CHAPTER 2

EXPERIMENTAL

2.1 Instrument and apparatus

1. Potentiostat: AUTOLAB® model PGSTAT 20 voltammetric analyzer (Eco Chemie, Utrecht, Netherlands), equipped with a VA Stand 663 (Metrohm, Herisau, Switzerland).
2. Flow-through thin-layer electrochemical cell: CC5; Bioanalytical Systems Inc.(BAS), USA.
3. Fluoride-selective solid-state membrane electrode (Metrohm, Herisau, Switzerland).
4. Pump; Binary LC Pump 250, Perkin-Elmer, England.
5. LC-95 UV/Visible spectrometer, λ=210 nm, 0.1 AUFS, Perkin-Elmer, England.
6. HPLC columns; Nava-Pack C18 (4μm), 3.9x150 mm, (Waters) and Spherisorb ODS (5 μm), 4.6x150 mm (Phenomenex).
7. Peristaltic pump: A single-channels, MP-3, EYELA, Japan.
8. Rotary injection valve: six-port; Pye Unicam, England.
9. Magnetic stirrer and 1.5 inch magnetic bar, IKA-Werke GmbH, Germany.
10. pH-meter, Russell RL150, Russell Inc., Boston, U.S.A.
11. Analytical balance, AA-200DS, Denver instrument company, Colorado, U.S.A.
12. Micropipette, *Transferpipette*®, 10-100 μl, Merck, Germany.

2.2 Chemicals and plant materials

Most chemicals used were of analytical reagent grade (except when specified) and used without further purification. Deionized water was used throughout which was prepared by passing distilled water through a Milli-Q system (Millipore, USA).

2.2.1 Chemicals

1. Acetic acid, glacial: CH_3COOH, (M.W.=60.05) ; BDH, England.
2. L-Arginine : $C_6H_{14}N_4O_2$, (M.W.=174.2) ; Fluka, Switzerland.
3. Ascorbic acid : $C_6H_8O_6$, (M.W.=173.16) ; Carlo Erba, Italy.
4. Boric acid : H_3BO_3, (M.W.=61.83) ; BDH, England.
5. Cadmium nitrate : $Cd(NO_3)_2.4H_2O$, (M.W.=308.47) ; Fluka, Switzerland.
6. Calcium nitrate : $Ca(NO_3)_2.4H_2O$, (M.W.=236.15) ; Fluka, Switzerland.
7. Catechol : $C_6H_6O_2$, (M.W.=110.11) ; Merck, Germany.
8. Chloroform : $CHCl_3$, (M.W.=119.38) ; Merck, Germany.
9. Chromium nitrate : $Cr(NO_3)_3.9H_2O$, (M.W.=400.15) ; Merck, Germany.
10. Citric acid : $C_6H_8O_7.H_2O$, (M.W.=210.14) ; Merck, Germany.
11. Cobalt nitrate : $Co(NO_3)_2.6H_2O$, (M.W.=291.03) ; Fluka, Switzerland.
12. Copper nitrate : $Cu(NO_3)_2.3H_2O$, (M.W.=241.6) ; Fluka, Switzerland.
13. Ferric nitrate : $Fe(NO_3)_3.9H_2O$, (M.W.=404.0) ; J.T.Baker, USA.
14. Ferrocene : $C_{10}H_{10}Fe$, (M.W.=186.04) ; Fluka, Switzerland.
15. Ferrous sulphate : $FeSO_4.7H_2O$, (M.W.=278.02) ; Fluka, Switzerland.
16. Formic acid : HCOOH, (M.W.=46.03) ; BDH, England.

17. Fructose : $C_6H_{12}O_6$, (M.W.=180.16) ; Fluka, Switzerland.
18. Glucose : $C_6H_{12}O_6$, (M.W.=180.16) ; Fluka, Switzerland.
19. Glutamic acid : $C_5H_9NO_4$, (M.W.=147.13) ; Fluka, Switzerland.
20. Glycine : $C_2H_5NO_2$, (M.W.=75.07) ; Carlo Erba, Italy.
21. Glycolic acid : $C_2H_4O_3$, (M.W.=76.05) ; Fluka, Switzerland.
22. Graphite powder ; Fluka, Switzerland.
23. L-Histidine : $C_6H_9N_3O_2$, (M.W.=155.2) ; Sigma, USA.
24. Hydrogen peroxide 40%(w/w) : H_2O_2, (M.W.=34.02) ; Carlo Erba, Italy.
25. Lactic acid : $C_3H_6O_3$, (M.W.=90.08) ; Fluka, Switzerland.
26. Lead nitrate : $Pb(NO_3)_2$, (M.W.=331.2) ; J.T.Baker, USA.
27. Magnesium nitrate : $Mg(NO_3)_2.6H_2O$, (M.W.=256.41) ; Merck, Germany.
28. Maleic acid : $C_4H_4O_4$, (M.W.=116.08) ; Fluka, Switzerland.
29. Manganese nitrate : $Mn(NO_3)_2.4H_2O$, (M.W.=251.01) ; Fluka, Switzerland.
30. Malonic acid : $C_3H_4O_4$, (M.W.=104.06) ; pract., Koch-Light, England.
31. Mineral oil, Fluka, Switzerland.
32. Nickel nitrate : $Ni(NO_3)_2.6H_2O$, (M.W.=290.81) ; Carlo Erba, Italy.
33. Oxalic acid : $C_2H_2O_4.2H_2O$, (M.W.=126.07) ; Fluka, Switzerland.
34. Phenol : C_6H_6O, (M.W.=94.11) ; Merck, Germany.
35. Potassium permanganate : $KMnO_4$, (M.W.=158.04) ; BDH, England.
36. Salicylic acid : $C_7H_6O_3$, (M.W.=138.12) ; BDH, England.
37. Silver nitrate : $AgNO_3$, (M.W.=169.88) ; BDH, England.
38. Sodium chloride : NaCl, (M.W.=58.44), Carlo Erba, Italy.
39. Sodium cyanide : NaCN, (M.W.=49.01) ; Fluka, Switzerland.

40. Sodium dihydrogen phosphate : $NaH_2PO_4.2H_2O$, (M.W.=156.01); BDH, England.
41. Sodium fluoride : NaF, (M.W.=41.99) ; Carlo Erba, Italy.
42. Sodium hydroxide : NaOH, (M.W.=40.0) ; Carlo Erba, Italy.
43. Sodium nitrate : $NaNO_3$, (M.W.=84.99) ; Carlo Erba, Italy.
44. Sodium nitrite : $NaNO_2$, (M.W.=68.99) ; Carlo Erba, Italy.
45. Sodium oxalate : $Na_2C_2O_4$, (M.W.=134.0) ; Fluka, Switzerland.
46. Sodium sulfate : Na_2SO_4, (M.W.=142.04) ; Merck, Germany.
47. Sodium sulfite : Na_2SO_3, (M.W.=126.04) ; Merck, Germany.
48. Sodium thiosulphate : $Na_2S_2O_3.5H_2O$, (M.W.=248.18) ; Carlo Erba, Italy.
49. Stannous sulphate : $SnSO_4$, (M.W.=214.75) ; Fluka, Switzerland.
50. Starch : $(C_6H_{10}O_5)_n$; Carlo Erba, Italy.
51. Succinic acid : $C_4H_6O_4$, (M.W.=118.0) ; BDH, England.
52. Tartaric acid : $C_4H_6O_4$, (M.W.=150.09) ; BDH, England.
53. Tran-1,2-Diaminocyclohexane-N,N,N',N'-Tetraacetic acid or cyclohexanediamine tetraacetic acid (CDTA) :$C_{14}H_{22}N_2O_8.H_2O$, (M.W.=364.44) ; Sigma, USA.
54. L-Tyrosine : $C_9H_{11}NO_3$, (M.W.=181.19) ; Carlo Erba, Italy.
55. Uric acid : $C_8H_4O_3N_4$, (M.W.=168.11) ; Fluka, Switzerland.
56. Zinc nitrate : $Zn(NO_3)_2.6H_2O$, (M.W.=297.48) ; Fluka, Switzerland.

2.2.2 Plant materials

1. Asparagus (*Asparagus officinalis*) was obtained daily from the market of Marketing Organization for Farmers, Chatuchak, Bangkok and stored at 4°C until used. Size of asparagus was approximately 1.5-2.0 cm diameter and 20-25 cm long. (Figure 2.1 a)
2. Sunflower (*Helianthus annuus L.*) was grown in chamber environment for the experiment. Sunflower seeds were purchased from a local market. Sunflower leaves of 1-2 month ages were used immediately after harvesting. (Figure 2.1 b).

(a) (b)

Figure 2.1 Asparagus (a) and sunflower leaves (b)

2.3 Preparation of standard solutions and reagents

Deionized water was used for all solutions.

1) Stock standard H_2O_2 0.10 M

This solution was prepared by dissolving 1.066 g of 31.9% (w/w) H_2O_2 (previously standardized with standard solution $KMnO_4$ s [65]) in water and diluting to 100.0 ml with deionized water. Then stored at 4°C until use.

2) Stock standard fluoride 1000 mg l^{-1}

This solution was prepared by dissolving 0.221 g of NaF (previously dried at 110°C for 2 h. and cooled in a desiccator) in water, diluted to 100.0 ml and storing in a polyethylene bottle.

3) Stock standard glycolic acid 0.10 M

This solution was prepared by dissolving 0.760 g of glycolic acid in water, diluting to 100.0 ml and storing in a polyethylene bottle.

4) Sodium dihydrogen phosphate solution (pH 5.0), 0.05 M

This solution was prepared by dissolving 7.800 g of NaH_2PO_4 in water (900 ml). The pH of this solution was adjusted to pH 5.0 with 0.05 M NaOH and diluted to 1000 ml with water [66].

5) Sodium dihydrogen phosphate solution (pH 8.0), 0.05 M

This solution was prepared by dissolving 7.800 g of NaH_2PO_4 in water (900 ml). The pH of the solution was adjusted to pH 8.0 with 0.05 M NaOH and diluted to 1000 ml with water.

6) Total ionic strength adjustment buffer (TISAB)

This solution was prepared by mixing 57.5 ml of glacial acetic acid, 58.4 g of NaCl, 5.0 g of cyclohexanediamine tetraacetic acid (CDTA) and 500 ml deionized water in a 1000 ml beaker. The content was cooled to room temperature and adjusted to pH 5.4 with 6 M NaOH, diluted to 1000 ml with water and stored in polyethylene bottle.

7) Fluoride tablets sample

Fluoride tablets containing 0.25 and 1.0 mg/tablet (*Zymafluor*®, Novartis Consumer Health, Switzerland) were purchased from drug store.

The fluoride tablet samples selected at random were accurately weighed and calculated for an average tablet weight. The tablets were ground to fine powder, followed by passing through a 60 mesh sieve. The drug powder equivalent to an average weighed tablet was taken and accurately weighed. This fluoride drug powder was completely dispersed in a conical flask that contained deionized water by heating on a steam bath and shaking intermittently. After filtration, the solution (40 ml) was allowed to room temperature, transferred into a 50.0 ml volumetric flask and diluted to the mark with deionized water. The solution was stored in a plastic bottle.

8) Human urine samples

The 10 ml of 10 fold-diluted human urine sample from a volunteer (kept at 4°C) was mixed with 300 mg of activated charcoal and the charcoal was removed by centrifugation. The supernatant was then used for the glycolic acid assay.

2.4 Batch system

2.4.1 Component of the batch system (Figure 2.2)

1) Potentiostat : AUTOLAB® model PGSTAT 20 voltammetric analyzer.

2) VA Stand 663 Electrochemical cell (Figure 2.3)

- Reference electrode, Ag/AgCl (3M KCl)
- Auxiliary electrode, Platinum auxiliary electrode (Pt-wire, 2 mm x 65 mm)
- Working electrode, carbon paste electrode consisted of a paste matrix, electrode body was made from a glass tube with a surface area of 3.14 mm^2. The carbon paste was packed firmly into the end of the electrode body. The electrode connection to the carbon paste was established *via* a copper wire.

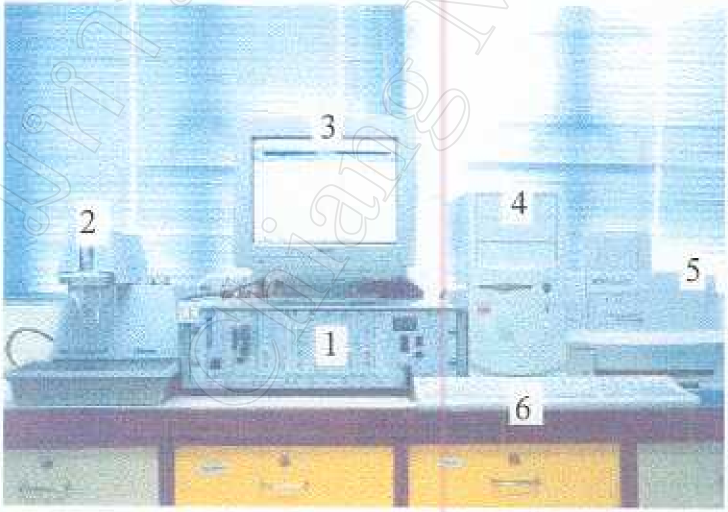

Figure 2.2 Experimental set-up of the batch system for fluoride determination

1) Potentiostate	2) Electrochemical cell
3) Monitor	4) Computer
5) Printer	6) Keyboard

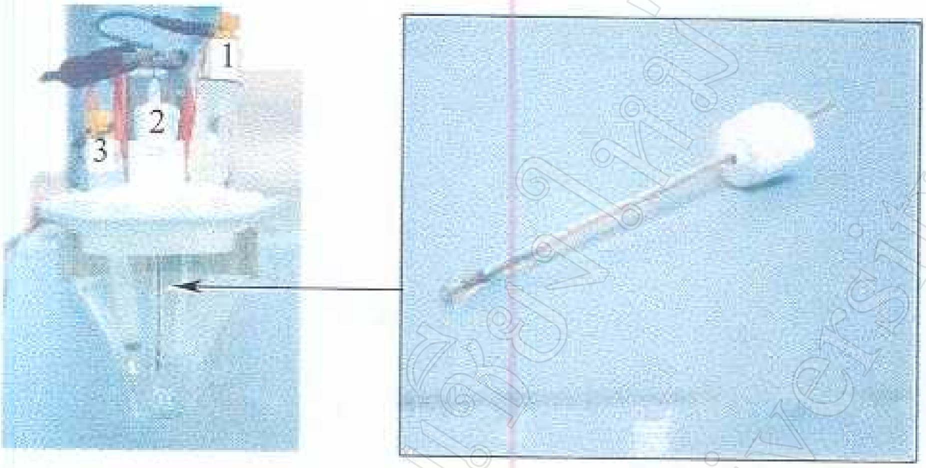

Figure 2.3 Electrochemical cell of the batch system

1) Reference electrode 2) Working electrode

3) Auxiliary electrode

2.4.2 Electrode construction of the batch system

The plant tissue modified carbon paste was prepared as described by Oungpipat *et al*. [30] with slight modification for fluoride determination. Initially, a 10.8% (w/w) ferrocene-modified graphite powder was prepared. This is done by mixing 0.892 g of graphite powder with 0.108 g of ferrocene dissolved in 10 ml of chloroform. The solvent was subsequently evaporated. Then, 0.600 g of this ferrocene-modified graphite powder was mixed with 0.400 g of mineral oil to yield ferrocene-modified carbon paste. This paste (0.930 g) was then thoroughly mixed with 0.070 g of ground tissue from the stem skin of asparagus stems. The resulting mixture which contained 6% ferrocene and 7% tissue was ultimately packed into the electrode body made from glass with a geometric surface area of 3.14 mm^2 and the surface was smoothed with a piece of paper. The electrode connection to the carbon paste was established via a copper wire. When not in use, the bioelectrode was stored in a refrigerator at 4°C.

2.5 FIA system

2.5.1 Components of the FIA system (Figure 2.4)

1) Potentiostat : AUTOLAB® model PGSTAT 20 voltammetric analyzer.

2) Flow-through thin-layer electrochemical cell: CC5 (Figure 2.5)

- Reference electrode, Ag/AgCl (3M KCl)
- Auxiliary electrode: A stainless steel electrode
- Working electrode: A dual carbon paste electrode in serial configuration with a jumper was built in-house but was similar in design to those available from BAS. The *Perspex* plastic body was 27 x 27 x 10 mm size with two circular cavities of the same size 2 mm in diameter and 2 mm deep each. A geometric surface area of 2 x 3.14 mm^2 and the surface was smoothed by mean of a piece of paper.

3) Peristaltic pump

4) Rotary injection valve

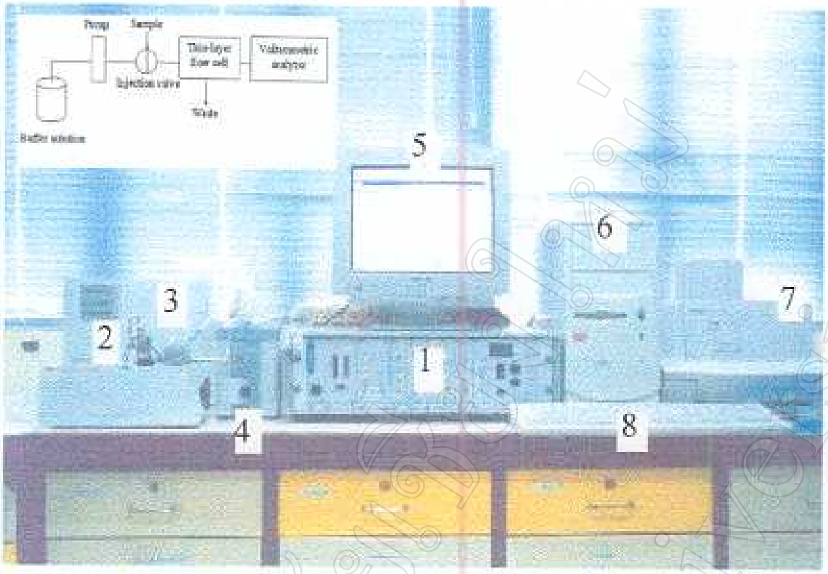

Figure 2.4 Experimental set-up of flow injection system for the glycolic acid determination

1) Potentiostat
2) Thin-layer flow cell
3) Injection valve
4) Pump
5) Monitor
6) Computer
7) Printer
8) Keyboard

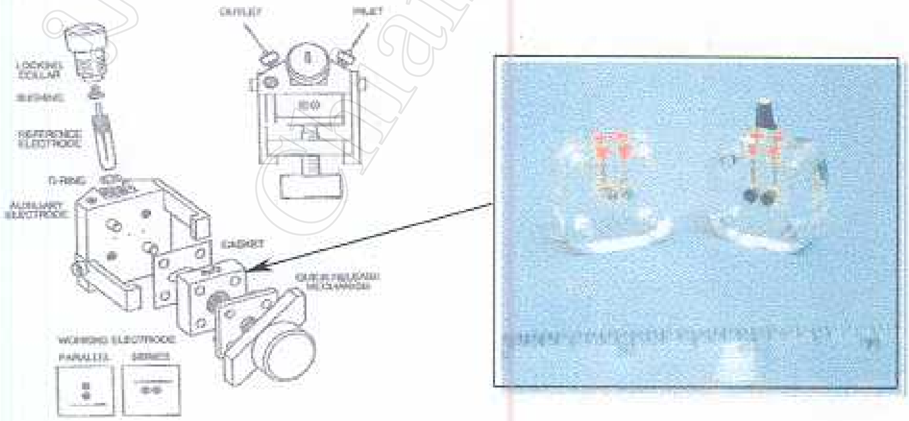

Figure 2.5 Flow-through thin-layer electrochemical cell with a home-made working electrode

2.5.2 Electrode construction of the FIA system

The plant tissue modified carbon paste was prepared as follows. A 10.5% (w/w) ferrocene-modified graphite powder was prepared. This was done by mixing 0.895 g of graphite powder with 0.105 g of ferrocene dissolved in 10 ml of chloroform. The solvent was subsequently evaporated. Then, 0.600 g of this ferrocene-modified graphite powder was mixed with 0.400 g of mineral oil to yield ferrocene-modified carbon paste. This paste (0.800 g) was then thoroughly mixed with 0.200 g of ground tissue of sunflower leaf. The resulting mixture which contained 5%(w/w) ferrocene and 20%(w/w) tissue was ultimately packed into the dual-series electrode body (27x27x10 mm size) made from *Perspex* plastic with two circular cavities of 2 mm in diameter and 2 mm deep each. A geometric surface area of 2 x 3.14 mm^2 and the surface was smoothed with a piece of paper. The signal from dual-series carbon paste was combined by a jumper. The electrode connection to the carbon paste was established *via* a copper wire. When not in use, the bioelectrode was stored in a refrigerator at 4°C.

2.6 BIA system

2.6.1 Component of the BIA system (Figure 2.6)

1) Potentiostat : AUTOLAB® model PGSTAT 20 voltammetric analyzer

2) A home-made BI electrochemical cell (Figure 2.7)

The cell made from a 1000 ml beaker. A hole, drilled at the bottom corner, was used to insert the inverted carbon paste electrode as a working electrode.

3) Ag/AgCl reference electrode (3M KCl)

4) Platinum auxiliary electrode (Pt-wire, 2 mm x 65 mm)

5) Working electrode consisted of a carbon paste electrode and a plastic jacket. The carbon paste electrode consisted of a paste matrix, electrode body was made from a glass tube with a surface area of 3.14 mm^2. The jacket was a PVC tube (6 x 8 mm, 4 mm i.d.) with four holes (2 mm diameter) on the jacket tube.

6) A home-made pipette stand (Figure 2.7)

The stand, made of *Perspex*, used for accommodating the Merck standard micropipette to ensure a reproducible and quick positioning.

7) Magnetic stirrer and a 1.5 inches magnetic bar

2.6.2 Electrode construction of the BIA system

Initially, a 10.0% (w/w) ferrocene-modified graphite powder was prepared. This is done by mixing 0.900 g of graphite powder with 0.100 g of ferrocene dissolved in 10 ml of chloroform. The solvent was subsequently evaporated. Then, 0.600 g of this ferrocene-modified graphite powder was mixed with 0.400 g of mineral oil to yield ferrocene-modified carbon paste. This paste (0.840 g) was then thoroughly mixed with 0.160 g of ground tissue of sunflower leaves. The resulting mixture which contained 5% ferrocene and 16% tissue was finally packed into the electrode body made from glass with a geometric surface area of 3.14 mm^2 and the surface was smoothed with a piece of paper. The electrode connection to the carbon paste was established *via* a copper wire. When not in use, the bioelectrode was stored in a refrigerator at 4°C.

Figure 2.6 Experimental set-up of the BIA system for glycolic acid determination

1) Potentiostat
2) BIA electrochemical cell
3) Micropipette and stand
4) Stirrer
5) Monitor
6) Computer
7) Printer
8) Keyboard

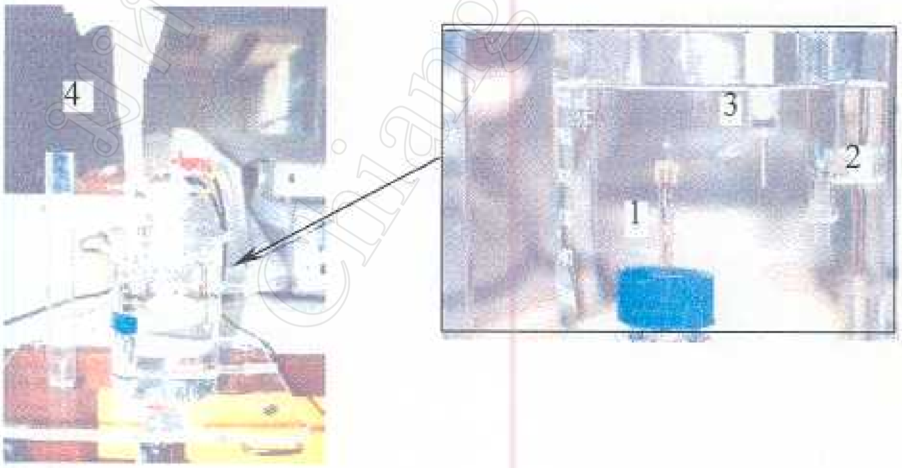

Figure 2.7 A home-made electrochemical cell of the BIA system

1) Working electrode
2) Reference electrode
3) Auxiliary electrode
4) A home made pipette stand and pipette

2.6.3 Construction of the BIA system

The BI electrochemical cell was shown in Figure 2.7. The cell, made of a 1000 ml-beaker, filled with 900 ml of blank solution. A hole, drilled at the bottom corner, was used to insert the inverted carbon paste electrode as a working electrode. The cell cover has three holes. The one located exactly opposite to the center of the working electrode which was used for accommodating the Merck standard micropipette. The pipette was held by an in-house pipette stand to ensure a reproducible and quick positioning (after each filling). Its tip was kept at a fixed distance, usually 1 mm from the center of the working electrode (this distance could be adjusted by moving the electrode up or down through a silicone adapter seal). To reduce the rapid loss of the analyte from the sensing area, a PVC tube (6 mm o.d., 4 mm i.d.) was used as a jacket. This jacket tube was 8 mm in length, 4 mm of which was fitted onto the working electrode. There were four holes (2 mm) on the jacket tube, 2 mm from the sensing surface of the working electrode. Two other holes at the cover were used to insert a platinum wire (2 x 65 mm) auxiliary electrode and Ag/AgCl reference electrode (3 M KCl) respectively. The electrodes were connected to an AUTOLAB® model PGSTAT 20 voltammetric analyzer (Eco Chemie, Utrecht, Netherlands) and monitored. The cell was placed on the magnetic stirrer.

2.7 Procedure

2.7.1 Asparagus based amperometric sensor for fluoride determination

2.7.1.1 Cyclic voltammetry

Cyclic voltammetry was performed in an unstirred 0.05 M NaH_2PO_4-NaOH buffer of pH 7.0. The test solutions were not deaerated. The voltammograms were recorded using a cyclic potential sweep between 1.00 and –1.00 V *versus* Ag/AgCl with an initial value of 0.00 V. The scan rate was 0.03 V s^{-1}.

2.7.1.2 Amperometric measurement

The amperometric measurements were performed either by single or successive injections of aliquots of stock fluoride solution. In both cases, the electrodes were immersed into an electrochemical glass cell containing 10.0 ml of NaH_2PO_4-NaOH buffer, pH 5.0 (unless otherwise mentioned). The test solutions were not de-aerated. After a constant potential of –0.05 V *versus* Ag/AgCl had been applied to the working electrode, the background current was allowed to decay to a steady-state. Subsequently, 0.1 mM of hydrogen peroxide was added into the measuring cell while the current-time data were recorded. When the signal approached the steady-state, aliquots of stock fluoride solutions were added into the cell. The net signal (ΔI) obtained by subtracting the background current from the sample current which was then used for subsequent interpretation of the data. Thus, the current labeled in all the plotting presented in this investigation refers to ΔI. The sensitivity is defined as the slope ($\Delta I/\Delta[F^-]$) of a plot of responses *versus* various fluoride concentration (unless otherwise specified). All experiments were performed at room temperature.

2.7.1.3 Optimization of experimental variables

The following parameters namely pH, applied potential, tissue loading, ferrocene loading and H_2O_2 concentration were studied. The experimental variables and their ranges studied are shown in Table 2.1.

Table 2.1 Experimental variables and their ranges studied of fluoride sensor in batch system

Experimental variables	**Range studied**
pH	4.0 - 8.0
Applied potential (V) (*versus* Ag/AgCl)	-0.2 to +0.1
Tissue loading (%, w/w)	3 - 11
Ferrocene loading (%, w/w)	0 - 8
H_2O_2 concentration (mM)	0.05 - 0.50

2.7.2 Sunflower based amperometric biosensors for glycolic acid determination incorporating with flow injection system

2.7.2.1 Cyclic voltammetry

Cyclic voltammetry was performed in an unstirred 0.05 M NaH_2PO_4-NaOH buffer of pH 8.0. The test solutions were not deaerated. The voltammograms were recorded using a cyclic potential sweep between 1.00 and –1.00 V *versus* Ag/AgCl with an initial value of 0.00 V. The scan rate was 0.03 V s^{-1}.

2.7.2.2 Amperometric measurement

Amperometric flow injection measurements were performed by successive injections of aliquots of stock glycolic acid solutions into the flow injection (FI) system. The electrode was conditioned in a solution of sodium dihydrogen phosphate (pH 8.0) pumped through the flow system with a constant flow rate of 0.3 ml min at 0.00 V(*vs*. Ag/AgCl) until a stable baseline was established. The sample was then injected into the FI system *via* an injection valve equipped with a fixed sample loop of 150 µl. The resulting peak was then recorded. The peak height (*ΔI*) was used for subsequent interpretation of the data. All experiments were performed at room temperature.

2.7.2.3 Optimization of experimental variables

The following parameters namely pH, applied potential, buffer system, tissue loading, ferrocene loading, carrier flow rate and injection volume were studied. The experimental variables and their range studied are shown in Table 2.2.

Table 2.2 Experimental variables and their ranges studied for glycolic acid biosensor in FIA system

Experimental variables	**Range studied**
Applied potential (V) (*vs*. Ag/AgCl)	-0.15 to +0.15
pH	5.0 - 10.0
Buffer system	NaH_2PO_4-NaOH, Na_2HPO_4-NaH_2PO_4, boric-Na_2HPO_4, tartaric-NaOH and tris-HCl
Tissue loading (%, w/w)	0 – 30
Ferrocene loading (%, w/w)	0 – 11
Carrier flow rate (ml min^{-1})	0.2 - 1.8
Injection volume (μl)	50 - 300

2.7.3 Sunflower based amperometric biosensors for glycolic acid determination incorporating with batch injection system

2.7.3.1 Cyclic voltammetry

As described earlier in section 2.7.2.1.

2.7.3.2 Amperometric measurement

The batch injection (BI) amperometric measurements were performed in BI electrochemical cell. After a constant potential of 0.00 V (*vs.* Ag/AgCl) had been applied to the bioelectrode, immersed in phosphate buffer solution (pH 8.0) with a stirring rate of 75 rpm. The background current was allowed to decay to a steady-state and a stable baseline was established. Subsequently, 30 μl of the analyte solution (sample or standard solution containing glycolic acid) was injected from micropipette tip with manual micropipette directly over the center of bioelectrode, while the resulting peak were recorded. The peak height (ΔI) was used for subsequent interpretation of the data. The sensitivity is defined as the slope ($\Delta I/\Delta[glycolic\ acid]$) of a plot of responses *versus* various glycolic acid concentration. All experiments were performed at room temperature.

2.7.3.3 Optimization of experimental variables

The following parameters namely applied potential, pH, buffer system, tissue loading, ferrocene loading, stirring rate, tip-electrode distances and injection volume were studied. The experimental variables and their ranges studied are shown in Table 2.3

Table 2.3 Experimental variables and their ranges studied for glycolic acid biosensor in BIA system

Experimental variables	**Range studied**
Applied potential (V *vs*. Ag/AgCl)	-0.15 to +0.15
pH	5.0 - 10.0
Buffer system	NaH_2PO_4-NaOH, Na_2HPO_4-NaH_2PO_4, boric-Na_2HPO_4, tartaric-NaOH and tris-HCl
Tissue loading (%, w/w)	0 - 24
Ferrocene loading (%, w/w)	0 - 9
Stirring rate (rpm)	50 – 300
Tip-electrode distances (mm)	1–5
Injection volume (μl)	10 – 50

2.8 Some of analytical characteristics of bioelectrode

1) Michaelis-Menten constant

The Kinetic parameters of bioelectrode, apparent Michaelis-Menten constant (K_m^{app}) and the maximum current density (I_{max}) can be determined from the electrochemical Eadie-Hofstee form of the Michaelis-Menten equation. [67, 68]

$$I = I_{max} - K_m^{app}\left(\frac{I}{C}\right) \qquad (2.1)$$

where I is the steady-state current, I_{max} is the maximum current measured under conditions of enzyme saturation and C is substrate concentration.

2) Sensitivity

Sensitivity is usually defined as the final steady state change in the magnitude of the biosensor output signal (ΔS) with respect to the change in the concentration of a specific chemical species (ΔC) [69].

$$\text{Sensitivity} = \frac{\Delta S}{\Delta C} \qquad (2.2)$$

Slopes of calibration curve was also reported to determine the sensitivity values [48].

2) Detection limit

The most well known one defines the detection limit as that concentration (or sample amount) for which the signal-to-noise ratio is given by $S / N = 3$. Evidently, detection limit is dependent only on baseline noise (N) and the signal (S)[70].

4) Precision

Precision describes the reproducibility of the results from replicate measurements using the same method. Generally, this characteristic is expressed as the relative standard deviation (RSD) and is given by equation 2.3 [71].

$$\%\text{RSD} = \frac{\text{Standard deviation}}{\text{Mean of replicate measurement}} \times 100 \qquad (2.3)$$

5) Response time

The response time (t_{90}) is the time taken to reach 90% of the response. The response time of a biosensor is normally the time taken for it to reach a steady state where it is no longer possible to detect any variation in the signal [7]. It is an important factor in bioelectrode assays, especially when the bioelectrode was expected to be used in continuous analysis.