Chapter 2

Materials and Methods

This chapter discusses in detail the synthesis of each of the ion associations used in the fabrication of the different sensors. It also describes the general method of fabrication of the two types of sensors viz; the PVC membrane sensor and the carbon paste electrode (CPE). Details about the general reagents and the instruments used in the investigations are also discussed in this chapter. The chapter also discusses the general procedure for the analysis of the pharmaceutical formulations and real samples employed in the studies.

2.1 Reagents

All reagents used were of analytical grade and distilled water was used throughout the studies. High molecular weight PVC, perchloric acid and all the metal salts were obtained from Merck, Germany and were used as received. The ion pairing reagents such as silicotungstic acid (STA), phosphotungstic acid (PTA), molybdophosphoric acid (MPA) and sodium tetraphenyl borate (NaTPB) were obtained from sd fine chem. India. The plasticizers Bis(2-ethyl hexyl) phthalate (BEP), Bis(2-ethyl hexyl) sebacate (BES), Di-n-butyl phthalate (DBP), Di-butyl sebacate (DBS) and Bis(2-ethyl hexyl) adipate (BEA) were all products of Lancaster UK and were used without any further purification. High purity graphite was purchased from Sigma Aldrich Corporation, USA and was used as received. Tetrahydrofuran
(THF), 2, 2-diphenyl-1-picrylhydrazyl, 5,5-diethylbarbituric acid, ethyl acetate, acids and indicators were products of sd fine chem. India. Pure drugs viz, tetracycline, dextromethorphan, mebendazole, ambroxol, sildenafil citrate and pefloxacin were obtained as gift samples. Pharmaceutical formulations containing the drugs were purchased from local drug stores.

2.2 Synthesis of the ion association complexes

The ion association complexes (ionophores) for each drug were prepared using the respective ion pairing reagents. The different ion pairing reagents employed were silicotungstic acid (STA), phosphotungstic acid (PTA), molybdophosphoric acid (MPA) and sodium tetraphenyl borate (NaTPB).

2.2.1 Mebendazole-STA ion association

0.29 g of mebendazole (MBZ) was dissolved in very dil. HNO₃ and made upto 100 mL with distilled water. 75 mL of the 10⁻² M MBZ solution thus obtained was mixed with 25 mL 10⁻² M STA solution. The resulting solution was stirred well for 10 minutes. The white coloured precipitate obtained was filtered and washed several times. The precipitate was dried at room temperature and stored in a desiccator.

2.2.2 Mebendazole-PTA ion association

10⁻² M MBZ solution was prepared by dissolving 0.29 g of pure drug in very dil. HNO₃ and made upto 100 mL with distilled water. MBZ-PTA ion association was prepared by mixing 75 mL of this drug solution with 25 mL of 10⁻² M PTA solution and stirred well for 10 minutes. The yellow coloured precipitate thus formed was filtered, washed, dried at room temperature and stored in a desiccator.
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2.2.3 Mebendazole-MPA ion association

MBZ-MPA ion association was prepared by mixing 75 mL 10⁻² M MBZ solution and 25 mL 10⁻² M MPA. The solution was stirred well for 10 minutes. The resulting yellow coloured precipitate was filtered and washed repeatedly. The ion association was dried at room temperature and stored.

2.2.4 Pefloxacin-STA ion association

0.33 g of pure pefloxacin (PEF) was dissolved in distilled water and made up to 100 mL to get 10⁻² M PEF solution. 75 mL of this solution was mixed with 25 mL of 10⁻² M STA and stirred well. The yellow coloured precipitate was filtered and washed. It was dried at room temperature and stored.

2.2.5 Pefloxacin-MPA ion association

A 10⁻² M PEF solution was prepared in 100 mL by dissolving 0.33 g of the pure drug in distilled water. 75 mL of this solution was mixed with 25 mL 10⁻² M MPA and stirred for 10 minutes. The brown coloured precipitate obtained was filtered, washed several times and dried at room temperature.

2.2.6 Ambroxol-mpa ion association

0.41 g of pure ambroxol (AMB) was dissolved in dil. methanol and made up to 100 mL to get a 10⁻² M AMB solution. 75 mL of this solution was mixed with 25 mL 10⁻² M of MPA and the brown coloured precipitate obtained was washed several times. The precipitate was dried at room temperature and stored in a desiccator.

2.2.7 Ambroxol-PTA ion association

75 mL 10⁻² M ambroxol (AMB) solution was mixed with 25 mL 10⁻² M PTA solution. The solution was allowed to stand for a few minutes and
the faint yellow coloured precipitate formed was filtered and washed. The precipitate dried at room temperature was stored in a desiccator.

2.2.8 Sildenafil citrate-PTA ion association

0.47 g of sildenafil citrate (SIL) was dissolved in dilute methanol and made up to 100 mL to prepare a 10^{-2} M SIL solution. 75 mL of this solution was mixed with 25 mL 10^{-2} M PTA and stirred well for 15 minutes. It was allowed to stand for a few more minutes and the resulting yellow coloured precipitate was washed repeatedly. The dried precipitate was stored in a desiccator.

2.2.9 Sildenafil citrate-STA ion association

The SIL - STA ion association was prepared by mixing 10^{-2} M (75 mL) SIL with 10^{-2} M (25 mL) STA. The solution was stirred well for 15 minutes and allowed to stand. The brown coloured precipitate formed was washed repeatedly, dried at room temperature and was stored in a desiccator.

2.2.10 Dextromethorphan-NaTPB ion association

10^{-2} M solution of dextromethorphan (DEX) was prepared in 100 mL by dissolving 0.37 g of the drug in distilled water. 100 mL of 10^{-2} M solution of NaTPB was prepared. The ion association of DEX with NaTPB was obtained by mixing 50 mL each of these prepared solutions and stirred well. The resulting white precipitate was filtered, washed with distilled water and dried at room temperature. The dried precipitate is stored in a desiccator for future use.

2.2.11 Dextromethorphan-PTA ion association

The DEX-PTA ion association was prepared by mixing solutions of DEX and the ion-pairing reagent PTA. 10^{-2} M solution of dextromethorphan (DEX) was prepared in 100 mL by dissolving 0.37 g of the drug in distilled
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water and $10^{-2}$ M solution of PTA was also prepared. The DEX-PTA ion association was prepared by mixing 75 mL of $10^{-2}$ M DEX with 25 mL of $10^{-2}$ M PTA. The mud coloured precipitate obtained was filtered, washed repeatedly, dried at room temperature and stored in a desiccator.

2.2.12 Tetracycline – NaTPB ion association

The ion association for tetracycline (TCE) was based on the ion-pairing reagent NaTPB. A $10^{-2}$ M solution of the drug (100 mL) was prepared by dissolving 0.44 g TCE in water. Similarly $10^{-2}$ M (100 mL) solution of NaTPB was also prepared. The ion association was prepared by mixing $10^{-2}$ M (50 mL) solution of the drug with $10^{-2}$ M (50 mL) of NaTPB. This was then stirred well. The resulting yellow coloured precipitate was filtered, washed several times with distilled water and dried at room temperature. The ion association thus obtained is stored in a desiccator.

2.3 Preparation of the drug solutions

A $10^{-1}$ M stock solution in suitable solvents was prepared for each of the drugs. The dilution series for the analysis was obtained by the serial dilution of $10^{-1}$ M stock solution.

2.4 Preparation of the buffer solutions

The buffer solutions were used to adjust the pH of the test solutions. The different buffer solutions were freshly prepared according the Robinson table.  

2.4.1 pH 1.0

To 50 mL 0.2 M potassium chloride solution, 134.0 mL of 0.2 M HCl solution was added to give the buffer having pH 1.0.
2.4.2 pH 2.0

To 50 mL 0.2 M potassium chloride solution, 13.0 mL of 0.2 M HCl solution was added to give the buffer having pH 2.0.

2.4.3 pH 3.0

To 100 mL 0.1 M potassium hydrogen phthalate solution, 44.6 mL of 0.1 M HCl solution was added to give the buffer having pH 3.0.

2.4.4 pH 4.0

To 100 mL 0.1 M potassium hydrogen phthalate solution, 0.2 mL of 0.1 M HCl solution was added to give the buffer having pH 4.0.

2.4.5 pH 5.0

To 100 mL 0.1 M potassium hydrogen phthalate solution, 45.2 mL of 0.1 M NaOH solution was added to give the buffer having pH 5.0.

2.4.6 pH 6.0

To 100 mL 0.1 M potassium dihydrogen phosphate, 11.2 mL of 0.1 M NaOH solution was added to give the buffer having pH 6.0.

2.4.7 pH 7.0

To 100 mL 0.1 M potassium dihydrogen phosphate, 58.2 mL of 0.1 M NaOH solution was added to give the buffer having pH 7.0.

2.4.8 pH 8.0

To 100 mL 0.025 M borax, 41.0 mL of 0.1 M HCl solution was added to give the buffer having pH 8.0.

2.4.9 pH 9.0

To 100 mL 0.025 M borax, 9.2 mL of 0.1 M HCl solution was added to give the buffer having pH 9.0.
2.4.10 pH 10.0
To 100 mL 0.05 M sodium bicarbonate, 21.4 mL of 0.1 M NaOH solution was added to give the buffer having pH 10.0.

2.4.11 pH 11.0
To 100 mL 0.05 M sodium bicarbonate, 45.4 mL of 0.1 M NaOH solution was added to give the buffer having pH 11.0.

2.4.12 pH 12.0
To 50 mL 0.2 M potassium chloride, 12.0 mL of 0.2 M NaOH solution was added to give the buffer having pH 12.0.

2.5 Analysis of the pharmaceutical formulations

2.5.1 Tablets for Tetracycline (Resteclin and Tetracycline)

The contents of ten capsules of each of Resteclin (NPIL - India) and Tetracycline (Dabur - India) were accurately weighed and powdered well in a mortar. About 250 mg of the powder was accurately weighed and dissolved in minimum amount of distilled water and filtered into a 100 mL volumetric flask. This was made up to the mark and shaken well. 10 mL of this solution was transferred into a 100 mL titrimetric flask and made up to the volume. The pH of the solution was adjusted to 6.0. 20 mL of this solution was transferred into a beaker and electrochemical studies were conducted.

2.5.2 Syrup for Dextromethorphan (TUSQ-DX)

10 mL of the syrup (Tusq-DX – Blue Cross - India) was transferred into a 100 mL volumetric flask. It was dissolved in minimum amount of distilled water by shaking well for 10 minutes. The pH of the solution was maintained at 5.0 using appropriate buffer solution. The solution was then
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Quantitatively diluted. 20 mL of this solution was taken in a beaker and electrochemical studies were carried out.

2.5.3 Tablet for Mebendazole (Mebex)

Ten tablets of Mebex (Cipla - India) were accurately weighed and finely powdered. The mass equivalent to the mass of one tablet was dissolved in dil. HNO₃ and filtered into a 100 mL volumetric flask. This was made up to the mark and shaken well. 10 mL of this solution was transferred to a 50 mL volumetric flask. The pH of the solution was adjusted to 6.0 by adding buffer solution and the solution was quantitatively diluted. 20 mL of this solution was transferred to a beaker and electrochemical studies were performed.

2.5.4 Tablet for Ambroxol (Ambrolite)

Ten tablets of Ambroxol (Ambrolite - Tablets India) were weighed, crushed and finely powdered. The mass equivalent to the mass of one tablet was accurately weighed and dissolved in dil. methanol. It was then made up to the mark in a 100 mL volumetric flask. The pH of the solution was maintained at 6.0. 20 mL of this solution was transferred to a beaker and the electrochemical response was investigated.

2.5.5 Tablet for Sildenafil citrate (Silagra)

The weight of ten tablets of Sildenafil citrate (Silagra - Cipla - India) was accurately determined and powdered well. The quantity of powder equivalent to the mass of one tablet was dissolved in minimum volume of distilled water and shaken well for 15 minutes. This was made up in a 100 mL titrimetric flask after adjusting the pH to 5.0. 10 mL of this solution was transferred to a 50 mL volumetric flask and made up. 20 mL of this solution was taken to carry out the electrochemical studies.
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2.5.6 Tablet for Pefloxacin (Pelox)

Ten tablets of Pelox (Wockhardt - India) were weighed and finely powdered. The mass equivalent to the mass of one tablet was dissolved in distilled water and made up in a 100 mL titrimetric flask. 10 mL of this solution was transferred into a 100 mL titrimetric flask and quantitatively diluted. The pH of the solution was maintained at 5.0. 20 mL of this solution was taken in a beaker and electrochemical studies were performed.

2.6 Standard methods
2.6.1 Tetracycline

The contents of ten capsules were accurately weighed and finely powdered. The mass equivalent to the mass of one capsule was accurately weighed and dissolved in minimum quantity of distilled water. 2 mL of 0.2 M calcium chloride and 2 mL of 0.5 M 5,5-diethylbarbituric acid were added and the undecomposed drug was extracted with three 10 mL portions of ethyl acetate. The organic phase was filtered through anhydrous sodium sulphate and the ethyl acetate was evaporated off. The residue was dissolved in methanol and made up to 100 mL. 1 mL of this solution was transferred to a 25 mL standard flask and 2 mL buffer solution (pH-6) and 2 mL of 2,2-diphenyl-1-picrylhydrazyl was added and mixed well. This solution was heated at 60 °C for 12 minutes. It was cooled and completed to the volume. The absorbance of the solution was measured at 520 nm against reagent blank. The amount of drug was calculated from the calibration graph.
2.6.2 Dextromethorphan \cite{171}

20 mL of the syrup was dissolved in 20 mL ethanol, made up to 100 mL in a volumetric flask and titrated with 0.1 M sodium hydroxide determining the end point potentiometrically.

2.6.3 Mebendazole \cite{172}

0.1124 g of Mebex was dissolved in 1.5 mL of anhydrous formic acid and 20 mL of anhydrous acetic acid was added. The solution was made up in a 100 mL volumetric flask. 10 mL of this solution was transferred to 100 mL titrimetric flask and made up. 20 mL of this solution was titrated with 0.1 M perchloric acid, determining the end point potentiometrically. 1 mL of 0.1 M perchloric acid is equivalent to 29.53 mg of MEB.

2.6.4 Ambroxol \cite{172}

The weight equivalent to the weight of five tablets was dissolved in 70 mL of alcohol and 5 mL of 0.01 M hydrochloric acid was added. It was made up to the volume in a 100 mL titrimetric flask. Potentiometric titration was carried out using 0.1 M sodium hydroxide.

2.6.5 Sildenafil citrate \cite{173}

0.1225 g of the tablet was dissolved in distilled water and taken in a separating flask. To this solution 2 mL of bromocresol green and 3 mL of buffer (pH 2) was added followed by 5 mL of chloroform. This was shaken well and kept aside for 1 minute. After the organic layer was transferred to a beaker, aqueous phase was again extracted with 5 ml of the same solvent. The successive organic layers were mixed and dried over anhydrous sodium sulphate and transferred to a 50 mL volumetric flask. The absorbance of the solution was measured at 415nm against a reagent blank.
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2.6.6 Pefloxacin

0.2864 g of pefloxacin was dissolved in 15 mL of anhydrous acetic acid and 75 mL of acetic anhydride was added. 10 mL of this solution was transferred to a 100 mL volumetric flask and made up. It was then titrated with 0.1 M perchloric acid and the end point was determined potentiometrically.

2.7 Analysis of urine sample

The developed sensors were used for the determination of the corresponding drugs in urine samples. Standard addition method was employed for this purpose. 20 mL of the urine sample containing the drug was taken in a beaker and the electrochemical studies were carried out. It was then spiked with 2 mL of known concentration of the drug solution and again the potential value was determined. The potential readings were noted and the amount of the drug present in the urine sample was calculated.

2.8 Fabrication of the sensors

The design of the electrochemical sensors plays a crucial role in determining the electrochemical response characteristics of the sensors. Two types of sensors were fabricated for the studies.

2.8.1 Fabrication of the PVC membrane sensor

The PVC membrane electrodes belong to the class of liquid membrane electrodes. PVC based sensor membranes contain a reagent dissolved in a suitable solvent, which selectively binds with the ion of interest, the ionophore. The general method of fabrication of the PVC membrane sensor was according to a procedure reported by Cragg's and
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Moody. The active membrane ingredients were varied to arrive at an optimum composition. Ionophore, plasticizer and PVC were taken in suitable percentage-weight ratios. The % w/w of these components vary approximately as 1-5 : 60-65 : 30-35 for ionophore, plasticizer and PVC respectively. The preliminary step involves dissolving the ionophore in THF followed by the plasticizer and the PVC in specific ratios. The resulting mixture was then poured into glass rings struck on a glass plate. It was then covered with a filter paper and left to dry allowing the slow evaporation of the solvent. Small disc shaped membranes thus obtained were cut out and glued to one end of a glass tube using Araldite and M-seal. This was then left to dry and the prepared membrane sensor was finally conditioned by dipping it in the drug solution of suitable concentration for 24 hrs. The internal filling solution consisted of a mixture of \( 1 \times 10^{-3} \) M drug and \( 1 \times 10^{-1} \) M NaCl solution.

2.8.2 Fabrication of the carbon paste sensor

The carbon paste electrodes (CPEs) belong to a special group of heterogeneous carbon electrodes. CPEs are represented by carbon paste, i.e.; a mixture prepared from graphite and ionophore with a suitable liquid binder packed into a suitably designed electrode body. One of the most significant advantages of the CPEs is that it does not require an internal filling solution. The ion association and high purity graphite were thoroughly mixed in a mortar with acetone in suitable proportions. It was homogenized and left at room temperature to evaporate off acetone. Then weighed amount of plasticizer was added to this carbon paste. This paste was then packed to one end of the Teflon holder in which electrical contact was made with a copper rod through the centre of the electrode. The electrode surface was polished.
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using a filter paper to produce reproducible working surface. The sensors were then conditioned by dipping it in suitable concentrations of the drug solutions.

2.9 Selectivity studies

If the full scope of a chemical sensor is to be fully realized, their selectivity in presence of various interferents demands proper and reliable assessment. The selectivity of the developed sensors for the drug in presence of various foreign ions has been determined using the Fixed Interference Method (FIM). The value of the selectivity coefficient $K_{A,B}^{pot}$ determines the extent of selectivity of the drug in presence of foreign ions. The potential of the cell comprising the sensor and the reference electrode was measured for solutions of constant activity of the interfering ion, $a_B$, and varying activity of the primary ion. The potentials obtained versus the logarithm of the activity of the primary ion were plotted and the intersection of the extrapolated linear portions of this graph indicates the value of $a_A^*$.

The $K_{A,B}^{pot}$ value is calculated using the following equation.

$$K_{A,B}^{pot} = \frac{a_A}{(a_B)^{z_A/z_B}}$$

$z_A$ and $z_B$ are charge numbers of the primary ion, A and of the interfering ion, B. The selectivity coefficient indicates the extent to which a foreign ion interferes with the response of an electrode to its primary ion.
2.10 Potential measurement and calibration.

The potential measurements were carried out at 25±1 °C on a Metrohm 781 ion meter. A saturated calomel electrode was used in conjunction with the developed sensors. The cell assembly for the potentiometric measurements can be represented as follows:

For PVC membrane sensor,

**Internal reference electrode | internal filling solution (1 × 10⁻³ M drug solution + 1 × 10⁻¹ M NaCl solution) | PVC membrane | test solution | external reference electrode.**

For carbon paste electrode (CPE),

**Reference electrode | test solution | Graphite electrode.**

The electrochemical response of the developed sensors was investigated by measuring the potential response of the test solutions. The solutions were stirred well and the stable potential readings were recorded.

2.11 Instruments used

The CHN analysis was done on a CHN analyzer, Elementar Vario EL III at Sophisticated Test and Instrumentation Centre (STIC), Kochi. Spectrophotometric measurements were carried out on a UV3500 Labomed Inc. spectrophotometer. All the potential measurements were carried out on a Metrohm 781 ion meter.