals selectively, a selective sensor is produced.
- Early work on the application of SAW instruments to CW agent detection reported the development of a temperature-controlled array of SAW devices with automated sample preconcentration and pattern recognition. Detection limits as low as 0.01 mg/m<sup>3</sup> for organophosphorus analytes and 0.5 mg/m<sup>3</sup> for organosulfur analytes were obtained within 2 min. Besides the difficulty of developing selective coatings for SAW sensors, polymer films can dewet the surface and produce isolated droplets of material, leading to a degradation of response.

## 2. Electrochemical sensors:

- Direct determination of organophosphate nerve agents can also be accomplished using the amperometric response of an enzyme biosensor. In this approach, the biosensor incorporated the enzyme organophosphorus hydrolase by covalently immobilizing it on glass beads. The enzyme hydrolyzes organophosphate to an electroactive species, which can be determined amperometrically. Best results were achieved with organophosphates containing a nitrophenyl group. This approach has been incorporated into a single-channel microchip for the rapid screening of organophosphate nerve agents.
- Chemiresistive vapor detectors for nerve agent simulants have also been reported. They use a conducting polymer composite that changes electrical resistance when an agent adsorbs onto the surface.
- The detection limit for this sensor is in the range of 47–240 µg/m<sup>3</sup> of dimethyl methyl phosphonate (DMMP) in air.

### 3. **ColorTest**

- The least expensive approach to chemical agent detection is the use of the color spot test. Chemical agent detector kits are available that can identify field concentrations of nerve agents (GB, GA, GC, GF, and VX), vesicant agents (mustard, phosgene oxime, mustard-lewisite, and lewisite), and blood agents (hydrogen cyanide and cyanogen chloride) in about 15–20 min. Chemical agents are adsorbed onto a pretreated test spot, and when a “developer” reagent is added, specific color changes indicate the presence or absence of chemical agents. The problem with the spot test approach is that it is too slow and insensitive for early warning detection and not sufficiently selective to provide reliable verification.
- Nevertheless, for CW verification, the spot test approach is a convenient method for initial screening.

### 4. **Immunochemicalsensors**

- Immunochemical sensors potentially offer a more specific alternative to the relatively nonspecific color reactions involved with spot tests. In this approach, a protein antibody is developed that binds specifically to the chemical agent and elicits some type of analytical response. Fluorescence and electrochemical responses have been used. However, enzyme-linked immunoabsorbent assays (ELISA) have complex bioengineered microorganisms.
- Traditional chemical agents—nerve, vesicant, and blood agents—have acute toxicities in the range of 10–3 g/person and are relatively easy to detect. Emerging chemical agents (toxic chemicals and aerosols) and bioregulators (neuropeptides and psychoactive compounds) are more varied in their chemical structure, requiring more sophisticated analytical methods for identification and detection. The most difficult chemical agents to detect are the cytotoxins and neurotoxins with chronic toxicities as low as 10–10 g/person .

### 5. **Gaschromatography(GC)**

- For vapor-phase chemical warfare agents, GC is usually the analytical separation method of choice. In general, a high-resolution gas chromatographic separation can require up to 30 min for complete separation and detection of a complex mixture. However, when selective detectors are used in combination with GC, the separation time can be reduced significantly. For example, using a pulsed-flame photometric detector, which is highly selective for phosphorus- and sulfur-containing compounds, detection of 20 ng/m<sup>3</sup> of organophosphorus compounds and 200 ng/m<sup>3</sup> for organosulfur compounds can be accomplished within 30 s

- Collection and concentration in tubes packed with selected adsorbents followed by thermal desorption of the agent and gas chromatographic separation proved to be useful for both high- and low-volatility analytes. Collection and concentration are also convenient for GC using solid-phase microextraction (SPME) methods. Selective SPME prior to sample introduction into a GC increases the selectivity of the analysis for sarin. Sulfur mustard has also been detected by SPME in soil samples

## 6. Liquid chromatography(LC)

- For compounds that are not volatile or thermally labile, LC rather than GC is often the method of choice for separation prior to detection. However, high pressures and small particle sizes limit column length and, therefore, the ultimate resolving power possible by LC. In addition, low diffusion rates in liquids make LC a relatively slow analytical method for the detection of CW agents.
- Nevertheless, a number of applications have been reported. For example, hydrolysis products of sulfur mustards can be determined at the ppm level in about 20 min per sample by reversed-phase liquid chromatography (RPLC) coupled with a sulfur flame photometric detector. Similarly, aqueous samples of alkylphosphonic acids can be analyzed at the ppt level in about 20 min/sample by reversed phase LC coupled with phosphorous selective flame photometric detection.

## 7. Capillary electrophoresis(CE)

- For higher resolving power than is possible with RPLC (or faster analysis times with similar resolving power), CE is the separation method of choice for ionic and ionizable compounds. Coupled with selective detectors such as the flame photometric detector, CE can detect alkylphosphonic acids in water at the 0.1–0.5 µg/ml level in less than 10 min. Indirect UV absorbance detection has also been reported for the detection of these compounds after CE. The use of electrokinetic injection improved detection limits for a number of degradation products. For example, methylphosphonic acid (MPA), ethylmethylphosphonic acid (EMPA), isopropylmethylphosphonic acid (IMPA), and pinacolylmethylphosphonic acid (PMPA) were detected at the ppb level in 3 min from water samples.
- A miniaturized analytical system has been reported for the separation and detection of nerve agents using CE microchips with amperometric detection

## 8. Mass spectrometry(MS)

- Unlike IMS, MS is an ion separation method in which gas-phase ions separate according to their mass to charge ratio as they move through a vacuum. Due to the lack of ion–molecule interactions and the rapid analysis time (on the order of  $\mu\text{s}$ ), typical mass spectrometers have resolving powers 1 to 2 orders of magnitude better than IMS and chromatographic instruments. However, the complexity of requiring a vacuum limits the practical application of MS as a field analytical technique.
- An ion trap mass spectrometer with a high-pressure negative ion source and selected reactant negative ions has been used to insure unambiguous detection of phosphorus- and nitrogen-containing

### 9. GasChromatography/massspectrometry(GC/MS)

- GC/MS is the only analytical method of analysis that has been approved for chemical warfare verification, but due to the complex nature of this instrument, it has not been employed for field verification of
- chemical agents. However, GC/MS can provide some of the most reliable analytical information. An excellent and thorough review of the combination of chromatography with mass for CW agent determination has been conducted . Currently, laboratory protocols for the GC/MS analyses of hydrolysis products of chemical warfare agents are available [41]. A recent discussion of the problems and possibilities associated with field-portable GC/MS has been reported based on environmental and forensic applications . Also, a field-portable, high-speed GC/TOFMS has been reported [43].

### 10. Liquidchromatography/massspectrometry(LC/MS)

In addition to GC/MS, LC is often employed to identify an unknown sample of chemical warfare agents . Degradation products of CW agents are particularly difficult to determine due their lack of volatility for gas chromatographic separation and lack of chromophores for detection. Several ionization

- methods after liquid chromatographic separation have been found to be useful for the determination of CW degradation products. These ionization methods include particle beam ionization (PBI), electrospray ionization (ESI), and atmospheric pressure chemical ionization (APCI) [45]. Each ionization method was found to provide complementary information. But it has been the introduction of ESI that has enabled the most successful applications of LC methods coupled with MS. Thus, the determination of sarin, soman, and their hydrolysis products is now possible at the 10  $\mu\text{g/g}$  level with liquid chromatographic separation followed by mass separation and detection.