CHAPTER 1

INTRODUCTION

There are numerous instrumental methods in analytical chemistry available for use in determining analytes of interest. These include spectrophotometic, electrochemical, chromatographic and titration methods. Of these, electrochemical method provides a high sensitivity and a rapid response. Potentiometry, voltammetry and amperometry are the common electrochemical methods. The amperometry is based on the measurement of the current crossing an electrochemical cell under an imposed potential. This current is a function of the concentration of the electrochemically active species in the sample. A working electrode generally performs oxidation or reduction of a species, and a second electrode acts as a reference electrode. During electrolysis, the working electrode may act as an anode or a cathode, according to the nature of the analyte. The working electrode may therefore be considered to be the most important component in these methods. As a consequence, much effort has been devoted to electrode fabrication.

1.1 Biosensors

1.1.1 History

In 1962, Clark and Lyons [1] first demonstrated the possibility of using enzyme-containing membranes to transform urea or glucose into a product that was detectable with pH or oxygen electrode. Later, in 1967, Updike and Hicks [2] prepared an enzyme electrode by polymerizing a gel containing glucose oxidase onto an oxygen electrode. When this electrode is

placed in contact with a biological solution containing glucose and oxygen, two compounds diffuse into the enzymatic membranes. The electrode then oxidizes the glucose into gluconic acid using the oxygen in the solution. The oxygen electrode measures the reduction in oxygen partial pressure, which is directly related to the glucose concentration. This discovery was a decisive step in biological analysis. In recent years, whole cell-based, including microbial biosensors, plant tissue-based biosensors have attracted considerable attention as the potential successors to a wide range of analytical techniques. This can be obviously seen by the increasing numbers of publications and monographs.

Rechnitz *et al.* [3] first reported the use of bacterial cells for the determination of L-arginine. The use of immobilized microorganism as biocatalysts in sensors offers advantages including low cost, prolonged electrode lifetime, regeneration of activity and the ability to effect complex reaction. This simplifies the analytical system and provides for induction of enzyme activity. However, such biocatalysts suffer disadvantages with respect to isolated enzymes in terms of lower selectivity, mechanical handling and sterility requirements. These shortcomings were the original reasons for investigating the use of tissue slice from animals and plants for the biosensor construction.

Animal tissues have been successfully employed for the development of novel biosensors since the first discovery of beef liver tissue and urease enzyme was isolated for the construction of an arginine bioelectrode by Rechnitz [4]. The use of animal tissue sliced as biocatalysts not only retains all the advantages offered by microorganisms but also adds other benefits of structural integrity and mechanical ruggedness. The comparison of enzyme, mitochondrial, bacterial and tissue electrodes for glutamine determination performed by Arnold and Rechnitz [5] showed that tissue electrode was superior in terms of life and electrode lifetime.

1.1.2 Definition

Biosensor is a device, which uses immobilized biomolecules combined with transducer to detect or respond to specific interaction with environmental chemicals. In order to achieve optimal signal transfer, the immobilized biocomponent is in close physical contact with the transducer unit [6].

1.1.3 Principle of biosensors [7, 8]

A biosensor can be considered as a combination of a bioreceptor, a biological component, and a transducer (the detection method). The total effect of a biosensor is to transform a biological event into an electrical signal. Figure 1.1 represents the principle of the operation of a biosensor which starting from the analyte, can provide all the information needed for its evaluation. This information can be processed and stored for later use.

1.1.3.1 Bioreceptor

Bioreceptor, a biological recognition element is the first component of a biosensor. Bioreceptor has a particularly selective site that identifies the analyte. The bioreceptor ensures molecular recognition, may transform the analyte in some way. This localized modification is generally made via an immobilized enzyme, which transforms the analyte into a product that is detectable by the transducer. This is the case for enzyme sensors. However, the enzyme is only stable in its natural environment, which cannot be modified, and the whole cell or microorganism is immobilized on the biosensor.

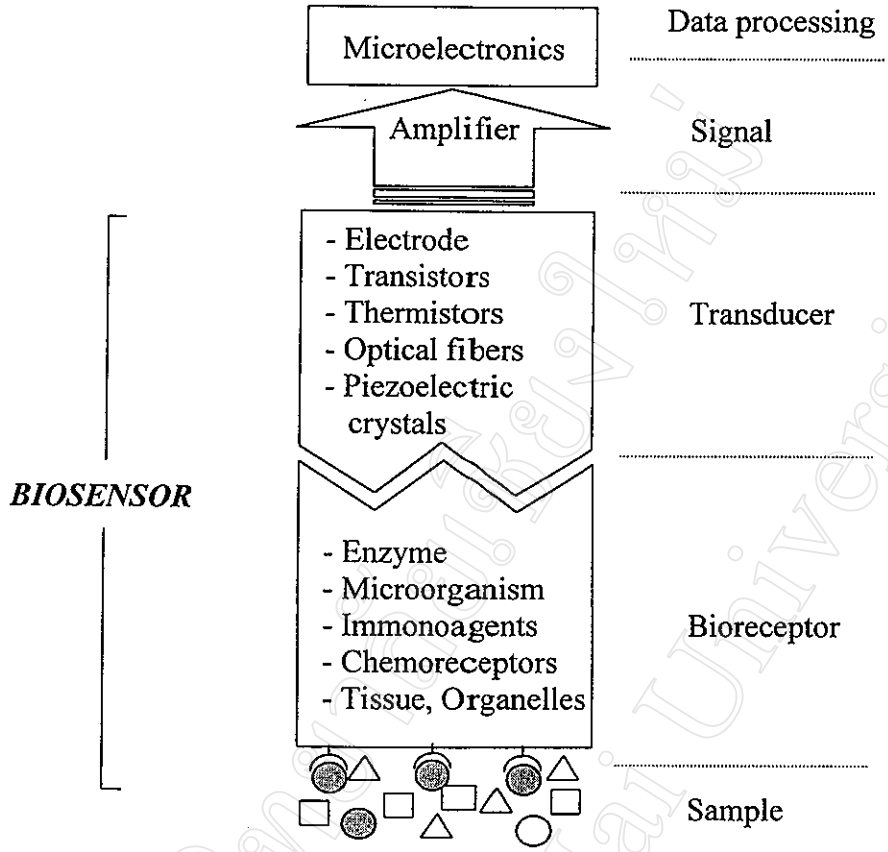

Figure 1.1 The various stages of determination with a biosensors [7]

The bioreceptor plays the role of molecular recognition device. In the presence of the substance under investigation, it must produce a physicochemical effect that is detectable by the transducer. The commonly used bioreceptor are:

1) Enzymes
2) Microorganisms
3) Plant and Animal tissues
4) Immuno agents
5) Chemoreceptors

The advantages and disadvantages of each bioreceptor are shown in Table 1.1.

Table 1.1 Bioreceptors for biosensors

Bioreceptors	Advantages	Disadvantages
Purified enzyme	High selectivity and activity	Expensive, limited availability and stability
Microorganism	Great variety, self-contained regeneration may be possible	Poor selectivity, limited lifetime
Plant and animal-tissue	High activity, natural configuration and inexpensiveness	Subject to interference and contamination
Immuno agent	Good selectivity	High cost, limited availability and non-catalytic behavior
Specialized organ or Structural element	High sensitivity	Limited lifetime, fragility, difficult to store, limited selectivity

1.1.3.2 Transducer

Transducer is used to convert the biological signal into an electrical signal. The choice of transducer depends on the type of biochemical modification. The range of transducers suitable for biosensor construction is summarized in Table 1.2.

Table 1.2 Examples of transducer types

Transduction technique	Example
Electrochemical	
- Potentiometry	Ion-selective electrodes, gas selective electrodes , modified metal electrode ,field effect transistors
- Amperometry	Oxygen electrodes
- Conductance	Conductometer
Optical	Fiber optic combined with photodiode detection of absorption or fluorescence, sensitized semiconductor
Calorimetry	Thermistor
Surface mass change	Piezoelectric crystal resonance

1.2 Plant tissue-based biosensors

1.2.1 History and principle

The specialized structures of plants have been used as alternative biocatalysts since it was found that the flowers, leaves, roots, seeds, and structures related to plant growth, reproduction and nutrient storage have high concentration of biocatalytic activity. The first-used of plant tissue from yellow squash for the construction of a glutamate bioelectrode was reported by Kuriyama and Rechnitz [9]. The highlight of the history of tissue-based biosensors are shown in Table 1.3

Table 1.3 Highlights of the history of plant tissue-based biosensors

Year	Authors	Events
1981	Kuriyama and Rechnitz [9]	The first of plant tissue from yellow squash for the construction of a glutamate bioelectrode was reported.
1984	Smith and Rechnitz [10]	The first use of leaves from cucumber for the construction of plant tissue-based electrode for cysteine analysis was reported.
1984	Schubert and Renneberg [11]	The first hybrid plant tissue-enzyme biosensor was reported.
1985	Sidwell and Rechnitz [12]	The first fruit (banana) tissue-based electrode for dopamine analysis was constructed.
1987	Uchiyama and Rechnitz [13]	The first fruit (chryanthenum & carnation) based electrode was developed.
1988	Wang and Brennsteiner [14]	The first mixed tissue-carbon paste biosensor was reported.
1989	Wang and Lin [15]	The plant tissue-based biosensors was used in a flow injection system for the first time
1990	Connor *et al.* [16]	A tissue-based electrode was used to eliminate interference from co-existing electroactive species.
1991	Wang and Naser [17]	The reticulated vitreous carbon-plant tissue composite bioelectrode was constructed
1991	Chen *et al.*[18]	The mediator was mixed with the tissue modified carbon paste for the first time.
1991	Wang *et al.*[19]	A plant-tissue based biosensor was used to eliminate protein interference.

Table 1.3 (continued)

Year	Authors	Events
1992	Sato *et al.* [20]	The first seed-based biosensor was constructed.
1992	Lin *et al.* [21]	Multi-enzyme activity in oat seeds was used for the first time in constructing a plant tissue-based biosensor for the determination of purinecine.
1992	Navaratne and Rechnitz [22]	A tissue culture was used for the first time for the construction of plant tissue-based biosensor.
1994	Botre *et al.*[23]	Plant tissue-based biosensor was used for the determination of fluoride by its inhibition effect on enzyme activities.
1995	He [24]	A plant tissue-based fiber-optic pyruvate sensor was used for the first time.
2000	Qin *et al.* [25]	A plant tissue-based chemiluminescence flow biosensor was used for the first time for the determination of urea.

1.2.2 Source of biocatalysts

Plants naturally contain a variety of enzymes in their physical structures. Recent research has demonstrated that the growing portions of plants (*e.g.* young leave, root, tips, blossom) or their nutrient storage structures (*e.g.* seeds, fruits, vegetable) are eminently suited for use as biocatalyst in biosensor construction. Table 1.4 summarizes examples of plants that have been used successfully in the construction of tissue based biosensors. Most of the sensors have been based on tyrosinase, polyphenol oxidase, peroxidase and catalase. This may be due to the fact that those enzymes occurs naturally in a variety of plants in high concentrations. The multi-enzymatic systems available in plants provide an interesting approach for use in the construction of a tissue-based biosensor for a suitable substrate. This avoids the complicated steps of immobilizing multienzyme onto a single transducer as in multi-enzyme sensor construction. Tissue culture technology can be applied to prepare a well defined source of plant tissue material with high enzymatic activity. This results in a reproducible sensor response and long life time in addition to other improved analytical parameters.

Table 1.4 Source of biocatalysts

Source of biocatalysts	Enzyme	Substrate	Ref.
apple (powder)	polyphenol oxidase	dopamine	26
apple (fresh and dry)	polyphenol oxidase	catechol	27
apple	polyphenol oxidase	flavanol	28
artichoke	peroxidase	hydrogen peroxide	29
asparagus	peroxidase	hydrogen peroxide	30
banana	tyrosinase	phenol, p-chlorophenol	31
banana	polyphenol oxidase	catechol	27

Table 1.4 (continued)

Source of biocatalysts	Enzyme	Substrate	Ref.
banana	oxalate oxidase	dopamine	32
banana	oxalate oxidase	oxalate	33
banana	polyphenol oxidase	dopamine	16
banana (powder)	polyphenol oxidase	dopamine	26
coconut	polyphenol oxidase	catechol	34
corn kernel tissue	pyruvate decarboxylase	pyruvate	24
cucumber	L-ascorbate oxidase	L-ascorbic acid	35
cucumber juice	ascorbate oxidase	L-ascorbic acid	36
cultured tobacco callus tissue	peroxidase	hydrogen peroxide	22
egg plant	polyphenol oxidase	catechol	37
grape tissue	catalase	hydrogen peroxide	38
horseradish	peroxidase	hydrogen peroxide	29, 17
horseradish	peroxidase	2-butanone peroxide	31
Latania sp. fruits	polyphenol oxidase	catechnol and dopamine	39
manioc	peroxidase	hydrogen peroxide	29
mushroom	tyrosinase	p-cresol	16
mushroom	polyphenol oxidase	dopamine	26
mushroom	tyrosinase	dopamine	17
mushroom	tyrosinase	phenol, p-chlorophenol	31
orange peel tissue	pectinesterase	pectin	40
papaya	papain protease	protein	19
pea seedling	diamine oxidase	speramine	41
peach	peroxidase	hydrogen peroxide	29
potato	acid phosphatase	glucose-6-phosphate	11

Table 1.4 (continued)

Source of biocatalysts	Enzyme	Substrate	Ref.
potato	polyphenol oxidase	catechol	27, 42
potato	polyphenol oxidase	dopamine	26
potato	tyrosinase	dopamine	17
soybean	urease	urea	25
spinach leaf	catechol oxidase	catechol	43
spinach leave	glycolate oxidase and peroxidase	glycolic acid, hydrogen peroxide	44
sweet potato, turnip, yam	peroxidase	hydrogen peroxide	29
yellow squash	glutamate decarboxylase	glutamate	45
zucchini	ascorbate oxidase	ascorbic acid	46
zucchini	peroxidase	hydrogen peroxide	29

1.2.3 Application of plant tissue-based bioelectrodes

Recently, the development of biosensors by the using novel biological materials as biocatalysts has received considerable attention for replacing isolated enzymes. These sensors offer several advantages over their enzymatic counterparts including higher biocatalytic activity, improved stability and low cost. Moreover, the plant tissue-based bioelectrodes are highly selective devices, which rely on the specific binding of the target analyte. The examples of such biosensors are shown in Table 1.5.

Table 1.5 A brief review of plant tissue-based bioelectrodes

Source of biocatalysts	Analytes	Method	Linear range, Detection limit	Ref.
apple	dopamine	amperometry	- 4-79.9 μM - Not presented	26
apple	catechol	amperometry	- 2-10 μM - $1.2x10^{-5}$ M	27
apple	flavanol	amperometry	- up to 0.3 mM - Not presented	28
asparagus	hydrogen peroxide	amperometry	- up to $6x10^{-5}$ M - $4x10^{-7}$ M	30
banana	catechol	amperometry	- 2-8 μM - Not presented	27
banana	phenolic and peroxide species	amperometry	- up to 5 mM - $3x10^{-5}$ M	31
banana	dopamine	amperometry	- up to $2x10^{-3}$ M - $6x10^{-4}$ M	16
banana tissue powder	dopamine	amperometry	- 4-40 μM - Not presented	26
banana skins	oxalate	potentiometry	- $1x10^{-4}$-$2x10^{-3}$ M - $2x10^{-5}$ M	33
		amperometry	- $2x10^{-5}$-$3x10^{-4}$ M - $2x10^{-5}$	
cultured tobacco callus tissue	hydrogen peroxide	amperometry	- $5x10^{-6}$-$1.1x10^{-4}$ M - $7.5x10^{-7}$ M	22
corn kernel tissue	pyruvate	fluorimetry	- $5x10^{-5}$-$1.5x10^{-3}$ M - $5x10^{-5}$ M	24
cucumber juice	ascorbic acid	amperometry	- $2.5x10^{-4}$-$1.6x10^{-3}$ M. - Not presented	36
egg plant	catechol	amperometry	- $5x10^{-6}$-$4.5x10^{-5}$ M - $1x10^{-6}$ M	37

Table 1.5 (continued)

Source of biocatalysts	Analytes	Method	Linear range, Detection limit	Ref.
grape tissue	hydrogen peroxide	amperometry	- $1x10^{-5}$-$5x10^{-4}$ M - Not presented	38
mushroom	dopamine	amperometry	- 4-79.9 μM - Not presented	26
mushroom	*p*-cresol	amperometry	- $5x10^{-4}$-$5x10^{-6}$ M - $1x10^{-4}$ M	16
mushroom	phenolic and peroxide species	amperometry	- up to 4 mM - $3x10^{-5}$ M	31
orange peel tissue	pectin	amperometry	- 0.1-0.9 g l^{-1} - Not presented	40
pea seedling	speramidine	amperometry	- 0.5-4 μM - 7.1 nM	41
potato	catechol	amperometry	- 2-15 μM - Not presented	27
potato	inorganic phosphate	amperometry	- up to $1.5x10^{-3}$ M - $2.5x10^{-5}$ M	11
	fluoride (inhibitor)	amperometry	- Not presented - $1x10^{-4}$ M	
potato	catechol	amperometry	- $2.5x10^{-5}$-$2.3x10^{-4}$ M - $1x10^{-5}$ M	42
potato tissue powder	dopamine	amperometry	- 4-40 μM - Not presented	26
spinach leaf	catechol	amperometry	- $2x10^{-5}$ – $8x10^{-4}$ M - $1x10^{-5}$ M	43
spinach leave	glycolic acid	amperometry	- up to $5x10^{-4}$ M - $1x10^{-6}$ M	44
yellow squash	L-glutamate	potentiometry	- $2x10^{-4}$-$1.3x10^{-2}$ M - $2x10^{-4}$ M	45

1.3 Flow injection analysis (FIA)

In 1957, Skeggs [47] proposed the principles of continuous flow analysis (CFA) based on segmentation of the sample by gas bubbles (air). Independently, in the mid 1970s, two groups of researcher, Ruzicka and Hansen in Denmark [48] and Stewart and co-worker [49] in the United State, proposed the possibility of unsegmented continuous flow analysis. This was based on injection of discrete microvolumes of sample into a continuously moving, laminar flow liquid carrier, giving rise to the formation of a controllable concentration gradient for the sample components in the flow stream. Ruzicka named this type of the technique as "flow injection analysis". In the first edition of their well-known published monograph, "flow injection". Ruzicka and Hansen [50] defined FIA as "A method based on the injection of a liquid sample into a moving, non-segmented continuous carrier stream of a suitable liquid". The injected sample forms a zone, which is then transported towards a detector that continuously records the absorbance, electrode potential, or other physical parameters as it continuously changes due to the passage of the sample material through the flow cell.

1.3.1 Theory and principle of FIA [51, 52]

FIA is based on a combination of three principles including

1) Reproducible sample injection

2) Controlled precise dispersion of the injected sample zone

3) Reproducible timing of the movement of the dispersed sample zone through the manifold system to the detection device

Dispersion was defined as the amount that the chemical signal is reduced by injecting the sample plug into a system. This is represented mathematically by :

$$D = C^o / C^{max} \qquad (1.1)$$

Where D is the dispersion coefficient at the peak maximum produced by the ratio between C^o, the sample concentration before and C^{max}, the sample concentration after transfer from the injection valve to the detector flow-cell [53]. Since the concentration of sample is directly proportional to the detector signal; peak height, the dispersion coefficient can be defined as the ratio between the signals produced before and after the sample disperses. Therefore, the previous concentration ratio becomes a height ratio, $D = h^o / h$ In order to study the factors influencing dispersion, the results are normally obtained by univariate method which monitor changes in, not only the peak height, but also its width, the residence time and the start-up time.

The FI experimental parameters or factors, which may influence dispersion including injection volume, carrier flow-rate, travelling distance (between the injection and detector), halting the flow (in order to increase the reaction yield and sensitivity and to determine the reaction rate), reaction rate, manifold components (geometrical dimensions and configurations), viscosity of the fluids and temperature. Under normal conditions, the last two factors have very limited effect on the dispersion, and, in most cases, may be neglected [54].

1.3.2 The basic components of a FIA system [55]

The basic components of a FIA system are shown in Figure 1.2. It includes the following essential parts:

1.3.2.1 Propelling system

Various mechanisms such as gravity-based, gas pressure-based, peristaltic pumps, high-pressure pump and an alternative type pump, electro-osmotic flow have been used to propel fluids through the FIA system. In FIA, the peristaltic pumps are the most frequently used types of propulsion systems.

1.3.2.2 Injection system

This system is intended to insert reproducible and accurate sample volumes into a carrier solution without altering its flow-rate. Syringe injection and rotary valve injection are commonly used in fixed volume method.

1.3.2.3 Sample transport and reaction system

This part is intended both to carry the flowing stream along the manifold and to interconnect the various parts making up the working system by means of tubing, connectors and reactors. The extent of mixing and dispersion is governed by the length and diameters of tubing and type of reactors.

1.3.2.4 Detection system

Optical detectors including UV-VIS, atomic absorption spectrophotometer, fluorescence and chemiluminescence detectors, infrared and near infrared spectrometers are the most commonly used in FIA system. Other detectors include electrochemical and radiometric.

1.3.2.5 Data acquisition and processing unit

A chart recorder and microprocessor or computer can be used to record the data output from the detection system.

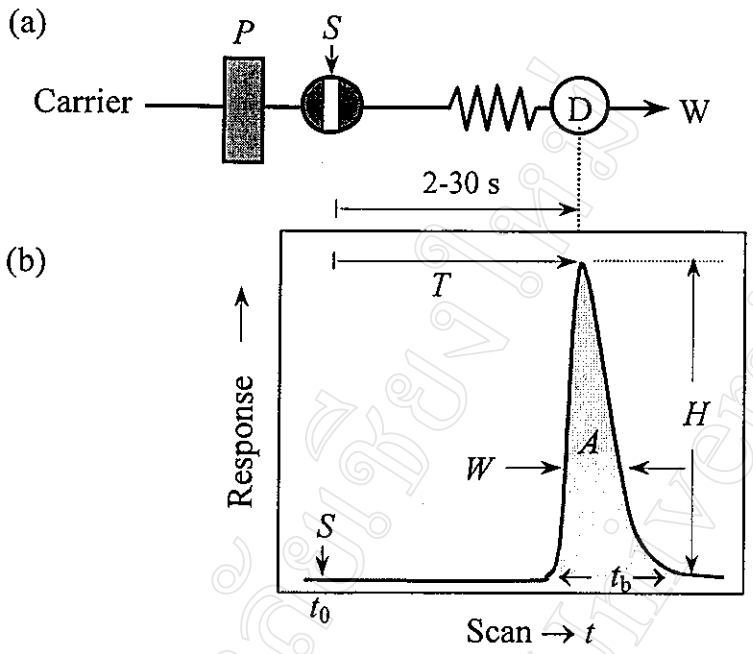

Figure 1.2 A single-line FIA manifold

(a) The simplest single-line FIA manifold utilizing a carrier stream of reagent, *P* is the pump, *S* is the injection port, *D* is the detector flow cell, and *W* is the waste

(b) The analog output has the form of a peak, the recording starting at *S* (time of injection t_o). *H* is the peak height, *W* is the peak width at a selected level, and *A* is the peak area, *T* is the residence time corresponding to the peak height measurement, and t_b is the peak width at the baseline [5 1].

1.3.3 Applications of plant tissue-base biosensors in FIA system

The examples of such plant tissue-based biosensors in a FIA system are shown in Table 1.6.

Table 1.6 A brief review of plant tissue-based biosensors in a FIA system

Source of biocatalysts	Analyte	Method	Linear range Detection limit	Ref.
banana	dopamine	amperometry	- up to $2.5x10^{-4}$ M - $1.9x10^{-6}$ M	32
cucumber	ascorbic acid	amperometry	- $5x10^{-4}$-$7x10^{-3}$ M - $1x10^{-4}$ M	35
coconut	catechol	amperometry	- $5x10^{-6}$-$8x10^{-4}$ M - $2x10^{-6}$ M	34
mushroom, potato and horseradish	dopamine	amperometry	- 0.2-1 mM - $1x10^{-5}$ M	17
zucchini, peach, yam, manioc, artichoke, sweet potato, turnip, horseradish	H_2O_2	spectrophotometry	- $1.6x10^{-5}$-$6.6x10^{-4}$ M - $2.1x10^{-6}$ M	29
Latania sp. fruits	catechol	amperometry	- $1x10^{-4}$-$1x10^{-3}$ M - $2.1x10^{-6}$ M	39
	dopamine	amperometry	- $5x10^{-6}$-$7x10^{-5}$ M - Not presented	
soybean	urea	chemiluminescence	- 4-400 μM - 2 μM	25

1.4 Batch injection analysis (BIA)

1.4.1 Principle of BIA

In 1991 Wang and Taha [56] introduced a new, non-flow injection-based technique, termed batch injection analysis (BIA). BIA is a non-flow technique involving the injection of a small amount of sample (less than 100 μl) from a micropipette tip directly onto the center of a flat sensor surface, immersed in a large-volume blank solution (stirred solution), and monitoring of the sharp transient response produced by the arrival, passage and dispersion of the sample zone over the sensor surface. The magnitude of the peak reflects the concentration of the injected analyte. Such dynamic measurements performed under batch operation yield an analytical performance (speed, sensitivity, reproducibility, simplicity, *etc.*) similar to that observed under well-established FIA conditions. However, unliked traditional FIA, which employs solution chemistry for homogeneous conversion of the analyte to a detectable species, BIA lacks a solution-handling capability and relies heavily on active or specific sensing surface. The use of active detectors that couple the analyte conversion and sensing functions is receiving increasing attention also in FIA.

1.4.2 The basic components of a BIA system

BIA is a recently developed technique. It can offer similar advantages to FIA without the problems associates with valves, tubing, detector flow cell and pumps. BIA reflects the same basic (impulse-response) thinking of FIA, namely a reproducible transport and washout of microliter sample plugs over the detector surface in the absence of carrier flow (for removing/pushing the sample from the detector).

The basic components of the BIA system is shown in Figure 1.3. It includes the following essential parts:

1.4.2.1 BIA cell

BIA cell is a container for containing a large volume of blank solution. A 1000 ml beaker is commonly used and modified to be used as on BIA cell. For example, it has a hole which is drilled at the bottom corner of the BIA cell, where the inverted detector such as working electrode was introduced, and a 3-holes cell cover. The one located exactly opposite to the center of the detector, which is used for accommodating the standard micropipette.

1.4.2.2 Injection system

A manual and electronic pipettes were used for sample injection of 10-100 μl (from a micropipette tip) onto a position nearby detector where is immersed in a large-volume of blank solution. The pipette was held by a clamp to ensure a reproducible and quick positioning (after each filling). Its tip is kept at a fixed distance, usually 1-2 mm, from the center of the detector.

1.4.2.3 Dispersion-controlled system

A magnetic stirrer and a stirring bar are used for reproducibly transport and washing out of microliter sample plug over the detector surface. The BIA cell is placed on a magnetic stirrer.

1.4.2.4 Detection system

Amperometric detector [56, 57], voltammetric detector [58, 59], ion-selective electrodes [60, 61], thermal sensor [62, 63] and optical sensor [64] have been used as the detectors in the BIA system.

1.4.2.5 Data acquisition and processing unit

A chart recorder, microprocessor or computer can be used to record the data output from the detection system.

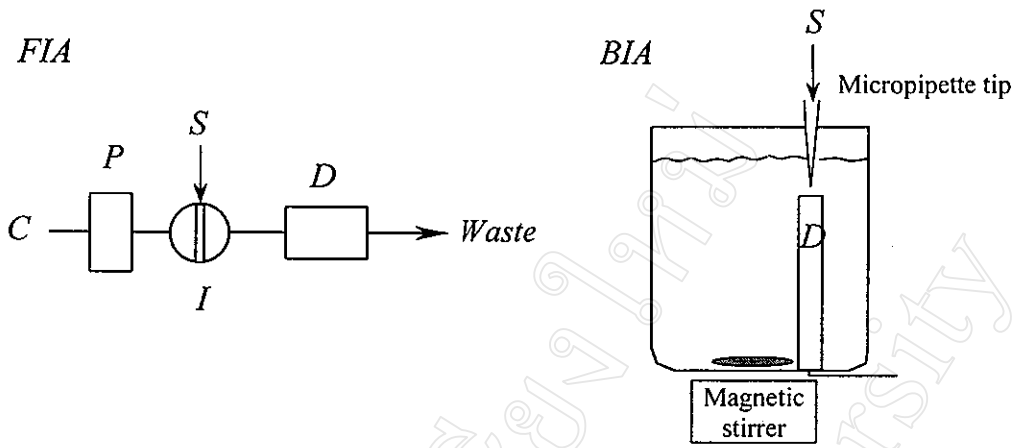

Figure 1.3 Comparison of FIA and BIA operation; *C*, carrier; *P*, pump; *S*, sample; *I*, injector and *D*, detector.

A comparison of the performance characteristics of BIA and FIA is presented in Table 1.7 [56].

Table 1.7 Comparison of the performance characteristics of BIA and FIA

Performance characteristics	**BIA**	**FIA**
Sampling frequency	high	high
Small sample size	yes	yes
Solution handling	no	yes
Controlled transport of samples	yes	yes
Sensitivity	yes	yes
Selectivity or reactive detectors	required	optional
Detection limit (amperometric detection)	nanomolar	nanomolar
Versatility	limited	yes
Low cost, simplicity	yes	yes
Reproducibility	yes	yes

1.5 Research aims

This research work consisted of three parts. Firstly, the determination of fluoride using a plant tissue-based bioelectrode obtained by incorporating asparagus (*Asparagus officinalis*) tissue into a ferrocene-mediated carbon paste was attempted. It is based on the amperometric determination of the inhibitory effect of fluoride on the asparagus peroxidase activity. Secondly, a new plant tissue-based bioelectrode obtained by incorporating sunflower (*Helianthus annuus L.*) leaf tissue as a source of glycolate oxidase and peroxidase into a ferrocene-mediated carbon paste electrode for the determination of glycolic acid using flow-injection amperometric measurements was developed. Finally, the sunflower based amperometric bioelectrode for glycolic acid determination in BIA system was constructed.

The aims of this research work were summarized as follows:

1. To design and construct the plant tissue-based bioelectrodes for static, flow injection and batch injection system based on amperometric measurements with a simple design, low cost, high sensitivity, low detection limit, and rapid response using asparagus and sunflower leave available in Thailand.

2. To apply the fabricated bioelectrodes for the determination of fluoride in fluoride tablets and glycolic acid in human urine samples.