CHAPTER 2

MATERIALS AND GENERAL EXPERIMENTAL PROCEDURES

This chapter deals with description of the materials used, experimental conditions employed, and general instrumental and electrode arrangements in protonation and metal–ligand complexation studies performed in this project by glass electrode potentiometry and sampled direct current polarography.

2.1 REAGENTS

The ligands glycine (Formula Weight (F.W.) 75.07, 99% pure) and sarcosine (F.W. 89.09, 99% pure) were purchased from Aldrich (Milwaukee, USA). All ligands were used as received and they were in the solid form as free acids. Cadmium nitrate tetrahydrate ($Cd(NO_3)_2.4H_2O$, F.W. 308.47, 99% pure), lead nitrate ($Pb(NO_3)_2$, F.W. 331.20, 99% pure) and zinc nitrate hexahydrate ($Zn(NO_3)_2.6H_2O$, F.W. 297.48, 98% pure) were obtained from Aldrich (Milwaukee, USA). Sodium nitrate ($NaNO_3$, F.W. 84.99, 99% pure), sodium hydroxide (NaOH, F.W. 40, 99% pure), potassium hydrogen phthalate (KHP) (F.W. 204.23, 99.5% pure), nitric acid (HNO_3, F.W. 63.01, 65%) and disodium tetraborate (borax) ($Na_2B_4O_7.10H_2O$, 99% pure) were obtained from Saarchem (Muldersdrift, South Africa). The metal salts were of analytical grade and were used without further purification. Before KHP was used, it was heated at 110 °C, cooled and stored in a dessicator.

De–ionized water was obtained by passing distilled water through a Milli–Q–water purification system (Millipore, Bedford, MA, USA).

2.2 PREPARATION AND STANDARDISATION OF SOLUTIONS

Stock solutions of about 0.1 M or 0.05 M NaOH were usually prepared by transferring the required mass of NaOH pellets to 250 or 500 mL volumetric flasks. An appropriate mass of $NaNO_3$ was also added to the volumetric flasks to ensure that subsequent solutions would be maintained at ionic strength of 0.5 M. De–ionized water was used for dissolving the reagents and dilution to the required final volume. The NaOH solutions were standardized against a weighed amount of the primary standard potassium hydrogen phthalate (KHP). Phenolphthalein was employed for end–point determination.

Stock solutions of about 0.1 M or 0.05 M HNO_3 (adjusted to ionic strength of 0.5 M with $NaNO_3$) were prepared by diluting with de–ionised water the appropriate volume of 65% HNO_3 in a 250 or 500 mL volumetric flask. The HNO_3 solutions were standardized by titration with disodium tetraborate (Borax) using methyl red as end–point indicator.

The standardization titrations were conducted manually using a 765 Dosimat digital burette for titrant delivery. The standardization procedures for NaOH and HNO_3 solutions involved at least five titrations of accurately weighed samples of KHP or borax (samples of between 0.1 to 0.2 g were used). The average concentration corresponding to the best four titration results was used in subsequent data analysis. The standardized solutions were used within three days after being standardized.

The stock solutions of the metal ions studied (Cd^{2+}, Pb^{2+} and Zn^{2+}) were prepared by weighing the required mass of the appropriate salt and dissolving it in de–ionised water in a 100–mL volumetric flask to give a solution of 5×10^{-2} M. Before dilution with de–ionised water, an appropriate mass of $NaNO_3$ was added to ensure solutions of ionic strength 0.5 M were used.

2.3 GLASS ELECTRODE POTENTIOMETRY

2.3.1 Electrodes and Instrumentation

Glass electrode potentiometric measurements were made using a Metrohm combination glass electrode (model 6.0234.100) connected to a 713 or 780 pH meter also from Metrohm (Herisau, Switzerland). Combination glass electrodes are electrodes in which an indicator (the glass electrode) and a reference electrode are combined in the same body. The indicator electrode provides a potential that depends on the composition of the sample solution. The task of the reference electrode is to supply a potential which is as independent as possible of the sample solution. The measured potential is the sum of all individual potentials produced by indicator and reference electrode [1]. Such combined electrodes are more convenient to handle than two separate electrodes. The built–in reference electrode for the combination glass electrode was a silver–silver chloride electrode (Ag/AgCl/3 M KCl). When not in use, the pH electrode was stored in 3 M KCl solution.

In all potentiometric titrations, titrant additions were performed with the use of a digital burette (Metrohm's 765 Dosimat) equipped with appropriate burette cylinders (Metrohm's Exchange units). Stirring operations of sample solutions were performed with a Metrohm 728 magnetic stirrer.

Temperature measurements were performed using a Metrohm Pt 1000 temperature probe (model 6.1110.100) connected to a 713 or 780 pH meter.

All automated potentiometric experiments were conducted utilizing a computer–controlled instrumentation developed in this project. In the instrumental set–up, the pH meter, the digital burette, and the stirrer have been interfaced to a personal computer equipped with dedicated software modules. The software modules, called virtual instruments (or VIs) have been developed using the LabVIEW programming package (National Instruments, Texas, USA). Detailed description of the instrumentation is discussed in Chapter 4.

2.3.2 Experimental set–up

All experiments were performed in a Metrohm double–walled glass vessel, equipped with a magnetic stirrer bar with thermostatted water at 25.0 ± 0.2 °C circulating in the space between the walls. The water was supplied to the vessel from a constant temperature water bath. The glass vessel was fitted on the vessel–holder of the top part of the 663 VA stand (Metrohm) incorporated in the instrumental set–up developed in this project (details will be described in Chapter 4). When fitted in this way the glass vessel is covered with a polytetrafluoroethylene (PTFE) cover which has a total of seven apertures that can be used to hold electrodes. One of the apertures on the PTFE cover was used to hold the combination glass electrode (CGE). Another opening was used to hold the temperature probe. A third inlet was used for fitting a burette tip used for titrant delivery to the sample solution. Moreover, there is an inlet for inert gas to de–aerate the solution under investigation. The unused apertures of the PTFE cover remained stoppered throughout the potentiometric experiments. The inert gas used in all experiments was ultra–high purity nitrogen (99.999% pure) obtained from Afrox (South Africa).

2.3.3 Glass Electrode Calibration

Accurate calibration of the glass electrode is crucial to the determination of stability constants or ligand protonation constants by potentiometric titrations, as any error in the calibration becomes a systematic error in the interpretation of titration data [2].

Starting with the Nernst equation, the response of a glass electrode, in a medium of constant ionic strength, can be generalized with the following relationship:

$$E = E^k + s' \operatorname{Log}[H^+] \qquad \textbf{(2.1)}$$

where E is a measured electrode potential, E^k and s' are parameters to be found during calibration and represent the effective standard electrode potential (a term

that also contains the junction potential) and the response slope of the glass electrode, respectively. [H^+] represents the hydrogen ion concentration.

Defining $\mathrm{pH} = -\mathrm{Log}\,[H^+]$[1], Equation 2.1 can be rewritten to express the measured potential E as a function of pH:

$$E = E^k + s\mathrm{pH} \qquad \textbf{(2.2)}$$

where $s = -s'$.

To establish the constant E^k and response slope s of a glass electrode, a potentiometric titration of a standardized strong acid with a standardized strong base at constant temperature of 25 °C and ionic strength of 0.5 M was performed. This method of calibration of glass electrodes, in terms of hydrogen ion concentration, has been widely recommended for metal–ligand equilibria studies at constant ionic strength [3–7].

The calibration titrations were performed automatically in this work using the instrumental set–up developed in this work whereby the virtual instrument for automated potentiometric titrations (*Autotitrator VI*), to be described in Chapter 4, was used. In a typical calibration experiment a 10–mL aliquot of a standardized HNO_3 solution (adjusted to ionic strength of 0.5 M with $NaNO_3$) was mixed with 20 mL of 0.5 M $NaNO_3$ in a thermostatted titration vessel and titrated with a standardized solution of NaOH (also adjusted to ionic strength of 0.5 M with $NaNO_3$). The titrant volume additions were typically set between 0.1 and 0.2 mL. To generate calibration curves, the spreadsheet program Microsoft Excel was used to calculate pH at each measured potential E corresponding to a specific volume of NaOH added. Linear regression analysis was employed to get the best fit of E versus pH. Points in the unbuffered region between pH 3.5 and 10 were not used in regression analysis. The combination glass electrode was calibrated before and

[1] Strictly, pH = – Log a_{H+}, where a_{H+} is the activity of hydrogen ions. Instead of activity, the concentration scale for hydrogen ions has been used in defining pH throughout this work as ionic strength was maintained constant in all experiments performed.

after a glass electrode potentiometric experiment performed in metal–ligand or protonation equilibria studies. The values of E^k and response slope s from the combined calibration results obtained with the two titrations (before and after main experiment) were used in subsequent data analysis (provided that they did not differ much). An example of a calibration plot obtained before a GEP experiment is shown in Figures 2.1.

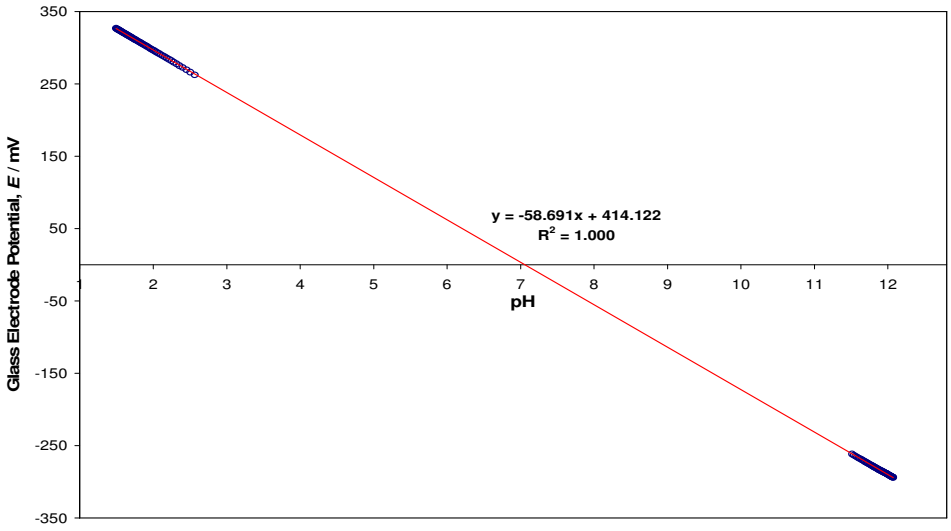

Figure 2.1: An example of a calibration curve obtained prior to performing a glass electrode potentiometric experiment for a metal–ligand system. The solid line is the best linear fit of the experimental points (o). *In the regression equation y = E and x =* pH. *In this example,* E^k *= 414.12 mV and the response slope s = 58.691 mV.*

2.3.4 Determination of ligand protonation constants

Protonation constants for two ligands, glycine and sarcosine, were determined by glass electrode potentiometry. At least two titrations were performed for each ligand. The general analytical procedure adopted for determination of protonation constants of the ligands is outlined here.

1. The titration glass vessel was initially cleaned with soapy water followed by several rinses with tap water. The cell was further cleaned with a solution of about 0.5 M HNO_3 followed by thorough rinsing with de–

ionised water. The vessel was dried using ashless 110 mm circular, qualitative filter papers (Whatman, Maidstone, England).

2. The combination glass electrode was then calibrated by strong acid/strong base titration (as described in section 2.3.3) in order to establish the E^k and response slope s of the electrode before the main experiment.
3. After calibration of the glass electrode, the cell was cleaned as described in point 1 above. Then, an appropriate volume of the background electrolyte (0.5 M $NaNO_3$) was transferred to the cell. Typical volumes were 20, 25 or 30 mL. In order to ensure complete de–aeration of the solution under investigation, N_2 gas was continuously purged into the cell.
4. An accurately weighed amount of the ligand under study (glycine or sarcosine) was added to the cell to give the required total ligand concentration. The typical ligand concentration in the protonation studies ranged from 5×10^{-3} to 2×10^{-2} M.
5. An appropriate amount of standardized HNO_3 solution (~ 0.05 M, adjusted to ionic strength of 0.5 M with $NaNO_3$) was added to the ligand solution to ensure initial pH of about 2.
6. A 5–mL burette cylinder (Exchange unit) containing a solution of standardized NaOH (~ 0.05 M, adjusted to ionic strength of 0.5 M with $NaNO_3$) was placed on a 765 Dosimat (the digital burette). The dedicated virtual instrument software module *Configure Dosimat and pH meter VI* (the software module for configuring the digital burette and the pH meter for automated titrations; detailed description is described in Chapter 4) was invoked to set the dosing mode of the 765 Dosimat to DIS C (Dispensing Cumulative Mode), the mode required for constant volume additions. The appropriate volume increment (0.1 – 0.2 mL) was also set.
7. The *Autotitrator VI* was employed to perform an automated potentiometric titration.
8. After the experiment, the glass electrode was calibrated again to check for any significant variation in the performance of the electrode.

Detailed experimental data for the specific ligands are provided in Appendix B.

2.3.5 Metal–Ligand Equilibria Studies by GEP

Glass electrode potentiometry was employed to study the metal–ligand systems Cd(II)–Glycine–OH, Cd(II)–Sarcosine–OH, and Zn(II)–Glycine–OH, at several total ligand to total metal ion concentration ratios (L_T : M_T ratios). The general experimental procedure adopted is described below.

1. The titration glass vessel was initially cleaned with soapy water followed by several rinses with tap water. The cell was further cleaned with a solution of about 0.5 M HNO_3 followed by thorough rinsing with de–ionised water. The vessel was dried using ashless 110 mm circular, qualitative Whatman filter papers.
2. The combination glass electrode was then calibrated by strong acid/strong base titration (as described in section 2.3.3) in order to establish the E^k and response slope s of the electrode before the main experiment.
3. After calibration of the glass electrode, the cell was cleaned as described in point 1 above. Then, an appropriate amount of the background electrolyte (0.5 M $NaNO_3$) was transferred to the cell. Typical amounts were 20, 25 or 30 mL. To ensure complete de–aeration of the solution under investigation, N_2 gas was continuously purged into the cell.
4. The required volume of the appropriate metal ion stock solution ($Cd(NO_3)_2$ or $Zn(NO_3)_2$) was added to give the sought total metal ion concentration (M_T), typically in the range 1×10^{-3} to 1×10^{-2} M, depending on the L_T : M_T desired.
5. The ligand under study was introduced to the titration vessel by transferring an appropriate volume from a stock solution of the ligand (typical concentration was 5×10^{-2} M adjusted to ionic strength of 0.5 M with $NaNO_3$) to give the required total ligand concentration (L_T). The typical ligand concentration range was 2×10^{-3} to 1.5×10^{-2} M, depending on the L_T : M_T desired.
6. In some cases it was necessary to adjust the pH to an appropriate starting value by addition of standardized HNO_3 solution (~ 0.05 M, adjusted to ionic strength of 0.5 M with $NaNO_3$).

7. A 5–ml burette (Exchange unit) containing a solution of standardized NaOH (~ 0.05 M, adjusted to ionic strength of 0.5 M with $NaNO_3$) was placed on a 765 Dosimat. The *Configure Dosimat and pH meter VI* was invoked to set the volume increment and the appropriate dosing mode for the 765 Dosimat. Typical volume increment values ranged from 0.010 to 0.070 mL.
8. The *Autotitrator VI* was employed to perform an automated potentiometric titration. Typical parameters for data acquisition were set as: (i) *Equilibration time* = 300 s; (ii) *Criterion of Stability* = 0.05 mV; (iii) *Sampling Rate of pH meter* = 3 s; (iv) *Max. Waiting Time* = 20 min. The specific parameters used are documented in the Appendices for experimental data of the individual metal–ligand systems studied (Appendices C, D, E, F and G).
9. After the main experiment, the combination glass electrode was calibrated again as described in section 2.3.3 to check for any variation in the performance of the electrode.

2.4 SAMPLED DIRECT CURRENT POLAROGRAPHY

2.4.1 Electrodes and Instrumentation

The instrumental set–up and the corresponding computer software modules (detailed descriptions are presented in Chapter 4 sections 4.5 and 4.6) were used for polarographic studies. Basically, the instrumentation incorporated commercial hardware components for polarographic and potentiometric measurements and these components have been interfaced to a personal computer equipped with dedicated virtual instrument software modules. The CV–27 Voltammograph (Bioanalytical Systems, Indiana, USA) was used as potentiostat in the instrumental set–up. Polarographic measurements were performed using three electrodes. A multi–mode electrode (MME) (Metrohm, model 6.1246.020) was employed as the working electrode (WE) and used in the dropping mercury electrode (DME) mode. The electrochemical process involving the analyte occurs at the working electrode.

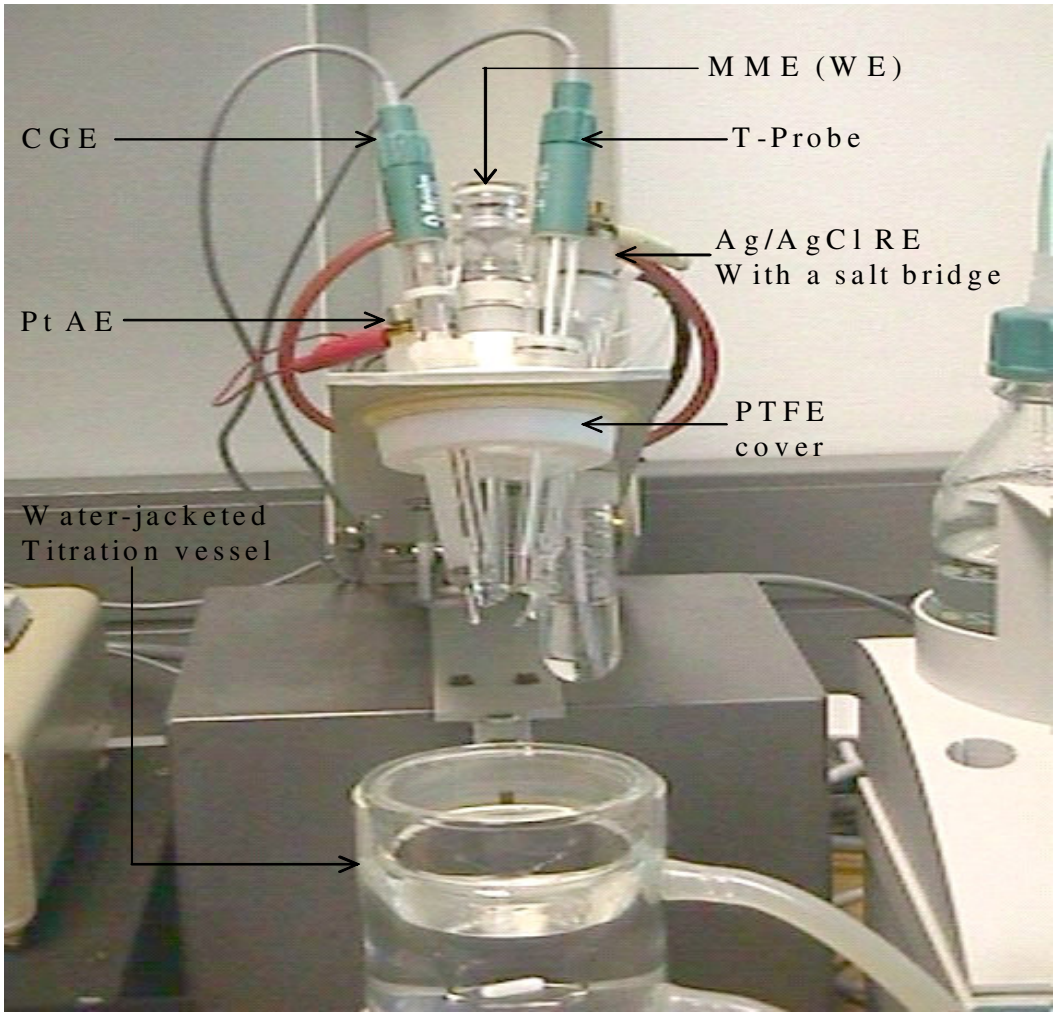

Figure 2.2: A photograph showing electrodes and probes used in studies of metal–ligand systems at fixed L_T : M_T and variable pH. MME = Multi-Mode Electrode, T–Probe = Temperature Probe, Pt AE = Platinum rod auxiliary electrode, WE = Working Electrode, RE = Reference Electrode, CGE = Combination Glass Electrode, PTFE cover = Polytetrafluoroethylene cover.

The MME consists of a glass capillary supplied with a mercury reservoir. A platinum rod electrode was used as the auxiliary electrode (AE) (also known as counter electrode). The function of the counter electrode is to supply the current that passes between itself and the working electrode. Ag/AgCl (3 M KCl) electrode was used as the reference electrode (RE) (Metrohm, model 6.0728.000). The reference electrode was connected to the solution under study by means of a glass salt bridge (Metrohm, model 6.1245.000). In electrochemical experiments, the function of the salt bridge is to allow for the flow of ions but prevent direct contact of the test solution with the electrode. The salt bridge electrolyte normally

has similar composition to the supporting electrolyte used in a voltammetric experiment [8]. In all polarographic experiments, the salt bridge electrolyte was 0.5 M $NaNO_3$. The electrode arrangement is shown in Figure 2.2.

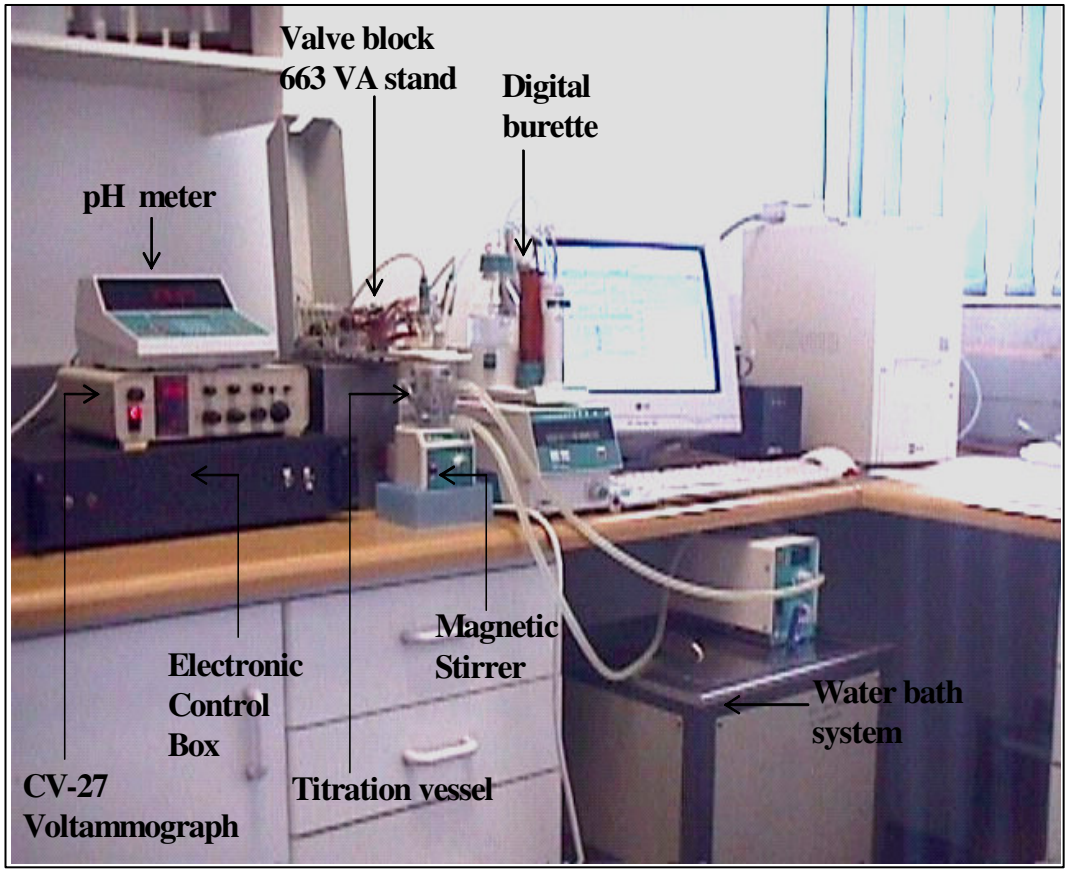

Figure 2.3: A photograph of the instrumental set–up in a typical automated experiment for a metal – ligand system at fixed L_T:M_T ratio and variable pH by sampled direct current polarography with glass electrode potentiometry as the leading technique.

2.4.2 Experimental Set–up

The experimental set–up for polarographic studies of metal–ligand systems involves usage of a combination of hardware components for glass electrode potentiometry as well as components typical for polarography. All polarographic experiments performed in this project required pH measurements. Components for GEP have been described in section 2.4. Additional components used for polarographic measurements are the Ag/AgCl reference electrode, the multi–mode electrode, and the platinum auxiliary electrode which were fitted into the

appropriate openings on the PTFE cover on the top part 663 VA stand for polarographic measurements. All experiments were performed in a titration glass vessel thermostatted at 25.0 ± 0.2 °C. Figure 2.3 shows the experimental arrangement.

2.4.3 Polarographic Studies of Metal – Ligand Equilibria

Sampled direct current polarography was employed in metal–ligand equilibria studies at fixed L_T : M_T ratios and varied pH. The metal–ligand systems investigated with this technique were: Cd(II)–Glycine–OH, Cd(II)–Sarcosine–OH, Pb(II)–Glycine–OH, and Zn(II)–Glycine–OH. An outline of the general experimental procedure adopted is presented here.

1. The titration glass vessel was initially cleaned with soapy water followed by several rinses with tap water. The cell was further cleaned with a solution of about 0.5 M HNO_3 followed by thorough rinsing with de–ionised water. The vessel was dried using ashless 110 mm circular, Whatman qualitative filter papers.
2. The combination glass electrode was then calibrated by strong acid/strong base titration (as described in section 2.3.3) in order to establish the E^k and response slope s of the electrode before the main experiment.
3. After calibration of the glass electrode, the cell was cleaned as described in point 1 above. Then, an appropriate amount of the background electrolyte (0.5 M $NaNO_3$) was transferred to the cell. Typical amounts were 20, 25 or 30 mL. A few corns of solid gelatine were placed into the solution. Gelatine was used to suppress polarographic maxima (which are peaks that interfere with the limiting diffusion current plateau) unusually appearing in polarographic curves due to some convection phenomena within and in the immediate vicinity of the dropping mercury electrode [9]. N_2 gas was purged into the cell for about 30 minutes to ensure sufficient removal of oxygen, which is electrochemically active and could interfere with the polarographic reduction processes of interest.

4. A DC polarogram was then recorded to check for the purity of the background electrolyte. For this purpose, the *DC* (*One Polarogram*) *VI* (description of the software module is provided in Chapter 4 section 4.5) was used to record the single DC polarographic scan. Typical potential ranges were 0 to –1 V for studies of Cd^{2+} and Pb^{2+} and –0.5 to –1.4 V for Zn^{2+} experiments. The drop time of 1 or 1.5 s and integration time of 80 ms were set. The step potential was always 4 mV.
5. The required volume of the appropriate metal ion stock solution ($Cd(NO_3)_2$, $Pb(NO_3)_2$ or $Zn(NO_3)_2$) was added to the cell to give the sought total metal ion concentration, typically in the range 8×10^{-5} to 1.5×10^{-4} M, depending on the L_T : M_T ratio. The volumes required were usually between 30 to 50 μL. For such minute volume additions, an appropriate micro–syringe (Hamilton, Bonaduz, Switzerland) was used.
6. At least three consecutive DC polarograms for the solutions of metal ions only, i.e., in the absence of ligand, were recorded using the *DC (One Polarogram) VI*. Data acquisition parameters for the specific experiments are documented in the Appendices C – G.
7. The ligand under study was then introduced to the titration vessel by transferring an appropriate weighed amount of the solid ligand to give the required L_T : M_T ratio.
8. In some cases it was necessary to adjust the pH to an appropriate starting value by addition of standardized HNO_3 solution.
9. A 1–mL or 5–mL burette cylinder (Exchange unit) containing a solution of standardized NaOH (~ 0.05 M, adjusted to ionic strength of 0.5 M with $NaNO_3$) was placed on a 765 Dosimat. The *Configure Dosimat and pH meter VI* was invoked to set the volume increment and dosing mode for constant volume additions (DIS C). Typical volume increment values ranged from 0.005 to 0.020 ml.
10. One of the virtual instruments (*Autotitrator–DC1*, *Autotitrator–DC2*, *Autotitrator–DC–Dynamic1*, or *Autotitrator–DC–Dynamic2* described in Chapter 4, section 4.3) was employed to perform an automated acid–base titration with acquisition of DC polarograms at appropriate pH values. The

pH step of 0.05 pH units was usually set to control acquisition of DC polarograms at appropriate pH values. The stop conditions were dependent on the metal–ligand system under study and the L_T : M_T ratio due to different pH values at which precipitation was expected to occur. The specific parameters for data acquisition are documented in the Appendices for experimental data of the individual metal–ligand systems (Appendices C, D, E, F and G).

11. After the main experiment, the glass electrode was calibrated again to check for any variation in the performance of the electrode during the titration of the metal–ligand solutions.

The experiments were usually conducted overnight and ran for total durations of between 12 to 20 hours. The set of polarographic data collected contained 50 to 90 curves.

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