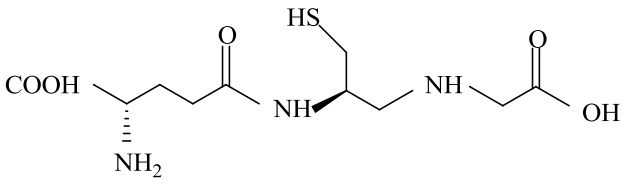
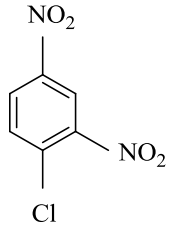
Chapter 2

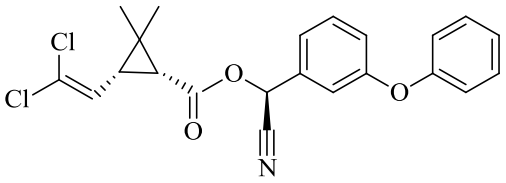
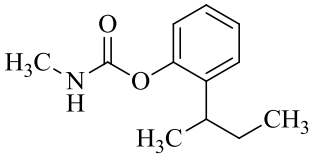
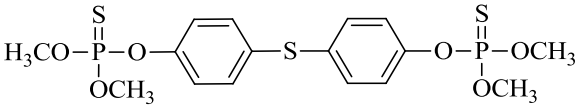
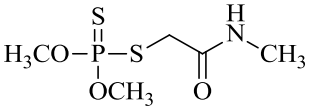
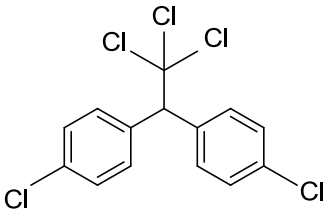
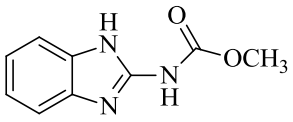
Materials and Methods

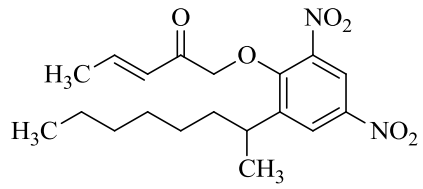
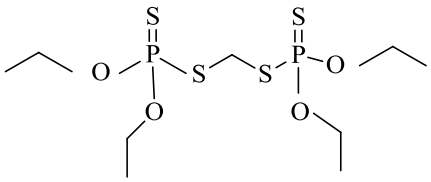
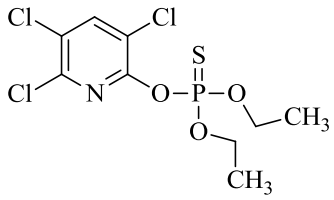
2.1 Reagents and materials

GST (from equine liver), CDNB, GSH, cypermethrin, temephos, fenobucarb and dimethoate (analytical standard) were purchased from Sigma–Aldrich. DDT and dinocap procured from Dr. Ehrenstofer; ethion, carbendazim, profenofos and chlorpyrifos from PESTANAL-Sigma. Phosphate buffer (PB) of 0.1 M (pH 6.5) was prepared by mixing KH_2PO_4 and K_2HPO_4 procured from Merck-Germany. Methanol, dichloromethane (DCM), acetonitrile, $NaNO_3$, $KMnO_4$, HCl and H_2SO_4 were of analytical reagent grade and purchased from Merck chemicals. Bondesil-NH_2 and carbon SPE bulk sorbent were purchased from Agilent technologies. The GST solution was prepared in PB containing 0.1 M KCl at pH 6.5 as the supporting electrolyte and stored at - 22 ºC. GSH stock solution was also prepared in PB (pH 6.5). CDNB solution was prepared in 50% aq. methanol so as to maintain the final percentage of methanol 25%. Distilled methanol was diluted to 50% using ultra-pure water from a Millipore Milli-Q system. The stock solutions of pesticides were prepared in methanol and diluted to the appropriate concentration for further experimental use. All the solutions except GST were prepared regularly before experiments.

2.2 Chemical structure of some compounds used in this study

Name of Chemicals	Molecular structures
Glutathione	
1-Chloro-2,4-dinitrobenzene	

Cypermethrin	
Fenobucarb	
Temephos	
Dimethoate	
DDT	
Carbendazim	

Dinocap	
Ethion	
Chlorpyrifos	

2.3 Instruments

PAR 273-A Potentiostate/Galvanostate and CHI 660A potentiostate (USA) were used for electrochemical measurements. UV-VIS spectrophotometer used was UV-2550, Shimadzu, Japan. FT-IR analysis was carried out in Perkin Elmer Frontier FIR-MIR spectrometer. SEM images were taken using JEOL, JSM Model 6390 LV. Chronoamperometric and impedance measurements were done using Potentiostate/Galvanostate SP 300 from Bio-Logics, France. GC-MS analysis was done using Agilent 7890A Gas Chromatogram. The X-ray diffraction (XRD) study was carried out using D8 Focus (Bruker AXS, Germany) in the range of $2\theta = 5°-70°$.

Electrochemical measurements were performed at 32 $(\pm 0.05)^0$C, using a standard electrochemical cell with three-electrode assembly. The working electrode was Pt electrode (3 mm diameter). A Pt wire was used as counter electrode and Ag/AgCl refilled with 0.1 M KCl was the reference electrode. Cleaning of all electrodes was done before each experiment. Prior to electrochemical measurement, the solution mixtures were mixed thoroughly in vortex shaker and then the measurements were made in static

solution condition. During the kinetic study using UV-visible spectrophotometer, the solutions were stirred constantly with magnetic needle.

2.3.1 Electrode surface preparation

Prior to use, Pt working electrodes were first polished on aqueous slurries of 1 µm, 0.3 µm and 0.05 µm alumina powder. After thorough rinsing in deionized water followed by acetone, the electrodes were dipped for about 5 minutes in a hot 'Piranha' solution {1:3 (v/v) 30 % H_2O_2 and concentrated H_2SO_4} and rinsed again with copious amounts of deionized water. The polished electrodes were then dipped in PB and cleaned electrochemically by cycling the potential scan between -0.1 to 0.1 V until it acquired at a steady state baseline or until the CV characteristics for a clean Pt electrode were obtained. The Pt counter electrode was regularly cleaned before and after synthesis and in between synthesis and analysis. KCl solutions of Ag/AgCl reference electrode were changed before each experiment.

2.4 Details of the techniques used

2.4.1 Cyclic voltammetry (CV)

In electrochemistry, cyclic voltammetry is a very popular technique. Its versatility combined with ease of measurement has resulted in extensive use of CV in the fields of electrochemistry, inorganic chemistry, organic chemistry and biochemistry. It is useful for obtaining information including modification of electrodes, reaction rates, reaction mechanisms and the concentration of an unknown concentration through calibration curve. Cyclic voltammetry is often the first experiment performed in an electrochemical study of a compound, a biological material or an electrode surface. The effectiveness of CV results from its capability for rapidly observing the redox behavior over a wide potential range [1]. It measures the current that develops in an electrochemical cell under conditions where voltage is in excess of that predicted by the Nernst equation. CV is performed by cycling the potential of a working electrode and measuring the resulting current. The potential of the working electrode is measured against a reference electrode which maintains a constant potential and the resulting applied potential produces an excitation signal. The resulting voltammogram is analogous to a conventional spectrum that conveys information as a function of an energy scan.

2.4.2 Ultraviolet-visible spectroscopy (UV-VIS)

The shorter wavelength, higher energy radiation in the UV (200-400 nm) and visible (400-700 nm) range of the electromagnetic spectrum causes many molecules to undergo electronic transitions that means when the energy from UV or visible light is absorbed by a molecule, one of its electrons jumps from a lower energy to a higher energy molecular orbital. Since the energy levels of matter are quantized, only light with the precise amount of energy that can cause transitions from one level to another will be absorbed. Such electron transfer processes may take place in transition metal ions (d-d transitions and ligand-to-metal or metal-to-ligand charge transfer transitions) and inorganic and organic molecules (mainly n - π^* and π - π^* transitions). They are responsible for the color of matter [2].

2.4.3 Fourier transformed infrared spectroscopy (FTIR)

Infrared spectroscopy is certainly one of the most important analytical techniques available with the great advantage of analyzing any sample in virtually any state. IR radiation is usually defined as that electromagnetic radiation whose frequency lies between 20 and 14500 cm^{-1}. Within this region of the electromagnetic spectrum, chemical compounds absorb IR radiation, provided there is a dipole moment change during a normal molecular vibration, molecular rotation and molecular rotation–vibration or from combinations, differences and overtones in the normal molecular vibrations. The infrared portion of the electromagnetic spectrum is usually divided into three regions: the far-infrared (FIR) region, mid-infrared (MIR) region and near-infrared (NIR) regions. Each region of the spectrum plays a distinct role in analysis according to the different character of the transitions involved in each case. The FIR region extends from 20 to 400 cm^{-1}. This region contains the bending vibrations of C, N, O and F with heavier atoms and additional bending motions in cyclic or unsaturated systems. However, the region between 400 and 4000 cm^{-1} is the most attractive one for chemical analysis. This MIR region corresponds to fundamental transitions in which one vibrational mode is excited from its lowest energy state to its first excited state. The NIR region lies between the visible and the MIR region of the electromagnetic spectrum and is defined as the spectral region spanning the wavelength range 4000–14500 cm^{-1}. This region arises from transitions in which a photon excites a normal mode of vibration from ground state to the

second or higher excited vibrational states (overtones) and transitions in which one photon simultaneously excites two or more vibrational modes (combinations). The NIR region is mainly used for quantitative analysis [3].

2.4.4 Scanning electron microscope (SEM)

Scanning electron microscopes scan a sample with a focused electron beam and deliver images with information about the samples' topography and composition. Because of its versatility and the wide range of information, SEM is often the preferred starting tool for analytic microscopy. In SEM, a focused beam of high-energy electrons is scanned over the surface of a material. The electron beam interacts with the material, causing a variety of signals; secondary electrons, backscattered electrons, X-rays, photons etc. each of which may reveal information about the sample including external morphology (texture), chemical composition, crystalline structure and orientation of materials making up the sample. Thus SEM can be used to characterize a material with respect to specific properties [4].

2.4.5 Chronoamperometry (CA)

Chronoamperometry is very powerful method for the study of kinetics of chemical reactions, diffusion processes and adsorption. In this electrochemical technique, potential of the working electrode is stepped and the resulting current from faradaic processes occurring at the electrode (caused by the potential step) is monitored as a function of time. Contrary to cyclic voltammetry where the potential is applied linearly, in chronoamperometry the potential is applied in steps from a value where the analyte is not oxidized or reduced (E_1) to a value where it is (E_2) [5].

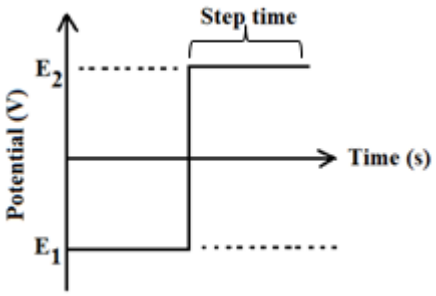


Figure 2.1. Potential applied to the cell vs time.

One advantage of chronoamperometry is that the current throughout the entire interval of the potential step is proportional to the concentration of the species electrolyzed. Thus, more data can be averaged and, in theory, signal-to-noise ratios can be improved [6].

2.4.6 Gas chromatography-mass spectrometry (GC-MS)

Gas chromatography-mass spectrometry begins with the gas chromatograph, where the sample is volatilized. This effectively vaporizes the sample (the gas phase) and separates its various components using a capillary column packed with a stationary (solid) phase and the mobile phase. Here, the mobile phase is a carrier gas, usually an inert gas such as argon, helium or nitrogen. Components in a sample are separated from each other because some take longer to pass through the column than others. As the components become separated, they elute from the column at different times, which is generally referred to as their retention times.

Once the components leave the GC column, it is fragmented by ionization and the fragments are sorted by mass to form a fragmentation pattern. It is here that ions are separated based on their different mass-to-charge (*m/z*) ratios. Like the retention time (RT), the fragmentation pattern for a given component of sample is unique and therefore is an identifying characteristic of that particular component. It is so specific that it is often referred to as the molecular fingerprint [7].

The final steps of the process include ion detection and analysis, with compound peaks appearing as a function of their *m/z* ratios. Peak heights, meanwhile, are proportional to the quantity of the corresponding compound present. A complex sample will yield several different peaks and the final readout will be a mass spectrum. Using computer libraries of mass spectra for different compounds, researchers can identify and quantitate unknown compounds and analytes.

2.4.7 Electrochemical impedance spectroscopy (EIS)

Impedance is simply a measure of the ability of a circuit to resist the flow of electrical current or in other words, it measures the resistive and capacitive properties of materials upon perturbation of a system by a small amplitude sinusoidal AC excitation signal typically of 2-10 mV [8]. The frequency is varied over a wide range to obtain the impedance spectrum. As already stated, it is usually measured by applying an AC

potential to an electrochemical cell. Impedance spectroscopy is mainly divided into two categories: electrochemical impedance spectroscopy and dielectric impedance spectroscopy. The principal difference lies in the material, device or system taken under consideration. As suggested by the name, the latter involves the analysis of dielectric materials, with predominant electronic conduction; on the other hand, electrochemical impedance spectroscopy applies on materials in which the ionic conduction is prevalent on the electronic one. Examples of such systems include solid and liquid electrolytes, glasses and polymers; but this technique has been predominantly employed also in the study of corrosion or electrochemical and photoelectrochemical cells, such as fuel cells, batteries, or solar devices [9].

2.4.8 X-ray diffraction (XRD)

X-ray diffraction is nowadays a common technique for the study of crystal structures and atomic spacing. X-ray diffraction is based on constructive interference of monochromatic X-rays and a crystalline sample. These X-rays are generated by a cathode ray tube, filtered to produce monochromatic radiation, collimated to concentrate and directed towards the sample. The interaction of the incident rays with the sample produces constructive interference (and a diffracted ray) when the conditions satisfy Bragg's Law ($n\lambda=2d\sin\theta$). This law relates the wavelength of electromagnetic radiation to the diffraction angle and the lattice spacing in a crystalline sample. These diffracted X-rays are then detected, processed and counted. By scanning the sample through a range of 2θ angles, all possible diffraction directions of the lattice should be attained due to the random orientation of the powdered material. Conversion of the diffraction peaks to d-spacing allows identification of the mineral because each mineral has a set of unique d-spacing. Typically, this is achieved by comparison of d-spacing with standard reference patterns.

Powder XRD is perhaps the most widely used x-ray diffraction technique for characterizing materials. As the name suggests, the sample is usually in a powdery form, consisting of fine grains of single crystalline material to be studied. The technique is used also widely for studying particles in liquid suspensions or polycrystalline solids. It has numerous advantages like non-destructive nature, high sensitivity, reliability and user-friendly operation [10].

References

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