CHAPTER 4

CONCLUSION AND SUGGESTION FOR FURTHER WORK

4.1 Conclusion

Plant tissue-based bioelectrode obtained by incorporating asparagus tissue and sunflower leaf tissue for the determination of fluoride and glycolic acid, respectively using batch system, FIA and BIA system have been obtained.

4.1.1 Asparagus based amperometric sensor for fluoride determination

A plant tissue-based bioelectrode based on the amperometric determination of the inhibitory effect of fluoride on the asparagus peroxidase activity for the determination of fluoride concentrations was developed by using batch measurements (Figure 2.2-2.3). The experimental variables had been studied. It was found that the optimum experimental variables were 0.05 M NaH_2PO_4-NaOH buffer (pH 5.0) containing 0.1 mM H_2O_2 at –0.05 V (*versus* Ag/AgCl) and the bioelectrode compositions were 7% (w/w) of asparagus tissue and 6% (w/w) of ferrocene. The resulting bioelectrode characteristics shown that the bioelectrode exhibits linear response up to a fluoride concentration of 14.0 mg l^{-1} with the regression equation of the calibration curve of $y = 8.2x10^{-3}x$ (r = 0.997) and a detection limit ($S/N=3$) of 0.5 mg l^{-1}. A response time (t_{90}) of 1 min and a relative standard deviation (R.S.D.) of 2.1% ($n=15$) were achieved. This bioelectrode was tested by assaying fluoride in tablet formulation and the result were compared favorably with potentiometric measurement (Table 3.16-3.17). Moreover, fluoride exhibits competitive inhibition to peroxidase. In fact, the inhibition mechanism is reversible. The results obtained confirm the feasibility of utilizing peroxidase from asparagus tissue for the monitoring of such an inhibitor. The results of interference studied are listed in Table 3.13. Of these possible interference studied, only cyanide, sulphite, ascorbic acid, catechol (each 5 mg l^{-1}) interfered to a significant extent. The use of plant tissue as a recognition element offers the advantages of high activity and stability, self-supported rigidity, extreme simplicity and low cost. These inherent advantages ensure good analytical characteristics and performance of the proposed bioelectrode. This concept could be extended to other sources of enzyme and to other inhibitors.

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

Sunflower leaf has been used as a source of peroxidase and glycolate oxidase for the construction of a new plant tissue-based bioelectrode in FIA system (Figure 2.4-2.5) for the determination of glycolic acid. The optimum of experimental variables of flow-injection amperometric measurements had been investigated. The optimum experimental variables were injection volume, 150 µl; 0.05 M of phosphate buffer carrier; pH, 8.0; flow rate, 0.3 ml min^{-1}. The bioelectrode consisted of 20% (w/w) of sunflower leaf tissue and 5% (w/w) of ferrocene at 0.00 V (*vs.* Ag/AgCl). The linear range of the bioelectrode response was up to $2x10^{-3}$ M with the linear regression equation $y = 1.92x10^{5}\,x$ ($r = 0.995$) and a detection limit ($S/N=3$) of $1x10^{-5}$ M. The sampling rate of 30 samples h^{-1} and a relative standard deviation of 1.67% ($n=15$) were achieved. The simple design and low cost of the bioelectrode construction are further distinctive features of the proposed electrode. It is typical for amperometric sensors based on mixed tissue carbon paste electrode to have high sensitivity and rapid response. Moreover, the use of a low operating potential together with the specific enzymatic reaction resulted in minimal interference effects.

Pretreatment of sample containing ascorbic acid such as human urine samples with activated charcoal is required to achieve bioelectrode accuracy.

4.1.3 Sunflower based amperometric biosensors for glycolic acid determination in batch injection system

A home-made batch injection (BI) electrochemical cell, micropipette stand and a bioelectrode for BIA system with simple designs and low cost had been constructed for the determination of glycolic acid (Figure 2.6-2.7). The batch injection amperometric measurements were performed by injection of a 30 μl of analyte solution from a micropipette tip directly over the center of the bioelectrode, immersed in 0.05 M of phosphate buffer solution (pH 8.0). The bioelectrode consisted of 16% (w/w) of sunflower leaf tissue and 5% (w/w) of ferrocene at 0.00 V (*vs*. Ag/AgCl). The linear response of the bioelectrode was up to a glycolic acid concentration of $8x10^{-4}$ M with a linear regression equation $y = 3.7x10^{5} x$ ($r = 0.997$) and a detection limit ($S/N=3$) of $1x10^{-5}$ mM. The sampling rate of 100 samples h^{-1} and a relative standard deviation (R.S.D.) of 2.4% ($n=15$) were achieved. The bioelectrode response decreased to 70% of the original value within 180 continuous injections. It offers advantages, which include speed of measurement, high activity, high stability, ease of preparation and low cost.

4.2 Suggestion for further work

With the respect to the interference studied of asparagus tissue-based amperometric sensor for fluoride determination (section 3.1), it was found that sulphite interfered to a significant extent. It indicated that sulphite is one of the inhibitors of peroxidase [74-76]. This concept could be extended to the determintion of sulphite concentration based on amperometric determination of the inhibitory effect of sulphite on the asparagus peroxidase activity or the other source of enzyme and/or to other inhibitors. The use of plant tissue as a recognition element offers the advantages of high activity and stability, self-supported rigidity, extreme simplicity and low cost. These inherent advantages ensure good analytical characteristics and performance of the proposed bioelectrode.

In section 3.2, a ferrocene-modified carbon paste bioelectrode based on sunflower leaf tissue has been developed. The proposed plant tissue bioelectrode was successfully used in conjunction with the home-made flow injection (FI) system with amperometric detection (the working electrode was build in-house but is similar in design to those available from BAS). It was found that the simple design and low cost of a home-made flow-through thin-layer electrochemical cell construction for an amperometric detection with flow injection system is further distinctive features of the proposed electrode. It can be constructed by means of *Perspex* plastics and comprising a Ag/AgCl reference electrode, Pt-wire auxiliary electrode and a carbon paste working electrode. This could lead to the development of low cost and efficient instrumentation in Thailand which could be used instead of the expensive ones.

In section 3.3, this work has seen successfully coupled with a novel plant tissues-based bioelectrode to the BIA system for determining glycolic acid. BIA can offer similar advantages to those of to those of FIA without any problems associated with valves, tubing, detector flow cell and pumps. So, the BIA system can be used incorporating with not only the voltammetric detector [58, 59], ion-selective electrode [60, 61], thermal sensor [62, 63] and optical sensor [64] but also can be used inconjunction with any plant tissue-based amperometric detector. The simple design and low cost of the BIA system with the simple bioelectrode construction are further distinctive features of the novel electrode. So, which can be applied to the determination of a wide range of real samples.