ENTIOMETRIC MEMBRANE SENSOR FOR DETERMINATION OF GLUTAMATE BASED ON [4](1)(2,3-DIAZABUTA-1,3-DIENE)FERROCENOPHANE

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ABSTRACT

tric membrane sensor has been developed and optimized based on
abuta-1,3-diene)ferrocenophane as an ionophore for high selective
of glutamate. The best performance was shown by a membrane with
tio of [4](1)(2,3-diazabuta-1,3-diene)ferrocenophane (ionophore): PVC
P (plasticizer) at 9: 36: 55 (% w/w). The sensor works well in a linear
10^{-5} to 1.0 x 10^{-1} M glutamate with a Nernstian slope of 57.6±1.0
d its detection limit is 7.95 x 10^{-6} M. The sensor shows working range
0 at temperature 25.0±1 °C and stable for a period of 3 months without
in potentials with response time equal or less than 30 seconds. The
fficient values determined by mixed solution method, indicate a good
glutamate over a wide variety of other tested anions.

PENGESAN MEMBRAN POTENSIOMETRI BAGI PENENTUAN GLUTAMAT BERASASKAN [4](1)(2,3-DIAZABUTA-1,3-DIENA)FEROSENOFAN

ABSTRAK

Pengesan membran potensiometri telah dibina dan dioptimum berasaskan [4](1)(2,3-diazabuta-1,3-diena)ferosenofan sebagai ionofor untuk penentuan glutamat berkepilihan tinggi. Keupayaan terbaik telah ditunjukkan oleh membran dengan nisbah komposisi [4](1)(2,3-diazabuta-1,3-diena)ferosenofan (inofor): PVC (pengikat): TEHP (pemplastik) pada 9: 36: 55 (% w/w). Pengesan ini bekerja dengan baik di dalam julat linear 1.0 x 10^{-5} hingga 1.0 x 10^{-1} M glutamat dengan kecerunan Nernstian 57.6±1.0 mV/dekad dan had pengesanannya ialah 7.95 x 10^{-6} M. Pengesan ini menunjukkan julat bekerja diantara pH 6-10 pada suhu 25.0±1 °C dan stabil untuk jangkamasa 3 bulan tanpa sebarang perubahan keupayaan dengan masa gerak balas 30 saat atau kurang. Nilai pekali kepilihan ditentukan dengan kaedah larutan bercampur, menunjukkan kepilihan yang baik terhadap glutamat mengatasi pelbagai jenis anion yang telah diuji.

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LIST OF ABBREVIATIONS

E_{cell}	Cell potential
E^o	Standard cell potential
E*	New constant of potential
G	Conductance
Ag/AgCl	Argentum/Argentum chloride
$K_{A,B}^{pot}$	Selectivity coefficient of primary ion, A and interference ion, B
IUPAC	International Union of Pure and Applied Chemistry
MPM	Matched potential electrode
SSM	Separate solution method
Emf	Electromotive force
$AlOSiO^-$	Aluminosilicate ions
ABS	Acrylonitrile butadiene styrene
NH_2	Amine group
PMMA	Poly(methyl methacrylate)
CWE	Coated wire electrode
o-NPOE	*ortho* 2-nitrophenyl octyl ether
MSG	Monosodium glutamate
FDA	Food and Drug Administration
FAO/WHO	Food and Agriculture Organization/World Health Organization
JECFA	Joint FAO/WHO Expert Committee on Food Additives
NMDA	*N*-methyl-D-aspartate receptor
ALS	Amyotrophic lateral sclerosis
NDA	Naphthalene-2,3-dicarboxaldehyde
CBI	1-cyanibenz[f]isoindole
mGluR	Metabotropic glutamate receptor

AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
GABA	γ-aminobutyric acid
CHO	Carbon hydrogen oxygen functional group
CP	Conducting polymer
GLOD	L-glutamate oxidase
FIA	Flow injection analysis
DCM	Dichloromethane
FTIR	Fourier transform Infra Red
CHNOS	Carbon, hydrogen, nitrogen, oxygen and sulphur
DOP	Diocthyl phthalate
Na_2HPO_4	Disodium hydrogen phosphate
ACN	Acetonitrile
FLD	Fluorescence detector
DAD	Diode array detector
DOPP	Dioctyl phenylphosphonate
GDH	Glutamate dehydrogenase
PhgAT	Phenylglycine aminotransferase
r^2	Correlation coefficient
Lys	Lysine
NaTPB	Sodium tetraphenyl borate

CHAPTER 1

INTRODUCTION

1.1 Electrochemical Sensor

Electrochemical sensors are based upon potentiometric, amperometric, or conductivity measurements. These 3 different principles have their own specific design of electrochemical cell.

1.1.1 Potentiometric Measurements

Potentiometric measurements are based on the determination of a voltage difference between two electrodes (reference electrode and working electrode) plunged into a sample solution with very small current is allowed (Rouessac & Rouessac, 2000). Each of these electrodes constitutes a half cell. The working electrode is in direct contact with the analyte solution and the reference electrode is usually separated from the analyte solution by a salt bridge of various forms (Figure 1.1). The electrode potential of the working electrode is normally directly proportional to the logarithm of the activity of the analyte in the solution (Kehlert, 2002; Evans, 1987). The potential difference is described by Nernst equation:

$$E = E^{o} + \frac{2.303\,RT}{zF} \log a_{cation} \tag{1.1}$$

$$E = E^{o} - \frac{2.303\,RT}{zF} \log a_{anion} \tag{1.2}$$

Where E° is the standard electrode potential of the sensor electrode, *a* is an ion activity, *z* is the charge of an ion, *R* is the gas constant (8.314 J K^{-1} mol^{-1}), T is the absolute temperature in Kelvin and F is Faraday constant (96500 coulombs). A common potentiometric sensor for the measurement of electrolytes is ion selective electrode (ISE). The ISE can be represented in the following way:

internal reference electrode||internal solution|membrane

1.1.2 Amperometric Measurements

Amperometric electrode is a type of electrochemical sensor, as potentiometric electrode discussed earlier. All chemical sensors consist of a transducer, which transforms the response into a signal that can be detected (current) and a chemically selective layer (Wang, Xu, Zhang, & Li, 2008).

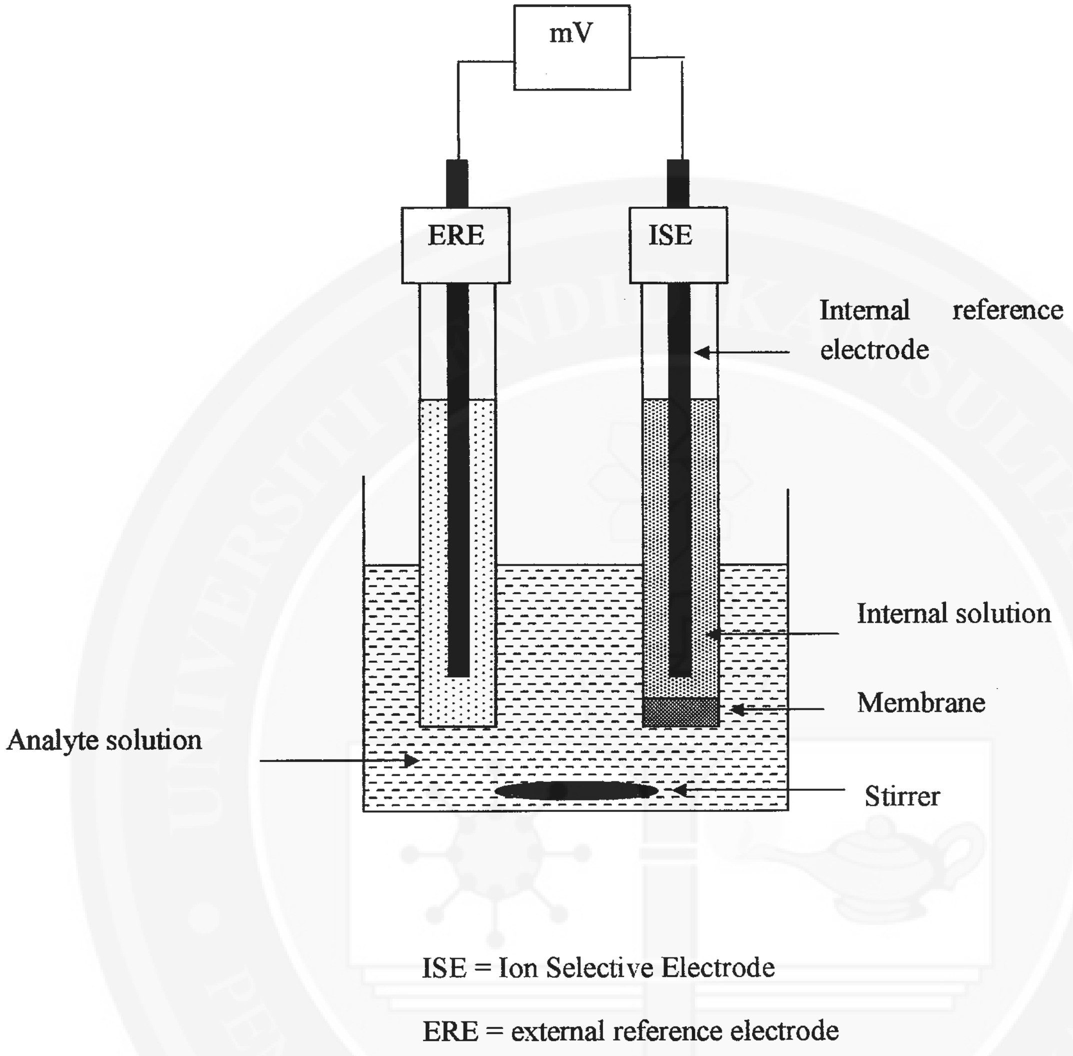

Figure 1.1. Schematic diagram of an electrochemical cell for potentiometric measurements

The transducer may be optical (example: fiber optic cable sensor), electrical (potentiometric, amperometric) and thermal. The signal from amperometric electrode is linearly dependent upon the activity of the analyte. As certain chemical species are oxidized or reduced (redox reactions) at inert metal electrodes, electrons are transferred from the analyte to the working electrode or from the working electrode to the analyte.

Enzyme electrodes make use of one of the types of amperometric electrodes. The enzyme is used to convert the species under test into an ion. As enzymes are specific in their reactions, the analytical process based on them should be highly selective (Evans, 1987). An example of an amperometric enzyme electrode is the glucose electrode (Figure 1.2). The enzyme glucose is immobilized in a gel (example, acrylamide) and coated on the surface of a platinum wire cathode. The gel also contains a chloride salt and makes contact with Ag/AgCl ring to complete the electrochemical cell. Glucose oxidase enzyme catalyzes the aerobic oxidation of glucose as follows:

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{glucose oxidase}} \text{gluconic acid} + H_2O_2$$

Glucose and oxygen from the test solution diffuse into the gel where their reaction is catalyzed to produce H_2O_2; part of this diffuses to the platinum cathode where it is oxidized to give a current on proportion to the glucose concentration. The remainder eventually diffuses back out of the membrane.

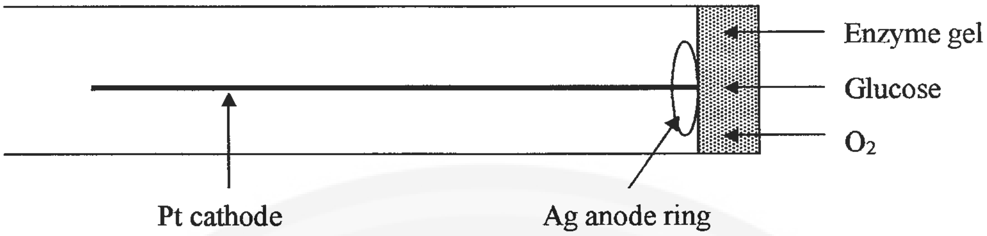

Figure 1.2. Glucose electrode: amperometric enzyme electrode

Other examples of amperometric enzyme electrodes based on the measurement of oxygen or hydrogen peroxide include electrodes for the measurement of galactose in blood (galactose oxidase enzyme), oxalate in urine (oxalate oxidase) and cholesterol in blood serum (cholesterol oxidase).

1.1.3 Conductivity Measurements

Conductometric sensors are based on the measurement of electrolyte conductivity. Conductivity measurements are generally performed with alternate current supply. The conductivity is a linear function of the ion concentration; therefore, it can be used for sensor applications. The conductance, G, measured between two electrodes of area, A, and spacing, d, inserted into a conducting medium is the reciprocal of resistance, R. For a given ion, the conductance of the solution will vary with the concentration of the electrolyte. This relationship is linear for very dilute solutions. A cell to measure the conductivity of electrolyte solutions usually comprises two parallel electrodes usually face to face made up of two planar and parallel electrodes (Wang, Xu, Zhang, & Li, 2008).

1.2 Ion Selective Electrode (ISE)

A high percentage of chemical analyses are based on electrochemistry. Electrochemical methods can be separated into two categories: those that measure voltages and those that measure currents. The first group uses ion selective electrodes (ISEs). Based on research conducted by Ganjali, Norouzi and Rezapour (2006) stated that ISEs have been widely used for more than thirty years and have been used in a wide variety of applications for determining the concentration of various ions in aqueous solution such as pollution monitoring (determining fluoride, chloride and nitrate in effluents and natural waters), agriculture (determining potassium, ammonium, cyanide and others in soils and fertilizers), food processing (determining nitrate and nitrogen dioxide in meat preservatives), corrosive effects of NO_3 in canned foods, determining fluoride in drinking waters and other drinks and last but not least in education and research.

There are many advantages of ISEs. ISEs are relatively inexpensive, simple to use, fast response, wide range of concentration and wide range of applications compared to many other analytical techniques. ISEs are also particularly useful in applications where only an order of concentration is required or it is only necessary to know that a particular ion is below certain level of concentration. Besides that, ISEs are particularly useful in medical and biological applications because they measure the activity of the ion directly rather than concentration. Since ISEs are one of the techniques which can measure both positive and negative ions, they are unaffected by turbidity and color of samples. In applications where interfering ions, pH levels or high concentrations are a problem, then, many manufacturers can supply a specialized experimental methods and special reagents to overcome many of these difficulties (Rundle, 2000).

1.2.1 ISE Classification

Depending on the nature of the membrane material used to impart the desired selectivity, ISEs can be divided into three groups: glass, liquid or solid electrodes. Based on research conducted by Wang in 2006, stated that more than three dozen ISEs are commercially available and are widely used.

1.2.1.1 Glass Electrodes

Glass electrodes are responsive to univalent cations. The selectivity for these cations is achieved by varying the composition of a thin ion-sensitive glass membrane (Wang, 2006). The most common potentiometric device is the pH electrode (Figure 1.3).

a) pH Electrode

This electrode has been widely used for pH measurements for several decades. Its success is attributed to its outstanding analytical performance, in extremely high

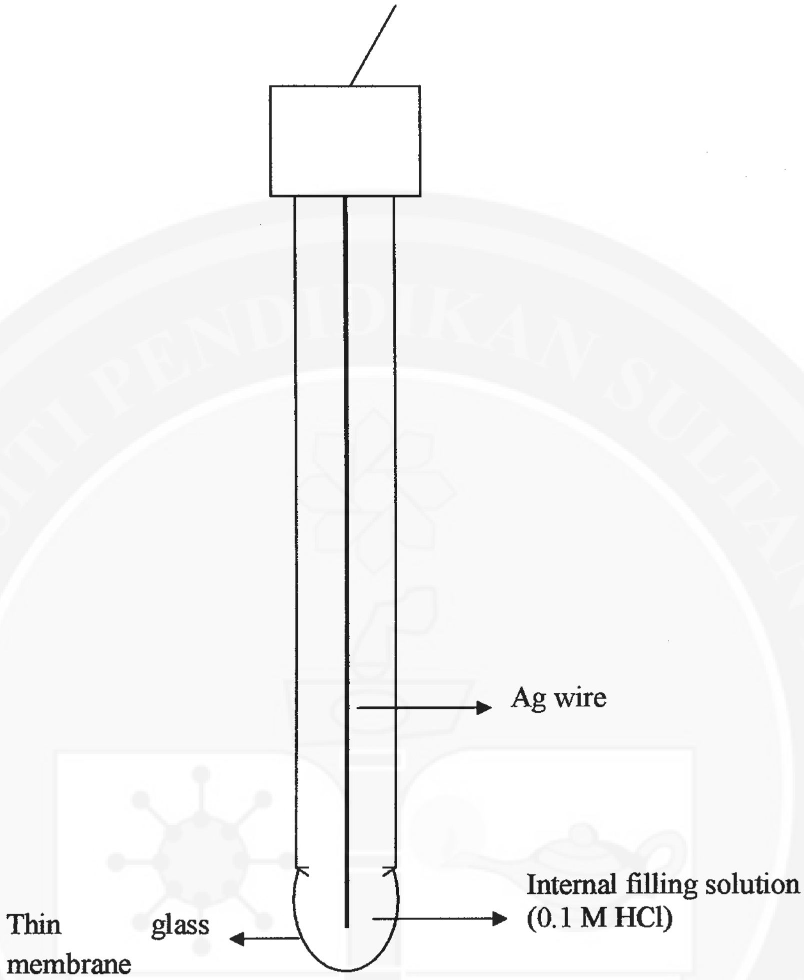

Figure 1.3. A glass pH electrode

selectivity for hydrogen ions, broad response range, and its fast and stable response (Rundle, 2000).

Various types of membrane electrodes have been developed in which the membrane potential is selective toward a given ion, just as the potential of the glass membrane of a conventional glass electrode is selective toward hydrogen ions. These electrodes are important in the measurement of ions, especially in small concentrations. Generally, they are not disturbed by the presence of proteins, as some other electrodes are, and so they are ideally suited to the measurements in biological media. None of these electrodes is specific for a given ion, but each will possess certain selectivity toward a given ion. So they are properly referred to as ion-selective electrodes.

This glass electrode is specific to H^+ ions. Glass in this case does not refer to the material of the electrode body but to the membrane that ensures contact with the solution. The membrane is a thin wall glass that has very high sodium content (25%). In the presence of water, hydration occurs and the membrane's surface becomes comparable to a gel while its interior corresponds to a solid electrolyte.

On a microscopic scale, the glass consists of a network of orthosilicate $Si(OH)_4$ whose open structure contains sodium cations that allow the movement of charges from one side of the membrane to the other. The outside of the membrane is in contact with the sample solution while the inside is in contact with the internal electrolyte, which has constant acidity (pH 7). The membrane is the seat of exchange between Na^+ and H^+ cations as follows:

H^+ (solution) + Na^+ (glass) $\rightleftarrows$ Na^+ (solution) + H^+ (glass)

When the concentration of H^+ is different on either side of the membrane, a potential difference is generated, which is related to the activity of H^+ ions in solution (i.e. pH). The latter is determined using an electronic milivoltmeter, the pH meter, which monitors the potential difference between the glass electrode and an internal reference electrode of Ag/AgCl (currently preferred to the mercurous chloride electrode for environmental purposes). When an H^+ ion forms a silanol bond, a sodium ion moves into the solution to preserve electroneutrality. Some of the more popular glasses have three component compositions of 72% SiO_2, 22% Na_2O, 6% CaO or 80% SiO_2, 10% LiO, 10% CaO (Wang, 2006).

Before using the pH electrode, it should be calibrated using two (or more) buffers of known pH. Many standard buffers are commercially available, with an accuracy of ±0.01 pH unit. Calibration must be performed at the same temperature at which the measurement will be made. The exact procedure depends on the model of pH meter used. The pH electrode must be stored in an aqueous solution when not in use, so that the hydrated gel layer of the glass does not dry out. A highly stable response can thus be obtained over a long time period. After calibration, the instrument will directly yield the pH of a solution.

For measurement, only the bulb needs to be submerged. There is an internal reference electrode and electrolyte ($Ag/AgCl/Cl^-$) for making electrical contact with the glass membrane. Its potential is constant and is set by the concentration of HCl. A complete cell is represented as:

$$\left.\begin{matrix}\text{reference}\\ \text{electrode}\\ \text{(external)}\end{matrix}\right|\ \text{H}^+\text{ (unknown)}\ \left\|\ \begin{matrix}\text{glass}\\ \text{membrane}\end{matrix}\ \right|\ \text{H}^+\text{ (internal)}\ \left|\ \begin{matrix}\text{reference}\\ \text{electrode}\\ \text{(internal)}\end{matrix}\right|$$

Where H^+_{int} is concentration of internal hydrogen ion and H^+_{unk} is concentration of unknown hydrogen ion. The potential of the glass membrane is given by;

$$E_{\text{glass}} = \text{constant} - \frac{2.303\ \text{R T}}{\text{F}} \log \frac{a_{\text{H}^+\ \text{int}}}{a_{\text{H}^+\ \text{unk}}} \tag{1.3}$$

And the voltage of the cell is given by;

$$E_{\text{cell}} = k + \frac{2.303\ \text{R T}}{\text{F}} \log a_{\text{H}^+\ \text{unk}} \tag{1.4}$$

k is a constant which include the potentials of the two reference electrodes, the liquid junction potential, a potential at the glass membrane due to H^+ (internal) and asymmetry potential. The asymmetry potential is a small potential across the membrane that is present even when the solutions on both sides of the membrane are identical. It is associated with factors such as nonuniform composition of the membrane, strains within the membrane, mechanical and chemical attack of the external surface, and the degree of hydration of the membrane. It slowly changes in time, especially if the membrane is allowed to dry out. For this reason, a glass pH electrode should be calibrated from day to day. The asymmetry potential will be varied from one electrode to another, owing to differences in construction of the membrane (Christian, 2004).

b) Glass Electrode for Other Cations

Alkaline solutions were noted to display some interference on the pH response for glass pH electrodes. Deliberate changes in the chemical composition of the glass membrane (along with replacement of the internal filling solution) have thus led to electrodes responsive to monovalent cations other than hyderogen, including sodium, ammonium and potassium (Wang, 2006). This usually involves the addition of B_2O_3 or Al_2O_3 to sodium silicate glasses to produce anionic sites of appropriate charge and geometry on the outer layer of the glass surface. Example, sodium selective glasses have the compositions 11% Na_2O, 18% Al_2O_3, 71% SiO_2 while ammonium selective glasses have the compositions of 27% Na_2O, 4% Al_2O_3, 69% SiO_2 (Wang,2006). These compositions are different from sodium silicate glasses which are used for pH measurements, it is because these sodium aluminosilicate glasses posses what may be termed $AlOSiO^-$ sites with weaker electrostatic field strength and a marked preference for cations other than protons.