CHAPTER II
LITERATURE SURVEYS

2.1 Polyaniline as Solution sensor

The use of polyaniline as a conductive polymer for biosensors has been reported first by Parente (1992). He used enzyme glucose oxidase immobilized on polyaniline and used glutaraldehyde as a bifunctional agent to bond between enzyme and polyaniline. This biosensor was used for the detection of glucose. The report showed a higher activity of immobilized enzyme between pH 6.0 and 7.5, and still retained 50% activity after 30 days of daily use.

Cooper and Hall (1993) created an enzyme-mediator-conducting polymer model which provided a convenient charge transport immobilization in the benzoquinone-polyaniline system. The current increased on polyaniline was related to Pt electrode and they used benzoquinone as an enzyme mediator. On Pt, one-electron equivalent was estimated. On polyaniline-modified electrode, the reduction of benzoquinone on polyaniline was nearly reversible for two-electron process.

Because the electronic conducting polymer such as polyaniline showed a strong dependence on the redox and protonation states of the polymer, Contractor (1994) used these properties to develop a genetic biosensor concept which involved the immobilization of enzyme in the conductive polymer matrix. Because of the enzyme catalysed reaction, a change in redox potential and/or pH of the microenvironment in the polymer matrix occurred leading to a change in electronic conductivity which was monitored. The systems chosen for investigation were glucose/glucose oxidase, urea/urease, neutral lipid/lipase and hemoglobin/peptin.

Polyaniline can also be used as a potentiometric glucose biosensor as Karyakina (1994) and co-workers reported. They used self-doped polyaniline instead of a common polymer as a pH transducer. A stable potentiometric response of 70 mV/pH was obtained. They showed that the polyaniline-based electrode passed three- to fourfold increase in potential relative to the glucose-sensitivity field-effect transistor. The sensitivity of the biosensor can be increased by using a thick ion-exchange membrane in order to concentrate the product near the electrode surface.

Ramanathan (1995) succeeded in depositing Langmuir-Blodgett films of the polyemeraldine base form of polyaniline on an indium tin oxide (ITO) coated glass surface. The enzyme glucose-oxidase was entrapped between the layers of the films. Then, the retention of its electroactivity was tested by using cyclic voltammetry (CV).

Beside its use as glucose biosensor, polyaniline can be synthesized by an electrochemical reaction of aniline in the presence of choline oxidase onto a Pt electrode for a choline biosensor, as reported by Hidaka and Aizawa (1995). Choline was amperometrically measured by monitoring the choline dependent O_2 reducing current of H_2O_2 oxidizing current.

Hydrogen peroxide was investigated via chemically modified electrode for cathodic determination, as reported by Mulchandani and Barrow (1995). The electrode was constructed by modifying the surface of a glassy carbon electrode with an electrochemically deposited ferrocene-modified polyaniline film from a solution of N-(ferrocenylmethyl)aniline monomer in acetonitrile. Hydrogen peroxide and oxygen were reduced at the electrode with an increasing response at higher cathodic potentials. The response of the electrode depended on the thickness of film and pH of the electrolyte.

Glucose, urea and triolein can be analyzed at the same time by immobilization of three different enzymes on three closely spaced

microelectrodes. The polymer deposition and enzyme immobilization were done electrochemically. This study was reported by Songokar (1996).

Glucose biosensors can be fabricated by both adsorption and electrochemical doping on/in polyaniline films. The study of Mu and Xue (1996) showed the binding force between glucose oxidase and polyaniline for electrochemical doping was stronger and had a higher operational stability and longer storage stability than that for adsorption. Glucose oxidase adsorbed on a polyaniline film desorbed more easily than electrochemically doped. This experiment used this biosensor for the determination of glucose in human blood.

The improvement of polyaniline glucose biosensor stability can be accomplished by suspending an enzyme in a water-ethanol mixture with a high (90%) ethanol content, mixing with polyelectrolyte solution, and drying at the target surface. Because perfluorosulfonate (Nafion) was deposited without excessive dissolving in water, the Nafion membrane was more uniform and stable. The stability of glucose oxidase in these suspensions was improved when compared with aqueous solution, as reported by Lukachova (1997).

A glucose amperometric sensor can be fabricated from a functionalized polyaniline film instead of polyaniline: poly-2-aminoaniline, via choranil on platinized platimum electrode. This study was reported by Losada and Armanda (1997). Glucose oxidase was covalently bound to poly-2-aminoaniline film formed onto a platinized Pt surface via teteacholro-1,4-benzoquinone linkage. The working potential for glucose determination was +250 mV and the biosensor exhibited a linear response in the range 0.2-2.0 mM with a response time that did not exceed 5 seconds.

Urea biosensor was developed by cross-linking urease onto a polyaniline-Nafion composite electrode which sensed the ammonium ion

effectively. A detection limit as low as 0.5 mu and a response time as short as 40 seconds were obtained. This study was reported by Cho and Huang (1998).

The sensitivity of glucose biosensor was improved by using anion self-exchange in electrochemically prepared polyaniline film. The exchange of the bulkier tosylate-ferricyanide ion with Cl-ion led to a change in porosity on polyaniline film. Scanning electron microscopy, which was used to delineate the surface morphology, indicated an enhanced loading of glucose oxidase after a self-ion exchange. This study has been reported by Vergnese (1998).

2.2 Applications of other polymers for Solution Sensors

The electrode used for an amperometric biosensor can be made from Prussian blue which is formed by cyclic voltametry onto the basal pyrolytic graphite surface to prepare a chemically modified electrode which provided excellent electrocatalysis for both oxidation and reduction of hydrogen peroxide. Moreover, the glucose oxidase and D-amino oxidase can be incorporated into the Prussian blue film during the electrochemical process. These two biosensors were fabricated by an elecrochemical codeposition, and the resulting sensors were protected by coverage with a Nafion thin film. This study was reported by Chi and Dong (1995).

In addition to polyaniline, polypyrrole can be used as a glucose biosensor. Vanos, Bult, and Vanbennekom (1995) entrapped the enzyme glucose oxidase into polypyrrole during electrochemical polymerization. In a flow-injection system, the glucose responses were measured amperometrically at a potential of +0.7 V vs SCE to detect hydrogen peroxide, which was generated by enzyme in the presence of oxygen. The best results were obtained when they used a current of 1 nA for 5 minutes, in the presence of 0.3 M pyrrole and glucose oxidase dissolved in 10 mM HEPES at pH 7. The stability of this electrode for ascorbic acid was also tested. Overtime, the

electrode was quite stable and gave very small response. Electrodes with higher glucose oxidase concentration showed less interference from ascorbic acid.

Uciyama and Sakamoto (1997) reported that uric acid sensor can be created by the electropolymerization of aniline and pyrrole solution containing uricase at a neutral pH which performed to immobilize the enzyme on the surface of the gas diffusion carbon felt. A selective uric acid sensor was fabricated by combining an immobilized enzyme carbon felt and an oxygen electrode with an oxygen permeable membrane. This carbon felt could be used in the uricase sensing because of an extremely efficient supply of oxygen for the enzymatic reaction due to its porosity permitting a transfer of oxygen.

Polypyrrole film can be used for electrochemical biosensor by incorporation of anti-human serum albumin (AHSA) antibody into polypyrrole film using galvanostatic polymerization. Barisci *et. al.* (1998) reported that the amount of antibody incorporated during polymer formation increased with increasing AHSA concentration and decreasing current density. At higher current density, the rate of polymerization reaction was such that the slow diffusing AHSA molecules had a smaller chance of being trapped into the growing polymer film. They also studied the effect of ionic species in order to improve the reproducibility of the PP/AHSA film preparation. Two supporting electrolytes, one containing a large anion (dextran), and another one containing a small anion (NO^-_3) were investigated. They reported that the smaller counterions were more readily incorporated into the polypyrrole film instead of the bulky AHSA. In contrast, dextran sulphate did not affect the incorporation. The detection of HSA by flow-injection analysis was possible using the PP/AHSA electrode operated under pulsed amperometric conditions in a $NaNO_3$ carrier solution. Pretreatment of the electrode with a bovine serum albumin solution was found to decrease the response due to non-specific binding.

Hydrogen peroxide determination is of importance in pharmaceutical and diary industries where it is employed as an anti-bacterial agent and for sterilization, respectively. Ferrocenes have been proven to be the most suitable mediators in horseradish peroxidase (HRP)-base electrode. Asok and Shengtian (1999) developed a reagentless amperometric enzyme biosensor based on HRP and an electrochemically polymerizable ferrocene-conjugated monomer for the determination of hydrogen peroxide and the other organic peroxides. The horseradish peroxidase/poly (m-aminoanilinomethyl ferrocene)-modified glassy carbon electrode reagentless biosensor was used to measured hydrogen peroxide and the other organic peroxides in both aqueous and organic mediums by the reduction at a low applied potential of –0.05 V (vs Ag/AgCl) without interference from oxygen. When this electrode was modified with glucose oxidase by entrapping glucose oxidase (GOX) in a o-phenylene diamine and resocinal copolymer, the new bioenzyme electrode measured glucose sensitively, and selectively, demonstrating the suitability of the peroxide biosensors for other oxidoreductase enzyme-based biosensors.

When the gold surface was exposed to the solution of dithiobis-N-succinimidyl propionate (DTSP), it gave rise to the modification of the surface with N-succinimidyl-3-thiopropionate (NSTP) which can react with amino groups allowing for the covalent immibilization of enzymes such as horseradish peroxide (HRP) (Darder, 1999). The coverage of NSTP has been estimated to be of the order of $1.3*10^{-3}$ from the charges consumed during the reductive desorption. The binding reaction of HRP with NSTP modified gold surface has been studied with Quartz Crystal Microbalance (QCM). The results suggested that the immobilization process involved two steps: the first step (faster) appeared to correspond to the rapid incorporation of the enzyme whereas the second step was likely due to the slow incorporation of additional enzyme and/or reorganization of the immobilized layers. Spectrophotometric and electrochemical assays indicated that the immobilized HRP retained its

enzymatic activity after immobilization onto the DTSP modified gold surface. A peroxide biosensor was developed making use of a gold surface modified with DTSP and HRP employing Os and Ru complexes of 1, 10-phenanthroline 5, 6-dione (phen-dione) as a mediator with the quinone moieties being the active component. The efficiency of the mediator increased with increasing number of phendione ligands. This study was reported by Darder *et al.* (1999).

The polyethylene glycol diglycidyl ether cross-linked with chitosan membrane was used to detect uric acid in serum by immobilizing uricase on a chitosan membrane. This immobilization occurred by the reaction of the amino groups of chitosan with the epoxy groups of polyethylene glycol diglycidyl ether. Then, the chitosan membrane was cast onto a rimmed glass plate. The numbers of ethylene glycol units were varied in order to determine their effects on immobilization. Suye and Mizusawa (1999) reported that when the number of polyethylene units was equal to 9, the optimum enzyme immobilization was achieved. The effect of enzyme concentration in the casting solution on immobilization was also investigated. They reported that the activities of immobilized uricase increased as the amount of uricase was increased, and reached a plateau at approximately $3.3*10^3$ units/cm^2. The value of enzyme activity in the uricase immobilized membrane by the present method was about 5 times that of the enzyme activity when uricase was entrapped in alkali solution, and the enzyme retained 100% of its initial activity for at least 30 days.

Rebecca, *et al.* (1999) immobilized alcohol oxidase covalently on a plasticized carboxylated poly(vinyl chloride) membrane, entrapped the oxygen-sensitive dye ion-pair in the same membrane and coated this membrane on overhead transparency film in order to detect the amount of alcohol in fermentation, beverages and blood. The sensing membrane related oxygen consumption, as a result of enzymatic oxidation, to alcohol concentration. The measurement was performed in air-saturated alcohol

standard solution of pH 7. The resulting enzyme membrane showed a good activity and possessed high stability, mechanical resistance and flexibility. Response time and reversibility of an alcohol sensing membrane were demonstrated by alternatively applying the membrane to solutions with two different ethanol concentrations. They achieved the full-steady state within 1 minute, which represented a fairly rapid response. The alcohol sensing membrane can be renewed easily by washing it with phosphate buffer.