CHAPTER 2

EXPERIMENTAL
2.1. Introduction

This chapter describes the instrumentation, devices, and the electrode systems with special emphasis on carbon paste electrodes, preparation and standardization of carbon paste electrodes, experimental techniques and procedures used in the present work. The basic equipment needed for electrochemistry is a potentiostat, a recording device, and an electrochemical cell. Everything inside the cell is chemistry and everything outside of it is electronics.

2.2. Potentiostat

The conventional three-electrode potentiostat is connected to the working, reference, and auxiliary electrodes immersed in the test solution placed in the cell. It controls the potential of the WE with respect to the RE while simultaneously measuring the current flowing between the WE and the AE. The potentiostat performs three functions:

(i) controls the applied potential, which is potential difference between the WE and RE (the applied potential controls what half reactions occur at the WE);
(ii) allows to pass current between the WE and AE without passing current through the RE (which would change its potential if current did pass through it) and
(iii) converts the cell current to a voltage for recording devices.

A potentiostat must be able to bring the potential of the WE (with respect to the RE) to the desired level in a short enough time. The time taken by the potentiostat for controlling the WE potential is called the rise time. The potentiostat's internal feedback circuits prevent all but a very small current from flowing between the WE and RE. Because the very basis of voltammetry is the control of electrode potential, a function generator is required to provide the potential sweep or pulse sequence to be applied to the WE. Most modern potentiostats include a built-in sweep and / or pulse generator, and those, which are interfaced to a computer usually, rely on the computer to generate the
desired waveform. The inputs to the potentiostat are the connections to the electrodes in the cell. The outputs from the potentiostat are signal lines reflecting the current and potential of the WE(s).

2.2.1. Potentiostat employed in the present work for CV and DPV experiments

The electrochemical experiments were carried out using potentiostat provided with the Data Acquisition PC interface Card Model EA-201 Electro Analyser fabricated by Chemi Link Systems, Trombay, Mumbai, India; compatible with an IBM PC and coupled to a printer (Fig. 2.1). This instrument is capable of performing more than six electroanalytical techniques. The instrument incorporates a high speed, high accuracy and an electrolysis mode that consists of high-gain operational amplifier with circuits for controlled potential.

The WE current signal is handled a bit differently. This signal line is also presented as a voltage signal, but the voltage level is actually proportional to the current flowing at the WE. The potentiostat has an internal ‘current converter’ circuit that performs the necessary current-to-voltage conversion automatically. The current converter has a number of ranges, and the operator is expected to choose the range most appropriate for the experiment being performed. Each range is associated with a particular proportionality constant, such as '100 mA/V' or '1 mA/V.'

2.2.2. Recording device

Computers entered into electroanalytical instrumentation in 1967¹ or even earlier. Computer applications in stationary electrode voltammetry²,³ and CV²⁶ were reported. Computers can be used to apply potential programme to the WE through the potentiostat. The initial potential, final potential, sweep rate, the nature of the pulse, current sensitivity etc, may be instructed to the computer in the digital form. Computers can be used very effectively in data acquisition. The applied potential values and the resulting current
values may be converted into digital information by an A/D converter and this improves the signal to noise ratio of the experimental cyclic voltammograms. Computers can repeat each experiment under identical conditions. Computers are used for the data analysis. It measures peak current or peak potential very accurately\(^7\text{,}^8\) by subtraction of background current\(^4\). Voltammetric curves may be differentiated to obtain peak potentials with greater precision\(^9\). The information thus obtained such as peak current, peak potential and peak width at various concentrations may then be correlated with theoretical predictions for establishing the nature of processes and for evaluating the rate parameters.

2.3. Apparatus (Electrolysis Cell)

In its simplest form, the electrolysis cell is a single piece of glassware capable of holding an appropriate volume of a test solution containing one or more electroactive analytes. The cell is then maintained oxygen free by passing nitrogen over the solution through nitrogen inlet. The electrochemical cell consists of three electrodes which are immersed in this solution and are electrically connected to the potentiostat. The RE used is SCE, which is often isolated from the solution by a salt bridge to prevent contamination by leakage from the RE. The AE (platinum foil) and WEs glassy carbon or carbon paste electrode are placed directly into the solution (Fig. 2.2). Custom glassware designs include convenient fittings for mounting electrodes, gas inlets and outlets for purging oxygen and temperature jackets. Since the limiting (peak) current in any type of voltammetry is temperature dependent, the cell is thermostated for the required temperature.

2.4. pH meter

A pH meter, manufactured by Systronic Digital model 335 was used for measuring and adjusting the pH of the solutions making use of a combination of glass and SCE.
2.5. Electrodes

In the present work three electrode system is used i.e. WE / AE / REs. The RE used is SCE which is often isolated from the solution by a salt bridge to prevent contamination by leakage from the RE. The platinum foil as AE and WEs are carbon paste electrode and glassy carbon.

2.5.1. Electrode pretreatment

Obtaining reproducible results and activating the electrode surface to obtain reproducible results are the two main objectives of a great variety of pretreatment procedures reported. Reproducible results can be obtained if the electrode is maintained in the same state of cleanliness. Mechanical polishing is carried out with metallographic papers of increasing fineness. The electrode may also be polished using alumina or diamond powder of 0.05 sizes. This would remove all surface impurities. However, care must be taken to clean the electrode thoroughly afterwards to ensure that the polishing materials are completely removed. Otherwise, these polishing materials themselves may show some specific catalytic or inhibitive effects. The most serious problem encountered in solid electrode methodology is the difficulty in understanding the true surface conditions and their possible effects on electrode processes. The surface oxidation of noble metal electrodes and the presence of adsorbed gas influence the electrode kinetics in a variety of ways. Thus, it becomes necessary to adopt some standard procedure that will produce identical surface conditions and hence enable one to interpret experimental observations in a useful way. It is possible to obtain reproducible results, provided the general pretreatment of the electrode is duplicated every time. Hence it is always essential to clean the electrode surface to rid it of the history effects before subjecting it to the appropriate pretreatment procedure.
2.5.1(a). Pretreatment of GCE

Glassy carbon was first used as an electrode material by Zittel and Miller and many workers continue to use the electrode. The properties of glassy carbon have been described by Yamada and Sato.

Before each measurement, the glassy-carbon surface (0.069 cm²) was polished with fine emery sheets upto 4/0 grade and then rinsed with purified water. The supporting electrolyte was placed in the cell and several potential sweeps were applied to obtain a low background. The electroactive species was added and the first potential sweep was registered.

2.6. Carbon Paste Electrodes (cpe)

2.6.1. Carbon paste electrodes: Important developments

Carbon paste electrodes (cpe) and related sensors underwent an attractive development. Its inspiring history, illustrating potentialities of electrochemistry as a whole reveal numerous connections with the current trends. In the initial stage cpe were employed mainly in studying the mechanisms of electrode reaction of various organic compounds. The first modification was done in 1964 in which an organic compound was dissolved with binder and this, which served to study the electrode behaviour of the substance itself, was considered as a pioneering step in the field of carbon paste electroactive electrodes. In 1965, cpe was prepared by rubbing a modifier into the paste had represented its case with which a cpe could be modified. The replacement of non electroactive pasting liquids by electrolyte solution in 1974 opened a new branch of carbon paste electroactive electrodes which at present belong to a special field of the so called solid state electrochemistry. The era of chemically modified electrodes culminated at the beginning of 80's. Modification of carbon paste by impregnating the carbon particles with methanolic solutions of dimethyl glyoxime represents another milestone in the history of cpe. The biologically modified cpe as enzymatic biosensors
were introduced in 1988 to monitor some enzymatically catalyzed reactions of biological substances. In 1988, a cpe with glucose oxidase blended into the paste was reported. The anchoring of enzymes to an electrode material attracted many biochemists and the field of cpe based enzymatic biosensors grew very fast. The robust carbon composite electrodes like screen printed electrodes are the newest trend in electrochemistry.

2.6.2. Carbon paste as electrode material

2.6.2(a). Unmodified carbon paste

Some carbon pastes may not be totally compact immediately after homogenization but the required consistency can be achieved by pressing such mixtures into electrode holders. Too dry or too liquid pastes surface is not renewable in reproducible way.

Binary Mixtures prepared from carbon powder and organic liquid of non electrolytic character are known as unmodified (‘virgin or bare’) carbon pastes. The electro-active moiety in cpe is still graphite powder with micrometric particles of high purity and distribution uniformity available as spectroscopic graphite. Non electrolyte binders Nujol and Silicone oil are non polar pasting liquids which fulfill all the important criteria like chemically inert, nonvolatile, water immiscible and also known to form paste mixtures of fine consistency. Silicone oil could be used in non-aqueous solvents also. Liquid organophosphates are also used. Though they have attractive property like ion-pairing ability, these are less stable and signal to noise characteristics require spectral pre treatment. The reasons for the very wide applications of cpe are their high mechanical stability, low porosity, inertness over a wide potential range and good conductivity, convenient modification and reproducibility.

2.6.2(b). Reason to modify carbon paste electrode

The primary reason to modify an electrode is to obtain qualitatively new sensor with desired, often pre-defined properties. There are four main reasons for modifying cpe
involving typical modifier-substrate interactions which increase the qualities of the electrodes: i) preferential preconcentration of components of the analyte, ii) exploitation of catalytic electrochemical responses, iii) immobilization of molecules involved in electrochemical reactions and iv) alteration of the physical properties of the electrode surface. Carbon pastes undoubtedly represent one of the most convenient materials for the preparation of modified electrodes.

2.6.2(c). Modified carbon pastes

The base of modified cpe is a mixture of powder graphite and non electrolytic binder \(^{22}\). The third constituent in the mixture is the modifier itself. Modifying agent is usually one substance; but, the pastes can also be modified with two or even more components, which is the case of carbon paste-based biosensors containing enzyme (its carrier) together with appropriate mediator \(^{25}\). Carbon paste represents one of the most convenient materials for the preparation of modified electrodes \(^{26}\). Depending on the character of modifying agent and its capability of forming enough active sites in modified paste, the amount of modifier in the paste usually varies between 10-30\% (w/w). In contrast to relatively complicated modifications of solid substrates, cpe can be modified by simple methods like, dissolving directly in the binder \(^{22,27,28}\) or admixed mechanically to the paste during its homogenization \(^{29,30}\). The various other methods used include soaking graphite particles with a solution of a modifier and after evaporating the solvent and thereby impregnating carbon powder \(^{18,21}\), \textit{in situ} \(^{31}\) modification of cpe also can be done which offer a possibility to employ the same carbon paste for repetitive modifications.

2.6.3. Some practical aspects on preparation of unmodified carbon paste electrode: carbon paste components and their choice

Optimal selection of both carbon powder and pasting liquid is the most important factors influencing the resultant behaviour of cpe \(^{22,26,32}\).
2.6.3(a). Choice of carbon powder

The carbon powders with high uniformity of particle size distribution, high purity and more or less suppressed adsorption capabilities \(^{22,32}\) are preferred in current measurements based techniques like voltammetry, coulommetry or amperometry. In contrast to this potentiometric sensors normally do not require the use of graphites with so strictly defined properties. The usual particle size of graphite materials reported in the literature ranges between 5 and 20 \(\mu m\) \(^{26}\) and 0.2 \(\mu m\) for the preparation of carbon paste micro electrodes \(^{33,34}\). The larger particles produce a rough texture and unfavorable mechanical and electrochemical properties \(^{35}\). Too porous materials like carbon black animal charcoal or similar products are not suitable for the preparation of carbon pastes.

2.6.3(b). Choice of pasting liquids or binders

The pasting liquids should be non conducting non volatile, immiscible with aqueous solutions and should exhibit a high chemical and electro chemical inertness \(^{22,26,32}\). Paraffin oil such as nujol and various silicone oils are most popularly used. Some organic esters like tri cresyl phosphate \(^{31,36,37}\) dioctyl phthalate or dinitophenyl octyl \(^{36}\) either which are found to have ion pairing capabilities are also being used. Binders give rise to hydrophobic character of the carbon paste surface, which is in principle the main reason for different behaviour of cpe compared to carbon solid electrodes \(^{22,26,32}\). The presence of pasting liquid at the surface decreases the transfer rate (slower kinetics) causing the higher over potential compared to homogeneous electrodes \(^{35}\). The increasing lipophility of the pasting liquid enhances the electrode overpotential (irreversibility). This is due to the marked hydrophobicity of the liquid which hinders the access of analyte towards the surface. The degree of surface hydrophobicity of the surface can be decreased by pretreatments.
2.6.3(c). Homogenization and preparation of carbon paste electrode for measurement

Preparation of carbon pastes can be done by simply hand mixing of carbon pastes. This is advantageous because the analyst can by himself choose the individual components as well as their mutual ratio despite the fact that ready-to-use carbon paste mixtures are commercially available.

Carbon powder and the pasting liquid can simply be mixed and homogenized using a pestle and agate. Porcelain dish is avoided due to the possible contaminations of the paste with porcelain particles originating from the rough rubbing wall. A clean glass rod is used to mix both the components carefully. The powder so mixed in an agate is then rubbed by intensive pressing with the pestle for effective homogenization. The paste is scrapped off the wall with a spatula and ultimately homogenized again and this step is repeated several times. The paste is kept for 24 hours for self homogenization. The ready prepared paste is then packed into the well (hole) in the electrode body. Its filling is made in small portions when each of them being pressed intimately before adding the next one.

2.6.4. Paste composition and surface states on electron transfer rate

Dry graphite gives electron transfer rates which give an almost Nernstian response and approach those obtained with platinum. The addition of any pasting liquid decreases these rates. Both electrochemical and chemical oxidative pretreatment of such paste increase the electron transfer in the direction of the ‘dry’ graphite limit.

2.6.4(a). Surface renewal

Mechanical renewal of cpe is one of the quickest and very effective method. Thus, easy and quick surface renewal is achieved by simple removal of the used carbon paste layer and renewing it with a fresh paste. Practically immediate surface renewal can be achieved by wiping some paste off using a wet filter paper. If being performed carefully, this procedure provides surface reproducibility nearly comparable to that
attained by rather time-consuming circle-like polishing of the electrode surface upon a paper pad \(^3\).

2.7. Basic Electrochemical Characteristic of Carbon Pastes Electrode

*Potential range and background currents*

The potential within which they can be operated is determined mainly by the quality of graphite and the binder as well as their ratio \(^22^,26^,32\). Polarizability of carbon pastes was long time believed to depend on the functioning of graphite and the role of pasting liquid was considered as less significant, influencing only the background currents level. Studies have shown that polarization limits of a CPE can be controlled effectively even via the choice of the binder. For e.g. tricresyl phosphate based carbon paste was found to exhibit extreme polarization limits and could be polarized from -2 to +2 V in an ammonia buffer \(^37\) and it is a typically broad for any electrode in aqueous media. A very wide potential window, especially in the negative potential range, was obtained with a carbon paste containing lipophilic phthalate \(^37\).

2.8. Preparation and Standardization of Carbon Paste Electrode Used in the Present Study

The carbon paste is prepared by intimately mixing spectroscopic grade carbon powder with a particle size of ~10 to 14 \(\mu\)m with the pasting liquid silicone (nujol \(^14^,26^,40^-\)). In the present study the ratio of carbon powder to silicone was optimized to 1g: 0.4 ml. An increase in the volume of the pasting liquid greatly affected the reversibility of the electrode. This was reflected in the greater peak separations \(\Delta E_p\) in potassium ferricyanide model system used for the study. The lower volumes of the binder affected the stability of the electrode. The paste was homogenized by careful grinding in an agate with the help of a pestle. The well or the cavity in the electrode was filled with the prepared paste. Fresh electrode surfaces were obtained by polishing on a weighing paper until they showed a smooth and shiny appearance. After every measurement, the
electrode surface was mechanically regenerated by removing some paste off (3 mm) and again polishing it on the weighing paper.

2.9. Construction of Carbon Paste Electrode

cpe holders are typically glass or Teflon rods whose end hole can be easily refilled with a new portion of carbon paste. The paste is tamped into a well like depression at one end of the Teflon or glass holder. At this same end, inside the Teflon tube, a graphite rod is placed and the other end of this graphite rod is connected to a copper wire which emerges out at the other end of the Teflon tube serves to establish electrical contact with the external circuit.

2.10. Storage of Carbon Paste Electrode

The cpe could be placed in a beaker containing distilled water and the tip filled with the paste is completely dipped down to the water level. Such storage prevents the desiccation of carbon paste. The cpe stored in this manner exhibit a very stable behaviour.

2.11. Procedure Used to Record Voltammograms

The solutions in the cell were thermostated to the required temperature. The electrodes were inserted into the arms of the cell. The supporting electrolyte was taken in the solvent without adding the analyte in order to record the voltammogram of the blank. If not mentioned otherwise, before voltammetric measurements the solution was purged with pure (99.99%) nitrogen for 20 minutes to remove dissolved oxygen and a stream of nitrogen gas was blanketed over the solution. The potentiostat is programmed for sweep rates for the measurements. The voltammogram of the blank was recorded. A fixed volume of the solution of the analyte was taken in the supporting electrolyte and the same procedure was repeated to record the voltammogram. The electrode surface of the cpe was renewed mechanically by smoothing some paste off and then polishing on a piece of transparent paper before conducting each of the experiments. The experiments were
performed in unstirred solutions. A similar procedure is adopted for differential pulse voltammetric recordings.

2.12. Model System for Basic Characterization of Carbon Paste Electrode in Voltammetry

2.12.1 (a) Potassium ferricyanide system

The surface of cpe can be studied by its effect on the rate of electron transfer. This can be judged qualitatively by examining the separation of the peak potentials in a cyclic voltammogram of a molecule whose electron transfer kinetics are known to be sensitive to the state of the surface. To evaluate the overall quality of the paste \([\text{Fe(CN)}_6]^{3-}/[\text{Fe(CN)}_6]^{4+}\) model system recommended is used \(^{46,47}\). The cyclic voltammograms obtained at cpe using EA-201 is depicted in (Fig. 2.3). Well-defined anodic and cathodic waves were obtained.

2.12.1(b) Calculation of surface area of the electrode

The surface area of cpe was determined using potassium ferricyanide (1 mM) system in 1 M KCl. The effect of scan rate on cyclic voltammograms of 1mM solution of ferricyanide has been studied at 50, 100, 150, and 200 mVs\(^{-1}\). For a reversible redox couple, the number of electrons transferred in the electrode reaction can be determined by the separation between the peak potentials \(\Delta E_p = (E_{pa} - E_{pc}) / n \approx 0.059\). The value found to vary from 0.061 to 0.066, which correspond to one electron transfer. It is found that the separation of the peak potentials is independent of the scan rate. Also, the ratio of \(i_{pa}/i_{pc}\) is found to be close to one (\(\approx 0.9855\)), which is a typical behaviour, exhibited by a reversible electrochemical charge transfer. On substitution of the diffusion-coefficient value \((12.2\times10^6\text{cm}^2\text{s}^{-1})\) \(^{48}\) in the following equation.

\[
i_p = 2.65 \times 10^{-5} n^{3/2} A C_o^*/D_o^{1/2} v^{1/2}
\]

Where \(i_p\) is the peak current in A, \(A\) is the area of the electrode in cm\(^2\), \(n\) the number of electrons, \(C_o^*/\) is the concentration in molcm\(^3\), \(D_o\) diffusion coefficient in cm\(^2\) s\(^{-1}\) and \(v\) is
the scan rate in Vs\(^{-1}\). The values of \(i_{pc}, i_{pa}\) obtained at different scan rates are substituted in the above equation and the average value of surface area is calculated. The surface area of the electrode was found to be 0.022 cm\(^2\).

### 2.13. Controlled Potential Electrolysis or Bulk Electrolysis

The principal behind the Controlled potential analysis (CPE) is if only either oxidized or the reduced species is initially present, then the potential is set at a constant value sufficiently negative or positive to cause rapid reduction or oxidation and is maintained at this value until only the reduced or oxidized species is present in solution. The products are then isolated for further analysis/characterize the product. The total charge passed during the CPE experiment (Q) is calculated by integrating the current and is related to the number of electrons transferred for molecule (n) and the number of moles of the oxidized or reduced species initially present (N) through Faraday's law \(Q = nFN\) where \(F\) is Faraday's constant (96500 C mol\(^{-1}\)). The potential required for a glassy carbon or cpe experiment is determined by the redox potential of the analyte using CV experiment and is set at 200 mV more negative or positive respectively for reduction or oxidation process. The rate of electrolysis is controlled by the rate of mass transport to the WE. Hence, the solution is stirred using a magnetic stirrer to increase the rate of mass transport. The cell required for CPE is different to that required for voltammetry experiments. H type of cell separating the anodic and cathodic compartments by means of a frit is used for bulk electrolysis. Pt gauze as AE, SCE as reference and cpe/glassy carbon paste electrode was used as the WE. The AE is separated from the WE to prevent species that are electrogenerated at the AE from interfering with electrolysis at the WE. The RE and WE are placed in one compartment of the H cell where as the AE was placed in the other compartment. The rate of electrolysis is enhanced by using a WE with a large surface area and also an AE of large surface area.
Fig. 2.1. Experimental set up of potentiostat for cyclic voltammetry.

RE – Reference electrode
WE – Working electrode
AE – Auxiliary electrode
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Fig. 2.2. A typical cell used in cyclic voltammetry experiments.

Fig. 2.3. Cyclic voltammograms of the model potassium ferricyanide system, scan rate, a=50, b=100, c=150, and d=200mV in 1M KCl at carbon paste electrode.
2.14. References


