DETECTION OF HUMAN IMMUNOGLOBULIN G BASED ON POLY(PYRROLE -3-CARBOXYLIC ACID) THIN FILM USING ELECTROCHEMICAL SURFACE PLASMON OPTICAL TECHNIQUE

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Rapiphun Janmanee
ABSTRACT

An electrochemically controlled PP3C-based surface plasmon optical technique was employed to study an optical and electrical property of the PP3C film and a label-free detection of the interaction between anti-IgG and IgG. In this study, electropolymerization was employed to fabricate poly(pyrrole-3-carboxylic acid) (PP3C) film on a gold surface of substrate using pyrrole-3-carboxylic acid (P3C) monomer solution in 0.5 M H₂SO₄. Electrochemical-surface plasmon resonance (EC-SPR) and electrochemical-transmission surface plasmon resonance (EC-TSPR) spectroscopy were carried out to study the kinetic property and electroactivity property of the PP3C film in a real-time. Various constant applied potentials were applied to the PP3C films in neutral phosphate-buffered saline (PBS) solution to study the electroactivity property. Moreover, Ultraviolet-visible (UV-vis) spectroscopy and
atomic force microscopy (AFM) were performed to characterize the PP3C films. The PP3C shows good stability and electroactivity in neutral PBS solution. It could be noted that the presented copolymer films could be employed to control the morphology of PP3C, which plays an important role in an effective immobilization in the PP3C-based immunosensor system by control the space for binding site in polymer chain.

The PP3C-based immunosensor was fabricated for a label-free detection of human IgG. In situ EC-SPR and EC-TSPR measurement were performed to monitor the probe immobilization of PP3C-based immunosensor and the immunoreaction between anti-human and human IgG with several concentrations of human IgG at different constant applied potentials. An electrochemically controlled PP3C-based surface plasmon optical technique shows a higher efficiency than that of the standard MUA system. The sensitivity of the sensor was improved by controlling the topology of the PP3C film by applying the potential. At a constant potential of 0.6 V showed the highest efficiency than that without applied potential is about 6 times, which was observed by EC-SPR measurement. Furthermore, electrochemically controlled P3C-based TSPR immunosensor could be represented a comparable performance or even better than that of EC-SPR immunosensor for detection of human IgG. The detection limit of the PP3C-based TSPR sensor is 1 ng/mL of human IgG. It could be concluded that an electrochemically controlled PP3C-based surface plasmon optical technique showed obvious advantages for the label-free detection of human IgG and may be possessed potential applications to study in various systems of the biosensor.
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<tr>
<td>Å</td>
<td>Angstrom</td>
</tr>
<tr>
<td>ATR</td>
<td>Attenuated Total Internal Reflection</td>
</tr>
<tr>
<td>A-IgG</td>
<td>Anti-human Immunoglobulin G (Feb specific)</td>
</tr>
<tr>
<td>°C</td>
<td>Celsius degree</td>
</tr>
<tr>
<td>CPs</td>
<td>Conjugated polymers</td>
</tr>
<tr>
<td>CV</td>
<td>Cyclic voltammetry</td>
</tr>
<tr>
<td>EC-SPR</td>
<td>Electrochemical-surface plasmon resonance spectroscopy</td>
</tr>
<tr>
<td>EC-TSPR</td>
<td>Electrochemical-transmission surface plasmon resonance spectroscopy</td>
</tr>
<tr>
<td>$E_g$</td>
<td>Energy gap</td>
</tr>
<tr>
<td>EA-HCl</td>
<td>Ethanolamine hydrochloride</td>
</tr>
<tr>
<td>EDC</td>
<td>1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride</td>
</tr>
<tr>
<td>e.g.</td>
<td>Exempli gratia (for example)</td>
</tr>
<tr>
<td>FETs</td>
<td>Field effect transistors</td>
</tr>
<tr>
<td>HOMO</td>
<td>Highest occupied molecular orbital</td>
</tr>
<tr>
<td>h</td>
<td>Hour</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
</tr>
<tr>
<td>ITO</td>
<td>Indium-Tin Oxide</td>
</tr>
<tr>
<td>LUMO</td>
<td>Lowest occupied molecular orbital</td>
</tr>
<tr>
<td>μm</td>
<td>Micrometer</td>
</tr>
<tr>
<td>mL</td>
<td>Mililiter</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>--------------</td>
<td>-----------</td>
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<tr>
<td>mV/sec</td>
<td>Milivolt/second</td>
</tr>
<tr>
<td>min</td>
<td>Minute</td>
</tr>
<tr>
<td>M</td>
<td>Molar concentration</td>
</tr>
<tr>
<td>NHS</td>
<td>N-hydroxysuccinimide</td>
</tr>
<tr>
<td>OLEDs</td>
<td>Light-emitting diodes</td>
</tr>
<tr>
<td>PANI</td>
<td>Polyaniline</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate-buffered saline</td>
</tr>
<tr>
<td>PP3C</td>
<td>Poly(pyrrle-3-carboxylic acid)</td>
</tr>
<tr>
<td>PEDOT</td>
<td>Poly(3,4-ethylenedioxythiophene)</td>
</tr>
<tr>
<td>PPy</td>
<td>Polypyrrole</td>
</tr>
<tr>
<td>PT</td>
<td>Polythiophene</td>
</tr>
<tr>
<td>sec</td>
<td>Second</td>
</tr>
<tr>
<td>SAM</td>
<td>Self-assembled monolayer</td>
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<td>SPR</td>
<td>Surface plasmon resonance spectroscopy</td>
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<td>TBAPF&lt;sub&gt;6&lt;/sub&gt;</td>
<td>Tetrabutylammonium hexafluorophosphate</td>
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<tr>
<td>TSPR</td>
<td>Transmission surface plasmon resonance spectroscopy</td>
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<td>UV-vis</td>
<td>Ultraviolet-visible</td>
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<tr>
<td>V</td>
<td>Volts</td>
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<td>λ</td>
<td>Wavelength</td>
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CHAPTER 1
GENERAL INTRODUCTION

1.1 Background

Surface plasmon resonance (SPR) is a powerful optical technique to monitor the change of the reflective index at a solid-liquid interface or the thickness of thin films [1–5] based on the excitation of surface charge-density wave, which can be produced at the interface between a metal (such as Ag, Au) and a dielectric and propagates along the interface. The change in the optical properties of the dielectric layer adjacent to the metal layer induces the excitation of the plasmon, thus forming the basis of SPR sensing [6]. SPR has been applied to characterization and study of ultrathin films, interfaces and kinetic processes at surfaces [7–11].

Recently, the great potential of SPR technology in biosensor application has become a widely accepted technique for analysis of interactions of numerous biomolecules including protein, DNA, antigen and antibody [12–16]. SPR-based biosensors offer the advantage of label free detection of biomolecular-binding events, the binding of biomolecules can be monitored in real-time and thus affinity constants can be determined from measured kinetics [14, 17–20]. Therefore, SPR technique is versatile for development in various fields of the study of biomolecular interactions, chemical detection and immunoassays. Today, SPR-based biosensors are increasingly employed in many important applications in food safety, biomedical, pharmaceutical and medical diagnostics [13, 16, 21].
Conducting polymers (CPs) are materials discovered over 20 years ago, which have attracted considerable interest on their electronic conducting properties, optical properties, chemical and biochemical properties [9, 22–25]. The most widely investigated CPs include polythiophene, polyaniline, poly(phenylene vinylene) and polypyrrole, poly(3,4-ethylenedioxythiophene) and their derivatives [26–35]. The structures of these molecules are shown in Table 1.1. CPs can be synthesized either chemically or electrochemically [36–41]. Today, electrochemical synthesis is a common alternative for making CPs, particularly because this synthetic procedure is relatively straightforward, ability of controlling thickness, shape and morphology [36, 42]. CPs have potential applications in various fields such as fuel cells, electrochromic displays, light-emitting diodes (LEDs), field effect transistors (FETs) and biosensor [43–49]. Biosensor based on CPs is considered to be the most promising material to detect several type of biomolecules including glucose, hormones, neurotransmitters, antibodies, and antigens [50–55] owning to its relative stability, good conductivity and ease of preparation [22, 25, 36, 56]. In particular, polypyrrole and its derivatives containing the carboxylic group were studied for their immunosensor applications because biomolecules can be immobilized with a covalent binding method, which has received considerable attention owing to their advantages of good stability and high immobilization density [9, 21, 35, 42, 53].

Based on the above considerations, it can be seen that CPs-based SPR biosensor is one of the special interesting techniques to generate novel and effective molecular recognition technology, especially in the purpose for study the optical and electrical properties of CPs and monitoring the interaction between biomolecules and electropolymerized of CPs-based biosensor [11, 12, 21, 42, 53].
Table 1.1 Chemical structures of some of the most common conjugated polymers [36, 49].

<table>
<thead>
<tr>
<th>Name structure</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyacetylene</td>
<td><img src="image" alt="Polyacetylene Structure" /></td>
</tr>
<tr>
<td>Polypyrrole</td>
<td><img src="image" alt="Polypyrrole Structure" /></td>
</tr>
<tr>
<td>Polythiophene</td>
<td><img src="image" alt="Polythiophene Structure" /></td>
</tr>
<tr>
<td>Polyaniline</td>
<td><img src="image" alt="Polyaniline Structure" /></td>
</tr>
<tr>
<td>Poly(3,4-ethylenedioxythiophene)</td>
<td><img src="image" alt="Poly(3,4-ethylenedioxythiophene) Structure" /></td>
</tr>
<tr>
<td>Poly(phenylene vinylene)</td>
<td><img src="image" alt="Poly(phenylene vinylene) Structure" /></td>
</tr>
</tbody>
</table>
1.2 Conducting polymers (CPs)

Conducting polymers (CPs) have attracted much attention because of their unique electrical, optical and structural properties [22, 26, 36]. Since the first pioneer works of Shirakawa, MacDiarmid and Heeger have discovered of the remarkably high electrical conductivity of iodine–treated polyacetylene thin film [57–58]. In the year 2000, the Nobel Prize in Chemistry was awarded to three men from the field of CPs widely recognized the importance of these materials and has prompted even more vigorous research in this field. CPs have both electrical and optical properties similar to those of metals and inorganic semiconductors as shown in Figure 1.1. However, they also exhibit the attractive properties associated with conventional polymers such as ease of synthesis and flexibility in processing. The polymer is called a ‘conducting polymer’ because of the alternating single and double bonds in the polymer chain. Due to the special conjugation in their chains, it enables the electrons to delocalize throughout the whole system and thus many atoms may share them. The delocalized electrons may move around the whole system and become the charge carriers to make them conductive. This polymer can be transformed into a conducting form when electrons are removed from the backbone resulting in cations or added to the backbone resulting in anions. Anions and cations act as charge carriers, hopping from one site to another under the influence of an electrical field, thus increasing conductivity [31, 60–63].
1.2.1 Conduction mechanisms [64–69]

To explain the mechanism of conductivity in CPs, a band theory has been used as shown in Figure 1.2. According to band theory [68], the electrical properties of direct gap conductive materials are determined by their electronic structures, and the electrons move within discrete energy states called bands. By analogy, the
bonding and antibonding π-orbitals of the $sp^2$ hybridized π-electron materials (e.g. polyenes) generate energy bands, which are fully occupied (π-band) and empty ($\pi^*$-band). The highest occupied band is called the *valence band*, and the lowest unoccupied band is the *conduction band*. The energy difference between them is called the *band gap*. Electrons must have certain energy to occupy a given band and need extra energy to move from the valence band to the conduction band. Moreover, the bands should be partially filled in order to be electrically conducting, as neither empty nor full bands can carry electricity.

**Figure 1.2** Band structure in an electronically conducting polymer [68].
Figure 1.3 A schematic description of the formation of polaron, bipolaron, and soliton pair on a trans-polyacetylene chain by doping [69].

The electronic phenomenon in these polymeric systems has been explained by the charged defects involving polaron, bipolaron and soliton. When an electron is added (removed) to the bottom of the conduction band (from the top of the valence band) of a conjugated polymer (Figure 1.3(a)), the conduction (valence) band ends up being partially filled and a radical anion (cation), commonly termed as a polaron, is created (Figure 1.3(b)). The formation of polarons causes the injection of states from the bottom of the conduction band and top of the valence band into the band gap. A polaron carries both spin (1/2) and charge (±1e). Addition (removal) of a second electron on a chain already having a negative (positive) polaron are results in the
formation of a bipolaron (spinless) through dimerization of two polarons, which can lower the total energy (Figure 1.3(c)). In conjugated polymers with a degenerate ground state (*i.e.* two equivalent resonance forms), like *trans*-polyacetylene, the bipolarons can further lower their energy by dissociating into two spinless solitons at one-half of the gap energy (Figure 1.3(d)). Solitons do not form in conjugated polymers with nondegenerate ground states, such as in polypyrrole, polythiophene and polyaniline. The population of polarons, bipolarons, and/or solitons increases with the doping level. At high doping levels, the localized polarons, bipolarons or solitons near to individual dopant ions could overlap, leading to new energy bands between and even overlapping the valence and conduction bands, through which electrons can flow.

It is this charge carrier mobility that leads to the high conductivity of these polymers. The conductivity, $\sigma$ of a conducting polymer is related to the number of charge carriers $n$ and their mobility, $\mu$ follow by equation 1.1:

$$\sigma \propto \mu n \quad (1.1)$$

Because the band gap of conjugated polymers is usually fairly large, $n$ is very small under ambient conditions. Consequently, conjugated polymers are insulators in their neutral state and no intrinsically conducting organic polymer is known at this time. A polymer can be made conductive by oxidation (p-doping) and/or, less frequently, reduction (n-doping) of the polymer either by chemical or electrochemical means, generating the mobile charge carriers as described earlier.
1.2.2 Synthesis of CPs [31, 36]

There are numerous synthetic techniques used in the synthesis of CPs. It can be synthesized either chemically or electrochemically, with each having advantages and disadvantages as summarized in Table 1.2 [36]. Different methods of chemical synthesis include step-growth (condensation) or chain-growth (addition) polymerization. Chemical synthesis not only provides many different possible routes to synthesize a variety of CPs, but also permits the scale-up of these materials, which is currently not possible with electrochemical synthesis. Electrochemical synthesis is a common alternative for making CPs, particularly because this synthetic procedure is relatively straightforward. The most significant difference between electrochemical and chemical methods of CP synthesis is that very thin CP films on the order of 20 nm can be produced using the electrochemical technique, whereas powders or very thick films are typically produced with chemical polymerization. All CPs can be synthesized chemically, but electrochemical synthesis is limited to those systems in which the monomer can be oxidized in the presence of a potential to form reactive radical ion intermediates for polymerization. The standard CPs (i.e., PPy, PT, PANI, PEDOT) can be polymerized both chemically and electrochemically; however, several novel CPs with modified monomers are only amenable to chemical polymerization.
**Table 1.2** Comparison of chemical and electrochemical CPs polymerization [36].

<table>
<thead>
<tr>
<th>Polymerization approach</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical polymerization</td>
<td>• Larger-scale production possible</td>
<td>• Cannot make thin films</td>
</tr>
<tr>
<td></td>
<td>• Post-covalent modification of bulk CP possible</td>
<td>• Synthesis more complicated</td>
</tr>
<tr>
<td></td>
<td>• More options to modify CP backbone covalently</td>
<td></td>
</tr>
<tr>
<td>Electrochemical polymerization</td>
<td>• Thin film synthesis possible</td>
<td>• Difficult to remove film from electrode surface</td>
</tr>
<tr>
<td></td>
<td>• Ease of synthesis</td>
<td>• Post-covalent modification of bulk CP is difficult</td>
</tr>
<tr>
<td></td>
<td>• Entrapment of molecules in CP</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Doping is simultaneous</td>
<td></td>
</tr>
</tbody>
</table>

1.2.2.1  **Step-growth polymerization** [31]

Condensation polymerization proceeds via the loss of small molecules, such as hydrochloric acid or water. The most general reaction for two molecules can be represented as:
and, in principle, any reaction that links two units together can be used to form a polymer. In general, we can write:

$$\text{A-A} + \text{B-B} \rightarrow [-\text{A-A-B-B-}]$$

or

$$n\text{A-B} \rightarrow [-\text{A-B-}]$$

A common example of this type of polymerization is the synthesis of polyesters which takes advantage of the fact that an alcohol and an acid can be condensed together (with the elimination of waters, thus the origin of the term “condensation” to form an ester.

$$n\text{HO-R-OH} + n\text{HOOC-R′-COOH} \rightarrow \text{H}(-\text{O-R-OOC-R′-CO-})_n\text{-OH} + (2n-1)\text{H}_2\text{O}$$

Polyamides, polyurethanes, polycarbonates, and many other polymers are routinely synthesized in this manner. This polymerization, in principle, is general since many synthetic organic reactions can be employed using difunctional units. This reaction take place in a number of statistical steps, and although the rate of bond formation can be very fast, the rate of molecular weight increase is initially very slow. First, most of the monomers will react to form dimers and statistically only after most of the dimers have formed will dimers start to react with monomers or dimers to form trimers or tetramers. Already 50% of the bonds have formed but the degree of
polymerization is only $n=2$. This slow buildup of molecular weight is the primary disadvantage of step-growth polymerization. Many conjugated polymer are synthesized by step-growth. If the polymer is soluble and the reaction employed is of high yield, high polymer can be expected. However, if oligomers precipitate before they react with one another, or if the yield of the reaction is low, the degree of polymerization can be very slow. This problem is often encountered in conjugated polymers synthesis.

1.2.2.2 Chain-growth polymerization [31]

The second mechanism of polymerization, chain-growth, involved three distinct steps: initiation, propagation, and termination. An initiator reacts with a monomer to produce a new species which can then react with another monomer, and another, until the monomer is depleted or growing polymer chain undergoes a termination reaction. This procedure is shown schematically below where * refers to a species that can add another monomer. This species is often a radical, cation, anion, or attached transition metal complex that can insert a monomer between itself and the growing polymer chain.

\[
\begin{align*}
I & \rightarrow I^* & \text{(initiation)} \\
I^* + M & \rightarrow I-M^* \\
I(-M-)_n^* + M & \rightarrow I(-M-)_n^* & \text{(propagation)} \\
I(-M-)_n^* + T & \rightarrow I(-M-)_n^*T & \text{(termination)}
\end{align*}
\]
This reaction is most commonly observed with vinyl monomers, yielding polyethylene, polystyrene, polyacrylates, etc.

\[
\begin{align*}
\text{H}_2\text{C}=\text{CH}_2 & \quad \text{Polyethylene} \\
\text{H}_2\text{C}=\text{CH} & \quad \text{Polystyrene (R=phenyl)} \\
\text{H}_2\text{C}=\text{C} & \quad \text{Poly(vinyl chloride) (R=Cl)} \\
\text{H}_2\text{C}=\text{C} & \quad \text{Polyacrylonitrile (R=CN)} \\
\text{CH}_3 & \quad \text{Poly(methyl methacrylate)}
\end{align*}
\]

**Figure 1.4** Common vinyl polymers synthesized by chain-growth polymerization [31].

The rate at which the molecular weight of chain-growth polymerization increase depends on three rates: the rate of initiation, propagation, and termination (\(R_i, R_p, R_t\)). In many cases, once initiated, the polymerization proceeds very quickly and the propagation chain end “lives” for only a short time before terminating (Table 1.3). Usually, the propagation spices forms a high polymer during this time. The most noticeable feature in this polymerization (\(R_p \gg R_i\)) is that high polymer forms very quickly, and advantage if the polymer is insoluble and precipitates from the reaction solution quickly as well.
Table 1.3 Modes of polymerization [31].

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Molecular weight control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\bar{M}_n = \bar{X}_n \times \text{Mol. Wt. Monomer}$</td>
</tr>
</tbody>
</table>

**Step growth**

- $\bar{X}_n = \frac{1}{1-\rho}$; $\rho = \%$ functional group reacted/100

**Chain growth**

- $\bar{X}_n = \frac{1}{1-\rho}$
- $\rho = \frac{R_p}{R_p + R_t + R_{ct}}$

**Living**

- $X_n = \frac{\text{moles monomer consumed}}{\text{moles of initiator}}$
- $R_i >> R_p ; R_i = 0$

---

* $M_n =$ Number Average Molecular Weight, $X_n =$ Degree of Polymerization
Not much control over molecular weight is available in this scenario. However, if \( R_i \gg R_p \), all the chains will initiate at about the same time and, if there is no chain termination, the molecules will continue to grow until all the monomer is depleted. At this time, since the propagating species is still active at the end of the polymer, a second monomer may be added which will then polymerize to give a polymer containing two “block” (a block copolymer). This special case, referred to as a “living polymerization”, allows the ultimate control over molecular weight and provides the most narrow molecular weight distribution available. A common example is the living anionic polymerization of styrene using alkyl lithium reagents (RLi).

1.2.2.3 Ring-opening polymerization [31]

Ring-opening polymerization can follow either a step-growth or chain growth mechanism and involves the breaking of bond in a ring to form an open-chain intermediate (Figure 1.5). In step-growth, these intermediates can react with each other to form polymer. In chain-growth, typically a few monomers ring-open initially, either with or without an initiator, to form a propagation species which then reacts with another monomer, opening it to form a new propagating species. Several living ring-opening polymerization are known.

![Figure 1.5 General schemes for a ring-opening polymerization [31].](image)
1.2.2.4 Electrochemical polymerization [36, 41, 70, 71]

Electrochemical preparation of CPs dates back to 1968 when ‘‘pyrrole black’’ was formed as a precipitate on a platinum electrode by exposing an aqueous solution of pyrrole and sulfuric acid to an oxidative potential. Today, electrochemical polymerization is performed using a three-electrode configuration (working, counter, and reference electrodes) in a solution of the monomer, appropriate solvent, and electrolyte (dopant) (Figure 1.6).

Current is passed through the solution and electrodeposition occurs at the positively charged working electrode or anode. Monomers at the working electrode surface undergo oxidation to form radical cations that react with other monomers or radical cations, forming insoluble polymer chains on the electrode surface is shown in

![Image of Electrochemical Synthesis](image-url)
Figure 1.7. A number of important variables must be considered, including deposition time and temperature, solvent system (water content), electrolyte, electrode system, and deposition charge. Each of these parameters has an effect on film morphology (thickness and topography), mechanics, and conductivity, which are properties that directly impact the utility of the material for biomedical applications. For example, a non-protic, non-nucleophilic solvent generates stronger and more conductive CPs because protic solvents, like nucleophilic solvents, can generate side reactions with the growing CP chain, limiting and disrupting chain growth.

**Figure 1.7** Mechanism for heterocycle polymerization via electrochemical synthesis. $X= \text{NH, S, or O}$. This pathway is initiated by the oxidation of a monomer at the working electrode to give a cation species, which can react with a neutral monomer species or radical cation oligomeric species to generate the polymer [36].
Electrochemical polymerization techniques are divided into controlled current and controlled potential in the experiments. In all electrochemical polymerizations, the usage of a potential is adequate for control in the electrochemistry. In simplified systems, such as a galvanostatic polymerization with two electrodes (no reference electrode), a current source can be used, though the lack of reference electrode will limit the understanding of the potential at the electrodes and its relation to the oxidation of the monomer. Potentiostats may also be constructed from simple circuit elements, though stand-alone instruments are quite common. Further customization can be obtained with a computer controlled data acquisition card and an analog controlled potentiostat.

1.2.2.4.1 Potentiostatic polymerization [41]

The potentiostatic polymerization offers a logical progression from the chemical polymerization in which an oxidizing agent of fixed strength is used. In a potentiostatic polymerization, the potential is fixed versus time and the current is measured. A likely scenario would have the potential fixed just at the oxidation potential of the monomer, mimicking the role of an oxidizing agent. This would generate oxidized monomer species available for coupling at the working electrode surface. As the monomer species oxidizes and reacts, more monomer diffuses toward the electrode from the bulk solution. In an electrochemical system, transport occurs by convection, migration, and diffusion. For an unstirred bath, the effects of convection can be neglected, especially as short time scales. The addition of an electrolyte in solution, typically an alkyl ammonium salt of hexafluorophosphate or tetrafluoroborate, overwhelms the concentration of oxidized or reduced reactive
species in solution and supports electrical current through the cell via migration. Diffusion is the key driving force affecting monomer concentrations near the interface during electrochemical oxidation.

At the working electrode surface monomer and dimmers continue to oxidize and react, forming tetramers and octamers and so on. In general, the additional conjugation of the oligomers stabilizes the oxidized form and lowers the oxidation potential. Thus at a potential sufficient for monomer oxidation, the oligomers will also oxidize, though they may react at a different rate. Once the polymer has a high enough molecular weight to affect solubility, it precipitates on to the electrode surface. Long polymerization times, however, may lead to trials of polymer or wisps of oligomer in solution away from the electrode, which can discolor the solution. This demonstrates the balance of solubility and transport for oligomers of increasing molecular weight. It is unclear whether the polymer coating the electrode continues to react with monomer and oligomer species in solution or with other polymer species in the bulk of the film. Unlike the chemical polymerization, this heterogeneous reaction is not limited by concentration as the bulk solution continually refreshes the monomer concentration near the electrode, provided sufficient monomer is available in the bath. Careful concentration of the bath and electrode sizes will maintain this condition, ensuring an even polymerization during the course of the experiment.

One additional feature of the electrochemical polymerization is the incorporation of anions into the film during polymerization. As the polymer is deposited on the electrode it maintains the potential of the electrode. This leads to the presence of positive charge along the backbone of the polymer and subsequent interaction with the counterion also attracted to the working electrode. This charge
incorporation is the equivalent of electrochemical doping, causing the electrochemically deposited polymer to be synthesized in a conductive rate. The advantage of synthesizing a material in the conductive state is that further electrochemistry is straightforward and does not require reoxidation of the polymer.

The measurement of an electrochemical polymerization is based on the current that is passed during the experiment. Each monomer or oligomer oxidation requires the removal of one electron from the working electrode. Thus, each coupling reaction requires two electrons and the reaction rate is directly to the current by Faraday’s law which states the equivalency of charge and reaction consumed as 96485.4 C/mol, or Faraday’s constant $F$. From this law, the rate equation is then described by

$$rate (mol \ s^{-1} m^{-2}) = \frac{i}{nFA} \quad (1.2)$$

Where $i$ is the current in the cell, $n$ is stoichiometric number of electrons consumed per reaction, $A$ is the electrode area. For the polymerization reaction, each monomer is oxidized twice, which sets $n=2$.

In addition to the current consumed by the polymerization reaction, the current required to dope the polymer is also passed through the electrochemical cell. With a typical dopant concentration between 0.25 and 0.33 for the oxidized polymer, it is estimated that charge is accumulated on the backbone at nearly one positive charge per three or four monomer units, increasing $n$ in the Equation 1.2 to 2.25 or 2.33. Other current in the electrochemical polymerization can be attributed to the initial charging of the electrode surface (which acts primarily as a capacitor) and the
leakage current that occur without reaction of monomer or polymer. Taking the charging current to be an initial and fast response (capacitances are on the order of 0.1 to 0.4 F/m$^2$) and the leakage current to be small relative to the polymerization current, the steady state current reached in a potentiostatic polymerization indicates the deposition rate of polymer at the electrode. The potential chosen for polymerization should be sufficient for monomer oxidation but not significantly higher for fear of over-oxidation and degradation of the polymer or solvent.

1.2.2.4.2 Galvanostatic polymerization [41]

Galvanostatic polymerization requires the application of a constant current to the electrochemical system, forcing oxidation and polymerization at the working electrode. One distinct advantage of the galvanostatic polymerization is the direct relationship between time and thickness of polymer on the electrode. Again assuming negligible effects from initial charging of the interface and leakage current in the cell, a constant current produces a linear increase in charge with time. A second advantage of the galvanostatic polymerization is the flexibility of potential versus time to accommodate charges and the solution concentration or passivation of the electrode surface. As polymer is deposited on the electrode, it is in the oxidized conductive state. The polymer possesses a finite conductivity, however, and at sufficient thicknesses the resistive drop through the thickness of the film may lower the potential at the surface sufficiently for the cessation of monomer oxidation. In a potentiostatic polymerization, this passivation leads to the end of the polymerization whereas in a galvanostatic system, the feedback required by current control causes the potential at the electrode surface to rise until the current density is restored. This makes
galvanostatic polymerization techniques superior to potentiostatic techniques for the growth of the thicker films, especially with less conductive materials.

The increase in potential versus time as is often seen in galvanostatic polymerizations may also lead to degradation of the polymer or reaction with solvent or impurities as the galvanostatic technique does not offer the same selectivity. Once the monomer concentration is exhausted, the potential will increase to accommodate a different reaction. The potentiostatic polymerization will stop in the absence of monomer. This elucidates an often-overlooked relationship in the galvanostatic polymerization design between the surface areas of the working electrode and the volume of solution. It is imperative that the solution contains sufficient monomer for complete polymerization on the electrode to a desired thickness. The effect of the polymerization on the bulk concentration should be negligible if the polymer is to be deposited uniformly. Taxing a system with a large working electrode and small volume will quickly exhaust the monomer supply and over-oxidize the polymer on the electrode, further inviting additional unwanted reactions.

1.2.2.4.3 Potentiodynamic polymerization [41]

Potentiodynamic conditions use a potential waveform that varies with time. Most often, this method employs a triangle wave in potential as is used in cyclic voltammetry. Cyclic voltammetry measures the oxidation and reduction of monomer, polymer, or a reference standard such as ferrocene. While small molecules often exhibit one or two oxidation and reduction peaks, polymers exhibit broad oxidation waves and reduction waves. During polymerization, the oxidation step is followed by a chemical coupling rather than a reduction, so each corresponding oxidation peak is
not balanced by a reduction peak. Polymer that is deposited on the electrode however, can also be reversibly oxidized and reduced. Thus the complex polymerization voltammogram exhibits large oxidation peaks superposed over a broad polymer oxidation and a corresponding polymer reduction peak. During the course of a polymerization, polymer accumulates on the electrode and the current magnitude corresponding to oxidation and reduction also increase. Therefore, an increase in current magnitude with each cycle is generally thought to be sufficient evidence for polymerization at the electrode. A sample polymerization voltammogram is presented in Figure 1.8, highlighting these observations.

![Polymerization Voltammogram](image)

**Figure 1.8** Potentiodynamic polymerization of 1.0 mM EDOT monomer in a solution containing 0.1M tetrabutylammonium hexafluorophosphate (TBAPF₆) in dichloromethane at scan rate of 50 mV/s. The first potential cycle is shown in red [41].

1.2.2.4.4 Galvanodynamic polymerization [41]
The limitations of potentiodynamic methods are overcome with a time varying current waveform, termed galvanodynamics. In formulation a galvanodynamic waveform, it is desirable to retain all of the advantages of the techniques described above. Specifically, it is useful to obtain a polymerization that is steady in time and does not require periodic increasing of the potential window to correct for electrode passivation.

The second objective in improving the current controlled polymerization is the periodic of the film that the improved proton elimination steps in the polymerization. The current controlled analog of the potentiodynamic sweep is not sufficient to meet these conditions. Even if the current magnitudes were kept low enough not to exhaust the monomer concentration, eventually enough polymers would be on the electrode to simply charge and discharge with the cycled current. In order to sustain polymerization, there must be more oxidation than reduction over the course of the experiment to account for the oxidation of addition monomer.

Combining the two objectives, a brief mode of constant current coupled with the swept current that charge and polymer are accumulated on the electrode over time. The shape of such a waveform is shown in Figure 1.9. The potential response to such a waveform, also shown in Figure 1.9, for a few cycles, is a combination of the galvanostatic response with a higher frequency oscillation superposed.
The oscillation in current allows for reduction and oxidation of the polymer during a polymerization. Furthermore, this technique has been successfully used to grow films of novel monomers, such as the one depicted in Figure 1.9, when no other technique listed above would work. The development of this polymerization technique demonstrates the combination of two different electrochemical techniques used in polymerization. The combination of constant current and swept current represents a new way to approach polymerization and one that highlights the availability of new techniques. The parameter space for electrochemical polymerization is already wide enough without changing the nature of the waveform, though selective waveform generation may enable the successful synthesis of numerous novels conducting polymer system.

**Figure 1.9** Galvanostatic deposition waveform and potential response [41].
1.3 Electrochemical setup [72–76]

An electrochemical cell must consist of at least two electrodes and one electrolyte. An electrode may be considered to be an interface at which the mechanism of charge transfer changes between electronic (movement of electrons) and ionic movement of ions. An electrolyte is a medium through which charge transfer can take place by the movement of ions. An electrochemical cell is containing a working electrode, a counter electrode, and a reference electrode. In all electrochemical experiments, the reactions occur at the surface of the working electrode. Therefore, in controlling the potential drop across the interface between the surface of the working electrode and the solution (i.e., the interfacial potential) are interested. However, it is impossible to control or measure this interfacial potential without placing another electrode in the solution. Thus, two interfacial potentials must be considered, neither of which can be measured independently. Hence, one requirement for this counter electrode is that its interfacial potential remains constant, so that any changes in the cell potential produce identical changes in the working electrode interfacial potential.

An electrode whose potential does not vary with current is referred to an ideal non-polarizable electrode, and is characterized by a vertical region on a current versus potential plot. However, there is no electrode that behaves in this way (although some approach ideal non-polarizable behavior at low currents). Consequently, the interfacial potential of the counter electrode in the two-electrode system discussed above varies as current is passed through the cell. This problem is overcome by using a three-electrode system as shown in Figure 1.10, in which the functions of the counter electrode are divided between the reference and auxiliary
electrodes; that is, the potential between the working and reference electrodes is controlled and the current passes between the working and auxiliary electrodes. The current passing through the reference electrode is further diminished by using a high-input-impedance operational amplifier for the reference electrode input. A current may flow between the working and counter electrodes, while the potential of the working electrode is measured against the reference electrode. This setup can be used in basic research to investigate the kinetics and mechanism of the electrode reaction occurring on the working electrode surface, or in electroanalytical applications.

Figure 1.10 Electropolymerization setup [76].
1.3.1 Working electrode or indicator electrode [73–76]

This is the electrode at which the electrochemical phenomena being investigated takes place. There are a number of noble metal electrodes currently available for voltammetric studies. The frequently use electrodes are platinum, gold and silver followed occasionally by palladium, rhodium and iridium. Various polycrystalline forms including sheets, rods and wires are commercially available in high purity and the materials are readily machined into useful shapes. All of the noble metals have an over potential for hydrogen evolution. The noble metal electrodes adsorb hydrogen on their surfaces although gold does so to a lesser extent. Palladium adsorbs hydrogen into the bulk metal in appreciable quantities and is not recommended for use as a cathode in protic solvents. As an inert electrode material, carbon is useful for both oxidation and reduction reaction either aqueous or non aqueous solutions. Only graphitic forms of carbon are therefore useful as electrode materials. Ordinary spectroscopic grade of graphite rods can be used for work in which the surface area of the electrode does not need to be well defined. Other types of carbon electrode include the vitreous (glassy) carbon electrode and the carbon paste electrode.

1.3.2 Counter or auxiliary electrode [73–76]

This electrode which serves as a source or sink for electrons so that current can be passed from the external circuit through the cell. In general, neither its true potential nor current is ever measured or known. That is used only to make an electrical connection to the electrolyte so that a current can be applied to the working electrode. The processes occurring on the counter electrode are not important; it is
usually made of inert materials (noble metals or carbon/graphite) to avoid its dissolution. This is the case for cells used for research or for electroanalytical purposes. Of course, for many practically used cells, the processes occurring on both electrodes can be very important and also called “auxiliary” electrode.

1.3.3 Reference electrodes [63–76]

This is the electrode whose potential is constant enough that it can be taken as the reference standard against the potentials of the other electrodes present in the cell can be determined. The ideal reference electrode should posses the following properties:

- it should be reversible and obey the Nernst equation with respect to some species in the electrolyte
- its potential should be stable with time
- its potential should return to the equilibrium potential after small currents are passed through the electrode
- if it is an electrode like the Ag/AgCl reference electrode, the solid phase must not be appreciably soluble in the electrolyte
- it should show low hysteresis with temperature cycling

1.3.3.1 Silver/silver chloride reference electrode

The redox process for this electrode is

\[ \text{AgCl} + e^- \rightarrow \text{Ag} + \text{Cl}^- \]  

(1.3)
This electrode consists of a silver wire, coated with silver chloride, which is immersed in a solution containing chloride ions. This electrode uses an aqueous solution containing 3M sodium, a porous frit is used for the junction between the reference electrode solution and the sample solution.

The potential $E$ for any electrode is determined by the Nernst equation, which relates $E$ to the standard potential $E^\circ$ and the activities of the redox components (the standard potential is the potential of the electrode at unit activity under standard conditions). The Nernst equation for the silver/silver chloride electrode is

$$E = E^\circ + \frac{RT}{nF} \ln \frac{1}{a_{Cl^-}}$$

(1.4)

(The activities of the solid silver and silver chloride under standard conditions are unities).

1.3.3.2 Saturated calomel reference electrode

The redox process for this electrode is

$$\text{Hg}_2\text{Cl}_2 + 2e^- \rightarrow 2\text{Hg} + 2\text{Cl}^-$$

(1.5)

The saturated calomel electrode (SCE) is an H-cell. One arm contains mercury covered by a layer of mercury (II) chloride (calomel). This is in contact with a saturated solution of potassium chloride; a porous frit is again used for the junction between the reference electrode solution and the sample solution at the end of the
other arm. Once assembled, the electrode should be stored with porous frit and immersed in a saturated solution of potassium chloride to maintain the chloride concentration in the reference electrode.

### 1.3.3.3 Pseudo–reference electrode

Pseudo-reference electrodes are simply metal wires (e.g., platinum or silver) immersed in the sample solution. Although such electrodes do provide a constant potential, the reference potential is unknown, and is dependent on the composition of the sample solution. Consequently, redox potentials measured using a pseudo-reference electrode should be quoted relative to redox potential of the internal reference compound. One advantage of pseudo-reference electrodes is its low impedance.

### 1.3.3.4 Silver/silver ion electrode

The redox process for this electrode is

\[
\text{Ag}^+ + e^- \rightarrow \text{Ag}
\]

This electrode is less stable than the aqueous electrodes discussed above (due to diffusion of silver ions out of the electrode and the photo reactivity of these ions), and must be prepared frequently. Bioanalytical system, inc. (BASI) provides a non-aqueous reference electrode kit, which requires assembly by the user. The BASI non-aqueous reference electrode consists of a silver wire immersed in a solution of silver nitrate or perchlorate (0.001M to 0.01M) and electrolyte (e.g., 0.1M tetrabutyl...
ammonium perchlorate, TBAP) in the desired organic solvent. Suitable organic solvents include acetonitrile, dimethylsulfoxide, methanol, ethanol and tetrahydrofuran. Silver ions are reduced by dimethylformamide and are insoluble in methylene chloride; these solvents are therefore not suitable for this reference electrode (acetonitrile can be used as the reference electrode solvent when one of these other two solvents is used for the sample solution). The potential for the silver/silver ion reference electrode depends on the solvent, the silver ion concentration the nature and concentration of the electrolyte. It is also changed by the introduction of salt bridges, which are used to decrease the contamination of the sample solution by the effect of silver ions.

1.3.4 Electrolyte solutions [62–73]

The medium is required for electrochemical experiment is electrolyte solutions which must be able to conduct the current. This can be achieved by using either a molten salt or an electrolyte solution. An electrolyte solution is made by adding an ionic salt to an appropriate solvent. The salt must be fully dissociated in the solvent in order to generate a conducting (i.e., ionic) solution. The electrolyte solution must also be able to dissolve the analyte, an electrochemically inert over a wide potential range (i.e., no current due to electrolyte solution oxidation/reduction), and must be pure (e.g., the presence of water decreases the size of the potential range). It is chemically inert, so that it will not react with any reactive species generated in the experiment (e.g., acetonitrile is nucleophilic, which can react with electrogene

cations). If the temperature is to be varied, the electrolyte solution must have an appropriate solubility range.
Electrolyte solutions can be aqueous or non-aqueous. A wide range of salts can be used for aqueous electrolyte solutions. Since the redox potentials of some compounds are pH sensitive, buffered solutions should be used for these compounds. Suitable non-aqueous solvents include acetonitrile, DMF, DMSO, THF, methylenechloride, and propylene carbonate. Salts for non-aqueous electrolyte solutions typically consist of a large cation (e.g., tetraalkylammonium cations), and large anions (e.g., hexafluorophosphate, tetrafluoroborate, and perchlorate) to ensure a full dissociation. Perchlorate salts should be handled with care, since they are potentially explosive.

1.4 Cyclic voltametry (CV) [76–79]

Cyclic voltammetry (CV) is an electrolytic method that uses microelectrodes and an unstirred solution so that the measured current is limited by analyte diffusion at the electrode surface. The electrode potential is ramped linearly to a more negative potential, and then ramped in reverse back to the starting voltage. The forward scan produces a current peak for any analyses that can be reduced through the range of the potential scan. The current will increase as the potential reaches the reduction potential of the analyst, but then falls off as the concentration of the analyte is depleted close to the electrode surface. As the applied potential is reversed, it will reach a potential that will re-oxidize the product formed in the first reduction reaction, and produce a current of reverse polarity from the forward scan. This oxidation peak will usually have a similar shape to the reduction peak. The peak current, \( i_p \), is described by the Randles-Sevcik equation:
\[ i_p = (2.69 \times 10^5)n^{3/2}A\ C\ D^{1/2}V^{1/2} \]  

(1.7)

where \( n \) is the number of moles of electrons transferred in the reaction

\( A \) is the surface area of the electrode

\( C \) is the analyte concentration (in mole/cm\(^3\))

\( D \) is the diffusion coefficient

\( V \) is the scan rate of the applied potential

The potential difference between the reduction and oxidation peaks is theoretically 59 mV for a reversible reaction. In practice, the difference is typically 70-100 mV. Larger differences, or non-symmetric reduction and oxidation peaks are an indication of a nonreversible reaction.

1.4.1 A cyclic voltametry primer [76, 78]

A simple potential waveform that is often used in electrochemical experiment is the linear waveform i.e., the potential is continuously changed as a linear function of time. The rate of change of potential with time is referred to as the scan rate (\( v \)).

The simplest technique that uses this waveform is linear sweep voltammetry. The potential range is scanned in one direction, starting at the initial potential and finishing at the final potential. A more commonly used variation of the technique is cyclic voltammetry, in which the direction of the potential is reversed at the end of the first scan. Thus, the waveform is usually of the form of an isosceles triangle. This has the advantage that the product of the electron transfer reaction that occurred in the
forward scan can be probed again in the reverse scan. In addition, it is a powerful tool for the determination of formal redox potentials, detection of chemical reactions that precede or follow the electrochemical reaction and evaluation of electron transfer kinetics.

An example wave form that can be used in cyclic voltammetry is shown in Figure 1.11 [72]. In this example it is assumed that only the reduced form of the species is initially present. Thus, a positive potential scan is chosen for the first half cycle during which an anodic current is observed. The reason by, the solution is quiescent; the product generated during the forward scan is available at the surface of the electrode for the reverse scan resulting in a cathodic current.

**Figure 1.11** Normal wave form of cyclic voltammetry [76].

Complex wave form composed of two isosceles triangles. The voltage is first held at the initial potential where no electrolysis occurs and hence no faradaic current flows. As the voltage is scanned in the positive direction, so the reduced compound is oxidized at the electrode surface. At a particular set value, the scan
direction is reversed and the material that was oxidized in the outward excursion is then reduced. Once the voltage is returned to the initial value, the experiment can be terminated. In this example however a further voltage excursion takes place to more negative (more reducing) values. This may be useful in probing for other species present in the sample or for investigating any electroactive products formed as a result of the first voltage excursion.

The basic shape of the current response for a cyclic voltammetry experiment is shown in Figure 1.12 [77].

![Figure 1.12](image_url)
At the start of the experiment, the bulk solution contains only the reduced form (R) of the redox couple so that at potentials lower than the redox potential, i.e. the initial potential, there is no net conversion of R into the oxidized form (O) at point A. As the redox potential is approached, there is a net anodic current which increases exponentially with potential. As R is converted into O, concentration gradients are set up for both R and O, and diffusion occurs down these concentration gradients. At the anodic peak (point B), the redox potential is sufficiently positive that any R that reaches the electrode surface is instantaneously oxidized to O. Therefore, the current now depends upon the rate of mass transfer to the electrode surface and so the time dependence is \( q_t \) resulting in an asymmetric peak shape. Upon reversal of the scan (point C), the current continues to decay with a \( q_t \) until the potential nears the redox potential. At this point, a net reduction of O to R occurs which causes a cathodic current which eventually produces a peak shaped response (point D). The situation is very different when the redox reaction is not reversible, when chemical reactions are coupled to the redox process or when adsorption of either reactants or products occurs. In fact, it is these "non-ideal" situations which are usually of greatest chemical interest and for which the diagnostic properties of cyclic voltammetry are particularly suited.

1.4.2 Mechanistic complication [76–77]

1.4.2.1 Nernstian (reversible) behavior

If a redox system remains in equilibrium throughout the potential scan, the electrochemical reaction is said to be reversible. In other words, equilibrium requires that the surface concentrations of O and R are maintained at the values required by the
Nernst Equation. Under these conditions, the following parameters characterize the cyclic voltammogram of the redox process.

- The peak potential separation ($E_{pa} - E_{pc}$) is equal to $57/n \text{ mV}$ for all scan rates where $n$ is the number of electron equivalents transferred during the redox process.
- The peak width is equal to $28.5/n \text{ mV}$ for all scan rates.
- The peak current ratio ($i_{pa}/i_{pc}$) is equal to 1 for all scan rates.
- The peak current function increases linearly as a function of the square root of $v$.

The system under investigation is a simple 1 electron reversible couple so under the conditions of the experiment, the above parameters are observed. Cyclic voltammograms for ferrocene carboxylic acid in an aqueous pH 7.0 phosphate buffer electrolyte showing typical Nernstian (reversible) behavior as show in Figure 1.13 [77].
As the voltage becomes more positive (oxidizing) value is reached where ferrocene carboxylic acid (reduced form) is converted to the oxidized ferricinium species. This results in the appearance of the anodic peak. Assuming that the reaction kinetics are very fast compared to the scan rate, the equilibrium involving the concentrations of reduced and oxidized species at the electrode surface will adjust rapidly according to the Nernst equation:

$$E = E^0 + \left(\frac{RT}{nF}\right) \ln \frac{C_0}{C_R}$$  \hspace{1cm} (1.8)
where \( C_0 \) and \( C_R \) represent the surface concentration of oxidized and reduced species, respectively. If the system is diffusion controlled (the normal situation for cyclic voltammetry) then Fick's law of diffusion holds for both O and R. Under these conditions, the peak current \( (i_p) \) is given by the Randles Sevcik equation:

\[
i_p = (2.69 \times 10^5)n^{3/2} A D_0^{1/2} \nu^{1/2} C_0
\]  

where \( A \) is the electrode area (cm\(^2\)), \( n \) is the number of electrons transferred, \( D_0 \) is the diffusion coefficient, \( C_0 \) is the concentration (mol.cm\(^{-3}\)) and \( \nu \) is the scan rate (volt/s).

1.4.2.2 The electrochemical chemical (EC) mechanism [52, 63]

The shape of a voltammogram can be significantly altered if there is a coupled chemical reaction either before or after the electrochemical process. Further complications attributed to the chemical nature of the reaction, the degree of reversibility, the rate and equilibrium constants of the process can all play a part in the final shape of the voltammogram and on the information that can be obtained from a set of experiments. In general terms, coupled mechanistic schemes are described by the letters E (electrochemical) and C (chemical). The order in which they are written denotes the order in which the processes occur. Thus an \( ECE \) mechanism describes a process in which an electrochemical step is followed by a chemical step which is then followed by an electrochemical step. A chemical step is a step where no electron-transfer to or from the electrode takes place. Such a step does not by itself produce a charge flow into or out of the electrode and thus is not directly observable by an
external measuring circuit. It may however influence charge flow because of other steps in the mechanism which can be detected indirectly. The chemical step is not directly influenced by the electrode potential.

**Figure 1.14** The basic shape of cyclic voltammograms of EC mechanism with different first-order rate constant ($k_f$) [77].

An electrochemical step on the other hand involves electron flow to and from the electrode and as such produces a flow of charge that can be monitored by the external measuring circuit. The example in Figure 1.14 deals with an EC mechanism, i.e. an electrochemical step followed by a chemical one. The following experimental parameters were used to obtain the simulated voltammograms shown: electrode area = 0.1 cm$^2$, $k_{het}$ = 1 cm s$^{-1}$, $v$ = 1 volt s$^{-1}$, $E^\circ$ = 0.5 V and $D_0 = D_R = 1 \times 10^{-5}$ cm s$^{-1}$. 
Consider the following generalized mechanistic scheme, this shows a typical EC mechanism. In the first step (E), a reduced species is oxidized at the surface of an electrode. The product of the reaction O is unstable and once formed, reacts chemically (C) for example with itself, neighboring molecules or with the solvent to give a new species A which is either electro-inactive or simply not electroactive within the potential window of interest.

\[ R \rightarrow O + ne \text{ (E)} \]

\[ O \rightarrow A \text{ (C)} \]

An example of this type of mechanism is the electrochemical oxidation of ascorbic acid (vitamin C) and its subsequent reaction with water (the solvent) to yield electrochemically inactive dehydroascorbic acid (Figure 1.15).

![Chemical structure of ascorbic acid and dehydroascorbic acid](image)

**Figure 1.15** Electrochemical oxidation of ascorbic acid [77].
The electrochemical reaction is characterized by the heterogeneous rate constant $k_{\text{het}}$ which we can assume to be very fast. The chemical reaction is characterized by a homogeneous first order rate constant $k_f$ for which the equilibrium constant $K$ is equal to;

$$K = \frac{[A]_0}{[O]_0} \quad (1.10)$$

where the concentrations of A and O are surface concentrations. The resultant voltammograms for such a process would be similar to those depicted above. Close inspection of the diagram reveals that the forward scan (the oxidation of R to O) is unaffected but the reverse scan (O to R) is altered.

An important parameter in determining the shape of the voltammogram is the dimensionless ratio $k_f/s^{-1}$. Because the homogeneous step has a finite rate constant associated with it, there will be a limiting sweep rate ($s^{-1}$) which is fast enough to have completed the reverse scan before any conversion of O to A has taken place. Under these conditions, the voltammogram will not be altered in any way and the ratio of the two peak currents will be unity.

This feature can be best understood by looking at the simulated voltammograms shown in Figure 1.16. In this case, the voltammograms for an EC reaction are recorded at increasing scan rates (1 to 10 Vs$^{-1}$). It is evident, that as the scan rate is increased, the contribution from the homogeneous reaction becomes less pronounced and the voltammogram approaches the shape of that for a normal, uncomplicated situation. The following experimental parameters were used to obtain
the simulated voltammograms shown; electrode area = 0.1 cm$^2$, $k_{\text{het}} = 1 \text{cm s}^{-1}$, $E^\circ = 0.5$ V, $D_0 = D_R = 1 \times 10^{-5} \text{ cm s}^{-1}$ and $k_f = 10 \text{ s}^{-1}$.

**Figure 1.16** The basic shape of cyclic voltammograms of EC mechanism with different scan rates [77].

### 1.4.2.3 The electrochemical chemical electrochemical (ECE) mechanism

Another complex mechanism which is ideally suited for study by cyclic voltammetry is the ECE mechanism. As the acronym suggests, this is a mechanism which occurs in three stages. The first step is an electrochemical process which involves the transfer of electrons at the electrode surface. This gives rise to a product
which is inherently unstable under the conditions of the experiments and is then converted via a chemical reaction into a second species which is itself electrochemically active within the potential window used in the experiment. As the voltage is scanned back to the initial value, a new peak is detected in the voltammogram attributed to the electrochemical activity of the new species. Alternatively, the second electrode reaction may be observed in the forward scan only if the new species are "more difficult" to oxidize than the parent compound. The normal sequence of events in studying an ECE reaction by cyclic voltammetry is as follows:

- The initial electron transfer is carried out
- A suitable "time gate" allowing the chemical reaction to occur is provided (set by the position of the switching potential)
- The effects of the chemical reaction on the initial and subsequent electron transfers are studied electrochemically

The following experimental parameters were used in the simulation of the cyclic voltammograms, electrode area = 0.1 cm$^2$, $k_{\text{het}} = 1 \text{ cm s}^{-1}$, $k_f = 1 \text{ s}^{-1}$, $E^\circ_1 = 0.5 \text{ V}$, $E^\circ_2 = 0.25 \text{ V}$ and $D_0 = D_R = 1 \times 10^{-5} \text{ cm s}^{-1}$.

The diagram in Figure 1.17 shows a family of cyclic voltammograms for an ECE mechanism obtained at different scan speeds. In this example, oxidation of the initial compound leads to a species which is unstable and is converted into a product which is itself electroactive and can be reduced at potentials lower than 0.3 volts resulting in the appearance of the second reduction wave. A chemical scheme which follows this type of mechanism is shown in Figure 1.18.
Figure 1.17 The basic shape of cyclic voltammograms of ECE mechanism with different scan rates [77].

![ECE mechanism diagram](image)

**Figure 1.17** The basic shape of cyclic voltammograms of ECE mechanism with different scan rates [77].

-2e, -2H$^+$ + 2H$^+$, -2NH$_3$ -2e, -2H$^+$

Phenylene-diamine  Phynylene-diimne  Benzoquinone  Hydroquinone

**Figure 1.18** A chemical scheme which follows ECE mechanism [77].
In the example, 1,4-phenylenediamine (PDD) undergoes a simple two-electron oxidation to the diimine 1,4-phenylenediimine (PHI). If the pH is less than 3, PHI is unstable and is rapidly hydrolyzed to 1,4-benzoquinone (BQ). BQ is itself electroactive and can be reduced in a two-electron process to hydroquinone (HQ). Since all the compounds in the above scheme are electroactive, they can be detected by CV. The exact position of the peaks will be dependent upon the individual $E^\circ$ values of the redox species. The extent of the distortion will be dependent in part on the magnitude of the homogeneous rate constant $k_f$ and on the scan rate used in the experiment.

1.5 Applications of CPs [49, 60, 80–81]

Potential applications for CPs are numerous, since metals are toxic and can damage the environment. Moreover, CPs offer many advantages over other materials because of their unique optical, electrical and structural properties. Below are some potential applications for conducting polymers.

1.5.1 Polymeric batteries [49, 82]

One of the first applications of CPs, that was the focus of attention worldwide, was that of light-weight batteries. The general design for a battery is shown in Figure 1.19. Batteries have several key components: the electrodes allow for collection of current and transmission of power; the cathode material becomes reduced when the anode material is oxidized and vice versa; the electrolyte provides a physical separation between the cathode and the anode, and provides a source of cations and anions to balance the redox reactions. Aside from picking the best
conducting polymer available, there are many other issues, not related to conducting polymers, that affect battery performance, such as electrolyte stability and stability of the counter half-cell reaction (which is at least as important as the conducting polymer electrode), and compatibility between the electrolyte and the materials.

![General battery design](adapted from Elsevier Science resources) [82].

For example, batteries made using either polypyrrole or polyaniline as the positive electrode (cathode) and lithium–aluminium alloy as the negative electrode (anode) exhibited much more respectable properties. The electrolyte in these cases was either LiClO$_4$ or LiBF$_4$ in propylene carbonate (a highly polar aprotic solvent, which is also fairly resistant to oxidation). During the battery discharge, electrons move from the lithium alloy (which gets oxidized) to the polyaniline cathode (which gets reduced), as Li$^+$ from the anode and BF$_4^-$ from the cathode enter the electrolyte.
There was a great deal of initial excitement about CPs as active materials in batteries. Owing to their low density, it was thought that battery with power densities much higher than those of the ordinary lead/acid battery could be readily obtained. Since the charge on a polymer backbone is distributed over three or four repeat units, the charge capacity per unit of mass for CPs is marginally better than that of metals.

CPs still has a potential use in lithium-based high-power density batteries, which use the high potential difference between lithium and the polymer to achieve high power densities, although stability and shelf life are still the issues. As more and more individuals utilize cellular phones, laptop computers, and cordless drills, the importance of batteries that will handle many deep cycles (at least 60% depth of discharge) becomes increasingly apparent.

### 1.5.2 Electrochromic displays [82–84]

Electrochromic display is another interesting application which utilizes the electrochemical doping and undoping of conducting polymers. The basic idea, in such devices, is to effect a significant change in the color (both the wavelength of absorption and its intensity) upon application of an electric potential. Depending on the conducting polymer chosen, either the doped or undoped state can be essentially colorless or intensely colored. In general, the absorption of the doped state is dramatically red-shifted (moves to longer wavelength) from that of the undoped state. Because of their very high absorption coefficients (ca. 105 cm\(^{-1}\)) in the visible range of the electromagnetic spectrum, only very thin films are required to provide display devices with high contrast and a very broad viewing angle. Polyaniline, polypyrrole, polythiophene and their derivatives have been successfully used to prepare prototypes.
of such display devices. The structure of an electrochromic cell is shown in Figure 1.20.

Figure 1.20 The structure of an electrochromic cell [83].

Thus, while these materials are yet to achieve the set target for use as electrochromic displays, other interesting and innovative applications, such as electrochromic windows and other applications in the automotive industry are being actively pursued. Electrochromic windows, for instance, are windows in buildings/automobiles which can be made to go from low transmitting (during the day) to high transmitting (during the night); the switching in such systems occurs upon application of an electric potential.

1.5.3 Light-emitting diodes (LEDs) [63, 82, 85]

Other exciting phenomena, that have caught the imagination of both scientists and technologists alike, are the phenomena of photoluminescence and
electroluminescence in conjugated polymers. Emission of light upon irradiation is termed as photoluminescence, while the emission on application of a voltage is termed electroluminescence. Light emitting diode is an example of utilization of the latter phenomenon.

A general design for LEDs is shown in Figure 1.21. A simplistic overview of the function of an LED is as follows: an electron is injected into the polymer from the cathode while a hole is injected from the anode; there is an oxidized polymer on one side of the polymer film and a reduced polymer on the other side. The hole and the electron then migrate towards the center of the film and, when they meet each other, they recombine and give off light. The frequency of the light emitted is roughly equal to the difference between the oxidation and the reduction potential of the polymer (the electrochemical band gap) and, therefore, is related to the electronic band gap. Polymers with a different band gap have distinct values for the difference between oxidation and reduction potential, and emit different wavelength of light.

Several articles on conducting polymer LEDs and the effect of various additives, electrode modifications, tuning emission, the effect of impurities, and discussions of hole tunneling, photoexcitation, and unusual symmetric bias, have been published. The efficiency of LEDs is constantly being improved along with novel developments such as flexible LEDs, polarized light-emitting LEDs and light emitting electrochemical cells. The emission of red, green, blue and white light have all been demonstrated, and so has brightness of the order of 400 cd m$^{-1}$, which is similar in brightness to fluorescent lights or computer displays.
1.5.4 Biosensor [36, 80, 86–88]

A biosensor is an analytical device, which converts a biological response into an electrical signal. It consists of two main components: a bioreceptor or biorecognition element, which recognizes the target analyte and a transducer, for converting the recognition event into a measurable electrical signal. A bioreceptor can be a tissue, microorganisms, organelles, cells, enzymes, antibodies, nucleic acids and biomimic and the transduction may be optical, electrochemical, thermometric, piezoelectric, magnetic and micromechanical or combinations of one or more of the above techniques.

Figure 1.22 shows schematic diagram of a biosensor. The bioreceptor recognizes the target analyte and the corresponding biological responses are then converted into equivalent electrical signals by the transducer. The amplifier in the
biosensor responds to the small input signal from the transducer and delivers a large output signal that contains the essential waveform features of an input signal. The amplified signal is then processed by the signal processor where it can later be stored, displayed and analyzed. Biosensors have been widely applied to a variety of analytical problems in medicine, the environment, food, process industries, security, and defense.

**Figure 1.22** Schematic diagram of a biosensor [80].

### 1.5.4.1 Generations of biosensor [80]

Depending on the level of integration, biosensors can be divided into three generations, i.e., the method of attachment of the biorecognition element or the bioreceptor molecule to the base of the transducer element. The three generations of a biosensor are depicted in Figure 1.23.
Figure 1.23 The three generations of a biosensor [80].

In the first generation, the biorecognition element or the bioreceptor molecule is either bound to or entrapped in a membrane, which in turn is fixed on the surface of the transducer. The mediated or second-generation biosensors use specific mediators between the reaction and the transducer to improve sensitivity. It involves the adsorption or covalent fixation of the biologically active component to the transducer surface and permits the elimination of semi-permeable membrane. In the case of third-generation biosensors or direct biosensors, it is the direct binding of the bioreceptor molecule to the sensor element, and thus the bioreceptor molecule
becomes an integral part of the biosensor. So no normal product or mediator diffusion is directly involved in this. Conducting polymer-based biosensors come under this category.

1.5.4.2 Classifications of biosensor [80]

Biosensors can be classified by their bioreceptor or their transducer type. Figure 1.24 shows the biosensor classifications.

**Bioreceptors:** Classified into five different major categories. These categories include antibody/antigen, enzymes, nucleic acids/DNA/RNA, cellular structures/cells, and biomimetic. The enzymes, antibodies, and nucleic acids are the main classes of bioreceptors which are widely used in biosensor applications. Though the enzymes are one of the biorecognition elements, they are mostly used to function as labels than the actual bioreceptor.

**Transducers:** The transducer plays an important role in the detection process of a biosensor. In case of conducting polymer polymerbased biosensor, the conductive polymer acts as a transducer that converts the biological signal to an electrical signal. Biosensors can also be classified based upon the transduction methods they employ. Wide varieties of transduction methods have been developed in the past decade for the detection of foodborne pathogens.

Although there are new types of transducers constantly being developed for use in biosensors, the transduction methods such as optical, electrochemical, and mass based are given importance here since these are the most popular and common methods. Each of these three main classes contains many different subclasses and they can be further divided into label and label-free (nonlabeled) methods, where, the
labeled methods depend on the detection of a specific label (e.g., fluorescence) and the label-free detection is based on the direct measurement of a product developing during the biochemical reactions on a transducer surface.

**Figure 1.24** The biosensor classifications [80].

1.5.4.3 **Significance of conducting polymers to biosensors [80]**

Conducting polymers have been used as a transducer in biological sensors due to its attractive properties and their use in biosensors has grown over the past decade. Some of the attractive features of conducting polymers for biosensor applications include:

- Availability of varied range of monomer types.
- Availability synthetic analogues of monomers.
• Composites can be prepared combing conducting polymers with nonconducting polymers or with nonpolymer materials such as carbon, carbon nanotubes, metals, etc.
• It can be prepared both electrochemically and chemically.
• It can be prepared in a range of soluble and insoluble forms.
• It has unique electrical, electronic, magnetic and optical properties.
• Compliance with micro and nanoscale fabrication.
• Compatibility with diverse range of fabrication techniques such as electrochemical, optical, mass-based, etc.
• Biomaterials such as enzymes, antibodies, whole cells, and nucleic acids can be incorporated into the polymer matrix.
• Strong biomolecular interactions.
• Low detection limits.
• Enhanced sensitivity (when used as a composite material with nanoparticles).
• Reversible responses at ambient temperatures.
• Cost effectiveness.

1.5.4.4 CPs based biosensor [36]

The first biosensing device was created by integrating an enzyme into an electrode, and since that time, much progress has been made in monitoring and diagnosing metabolites (e.g., glucose, hormones, neurotransmitters, antibodies, antigens) for clinical purposes. A biosensor is composed of a sensing element (i.e., biomolecule) and a transducer. The sensing element interacts with the analyte of
interest producing a chemical signal that is transmitted to the transducer, which ultimately transforms the input into an electrical signal, is shown in Figure 1.25.

**Figure 1.25** Schematic of a biosensor. A biological sensing element (e.g., enzyme, antibody) detects a specific analyte producing a biochemical signal that is transferred to the transducer (e.g., conducting polymer), which ultimately produces a digital electronic signal that is proportional to the amount of analyte present [36].

CPs is extensively used as transducers that integrate the signals produced by biological sensing elements such as enzymes (Table 1.4). Depending on how the chemical signal is sensed and transmitted, biosensors can be divided into several categories: amperometric (measures current), potentiometric (measures potential), conductometric (measures change in conductivity), optical (measures light absorbance or emission), calorimetric (measures change in enthalpy), and piezoelectric (measures mechanical stress).
Table 1.4 Examples of biosensors using CPs [36].

<table>
<thead>
<tr>
<th>Analyte (sensing element)</th>
<th>Types of sensor</th>
<th>Polymers explored [Ref.]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (glucose oxidase)</td>
<td>Amperometric</td>
<td>PPy [89]</td>
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<tr>
<td></td>
<td>Potentiometric</td>
<td>PANI [90]</td>
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<td></td>
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<td>PT [91]</td>
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<tr>
<td>Cholesterol</td>
<td>Amperometric</td>
<td>PPy [92]</td>
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<tr>
<td>(cholesterol oxidase/esterase)</td>
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<td>PANI [93]</td>
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<tr>
<td>L-lactate</td>
<td>Amperometric</td>
<td>PPy [94]</td>
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<tr>
<td>(lactate oxidase/dehydrogenase)</td>
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<td>PANI [95]</td>
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<td>PT [96]</td>
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<tr>
<td>Urea (urease)</td>
<td>Amperometric</td>
<td>PPy [97]</td>
</tr>
<tr>
<td></td>
<td>Potentiometric</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Conductometric</td>
<td></td>
</tr>
<tr>
<td>DNA (DNA hybridization)</td>
<td>Amperometric</td>
<td>PPy [98]</td>
</tr>
<tr>
<td></td>
<td>Gravimetric</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PT [99]</td>
</tr>
</tbody>
</table>

The most common types of transducers are amperometric and potentiometric. An amperometric biosensor measures the current produced when a specific product is oxidized or reduced (e.g., redox reaction of a substrate in an enzyme) at a constant applied potential (Figure 1.26). The CP mediates the electron transfer (e.g., via hydrogen peroxide) between an enzyme, such as an oxidase or dehydrogenase, and the final electrode; however, the exact mechanism is not completely understood. Redox mediators such as ferrocene, viologen, Prussian Blue, or their derivatives are
used to improve electron transfer from the biochemical reaction to the CP and therefore improve sensor sensitivity and selectivity. These redox mediators can be entrapped, incorporated as dopants, or chemically conjugated to the monomer.

**Figure 1.26** Schematic of electron transfer in an amperometric biosensor. An enzyme catalyzes a redox reaction of a specific analyte, which results in the reduction of the conducting polymer (transducer) and the measurement of a current [36].

Potentiometric biosensors use ion-selective electrodes as physical transducers. For example, detection of urea by ureases is performed via the production of NH$_3$, which interacts with PPy to produce an electrical signal. This signal could be a product of a change in pH and the subsequent ion mobility in the polymer matrix triggered by an equilibration of the dopants with the free ions in solution.

A key aspect in biosensor applications is the integration of the electrical component (i.e., CPs) with the biological recognition components. The
immobilization of bioactive macromolecules in or on electrically conductive polymers has been extensively explored in an effort to provide intimate contact between these two elements. This section focuses on describing the different available techniques for the immobilization of biologically active molecules on CPs. For this immobilization, it is critical to maintain the activity of the molecules, increase stability, and ensure accessibility of the analyte to perform biological events such as hybridization of complementary oligonucleotides, antigen–antibody binding, or enzyme-catalyzed reactions.

Table 1.5 summarizes the main categories of immobilization techniques of biological sensing elements on CPs. Two main classes are distinguished: non-covalent and covalent modifications. Non-covalent modifications include adsorption, physical entrapment, and affinity binding. Covalent immobilization includes all techniques that create a covalent bond between the conducting substrate and the biomolecule via functional moieties.
Table 1.5 Immobilization techniques of biomolecules on CPs for biosensing devices [36].

<table>
<thead>
<tr>
<th>Immobilization technique</th>
<th>Principles of immobilization</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non-covalent techniques</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adsorption</td>
<td>Electrostatic forces, hydrogen bonding, Van der Waal’s forces, etc.</td>
<td>Simple</td>
<td>Biomolecule loss (desorption) over time, Limited control over immobilization, Random orientation on surface</td>
</tr>
<tr>
<td>Entrapment</td>
<td>Molecule incorporation during electropolymerization</td>
<td>Simple, Good proximity between elements</td>
<td>Potential loss of biomolecule activity, Steric and diffusion constraints, Requires high biomolecule concentration</td>
</tr>
<tr>
<td>Affinity binding</td>
<td>High affinity interactions such as avidin-biotin</td>
<td>Control over molecule orientation, High accessibility of analytes, Minimal loss of biomolecule activity</td>
<td>Requires pre-immobilization of one of the affinity molecules (e.g., biotin)</td>
</tr>
<tr>
<td><strong>Covalent techniques</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemical conjugation</td>
<td>Surface chemical reaction between functional groups</td>
<td>Tighter control over immobilization, High accessibility of analytes, Minimal biomolecule loss over time, Control over biomolecule orientation</td>
<td>Complex, Conditions are not always appropriate for biomolecules, Potential loss of biomolecule activity</td>
</tr>
</tbody>
</table>
1.6 **Surface plasmon resonance (SPR) spectroscopy** [1, 2, 13, 100–102]

The first documented observation of surface plasmons dates back to as early 1902, when R. W. Wood illuminated a metallic diffraction grating with polychromatic light and noticed narrow dark bands in the spectrum of the diffracted light, which he referred to as anomalies [13, 102]. This anomalous phenomenon, the excitation of electromagnetic surface waves on the surface of the diffraction grating was explained in terms of surface plasmon resonance (SPR) in 1968. In the same year, optical excitation of surface plasmon by attenuated total reflection (ATR) was reported by Otto, Kretschmann and Raether [1, 2, 13, 102]. In the late 1970s, SPR were first employed for the characterization of thin films and the study of processes at metal boundaries [102]. The application of SPR for gas detection and biosensing was later demonstrated in 1983 [13].

### 1.6.1 Physics of surface plasmons [13]

Surface plasmons are electromagnetic excitations in the form of charge density oscillations propagating at the interface between a dielectric and a metal, evanescently confined in the perpendicular direction (Figure 1.27), which are known in termed surface plasmon polaritons (SPPs) or surface plasma waves (SPWs).

Surface plasmons are longitudinal waves with magnetic vector perpendicular to the plane of incidence (i.e. transverse-magnetic (TM) or p-polarized) whose dispersion relation is described by:

\[
k_{SP} = \frac{\omega}{c} \left[ \frac{\varepsilon_m \varepsilon_d}{\varepsilon_m + \varepsilon_d} \right]^{1/2} \tag{1.11}
\]
where $k_{sp}$ is the propagation constant of the surface plasmons, $\omega$ is the angular frequency, $c$ is the speed of light in vacuum, and $\varepsilon_m$ and $\varepsilon_d$ are the dielectric constants of the metal and dielectric, respectively. The schematic illustration of surface Plasmon dispersion relation is shown in Figure 1.28 where $k_x$ is the component along the x-axis of the incident light wave-vector.

**Figure 1.27** Schematic illustrations of charge density oscillations at a metal-dielectric interface propagating in the x direction [13].

**Figure 1.28** Schematic illustration of surface plasmons dispersion relation (blue line). The corresponding light line in the dielectric is also shown (red line) [13].
Since $k_{SP}$ and $\varepsilon_m$ are a complex entity. Its real part is related to the effective refractive index of the surface plasmons while the imaginary part is associated with the attenuation of the surface plasmons in the direction of propagation.

1.6.2 Optical excitation of surface plasmons [13]

Surface plasmons can be optically excited with special light-plasmon couple conditions. Methods of optical excitation of surface plasmons include prism, grating, and waveguide coupling.

1.6.2.1 Prism coupling

The optical excitation of surface plasmons can be accomplished by passing the light wave through an optically denser medium in the attenuated total reflection (ATR) method. This can be increase the wave vector of the incident light must be increased larger than the wave vector of surface plasmons at a metal-dielectric interface to allow optical excitation of surface plasmons (Figure 1.29 (b)).

Figure 1.29 (a) shows the excitation of surface plasmons by ATR. A light wave passing through a high refractive index prism and totally reflected, for a specific angle, at the prism base generates an evanescent wave penetrating the thin metal film. If the thickness of the metal film is properly chosen, the evanescent wave can then tunnel through the metal film to excite surface plasmons at the other metal-dielectric interface. The resonance condition can be given by:

$$k_x = \frac{2\pi}{\lambda_0} n_p \sin \theta = Re\{k_{SP}\}$$  \hspace{1cm} (1.12)
where $k_x$ is the component along the x-axis of the incident light wave-vector, $\lambda_0$ is the wavelength in vacuum, $n_p$ is the prism refractive index, $\theta$ is the incident angle, and $\text{Re}\{\}$ corresponds to the real part.

The optical excitation of surface plasmons is accompanied by the transfer of the light wave energy into the surface plasmons and its subsequent dissipation in the metal film. This process results in a drop in the intensity of the reflected light.

Figure 1.29 (a) Excitation of surface plasmons by the attenuated total reflection (ATR) method. (b) The light line in the denser medium (green line) is tilted to the right relative to that in the dielectric (red dashed line) resulting in an increase of the incident light’s wave vector. For a given wavelength, resonance takes place at a specific angle of incidence when this light line intersects the dispersion curve of the surface plasmons (blue line) [13].
1.6.2.2 Grating coupling

The in-plane wave vector of the incident light wave impinging on a dielectric-metal interface can be increased to match that of the associated surface plasmons by a diffraction grating (Figure 1.30). The diffraction grating scatters the incident light wave and consequently modifies its $k_x$ by an integer multiple of the grating wave number $G$ depending on the diffraction order. The coupling condition when the $m$th diffraction is coupled to the surface plasmons can then be expressed by:

$$k_d = k_x + mG = \frac{2\pi}{\lambda_0} n_d \sin \theta + m \frac{2\pi}{\Lambda} = Re\{k_{SP}\}$$  \hspace{1cm} (1.13)

where $k_d$ is the propagation constant of the diffracted light, $G$ is the wavenumber of the grating, $m = 0, \pm 1, \pm 2, \ldots$ is the diffraction order, $\Lambda$ is the grating period, and $n_d$ is the dielectric refractive index. The detection in this case can be made for example by measuring the reflected intensity from a monochromatic light wave as a function of the angle of incidence (angular modulation).

1.6.2.3 Waveguide coupling

Surface plasmons can also be excited by guided modes of a dielectric waveguide (Figure 1.31). When a guided mode propagating along the dielectric waveguide enters the region covered by a thin metal film, its evanescent field can penetrate through the metal film. Resonance can then take place if the wavelength-dependent propagation constant of the guided mode matches that of the surface plasmons at the outer metal-dielectric boundary. As this phase-matching condition is
only satisfied for a narrow wavelength range, a dip in the transmitted spectrum can be observed.

**Figure 1.30** (a) Excitation of surface plasmons by the diffraction grating method. (b) Dispersion diagram illustrating the phase-matching condition. The original $x$ component of the wave vector of the incident photons $k_x$ is increased by $G$ through $m = +1$ diffraction order to match that of the surface plasmons [13].

**Figure 1.31** (a) Excitation of surface plasmons by waveguide coupling. Dark blue layer indicates substrate, gray layer indicates waveguide, yellow layer indicates metal film, light blue layer indicates dielectric. (b) Schematic illustration of the transmitted spectrum showing a resonance dip [13].
1.6.3 Theoretical of SPR [1, 100–101]

SPR is a technique which associated with the total internal reflection of light (evanescent wave) at the boundary between two media of different optical properties described by their different dielectric function, $\varepsilon_i$ [1]. The example of this observation is shown in Figure 1.32 (a) which is the boundary between a glass prism and water. A plane wave from a laser light source (wavelength $\lambda$) or incoming light impinging upon the interface from glass side, i.e. from material with higher refractive index, will be totally (internally) reflected if the angle of incidence exceeds a critical value, $\theta_c$. This can be observed by recording the reflectivity, $R$ (the ratio between reflected and incoming intensity) with a diode detector as a function of the angle of incidence, $\theta$. In typical experiment, at angles of incidence smaller than $\theta_c$, most of incoming light is transmitted and therefore the reflectivity is low. When the angle of incidence approaches $\theta_c$, the reflectivity reaches unity as shown in Figure 1.32 (b). The evanescent wave is an electromagnetic field which the electric field perpendicular to the interface ($E_Z$) does not fall to zero abruptly but decays exponentially with a decay length, $l$. This decay length is a function of the angle of incidence. On the other hand, the component along the propagation direction ($E_X$) had the usual oscillatory character of an electromagnetic mode. The evanescent wave is formed at the angle greater than critical angle. When the interface between a metal and a dielectric material is considered, the term “plasmon surface polaritons (PSP) or surface plasmons” for short will be described [1, 100]. The coupling of the collective plasma oscillations (called “plasmon”) of the nearly free electron gas in a metal to an electromagnetic field has been shown to produce the surface plasmon. This surface plasmon propagates at the metal/dielectric material with the coupling angle which can be excited with photons
when the energy and momentum matching conditions between photons and surface plasmons has reached [1, 100–101].

**Figure 1.32** (a) Total internal reflection of a plane wave at the base of glass prism in contact with dielectric medium (b) The curve showing reflectivity as a function of incident angle [1].
1.6.4 The architecture of experimental setup [100, 103–104]

The three different coupling schemes had been proposed among which are grating, edge, and prism [100, 103–104]. The different schemes by using prism have been widely used for many applications. In principle, there are two concepts for this experimental setup as shown in Figure 1.33: Otto-configuration and Kretschmann configuration. The latter one is the most widely used and convenient configuration because the resulting plasmon can be observed directly through the metal. In the Otto-configuration as shown in Figure 1.33 (a), photons are not coupled directly to the metal/dielectric interface, but via the evanescent tail of light totally internally reflected at the base of a high-index prism ($\varepsilon_p > \varepsilon_d$). By choosing the appropriate angle of incidence, resonant coupling between evanescent photons and surface plasmons can be obtained. This resonant coupling is observed by monitoring the laser light, which is reflected by the base of prism, as a function of the incident angle. However, since the major technical drawback of this configuration is the need to obtain the metal surface closes enough to the prism base, typically ~ 200 nm. This means even a few dust particles can be the spacers preventing the efficient coupling. As this drawback, the Otto-configuration has not gained any practical importance despite its potential importance for the optical analysis of polymer-coated bulk metal samples.
Figure 1.33 Two concept for experimental setup of surface plasmon resonance spectroscopy (a) The Otto-configuration (b) The Kretschmann configuration with attenuated total internal reflection (ATR) construction [1].
On the other hand, the experimentally easier and hence the most widespread configuration, Kretschmann configuration, as shown in Figure 1.33 (b) has the similar scheme for exciting surface plasmons to Otto-configuration. In Kretschmann configuration, photons in the prism couple through a very thin metal layer (typically ~ 45-50 nm thick), which is deposited directly onto the base of the prism or onto a glass slide, to surface plasmons at the other side in contact with the dielectric medium. In qualitative, the same consideration for energy and momentum matching are applied as discussed in Otto-configuration. Quantitatively, however, the finite thickness of the metal layer causes some modification of the dispersion behavior at surface plasmons. By solving Maxwell’s and/or Fresnel’s equations for the layer architecture of glass/Ag-layer/dielectric, the angular dependence of the reflectivity can be described. An example is shown in Figure 1.34 based on the known parameters $\varepsilon_p$, $\varepsilon_d$, $\varepsilon_m$ and the metal layer thickness, d. The curve as shown in Figure 1.34 is calculated by the assumption of a perfect interface with perfectly flat interfaces exhibiting no roughness or other imperfections, enhancement factors of more than 80 for the surface plasmon light compared to the incoupling laser light. The enhancement might be more moderate in the real experimental conditions, but still exists.
1.6.5 SPR for investigation of the adsorption processes [1, 101, 105–106]

SPR has been shown to be a technique which has high sensitivity for characterization of ultrathin film, interfaces, and kinetic processes at the nanometer scale [47, 77, 80–81]. The experimental SPR system for characterization of ultrathin films is shown in Figure 1.35(a) which is relatively simple. A laser beam of wavelength $\lambda$ incidents at angle $\theta$ on the noble-metal coated base of the prism, which is covered with the thin film of interest material, is reflected. The intensity of the reflected light is then monitored with a detector as function of $\theta$. A typical reflectivity curve is shown in Figure 1.35(b). The curve labeled a was taken in air on a bare Au-film evaporated-deposited onto the prism base. The deposition of an ultrathin organic layer of interest molecules which can be prepared by spontaneous self-assembly
process, LB technique, LbL deposition method or even simple technique; spin-coating, from solution to Au-surface results in a shift of the curve for SPR running along this modified interface and hence in a shift of the resonance angle (from $\theta$ to $\theta_1$) as shown in the curve labeled b.

\[ \theta_0 \]

\[ \theta_1 \]

\[ \theta \]

\[ \theta_0 \]

\[ \theta_1 \]

\[ \theta \]

**Figure 1.35** (a) Schematic of the experimental system for SPR (b) Reflectivity curve obtained from a bare Au-film and self-assembled monolayer [1].
The example for using of SPR to *in situ* investigation of the self-assembly polymer solution adsorption process is shown in Figure 1.36 was studied by Knoll group [106]. The experimental setup in this study is Kretchmann configuration with ATR condition. The monolayers of each interest materials were stepwisely deposited by LB process onto the high refractive index glass/Au/octadecyl-thiol layer. A sequence of reflectivity data taken after consecutive depositions showed the linear increases of the multilayer thickness after analysis with Fresnel equation.

![Schematic diagram of the experimental setup](image.png)

**Figure 1.36** Schematic diagrams showing the experimental setup for study the *in situ* self-assembly adsorption process [106].
In addition, the other information, which can be studied by using SPR, is the kinetic information on the interfacial of the multilayer. The kinetic information on any changes of the interfacial architecture is the time-dependant process which can be obtained by monitoring the reflectivity at a fixed angle of observation, \( \theta_{\text{obs}} \) (as shown in Figure 1.37 (a)) as a function of time. The time dependence of the adsorption process is shown in Figure 1.37 (b). At \( t=0 \), the solution is injected and the adsorption followed in real time as a change in reflectivity. The adsorption process for each layer is complete after several minutes, thus giving the important information for the subsequence of the alternating multilayers preparation.

![Figure 1.37](image)

**Figure 1.37** (a) Angular scans of SPR (b) The corresponding kinetic mode [106].
1.6.6 SPR-based optical sensor [13, 102]

The promising potential of SPR sensors lies in the very high sensitivity of surface plasmons excited at a metal-dielectric interface to a change in the refractive index of the dielectric. A change in the refractive index of the sensed medium (the dielectric) results in a change of the propagation constant of the surface plasmons. This change subsequently alters the resonance condition between the surface plasmons and the interacting optical wave. Based on the measured characteristics of the optical wave interacting with the surface plasmons, SPR sensors can be categorized as sensors with angular, wavelength, intensity, or phase modulation. In SPR sensors with angular modulation, a monochromatic light wave with a variable angle of incidence is used to excite surface plasmons. The excitation of surface plasmons is characterized by a dip in the angular spectrum of the reflected light at the angle of resonance. The shift of the angle of resonance is monitored as the refractive index change of the sensed medium (Figure 1.38); typically, this is the working scheme of configurations using prism coupling. In SPR sensors using wavelength modulation, a polychromatic optical wave is employed and the resonance wavelength corresponding to the surface plasmons excitation is monitored; this is, for example, the case for waveguide coupling configurations. Finally, intensity and phase shift of the interacting optical wave at fixed wavelength and angle of incidence is monitored in SPR sensors with intensity and phase modulation, respectively. Of these different modulation schemes, the angular and wavelength modulations are the most commonly employed in SPR sensors owing to their high resolution and relatively simple instrumentation. But intensity modulation can be also used, mainly in systems
devoted to multiple analyses, under the SPR-i (surface plasmon resonance imaging) acronym.

**Figure 1.38** Illustration of angular and wavelength modulation. The wavelength or angle of resonance shifts as the refractive index of the sensed dielectric changes from \( n \) (solid line) to \( n + \Delta n \) (dashed line) [13].

1.6.7 **SPR-based biosensor [13, 42, 53, 102]**

A SPR-based biosensor is made up of a SPR sensor and suitable surface functionalization acting as the biorecognition element (Figure 1.39). When a biomolecular interaction (e.g. specific binding of analytes) takes place, the refractive index near the surface is altered. This modification of refractive index can then be detected by the SPR sensor. As a SPR sensor and appropriate surface functionalization form the building blocks of a SPR biosensor, it is clear that the overall performance of a SPR biosensor depends on both the intrinsic optical
performance of the SPR sensor and the characteristics of the surface functionalization. In what follows, both of these factors are separately discussed.

![A SPR sensor equipped with suitable surface functionalization as biorecognition element is transformed into a SPR biosensor. Biological analytes, represented as green dots, are shown to interact with the biorecognition elements, represented as brown Y. The large blue arrow indicates the flow of the solution to be analyzed; practically this flow is generated by a microfluidic system [13].](image)

**Figure 1.39** A SPR sensor equipped with suitable surface functionalization as biorecognition element is transformed into a SPR biosensor. Biological analytes, represented as green dots, are shown to interact with the biorecognition elements, represented as brown Y. The large blue arrow indicates the flow of the solution to be analyzed; practically this flow is generated by a microfluidic system [13].

### 1.7 Electrochemical-surface plasmon resonance spectroscopy (EC-SPR) [1, 12, 21, 101, 106–107]

The combination of SPR, particularly in the Kretschmann configuration, with electrochemical measurements has become a powerful technique for simultaneous characterization and manipulation of an electrode/electrolyte interface. This combination has been known as *Electrochemical-Surface Plasmon Resonance Spectroscopy (EC-SPR)*. A scheme for EC-SPR setup is shown in Figure 1.40.
The gold substrate which carries the optical surface mode is simultaneously used as the working electrode in electrochemical experiments. One advantage of EC-SPR is that the electrochemical and optical properties can be obtained simultaneously during film forming on the nanometer thickness scale. Recently, EC-SPR was applied for characterization of a number of conducting polymer films including polyaniline and poly(3,4-ethylenedioxythiophene). The time-dependent processes could be induced by a potential sweep which the setup allows simultaneously record the reflectivity and the flow of charges through the electrical circuit, e.g. a classical cyclic voltammogram. The obtained data are the data as shown in Figure 1.37 with the
corresponding cyclic voltammogram. In addition, the EC-SPR technique had also been applied to many applications including biosensor development [42, 53, 108].

1.8 Electrochemical-transmission surface plasmon resonance spectroscopy (EC-TSPR) [109–111]

An optical measurement based on surface plasmon resonance spectroscopy as termed transmission surface plasmon resonance (TSPR) spectroscopy is the powerful technique for monitoring the molecular adsorption/desorption events at the metal film surface. The detection principle relies on the transmission spectra of p-polarized light are observed when light passing through at the thin gold film coated on a grating substrate under specific conditions. The interactions between biomolecules are measured as a change in the intensity of the transmission spectra. Moreover, TSPR spectroscopy is applicable to the measurements carried out either ex situ or in situ measurements. Moreover, TSPR spectroscopy combined with an EC method called EC-TSPR was considered for in situ investigation of the optical and electrochemical properties of CPs films and the biomolecular interactions. A scheme for EC-TSPR setup is shown in Figure 1.41. Recently, TSPR-enhanced optical transmission was actively controlled by an electrochromism of polyaniline and poly(3,4-ethylenedioxythiophene) thin films were deposited on a thin gold grating surface [109] and the detection of human IgG based on PP3C immunosensor was also studied by EC-TSPR spectroscopy [111].
1.9 UV–visible absorption spectroscopy [112–114]

Many molecules absorb ultraviolet or visible light. The absorbance of a solution increases as attenuation of the beam increases. Absorbance ($A$) is directly proportional to the path length, $b$, and the concentration, $c$, of the absorbing species. *Beer's Law* states that

$$A = \varepsilon bc,$$  \hspace{1cm} (1.14)

where $\varepsilon$ is a constant of proportionality, called the *absorbtivity*. In Beer's law, $\varepsilon$ is the most important sensitivity indicator. When $b$ is 1 cm and $A$ is plotted as a function of $c$, a straight line relationship is obtained as in Figure 1.42.

*Figure 1.41* Schematic of EC-TSPR setup [109].
**Figure 1.42** A straight line relationship between absorbance ($A$) and concentration ($C$). Molar absorptivity is the slope when $b$ equals to 1 cm. [114].

When the concentration is expressed in mol/L and is plotted on the X-axis, $\varepsilon$ is the slope and its value is an indication of the sensitivity of the method.

Different molecules absorb radiation of different wavelengths. An absorption spectrum will show a number of absorption bands corresponding to structural groups within the molecule. For example, the absorption that is observed in the UV region for the carbonyl group in acetone is of the same wavelength as the absorption from the carbonyl group in diethyl ketone.

### 1.9.1 Electronic transitions

The absorption of UV or visible radiation corresponds to the excitation of outer electrons. There are three types of electronic transition which can be considered;

1. Transitions involving p, s, and n electrons
2. Transitions involving charge-transfer electrons
3. Transitions involving $d$ and $f$ electrons (not covered in this Unit)
When an atom or molecule absorbs energy, electrons are promoted from their ground state to an excited state. In a molecule, the atoms can rotate and vibrate with respect to each other. These vibrations and rotations also have discrete energy levels, which can be considered as being packed on top of each electronic level. The electronic transition diagram is shown in Figure 1.43 [113].

Absorbing species containing p, s, and n electrons absorption of ultraviolet and visible radiation in organic molecules is restricted to certain functional groups (chromophores) that contain valence electrons of low excitation energy. The spectrum of a molecule containing these chromophores is complex. This is because the superposition of rotational and vibrational transitions on the electronic transitions gives a combination of overlapping lines. This appears as a continuous absorption band. Possible electronic transitions of p, s, and n electrons are shown in Figure 1.44 [113].

![Electronic transition diagram](image_url)

**Figure 1.43** Electronic transition diagram [113].
Figure 1.44 Type of transition state [113].

σ-σ* Transitions; An electron in a bonding s orbital is excited to the corresponding anti-bonding orbital. The energy required is large. For example, methane (which has only C-H bonds, and can only undergo σ-σ* transitions) shows an absorbance maximum at 125 nm. Absorption maxima due to σ-σ* transitions are not seen in typical UV-Visible spectra (200–700 nm).

n-σ* Transitions; saturated compounds containing atoms with lone pairs (nonbonding electrons) are capable of n-σ* transitions. These transitions usually need less energy than σ-σ* transitions. They can be initiated by light whose wavelength is in the range 150–250 nm. The number of organic functional groups with n-σ* peaks in the UV region is small.

n-π* and π-π* transitions; most absorption spectroscopy of organic compounds is based on transitions of n or π electrons to the π* excited state. This is because the absorption peaks for these transitions fall in an experimentally convenient region of the spectrum (200–700 nm). These transitions need an unsaturated group in the molecule to provide the p electrons. Molar absorptivities from n-π* transitions are
relatively low, and range from 10 to 100 L mol\(^{-1}\) cm\(^{-1}\). \(\pi-\pi^*\) transitions normally give molar absorptivities between 1000 and 10,000 L mol\(^{-1}\) cm\(^{-1}\). The solvent in which the absorbing species is dissolved also has an effect on the spectrum of the species. Peaks resulting from \(n-\pi^*\) transitions are shifted to shorter wavelengths (blue shift) with increasing solvent polarity. This arises from increased solvation of the lone pair, which lowers the energy of the \(n\) orbital. Often (but not always), the reverse (i.e. red shift) is seen for \(\pi-\pi^*\) transitions. This is caused by attractive polarization forces between the solvent and the absorber, which lower the energy levels of both the excited and unexcited states. This effect is greater for the excited state, and so the energy difference between the excited and unexcited states is slightly reduced—resulting in a small red shift. This effect also influences \(n-\pi^*\) transitions but is overshadowed by the blue shift resulting from solvation of lone pairs.

Charge-transfer absorption; many inorganic species show charge-transfer absorption and are called charge-transfer complexes. For a complex to demonstrate charge-transfer behavior one of its components must have electron donating properties and another component must be able to accept electrons. Absorption of radiation then involves the transfer of an electron from the donor to an orbital associated with the acceptor. Molar absorptivities from charge-transfer absorption are large (greater than 10,000 L mol\(^{-1}\) cm\(^{-1}\)).

1.9.2 Instrumentation [114]

In this section, only a brief description of instrumental features will be mentioned. Two types of instruments are available according to the wavelength selector used.
1.9.2.1 Filter photometer

This uses filters for the selection of working wavelengths. Photometers are cheap machines that are widely used in most primitive analytical laboratories. The optical system and instrumental components can be represented by Figure 1.45. As can be seen from the figure, light is emitted from the source passing through a suitable filter for wavelength selection. Part of the light at the selected wavelength is absorbed by the sample and the transmitted light hits the phototube detector resulting in a signal that is displayed by the instrument as absorbance.

![Schematic diagram of a photometer](image)

**Figure 1.45** Schematic diagrams of a photometer [114].

1.9.2.2 Dispersive spectrophotometers

These use either prisms or gratings for wavelength selection. Prisms and gratings are excellent wavelength selectors where a very narrow band of light at specific wavelength can be chosen especially with good gratings. Dispersive instruments are divided into two types:
• **Single beam spectrophotometers**

This is similar to the photometer design but the wavelength selector is either a prism or grating instead of the filter. Usually, single beam instruments are of moderate price and require adjustment to zero using a blank before sample measurement. As the instrument is kept in the operational mode, multiple zero adjustments should be undertaken because there is always some drift in response with time.

• **Double beam spectrophotometers**

These incorporate places for two cells one for the blank and the other for the sample. The instrument automatically subtracts the absorbance of the blank or reference from that of the sample. The different optical components of the instrument can be seen in Figure 1.46.

![Figure 1.46 Schematic diagrams of a double beam spectrophotometer](114)
**Light sources:** The most commonly used sources are deuterium lamps in the ultraviolet region and tungsten-halogen lamps in the visible region.

**Cells:** Glass cells are adequate for measurement of absorbance in the visible region while quartz cells are adequate through the whole UV-Vis range.

### 1.10 Atomic force microscopy (AFM) [115–117]

The atomic force microscope (AFM), or scanning force microscope (SFM) was invented in 1986 by Binnig, Quate and Gerber. Like all other scanning probe microscopes, the AFM utilizes a sharp probe moving over the surface of a sample in a raster scan. In the case of the AFM, the probe is a tip on the end of a cantilever which bends in response to the force between the tip and the sample. The first AFM used a scanning tunneling microscope at the end of the cantilever to detect the bending of the lever, but now most AFM employ an optical lever technique. The diagram illustrates how this works; as the cantilever flexes, the light from the laser is reflected onto the split photo-diode. By measuring the difference signal (A-B), changes in the bending of the cantilever can be measured (Figure 1.47) [117].

Since the Cantilever obeys Hooke's Law for small displacements, the interaction force between the tip and the sample can be found. The movement of the tip or sample is performed by an extremely precise positioning device made from piezoelectric ceramics, most often in the form of a tube scanner. The scanner is capable of sub-angstrom resolution in x-, y- and z-directions. The z-axis is conventionally perpendicular to the sample. The AFM can be operated in two principal modes:
• with feedback control
• without feedback control

Figure 1.47 AFM tip and cantilever [117].

If the electronic feedback is switched on, then the positioning piezo which is moving the sample (or tip) up and down can respond to any changes in force which are detected, and alter the tip-sample separation to restore the force to a predetermined value. This mode of operation is known as constant force, and usually enables a fairly faithful topographical image to be obtained (hence the alternative name, height mode). If the feedback electronics are switched off, then the microscope is said to be operating in constant height or deflection mode. This is particularly useful for imaging very flat samples at high resolution. Often it is best to have a small amount of feedback-loop gain, to avoid problems with thermal drift or the possibility of a rough
sample damaging the tip and/or cantilever. Strictly, this should then be called *error signal* mode. The error signal mode may also be displayed whilst feedback is switched on; this image will remove slow variations in topography but highlight the edges of features. The way in which image contrast is obtained can be achieved in many ways. The three main classes of interaction are contact mode, tapping mode and non-contact mode.

1.10.1 Contact mode

This is the most common method of operation of the AFM. As the name suggests, the tip and sample remain in close contact as the scanning proceeds. By "contact" it means in the repulsive regime of the inter-molecular force curve (Figure 1.48). The repulsive region of the curve lies above the x-axis. One of the drawbacks of remaining in contact with the sample is that there exist large lateral forces on the sample as the drip is "dragged" over the specimen.

![Inter-molecular force curve](image)

*Figure 1.48* Inter-molecular force curve [116].
1.10.2 Tapping mode

This is the next most common mode used in AFM. When operated in air or other gases, the cantilever is oscillated at its resonant frequency (often hundreds of kilohertz) and positioned above the surface so that it only taps the surface for a very small fraction of its oscillation period. This is still contact with the sample in the sense defined earlier, but the very short time over which this contact occurs means that lateral forces are dramatically reduced as the tip scans over the surface. When imaging poorly immobilised or soft samples, tapping mode may be a far better choice than contact mode for imaging.

1.10.3 Non-contact

This operation is another method which may be employed when imaging by AFM. The cantilever must be oscillated above the surface of the sample at such a distance that there is no longer in the repulsive regime of the inter-molecular force curve. This is a very difficult mode to operate in ambient conditions with the AFM. The thin layer of water contamination which exists on the surface on the sample will invariably form a small capillary bridge between the tip and the sample and cause the tip to "jump-to-contact". Even under liquids and in vacuum, jump-to-contact is extremely likely, and imaging is most probably occurring using tapping mode. A different geometry is possible using the shear-force microscope (SHFM), and here true non-contact operation is possible.
1.10.4 Image display

Height image data obtained by the AFM is three-dimensional display. The usual method for displaying the data is to use a colour mapping for height, for example black for low features and white for high features. A popular choice of colour scheme is shown in Figure 1.49 [116]. Similar colour mappings can be used for nontopographical information such as phase or potential.

Figure 1.49 Color schemes mapping for displaying the height data [116]

1.11 Literature reviews

In recent years, conducting polymers (CPs) have been used in wide range of applications including battery technology, photovoltaic devices, light emitting diodes, electrochromic displays and more recently in biological application owing to their electronic conducting properties, optical properties, chemical and biochemical properties [22, 25, 43, 45]. Several techniques have been used for study the properties of polymer films such as quartz crystal microbalance (QCM) [118], Fourier transform-infrared (FT-IR) spectroscopy [119], electron spin resonance (ESR) [120], and surface plasmon resonance (SPR) spectroscopy [121–122]. SPR spectroscopy is one of the powerful technique has been widely used for the characterization and study of polymer films, interfaces and kinetic processes at surfaces [7–11].

In 2003, the properties of poly(3,4-ethylenedioxythiophene) (PEDOT) ultrathin films investigated by the combination of surface plasmon resonance (SPR)
and surface plasmon enhanced photoluminescence spectroscopy (SPPL) with electrochemical techniques, known as EC-SPPL, were reported by Baba and Knoll [7]. The electrochromic properties and the detection of photoluminescence in PEDOT ultrathin films were observed. The photoluminescence of PEDOT was observed when the polymer was dedoped under the applied potential. The photoluminescence intensity was controlled by the potential and dependent on the angular position in an SPR reflectivity experiment. The SPR characterization of the PEDOT film is corresponding with the PEDOT bulk electrochromic properties obtained from UV-vis-NIR spectra. The EC-SPPL method can be acted as a sensitive tool for detection the photoluminescence from a conjugated polymer film and could have potentials for sensors and optoelectronic devices applications.

An electroactivity of polyaniline (PANI) films in neutral pH condition and their electrocatalyzed oxidation of \( \beta \)-nicotinamide adenine dinucleotide (NADH) were also reported by Tian et al. [23]. Self-assembled PANI multilayer films by forming with poly(anion) such as sulfonated polyaniline (SPANI), poly(acrylic acid) (PAA), poly(vinyl sulfonate) (PVS) and poly(styrene sulfonate) (PSS) were prepared using layer-by-layer (LBL) method. The combination of EC-SPR and quartz crystal microbalance (QCM) techniques was used to monitor the electrochemical behavior and catalytic ability for the oxidation of \( \beta \)-nicotinamide adenine dinucleotide (NADH) in neutral solution of PANI multilayer films. The self-assembled PANI multilayer films prepared by LBL method showed very good stability, reversible, and electroactive in neutral solution. Moreover, the results of the electrocatalytic activity of PANI multilayer films indicated that the PANI copolymers can electrocatalyze the oxidation of NADH in neutral solution but their potential to electrocatalyze NADH
oxidation was quite different under the same condition. The catalytic ability of PANI/SPANI is better than the other assemblies under the same conditions due to both PANI and SPANI monolayers of PANI/SPANI system are electroactive, while for the other systems only the PANI layer is electroactive.

The investigation of the formation and effects of doping/dedoping processes on the optical properties associated of ultrathin polypyrrole (PPy) film using EC-SPR were studied by Damos et al. in 2006 [5]. The electropolymerization of pyrrole was performed by potentiostatic, potentiodynamic and galvanostatic methods. The in situ EC-SPR was monitored the nanometric films of PPy. The results showed the electropolymerization mode was essential to the stability of polymer and the reversibility of its optical properties during the doping and dedoping processes. The changes in the electrochemical and optical properties of the thin polypyrrole films produced the changes of the SPR angle position due to the changes in the values of the real and imaginary parts of the dielectric constant for PPy films during doping/dedoping processes. The results from the QCM measurement to monitor the correlation between doping/dedoping processes and the changes in the real and imaginary parts of the dielectric constant of the polypyrrole film were consistent with the results from EC-SPR technique.

In 2009, an electrochemical surface plasmon excitation and emission light properties in poly(3-hexylthiophene) (P3HT) thin films was investigated by Kato et al. [10]. The electrochemical change of P3HT thin films was in situ measured by the attenuated total reflection (ATR) and emission light properties utilizing surface plasmon (SP) excitations. The ATR and SP emission light properties were observed for the doping–dedoping states of P3HT thin film. The ATR and SP emission light
properties were remarkably changed with the electrochemical doping and dedoping. The SP emission light also decreased by decreasing the molecular luminescence of P3HT by doping. For the dedoped-state P3HT thin film, SP emission light also increased by increasing the molecular luminescence. The SP emission light excited by molecular luminescence can be controlled by controlling the doping–dedoping states.

Conducting polymers in the area of biosensors have attracted much attraction. Biosensors based on conducting polymers were used to detect the biomolecules including glucose, hormones, neurotransmitters, antibodies and antigens [50–55]. Recently, electrochemical surface plasmon optical techniques have been evolved only the in situ investigation of optical and electrical properties of conducting polymer films but also the biomolecular interaction on CPs-based biosensor [21, 42, 111]. These techniques offer the advantages of direct detection of biomolecular-binding events without the need of molecular lables and can be monitored in real-time [20].

SPR immunosensor for detection of insulin based on oligo(ethyleneglycol)-dithiocarboxylic acid (OEG-DCA) monolayer was studied by Gobi et al. in 2007 [8]. The SPR chip was prepared by a heterobifunctional oligo(ethyleneglycol)-dithiocarboxylic acid derivative (OEG-DCA) containing dithiol and carboxyl end groups to functionalize the thin Au-film. Insulin was covalently bound to the Au-thiolate monolayer of OEG-DCA for activating the sensor surface to immunoaffinity interactions. The sensor chip was monitored in real-time for detection of insulin at various concentrations. The lowest detectable concentration of insulin was 1 ng/ml with a response time of less than 5 min and the determination range covered a wide concentration range of 1–300 ng/ml. The developed OEG-monolayer based sensor
chip provided exceptional sensor performance characteristics, reproducibility, stability, and high resistivity to non-specific adsorption of proteins. The reusability of sensor chip for detection of insulin was more than 25 cycles without an appreciable change in the original sensor activity.

In 2008, the study of protein immobilization on poly(pyrrrole-co-pyrrole propylic acid) (PPy/PPa) for immunosensing application was investigated by Hu et al. [21]. Goat IgG as a model protein was covalently immobilized on the carboxyl-containing of PPy/PPa copolymer film through EDC/NHS as the coupling reagents. An electropolymerization process, protein covalent immobilization, and kinetics of antibody–antigen were in situ studied by SPR technique. Attenuated total reflection-Fourier transforms infrared (ATR-FTIR) and atomic force microscopy (AFM) were applied to characterize the prepared film. The evident peak around 1714 cm\(^{-1}\) on the ATR-FTIR spectra confirmed the existence of carboxyl groups in the prepared PPy/PPa films. The maximum immobilization capability (~8 ng/mm\(^2\)) was achieved by an optimal monomer solution containing 10% pyrrole propylic acid. The initial reaction between the antibody and antigen followed the first-order kinetics and the diffusion limitation was present during the detection. The SPR label-free detection of anti-goat IgG demonstrated the power of SPR for in situ monitoring the polymer deposition, protein immobilization and sensing process, and the feasibility to fabricate a label-free SPR immunosensor.

The polypyrrole propylic acid (PPA) based on EC-SPR immunosensor was then reported by Dong et al. [12]. The polymer formation, probe immobilization, antigen–antibody interaction and protein immunosensing process were in situ monitored by the EC-SPR measurement. The schematic diagram of in situ EC-SPR
instrument for construction of immunosensor based on polyporrole propylic acid (PPA) film is shown in Figure 1. PPA film was prepared by electropolymerization of pyrrole propylic acid monomer. Mouse IgG was used as a model analyte. Probe proteins were covalently immobilized with EDC/NHS as the coupling reagents. The modified sensor chip was in situ investigated by SPR instrument with different concentrations of mouse IgG. The results showed that the introduction of captured antibody conjugated enzyme not only enhanced the current responses but also increased the SPR angle shift. An approximate linear relationship could be obtained by plotting the data in semi-logarithmic reference frame. The in situ EC-SPR immunosensor described herein could have important potentials for diagnostics and medicine applications.

In 2010, the electrochemical controlled SPR immunosensor based on carboxylated polyaniline for the detection of human immunoglobin G (IgG) without using further label molecule was reported by Sriwichai et al. [42]. Poly(3-aminobenzoic acid) (PABA) film was fabricated by electropolymerization of carboxylated aniline monomer (3-aminobenzaic acid) on gold-coated high reflective index glass slide. The in situ EC-SPR was performed to study the kinetic property and electroactivity property of the polymer film. The SPR immunosensor based PABA film was constructed by using monoclonal anti-human IgG produced by mouse as a model analyte. The immunosensor electrode was used to probe the binding reaction of anti-human/human IgG with various concentrations of human IgG at different constant applied potentials. The results showed that higher binding amount of human IgG was obtained at applied potentials of −0.2 and 0.4 V. The conducting polymer-
based EC-SPR sensor could control the surface morphology and was a sensitive tool for enhancing the binding of biological molecules.

A novel method to detect adrenaline on poly(3-aminobenzylamine) (PABA) ultrathin films by EC-SPR spectroscopy was reported Baba et al. in 2010 [11]. The PABA film was prepared by electropolymerization of 3-aminobenzylamine (ABA) monomer on a gold substrate. The specific reaction of benzylamine within the PABA structure with adrenaline was studied by XPS, UV-vis spectroscopy, and EC-SPR techniques. A real-time detection of optical and electrochemical signals from the specific reaction of adrenaline on PABA was monitored using EC-SPR measurement. It was found that the number of changes in both current and SPR reflectivity on the injection of adrenaline showed the linear relation to the concentration, and the detection limit was 100 pM. The responses of adrenaline on PABA were compared with uric acid and ascorbic acid, which are major interferences of adrenaline. The reflectivity changes in the uric acid and ascorbic acid were obviously smaller than the reflectivity changes in adrenaline indicate that the selective detection of adrenaline on PABA.

Moreover, the surface plasmon optical technique termed transmission surface plasmon resonance (TSPR) has become a widely interesting tool to monitor the optical and electrical properties of CPs on metal supports, particularly for the detection of biomolecular interactions. The utility of this technique over other techniques including simple instrumentation and a simple platform based upon an inexpensive and commercially available diffraction grating, etc.

In 2008, the performance of grating-based SPR sensing platform for ex situ sensing by measuring thin films of various thicknesses and detecting the formation of
immunocomplexes between bovine serum albumin (BSA) and anti-BSA was demonstrated by Singh et al. [110]. This grating-based transmission surface plasmonic device was represented the ability to measure the thickness of hexanethiol (HT), decanethiol (DT) and octadecanethiol (ODT) films, which was used as adsorbates on the gold grating. The measured thicknesses of the HT, DT, and ODT films were 0.60, 1.04, and 2.14 nm, respectively. Moreover, the grating-based SPR sensing technique was monitored the immunoreactions between BSA and anti-BSA in order to represent the utility of the grating-based transmission SPR sensor. Therefore, the sensor platform based upon surface plasmon resonance enhanced transmission of light at gold-coated grating surfaces exhibited a simple and sensitive platform, which can easily be extended in the study of a variety of surface adsorption processes or other systems for biomolecular detection.

Recently, TSPR-enhanced optical transmission was actively controlled by an electrochromism of polyaniline and poly(3,4-ethylenedioxythiophene) thin films was reported by Baba et al. in 2012 [109]. A novel method of tuning and controlling SPR-enhanced transmission light by using electrochemically controlled Polyaniline (PANI) and poly(3,4-ethylenedioxythiophene) (PEDOT) thin films deposited on a thin gold grating surface. The TSPR peak switching and wavelength tuning could be observed through the TSPR measurement of PANI and PEDOT, respectively. The tunability SPR-enhanced transmission of light using electrochemically controlled conducting polymers could be provided novel opportunities for study in active plasmonic device applications.
1.12 Research objectives

1.12.1 Preparation of polypyrrole derivatives ultrathin film by electrochemical method

1.12.2 Characterization and study the properties of polypyrrole derivatives ultrathin film by electrochemical surface plasmon optical techniques

1.12.3 Construction of polypyrrole derivatives-based immunosensors

1.13 Usefulness of the research (Theoretical and/or Applied)

Novel SPR immunosensors-based of polypyrrole derivative for detection of human IgG will be obtained.

1.14 Research plan, methodology and scope

1.13.1 Literature review

1.13.2 Fabrication of polypyrrole derivatives ultrathin film by electrochemical method

1.13.3 Characterization and study the properties of polypyrrole derivatives ultrathin film by electrochemical surface plasmon optical techniques

1.13.4 Construction of polypyrrole derivatives-based immunosensors

1.13.5 Characterization and study the properties of polypyrrole derivatives-based immunosensors by electrochemical surface plasmon optical techniques

1.13.6 Discussions and conclusions
1.15 References


CHAPTER 2
EXPERIMENTAL SECTION

2.1 Materials

All chemicals used in the experiments are shown in Table 2.1.

Table 2.1 Chemicals, molecular formula, molecular weight, purity and company

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
<th>Purity</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride</td>
<td>C₈H₁₇N₃·HCl</td>
<td>191.70</td>
<td>&gt; 98%</td>
<td>Tokyo Chemical Industry. Co. Ltd.</td>
</tr>
<tr>
<td>11-Mercaptoundecanoic acid</td>
<td>HS(CH₂)₁₀CO₂H</td>
<td>218.36</td>
<td>98%</td>
<td>Sigma-Aldrich, USA</td>
</tr>
<tr>
<td>Ethanol</td>
<td>CH₃CH₂OH</td>
<td>46.07</td>
<td>99.5%</td>
<td>Kanto Chemical Co., Inc, Japan</td>
</tr>
<tr>
<td>Ethanolamine hydrochloride</td>
<td>NH₂CH₂CH₂OH·HCl</td>
<td>97.54</td>
<td>99%</td>
<td>Tokyo Chemical Industry. Co. Ltd.</td>
</tr>
<tr>
<td>Human IgG</td>
<td>–</td>
<td>–</td>
<td>95%</td>
<td>Sigma-Aldrich, USA</td>
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</table>
Table 2.1 (Continued)

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
<th>Purity</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monoclonal anti-human IgG (Feb specific)</td>
<td>–</td>
<td>–</td>
<td>95%</td>
<td>Sigma-Aldrich, USA</td>
</tr>
<tr>
<td>N-hydroxysuccinimide</td>
<td>C₄H₅NO₃</td>
<td>115.09</td>
<td>98%</td>
<td>Wako Pure Chemical Industries. Ltd.</td>
</tr>
<tr>
<td>Nitric acid</td>
<td>HNO₃</td>
<td>63.01</td>
<td>60%</td>
<td>Kanto Chemical Co., Inc, Japan</td>
</tr>
<tr>
<td>Phosphate-buffered saline</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Sigma-Aldrich, USA</td>
</tr>
<tr>
<td>Pyrrole-3-carboxylic acid</td>
<td>C₅H₅NO₂</td>
<td>111.10</td>
<td>≥ 96%</td>
<td>Sigma-Aldrich, USA</td>
</tr>
<tr>
<td>Sulfuric acid</td>
<td>H₂SO₄</td>
<td>98.08</td>
<td>98%</td>
<td>Sigma-Aldrich, USA</td>
</tr>
</tbody>
</table>
2.2 Instruments

The instruments used in the experiments are summarized in Table 2.2.

Table 2.2 Instrument used in the experiments

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Model</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atomic Force Microscopy</td>
<td>SPM-9600</td>
<td>Shimadzu</td>
</tr>
<tr>
<td>Vacuum Oven</td>
<td>AVO-250N</td>
<td>AS ONE Corp.</td>
</tr>
<tr>
<td>Potentiostat</td>
<td>HZ-5000</td>
<td>Hokuto Denko</td>
</tr>
<tr>
<td>Surface Plasmon Resonance Spectrometer</td>
<td>~</td>
<td>Home-built instrument</td>
</tr>
<tr>
<td>Transmission Surface Plasmon Resonance Spectrometer</td>
<td>~</td>
<td>Home-built instrument</td>
</tr>
<tr>
<td>UV-Vis spectrophotometer</td>
<td>V-650 Spectrophotometer</td>
<td>Jasco</td>
</tr>
</tbody>
</table>

2.3 Substrate preparation

All substrates play an important role in the present study. It was used not only to couple the surface plasmons in surface plasmon resonance (SPR) and transmission-surface plasmon resonance (T-SPR) measurements, but also as the working electrode in the electrochemical experiments. The preparation and processing of the substrates used in the present study are described with the following procedure.
2.3.1 Indium tin oxide (ITO) substrate

ITO substrates were cleaned with the following procedure:

- Washing with distilled water for 15 min
- Washing with detergent for 15 min
- Washing with distilled water and deionized water for 15 min each
- Drying in an oven at 120°C for 1–2 h

2.3.2 Gold-coated high reflective index glass substrate

All substrates were cleaned with the following procedure:

- Washing with distilled water for 15 min
- Washing with detergent for 15 min
- Washing with distilled water and deionized water for 15 min each
- Drying in oven at 120°C for 1–2 h

Gold-coated high reflective index glass substrates were prepared by the successive vacuum evaporation of an approximately 3 nm Cr layer followed by a 47 nm Au layer onto a clean high reflective index glass substrate at an evaporation rate of ~0.1 nm/s.

2.3.3 Gold-coated transparent grating substrate

DVD-R (Taiyo Yuden) was used as the diffraction grating substrate in this present study. Initially, the DVD-R was cut into several pieces and then grooved polycarbonate pieces were manually split from the polymer coating layer. All pieces of the substrate were cleaned with the following procedure:
• Soaking in concentrated nitric acid for 15 min to the layer of dye on the grooved polycarbonate side
• Washing with distilled water for 15 min
• Washing with deionized water for 15 min
• Washing with ethanol for 15 min
• Washing with distilled water and deionized water for 15 min each
• Drying with pure N\textsubscript{2} gas stream

Gold-coated transparent grating substrate were prepared by the successive vacuum evaporation of an approximately 3 nm Cr layer followed by a 47 nm Au layer onto a clean transparent grating substrate at an evaporation rate of ~0.1 nm/s.

2.4 Measurement and instrumentation

In this present study, Poly(Pyrrole-3-carboxylic acid) (PP3C) film of was prepared by electropolymerization. The combination of electrochemistry with surface plasmon resonance (EC-SPR) spectroscopy and electrochemical transmission surface plasmon resonance (EC-TSPR) spectroscopy were performed to study the kinetic property and electroactivity property of PP3C film. Besides, these techniques were also used as main detection method in order to monitor the probe immobilization and immunosensing process of the immunosensors. Other techniques used include UV-vis spectroscopy and atomic force microscope (AFM) was performed to characterize the PP3C film and PP3C-based immunosensor.
2.4.1 Electrochemical measurements

All cyclic voltammetry (CV) and potentiostatic measurements were carried out using a three electrode cell driven by a potentiostat HZ-5000 model (Hokuto Denko), connected to a personal computer. The electrochemical cell [1, 2], a platinum wire and Ag/(AgCl, 3M NaCl aqueous) were used as the counter and reference electrodes, respectively. ITO substrate, gold-coated high reflective index glass substrate and gold-coated transparent grating substrate were used as the working electrode in this present study. The scheme showing the electrochemical setup is shown in Figure 2.1. All potentials were recorded with respect to this reference electrode. All measurements were performed at room temperature.

![Electrochemical setup](image)

Figure 2.1 Electrochemical setup [1].

2.4.2 Electrochemical surface plasmon resonance (EC-SPR) measurements

*In situ* EC-SPR measurement was performed to study the kinetic property during the electropolymerization and electroactivity property of deposited films [3, 4] using a SPR setup combined with single compartment three-electrode electrochemical...
cell with a Kretschmann configuration [5] for excitation of surface Plasmon resonance. Details of the SPR setup have been well described [6]. The scheme showing experimental EC-SPR setup for this study is shown in Figure 2.2. Surface plasmons are excited by reflecting p-polarized laser light off the Au-coated base of the prism. The He–Ne laser (\(\lambda=632.8\) nm) was used as an excitation source in this study. The kinetic measurement was performed to monitor the electrochemical properties and doping/dedoping processes of deposited films via reflectivity changes as a function of time. The reflectivity-angular measurements were also performed by scanning an incident angle range before and after electropolymerization. For these experiments, the evaporated gold film (thickness of 47 nm) on the high reflective index glass substrate was used as both surface plasmon medium and working electrode. The electrode surface area was 0.635 cm\(^2\).

**Figure 2.2** Schematic representation of the EC-SPR setup.
2.4.3 Electrochemical transmission surface plasmon resonance (EC-TSPR) measurements

In situ EC-TSPR measurement was obtained using a TSPR setup combined with a three-electrode electrochemical cell is shown in Figure 2.3 [7, 8]. The TSPR setup was carried out in a collinear geometry. The white light from a halogen source (model LS1; Ocean Optics, Dunedin, FL, USA) was collimated using a convex lens with focal length of 150 mm (Newport Corporation, Irvine, CA, USA). The resulting beam was passed through a linear polarizer, which was captured using p- and s-polarized light before illuminating the grating sample through an aperture (diameter of 2 mm). The sample was mounted on a rotating sample holder for manual alignment. The transmitted light was collected by a 600 µm optical fiber and recorded with a Fiberoptic Spectrometer (SD2000; Ocean Optics). OP Wave software was used for all measurements, storage, and processing of data. For the electrochemical system, the electrochemical cell consisted of a three-electrode cell with platinum wire and silver wire used as the counter and reference electrodes, respectively. The gold-coated transparent grating substrate was used as a working electrode. All the potentials reported in this work are relative to this reference electrode. The electrochemical experiments were measured in a single-compartment three-electrode electrochemical cell with a computer-controlled potentiostat HZ-5000 model (Hokuto Denko).
Figure 2.3 Schematic illustration of the EC-TSPR setup [7].

2.4.4 Characterization of deposited films

2.4.4.1 UV-vis measurement

The UV-vis spectra of the deposited film on an ITO glass electrode were recorded using a Jasco V-650 spectrophotometer. The deposited film was placed in the supporting electrolyte solution in a standard 10 mm cuvette as the simple beam. The bare ITO glass was placed in the electrolyte as the reference beam. In addition, the obtained polymer films with applied constant potentials at -0.2, 0, 0.3 and 0.6 V in Phosphate-buffered saline (PBS) solution to study the electroactivity property were monitored by UV-vis absorption spectroscopy.
2.4.4.2 Atomic force microscopy (AFM) measurement

AFM measurement was employed to characterize the topology of the PP3C films and PP3C-based immunosensor, the measurements were conducted with tapping mode at ambient temperature.

2.5 Eletropolymerization of pyrrole-3-carboxylic acid (P3C)

Initially, a 0.1 M P3C monomer in 0.5 M H$_2$SO$_4$ solution was used for electropolymerization of the PP3C film on the substrate. The electropolymerization was performed by CV from 0.0 V to 1.0 V at a scan rate of 20 mV/s. The electropolymerized electrode was then rinsed with 0.5 M H$_2$SO$_4$ and deionized water. The electropolymerization process was monitored in situ by EC-SPR and EC-TSPR measurement. The scheme for the electropolymerization of PP3C film is shown in Figure 2.4.

![Electropolymerization Scheme](Figure 2.4)

Figure 2.4 Schematic representation of the electropolymerization of PP3C.

2.6 Fabrication of immunosensor

The fabrication of SPR immunosensor for the detection of humam IgG was performed after the electropolymerization of the conducting polymer films [9, 10]. After obtaining the SPR baseline in PBS solution, a 1:1 aqueous mixture solution of
0.4 M 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and 0.1 M N-hydroxysuccinimide (NHS) was injected for 10 min to activate the carboxylic group on the surface of the PP3C film to N-hydroxysuccinimide ester. The PBS solution was then injected as a rinsing solution followed by the addition of 100 µg/mL anti-human IgG for 10 min. After rinsing with PBS solution, ethanolamine hydrochloride (EA-HCl) buffer solution was injected for 10 min to avoid any nonspecific binding by deactivating the remaining unreacted NHS groups before the detection of human IgG. A schematic diagram of the fabrication of the PP3C-based immunosensor for the detection of human IgG is shown in Figure 2.5.

2.7 Detection of human IgG

The obtained PP3C-based immunosensor was applied at different constant potentials of -0.2, 0.3, and 0.6 V and open circuit potential for the detection of various concentrations of human IgG (1, 2.5, 5, and 10 µg/mL). The probe immobilization and immnosensing process of the polypyrrole derivatives-based immunosensors were in situ monitored by EC-SPR and EC-TSPR measurement.

2.8 Self-assembled monolayer of 11-mercaptoundecanoic acid (MUA)

Thin layer of MUA on Au substrate was prepared by self-assembly monolayer [11, 12]. The clean Au substrate was immersed into 10 mM MUA in ethanol solution for 4 h at room temperature. Then, the substrate was removed out from solution, rinsed several times with ethanol and deionized water. After rinsing, the substrate was dried with pure N$_2$ gas stream. The thin layer of MUA on Au substrate was employed to construct an immunosensor-based self-assembled MUA
monolayer with the same procedure of the fabrication of PP3C-based immunosensor for the detection of human IgG.

Figure 2.5 Schematic representation of the fabrication of polypyrrole derivatives -based immunosensor for the detection of human IgG [9].
2.9 References


CHAPTER 3

DETECTION OF HUMAN IgG ON POLY(PYRROLE-3-CARBOXYLIC ACID) THIN FILM BY ELECTROCHEMICAL-SURFACE PLASMON RESONANCE SPECTROSCOPY

3.2.1 Introduction

Conducting polymers (CPs) have been attracting considerable attention owing to their electronic conducting, optical, chemical, and biochemical properties [1–5]. These unique properties of CPs have been used in a wide range of applications including battery technology, photovoltaic devices, light-emitting diodes, electrochromic displays, and more recently in biological applications [6–9]. Typical conducting polymers such as polyaniline, poly(3,4-ethylenedioxythiophene), polypyrrole, and their derivatives have been widely studied [10–12]. Conducting polymers based on polypyrrole (PPy) have been extensively investigated owing to their good electrical conductivity, environmental stability to air and water, and ease of synthesis through electrochemical and chemical routes [6, 13, 14]. Biosensors based on conducting polymers were used to detect biomolecules including glucose, hormones, neurotransmitters, antibodies, and antigens [15–18]. In particular, polypyrrole and its derivatives containing the carboxylic group were studied for their immunosensor applications because biomolecules can be immobilized with a covalent binding method, which has received considerable attention owing to their advantages of good stability and high immobilization density [19, 20]. Recently, the combination of surface plasmon resonance (SPR) and electrochemical measurement has been use
for *in situ* real-time investigation of optical and electrical properties of conducting polymer films at solid/liquid interfaces [21–25]. Moreover, electrochemical-surface plasmon resonance (EC-SPR) spectroscopy has been applied for monitoring the interaction between biomolecules and electropolymerized conjugated polymer films in biosensor and immunosensor applications [26–29].

In this study, the fabrication of a poly(pyrrole-3-carboxylic acid) (PP3C)-based EC-SPR immunosensor for the detection of human immunoglobulin G (IgG) without using further label molecule was reported. PP3C film was fabricated by electropolymerization of a pyrrole-3-carboxylic acid monomer on a gold-coated high-reflective-index glass slide. *In situ* EC-SPR spectroscopy was performed to study the kinetic property and electroactivity property of the deposited PP3C film. Moreover, UV–vis measurement was employed to characterize the PP3C film. The EC-SPR immunosensor with the PP3C film was fabricated using monoclonal anti-human IgG produced in mouse as a model analysis. The immunosensor electrode was used to probe the binding reaction of anti-human/human IgG with various concentrations of human IgG at different constant applied potentials. Furthermore, atomic force microscopy (AFM) was carried out to characterize the topology of the PP3C-based immunosensor.

### 3.2.2 Preparation and characterization of PP3C film

#### 3.2.1 *In situ* EC-SPR monitoring of electropolymerization of PP3C film

Electropolymerization of PP3C on the gold surface which was used as the working electrode in the electrochemical experiment was achieved by applying
potential cycling between 0 to 1.0 V at a scan rate of 20 mV/s with Ag/AgCl as a reference electrode and Pt wire as a counter electrode. The CV obtained during the electropolymerization of PP3C is shown in Figure 3.1. From the CV trace, the anodic scan increased at about 0.5 V, indicating the beginning of the formation of PP3C on the gold substrate. The dramatic increase in current and SPR angle after 0.5 V demonstrated that PP3C was deposited on the gold substrate. The current slightly decreased at about 0.2 V for the cathodic scan, which indicates the dedoping process of the deposited PP3C film. The SPR angular measurements before and after electropolymerization are also shown in Figure 3.2. The SPR dip angle was shifted to a higher angle after the electropolymerization, indicating the formation and deposition of PP3C on the gold substrate. The thickness of the PP3C film was calculated by fitting the obtained SPR curves by a Fresnel equation algorithm and was estimated to be about 16 nm.

![Figure 3.1](image)

**Figure 3.1** CV trace of electropolymerization of pyrrole-3-carboxlic acid in 0.5M H₂SO₄ at scan rate 20 mV/s.
3.2.2 Potentiostatic measurement in neutral PBS solution

The properties of a conducting polymer film used to fabricate immunosensors are essential for the sensing performance. Therefore, the properties of the PP3C films used for fabricating the immunosensors were also studied using EC-SPR measurement. The angular curves of PP3C thin film were studied in PBS at several constant applied potentials of -0.2, 0.0, 0.3, 0.6 V and the open-circuit potential. Figure 3.3 shows the SPR angular curves of PP3C in PBS solution at several constant applied potentials. The dip angle increased with increasing the constant applied potential, which indicates the change in thickness or dielectric constant. One possible reason is the topological change of PP3C film; the film might swell owing to the doping effect induced by applying the potential [30]. This is reasonable because the PP3C film can be electroactive in neutral solution owing to the
self-doping effect from the functional group. The constant applied potential plays an important role in controlling the topology of the PP3C film. This result suggests that the PP3C film can be used to control the space for the binding site in the polymer chain, which leads to an effective immobilization in the PP3C-based immunosensor system.

3.2.3 UV–vis measurement

The UV–vis spectra of the PP3C film deposited on an ITO glass substrate at constant applied potentials of -0.2, 0.1, 0.6, 0.8, and 1.0V are shown in Figure 3.4. The absorbance in the visible region of the PP3C film increased with decreasing potential. This is caused by the $\pi-\pi^*$ transition due to the dedoping of the PP3C film.

![Figure 3.3 SPR angular curves of PP3C film in PBS solution at several constant applied potentials.](image)

**Figure 3.3** SPR angular curves of PP3C film in PBS solution at several constant applied potentials.
Figure 3.4 UV–vis spectra of PP3C film on ITO glass at different constant applied potentials.

3.3 Fabrication and characterization of PP3C-based immunosensor

Figure 3.5 shows the real-time SPR kinetic curve during the fabrication of PP3C-based immunosensor. A PBS solution (pH 7.4) was employed to obtain the baseline of the SPR kinetic curve before the activation of the carboxylic group of PP3C with EDC/NHS. Following the injection of the mixture of EDC/NHS solution as a coupling reagent, SPR reflectivity increased, indicating the activation of carboxylic groups and the formation of a stable reactive intermediate, then the reflectivity decreased and became stable after rinsing with PBS. A solution of 100 µg/mL anti-human IgG was then injected to immobilize the activated surface. SPR reflectivity increased again owing to the amide bonding of antihuman IgG onto the activated surface. To block the remaining free binding sites, EA-HCl solution was
employed. After rinsing with PBS, a constant potential was applied for 1 min to obtain the baseline before adding human IgG. SPR reflectivity increased after the injection of human IgG, which is associated with the binding process of anti-human IgG and human IgG. Finally, SPR reflectivity decreased owing to some dissociation of IgG after the injection of PBS solution and remained stable. For the comparison of SPR reflectivity, the PPy film without the carboxylic group was also tested for the detection of human IgG under the same condition. In this case, no reflectivity increase was observed, indicating that the PP3C film is indeed useful for the immobilization of anti-human IgG.

![Figure 3.5 SPR responses during the fabrication of PP3C-based immunosensor for detection of human IgG (10 µg/mL) at constant applied potential of 0.3 V.](image-url)
3.4 Performance of PP3C-based immunosensor for detection of human IgG

First, the sensitivity signal of the PP3C-based immunosensor was compared with 11-mercaptopoundecanoic acid (MUA)-based self-assembled monolayer (SAM)-based immunosensor, which is a standard system, as shown in Figure 3.6. It was found that the SPR response during the binding process of anti-human IgG and human IgG on the PP3C film was greater than that of the standard MUA system, indicating that the PP3C film shows a higher efficiency for the detection of anti-human IgG–human IgG. Moreover, the detection sensitivity of the PP3C-based immunosensor is even better than that obtained in several previous studies if the systems were compared in the same range of the concentrations of the analyzed antibody [31, 32].

![Figure 3.6 SPR responses during binding process of human IgG (100 µg/ml) on anti-human IgG–PP3C film with comparison with anti-human IgG–MUA SAM system at open circuit.](image-url)
Figure 3.7 shows the SPR responses of anti-human IgG/human IgG binding and dissociation process at different concentrations of human IgG in PBS solution without applying potentials. It can be seen that the reflectivity increased as the concentration of human IgG increased, indicating that the amount of human IgG binding to antihuman IgG increased with increasing concentration of human IgG.

To study the association and dissociation kinetics of the anti-human IgG and human IgG, the association and dissociation rate constants were obtained by fitting experimental SPR curves. The association curves could be fitted by using the following equation:
\[ R = R_{eq} \left\{ 1 - \exp\left[-(k_{on}c + k_{off})t\right]\right\}, \]  

(1)

where \( R \) is the reflectivity response, \( R_{eq} \) is the equilibrium reflectivity response at the target concentration, and \( k_{on} \) and \( k_{off} \) are the association and dissociation rate constants, respectively. With \( c = 0 \), the dissociation curves could be fitted by using the following equation:

\[ R = R_{eq} \exp\left(-k_{off}t\right). \]  

(2)

\( k_{on} \) and \( k_{off} \) at different concentrations of the interaction of anti-human IgG-human IgG are shown in Table 3.1. The decrease in \( k_{on} \) value with increasing concentration of human IgG indicates that the degree of association of human IgG with anti-human IgG increased.

**Table 3.1** \( k_{on} \) and \( k_{off} \) at different concentrations of human IgG.

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>( k_{on} ) (M(^{-1}) S(^{-1}))</th>
<th>( k_{off} ) (S(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>( 4.94 \times 10^3 )</td>
<td>( 5.97 \times 10^{-4} )</td>
</tr>
<tr>
<td>2.5</td>
<td>( 1.42 \times 10^3 )</td>
<td>( 6.23 \times 10^{-4} )</td>
</tr>
<tr>
<td>5.0</td>
<td>( 4.39 \times 10^3 )</td>
<td>( 9.16 \times 10^{-4} )</td>
</tr>
<tr>
<td>10.0</td>
<td>( 1.79 \times 10^3 )</td>
<td>( 1.96 \times 10^{-3} )</td>
</tr>
</tbody>
</table>
3.5 Detection of human IgG by controlling potential

SPR responses of the human IgG/anti-human IgG binding process were in situ investigated by EC-SPR measurement at different concentrations of human IgG in PBS solution with applied potentials of -0.2, 0.3, and 0.6 V and open circuit potential (no potential). As shown in Figure 3.8, the reflectivity indeed increased by the applying potentials, indicating that the amount of human IgG binding to anti-human IgG increased as the applied potentials increase. The change in the reflectivity at a constant potential of 0.6 V was about 6 times more than that without applying potential (open circuit), which indicates that the sensitivity of the system can be improved by applying potential.

![SPR responses at different applied potentials for detection of human IgG (10 µg/mL) at several constant potentials.](image.png)
The plot between the shift of reflectivity (Y-axis) and the concentration of human IgG (X-axis) at constant applied potentials is shown in Figure 3.9. The values of the shift of the reflectivity were determined by the change in the SPR response before and after human IgG were injected. The SPR reflectivity shifts show a linear relationship with the concentrations of human IgG. Moreover, the difference in the SPR reflectivity shift was obtained with respect to the applied potentials. These results indicated that the ability of the binding process between anti-human IgG and human IgG could be enhanced with electrochemically controlled potential. This phenomenon is probably attributed to the change in the morphological property of the PP3C film when the constant potential was applied [29].

**Figure 3.9** The plot of the shift of reflectivity during binding process with concentration of IgG at different constant applied potentials.
In order to investigate the mechanism, the Freundlich model was applied to study the heterogeneity in the system. The Freundlich model is the most popular adsorption model based on the distribution of the solute between the solid phase and the aqueous phase at equilibrium [33]. The basic Freundlich equation is

\[ q = K_F C_e^n, \]  

(3)

where \( q \) is the shift of reflectivity and \( C_e \) is the concentration of human IgG solution. \( K_F \) and \( n \) are the Freundlich constants. Equation (1) can be rearranged into the linear form as

\[ \log q = \log K_F + n \log C_e. \]  

(4)

\( K_F \) and \( n \) can be determined from the linear plot between \( \log q \) (y-axis) and \( \log C_e \), the slope is \( n \) and the intercept is \( \log k \). \( K_F \) and \( n \) are the Freundlich constants indicating the adsorption capacity and intensity of the adsorption process. A higher value of \( n \) indicates a greater homogeneity (more order) of the adsorbent [34].

The Freundlich constants (\( K_F \) and \( n \)) and correlation coefficient (\( R \)) for the linear relationship of the adsorption of human IgG at different constant applied potentials are reported in Table 3.2. The correlation coefficient (\( R \)) of the linear relationship could be used to determine how well the Freundlich model represents the data. It was found that at the 99% level of significance, the Freundlich model adequately represents the data. The values of \( K_F \) and \( n \) for the binding process of human IgG at a constant applied potential at 0.6 V showed the highest effectiveness,
indicating the highest adsorption capacity and degree of heterogeneity. Therefore, it is concluded that the PP3C film electrochemically controlled by applying a constant potential could enhance the binding of immobilized of human IgG on the PP3C film.

**Table 3.2** Freundlich constants for human IgG binding process.

<table>
<thead>
<tr>
<th>Applied potential</th>
<th>$K_F$</th>
<th>$n$</th>
<th>$R$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open circuit</td>
<td>0.00522</td>
<td>0.80180</td>
<td>0.99623</td>
</tr>
<tr>
<td>-0.2 V</td>
<td>0.00625</td>
<td>0.89443</td>
<td>0.99157</td>
</tr>
<tr>
<td>0.3 V</td>
<td>0.02625</td>
<td>0.54185</td>
<td>0.99026</td>
</tr>
<tr>
<td>0.6 V</td>
<td>0.03563</td>
<td>0.50841</td>
<td>0.99515</td>
</tr>
</tbody>
</table>

### 3.6 AFM analysis

To further study the mechanism of improved sensitivity, two-dimensional views of $2 \times 2 \, \mu m^2$ AFM images of immobilized human IgG at several applied constant potentials are shown in Figure 3.10. The roughness of the PP3C film for the binding of human IgG at constant potentials of $-0.2$, $0.3$, and $0.6$ V and open circuit potential were $1.959$, $4.781$, $7.838$, and $2.237$ nm, respectively. The highest values of the roughness at an applied potential of $0.6$ V exhibited the highest degree of disorder of the film, which supports the explanation for the enhanced binding of human IgG. As shown in Figures 3.10(b) and 3.10(c), the surface morphology of immobilized human IgG seems to show aggregates. It can be expected that the PP3C film swells in
PBS at an applied potential. Hence, the film showed an aggregated morphology after the film was placed in air for AFM measurement, because the film shrinks and might have some aggregates upon exposure to air after the swelling in PBS.

**Figure 3.10** AFM images of PP3C after immobilization of human IgG with applied constant potential at (a) -0.2, (b) 0.3, (c) 0.6 V, and (d) open circuit.

A schematic diagram of the binding process of anti-human IgG to human IgG on the PP3C film with applied potential and without applied potential is shown in Figure 3.11. In the case of without applying the potential, the surface seems to be flat.
and less flexible, so that both anti-human IgG and human IgG cannot penetrate into the polymer matrix. On the other hand, if potential was applied to the PP3C film, the PP3C film swelled and the surface seemed to be rugged, leading to have more space inside the polymer chain for the binding of anti-human IgG and human IgG. Therefore, the change in the sensor signal with applied potential was greater than that without applied potential. These results indicate that the sensing system, i.e., on-off, might be obtained by controlling the potential.

**Figure 3.11** Schematic diagram of the binding process of antihuman IgG to human IgG on PP3C film (a) without applying potential and (b) with applying potential.
3.7 Conclusions

The PP3C film was successfully prepared by electropolymerization, which was used for the detection of human IgG. The kinetic property during electropolymerization of PP3C was in situ monitored by EC-SPR. The thickness of the polymer film was about 16 nm. The PP3C film showed electroactivity in neutral PBS solution. UV–vis spectra exhibited π-π* transition due to the dedoping of the PP3C film. The PP3C-based immunosensor was successfully fabricated for the detection of human IgG. The immobilization of anti-human IgG and its interaction with human-IgG were investigated in situ by using the EC-SPR technique. The SPR reflectivity change increased following the injection of human IgG on the functionalized surface, indicating the binding of human IgG to anti-human IgG. The applied potentials of 0.3 and 0.6 V exhibited the higher amount of human IgG binding. The Freundlich isotherm was determined from the linear plot of the log shift of the reflectivity versus log concentration of human IgG at different constant applied potentials. It was found that at an applied potential of 0.6 V showed the highest adsorption capacity and degree of heterogeneity, which corresponded to the observations of roughness in AFM images. Therefore, it can be concluded that an electrochemically controlled PP3C-based SPR sensor is a sensitive tool for enhancing the binding of biological molecules.
3.8 References


4.1 Motivation

As mentioned before, Surface plasmon resonance (SPR) is a powerful technique to monitor interfacial reactions and thin film deposition in real time [1−3]. Recently, an optical biosensor-based technique using SPR has been explored to study the interaction of numerous biomolecules including deoxyribonucleic acid (DNA), proteins, enzyme, antibodies, and antigens [4−7]. This is because of its characteristic of being a label-free analytical method with online monitoring and high selectivity [8]. Surface plasmon waves can be excited by the attenuated total reflection (ATR) method with a prism [1] or a diffraction grating coupling configuration [9, 10]. The advantages of grating-based SPR technique include the fact that it is prism-less, convenient, and propagating SPR excitation method [11−13]. More recently, several researchers have improved the sensitivity of biosensors and other immunosensor-based grating-coupled SPR methods [14−16].

The transmission of a specific wavelength of light which can be enhanced by SPR has been discovered [17] and is termed transmission-surface plasmon resonance (TSPR). The principle of detection relies on the transmission spectra of p-polarized light, which is observed when the light passes through the thin gold film coated-grating substrate under specific conditions [18, 19]. The TSPR-based sensor is an
attractive platform in label-free biosensor and immunosensor applications because it is applicable to simple *in situ* sensing systems.

Conducting polymers (CPs) in the area of biosensors have been attracting considerable attention [20−24]. Biosensors based on conducting polymers have been used to detect biomolecules such as glucose, hormones, neurotransmitters, antibodies, and antigens [25–30]. Immunosensors are biosensors based on specific antigen–antibody interactions in which the transducer directly or indirectly detects immunochemical reactions [31, 32]. In particular, polypyrroles (PPy) and their derivatives containing the carboxylic group have been the most promising conducting polymers studied for immunosensor applications [33–35]. This is because of the possibility of grafting biologically active molecules through a covalent bond with carboxylic functionalities. Also, they have received considerable attention because of their advantages of good stability and high immobilization density [35, 36].

In recent years, a combination of SPR and electrochemical measurement has been reported for the study of CPs [37−39] and has been adapted for monitoring the interaction between biomolecules and electropolymerized conjugated polymer films in several groups [26, 35, 40−43]. However, a study using TSPR spectroscopy combined with an electrochemical (EC) method for *in situ* investigation of the optical and electro-chemical properties of CPs films and the biomolecular interactions has not been reported. To our knowledge, this is the first study on biosensors using in situ electrochemical-transmission surface plasmon resonance (EC-TSPR) technique to simultaneously measure optical and electrical responses with CPs. We demonstrated that the sensitivity and utility of a TSPR technique and the possibility to control the morphology, optical properties, and stability of polymer film by an electrochemically
controlled method are key issues regarding possible applications of this new technique.

In this chapter, the EC-TSPR biosensors for the detection of human IgG based on a poly(pyrrrole-3-carboxylic acid) (PP3C) film was reported [44]. The EC-TSPR technique is a promising candidate to generate an efficient tool to monitor the optical and electrical properties of conducting polymer films on metal supports, particularly for the detection of biomolecular interactions. This is because of its high affinity and inherent advantages over other techniques (e.g., simple instrumentation and a simple platform based upon an inexpensive and commercially available diffraction grating). PP3C is a particularly promising material for immunosensor applications due to its unique optical, electrical, and structural properties. An electrochemical immunosensor could have an electrochemically controlled surface morphology to enhance the sensitivity of the sensing signal. Herein, the PP3C film was fabricated by electropolymerization of a pyrrole-3-carboxylic acid (P3C) monomer on a gold-coated transparent grating substrate. In situ EC-TSPR spectroscopy was performed to study the kinetic properties during electropolymerization of the deposited PP3C film and the electroactivity of PP3C film in neutral phosphate-buffered saline (PBS) solution. The EC-TSPR immunosensor-based PP3C film was fabricated using monoclonal antihuman IgG produced in mice as a model analysis. The real-time TSPR response during the fabrication of the PP3C-based immunosensor and the binding process of antihuman/human IgG with various concentrations of human IgG were monitored in situ by EC-TSPR measurement. Moreover, to investigate the electrochemically enhanced immunosensor sensitivity,
the TSPR responses at several constantly applied potentials compared with the open-circuit condition were measured.

### 4.2 TSPR properties on gold-coated grating substrates.

The gold-coated grating substrates used in this study were prepared by vacuum evaporation of approximately 3 nm Cr and 47 nm gold on the grating DVD-R [19]. The surface morphology of gold-coated grating substrate was characterized by atomic force microscopy (AFM) to investigate the homogeneity of the film. The obtained results indicated a grating pitch and amplitude of gold-coated grating substrate were 670 and 136 nm, respectively. A roughness analysis revealed a smooth surface with a roughness of 7.1 nm over an area of two-dimensional views of $2 \times 2 \, \mu m^2$. AFM image is shown in Figure 4.1.

![AFM image (2×2 µm²) of gold-coated grating substrate.](image)

**Figure 4.1** AFM image ($2 \times 2 \, \mu m^2$) of gold-coated grating substrate.
The transmission spectra measured through gold-coated grating substrates for s- and p-polarized light at an incident angle of 35° are shown in Figure 4.2. The transmission spectra for s- and p-polarized light showed a broad peak at approximately 550 nm, which was related to the green transmitted color of gold films. Furthermore, an additional peak for p-polarized light transmission was observed at approximately 750 nm. The increased light transmission represented was due to the decoupling of excited surface plasmon on the gold-coated grating substrate.

![Figure 4.2 TSPR spectra for s-polarized and p-polarized light on a gold-coated grating substrate at an incident angle (θ) of 35°.](image)

Moreover, to study the peak intensity at different incident angles, the sample on a rotating sample holder was rotated from 0 to 40°. The transmission spectra intensity increased as the TSPR angle was increased from 0 up to 35°, and then...
decreased after the TSPR angle of 40° as seen in Figure 4.3. Maximum transmission intensity was observed at an incident angle of 35°. Consequently, the obtained transmission spectra were evaluated by measuring the p-polarized light compared with s-polarized light at a fixed incident angle of 35° in which s polarized light was used as a baseline.

![TSPR spectra at several angles of incidence.](image)

**Figure 4.3** TSPR spectra at several angles of incidence.

### 4.3 In situ EC-TSPR monitoring of the electropolymerization of P3C

In the present study, PP3C was employed as the promising conducting polymer to fabricate immunosensors, which was prepared by electropolymerization on a gold-coated grating substrate from a 0.1 M P3C monomer in 0.5 M H₂SO₄ solution by cyclic voltammetry with a potential range from 0.0 to 1.0 V at a scan rate of 20 mV/s. Figure 4.4 shows the cyclic voltammetry properties during the electropolymerization of P3C. The anodic scan began to increase at about 0.5 V,
dramatically increased after 0.5 V, and then the current slightly decreased at about 0.2V in the cathodic scan. The corresponding relationship of the transmission kinetic data with the cyclic voltammogram as a function of time during the electropolymerization of P3C is shown in Figure 4.5. From the TSPR responses, the transmission light intensity increased during doping and decreased during dedoping. This is probably due to the formation of PP3C film deposited on the gold-coated grating substrate and the change in dielectric constants or thickness of the deposited film. Kinetic properties during electropolymerization of P3C monomer to PP3C on the substrate was monitored in situ using EC-TSPR measurement.

**Figure 4.4** Cyclic voltammograms of the electropolymerization of P3C.
Figure 4.5 The transmission kinetic data corresponding to the cyclic voltammetry scans with a potential range of 0.0 to 1.0 V as a function of time during the electropolymerization of P3C.

The results of the transmission spectra carried out before and after electropolymerization are shown in Figure 4.6. The increase in transmission intensity obtained after the electropolymerization was in good agreement with the formation and deposition of PP3C on the substrate, as discussed above. Therefore, the usefulness of the TSPR technique as a promising candidate allowing in situ investigation of electropolymerization properties was noted.
4.4 Potentiostatic measurement in neutral PBS solution

The properties of a polymer film used to fabricate immunosensors are essential for sensing efficiency. One strategy is the adjustment of polymer morphology under an electrochemically controlled potential. It was previously shown that the morphology of PP3C films can be changed by changing the potential in PBS solution [43]. The morphological change can be obtained by doping/dedoping in PBS solution. Here, the TSPR response of PP3C in PBS solution at constant potentials of −0.2, 0.0, 0.3, and 0.6 V and the open-circuit potential condition were measured. Figure 4.7 shows the TSPR spectra of PP3C in PBS solution at several constant applied potentials. Shifts in the TSPR curve to longer wavelengths and decreases in TSPR intensity were observed with increasing constant applied potential. This behavior indicated an increase in the thickness and imaginary part of the dielectric constant of the PP3C film because of the doping effect induced by applying the

![Figure 4.6 TSPR spectra before and after the electropolymerization of P3C.](image)

**Figure 4.6** TSPR spectra before and after the electropolymerization of P3C.
potential. This is reasonable because the PP3C film shows electroactivity in neutral PBS solution owing to the self-doping effect from the functional group [3]. The polymers can be swelled as the solution is also penetrated into the film during the doping. Therefore, PP3C film can be used to control the space for the binding site in the polymer chain based on the swelling and shrinking of polymer morphology. The swelling of the film should provide more space for the effective immobilization, which plays an important role in the sensing performance in the PP3C-based immunosensor system.

Figure 4.7 TSPR spectra of PP3C film in PBS solution at several constant applied potentials.
4.5 Fabrication of a PP3C-based immunosensor

The electrochemically controlled TSPR technique was used to monitor the immobilization of antihuman IgG on the PP3C surface and the binding of antihuman IgG and human IgG in real time. Figure 4.8 shows the real-time TSPR kinetic curve during the fabrication of the PP3C-based immunosensor. The surface-modification steps led to an increase in the intensity of the transmission response. Initially, PBS solution (pH 7.4) was used to obtain the TSPR baseline. The deposited PP3C film introduced COOH groups to the substrate, which could then be activated for anti-IgG immobilization. To activate the surface COOH group, we injected a mixture of EDC/NHS solution as a coupling reagent for 10 min to promote covalent linkages on the surface of the PP3C film by forming the N-hydroxysuccinimide ester [6]. TSPR intensity increased during the activation process indicating the activation of carboxylic groups and formation of a stable reactive intermediate; however, the intensity decreased and became stable after rinsing with PBS solution. Amide bonding of antihuman IgG onto the activated surface was also obtained as the TSPR intensity increased again after a solution of 100 µg/mL antihuman IgG was injected. Upon rinsing by PBS solution, the TSPR intensity showed a gradual increase after the modified surface was treated with EA–HCl solution, indicating deactivation of the remaining free binding sites. After rinsing with PBS solution, a constant potential was applied for 1 min to obtain the baseline before the detection of human IgG. The TSPR response increased after the injection of human IgG, which indicated the binding process of antihuman IgG and human IgG. Finally, the TSPR signal decreased due to some dissociation of IgG after the injection of PBS solution and remained stable.
Figure 4.8 TSPR binding curve during the fabrication of a PP3C-based immunosensor for the detection of human IgG (10 µg/mL) at an open circuit.

4.6 Detection of human IgG

The sensitivity of the PP3C-based immunosensor was compared with a standard system. Mercaptoundecanoic acid (MUA) was employed to fabricate an immunosensor-based self-assembled monolayer, which was attributed to be the standard system in this experiment. TSPR responses for the detection of human IgG on antihuman IgG–PP3C film in comparison with the antihuman IgG–MUA SAM system are shown in Figure 4.9.

The relationship between the shift in transmission intensity and human IgG concentration on the PP3C film showed a higher sensitivity than that of the standard MUA system, and the dynamic range was from 1 to $1 \times 10^3$ µg/mL. Furthermore, the concentration of human IgG at 1 ng/mL could be still detectable with this system is shown in Figure 4.10, although the linearity could not be obtained in this range. On
the basis of our previous study, the detection limit and dynamic range with the EC-TSPR technique is comparable or even better than that with conventional EC-SPR technique [43].

**Figure 4.9** Plots of human IgG concentration and the shift in TSPR responses during the binding process of human IgG on antihuman IgG–PP3C film in comparison with the antihuman IgG–MUA SAM system at an open circuit.
Figure 4.10 TSPR responses at the concentration of 1 ng/ml human IgG.

In addition, to enhance EC-TSPR sensitivity, the human IgG/antihuman IgG binding process and the detection of human IgG at different concentrations of human IgG by controlling the potential of −0.2, 0.3, and 0.6 V were investigated by EC-TSPR measurement. The corresponding relationship of the shift in transmission intensity with the concentration of human IgG at different constant applied potentials is shown in Figure 4.10. Linearity was obtained in each applied potential with the concentrations of human IgG. This observation implied that the ability of the binding process between antihuman IgG and human IgG could be enhanced with electrochemically controlled potentials. This is because, by applying a constant potential, the electrochemically controlled PP3C film swells and opens more binding sites inside the polymer chain for the binding of antihuman IgG and human IgG, which can enhance the binding efficiency for the detection of human IgG.
**Figure 4.11** Plot of the shift in transmission sensitivity during the binding process with concentration of human IgG at different constant applied potentials.

### 4.7 Conclusions

The performance, sensitivity, and utility of EC-TSPR spectroscopy for investigation of the properties of PP3C film and detection of human IgG were demonstrated. This is the first report using a novel *in situ* EC-TSPR technique to monitor the electropolymerization and formation of PP3C film. The TSPR spectra shifted to higher wavelengths with an increase in the constant applied potential due to the swelling of PP3C films. Immobilization of antihuman IgG and its interaction with human-IgG were investigated in situ using EC-TSPR measurements. TSPR intensity increased with increasing human IgG concentration and constant applied potential on the modified surface. The binding process of the electrochemically controlled system was better than that of a standard MUA system. Enhanced binding of human IgG on electrochemically controlled PABA film could be created by morphological changes...
at constant applied potentials, swelling/shrinking, and open/closed spaces of the binding site inside the polymer chain. Hence, this result clearly demonstrated that an electrochemically controlled PP3C-based TSPR sensor is a sensitive tool to enhance the binding of biological molecules. We conclude that this technique can be applied to the study of biomolecular interactions in various systems, and that diagnostic tools in many formats can be developed.

4.8 References


CHAPTER 5

SUMMARY

An electrochemically controlled CPs-based surface plasmon optical technique was employed to study an optical and electrical property of the conducting polymer and a label-free detection of the interaction between biomolecule and electropolymerized of CPs-based sensor. The combination of electrochemical and surface plasmon optical techniques have been represented in this study is electrochemical-surface plasmon resonance (EC-SPR) and electrochemical-transmission surface plasmon resonance (EC-TSPR). Poly(pyrrole-3-carboxylic acid) (PP3C) is a conduction polymer coated on a gold substrate to construct an immunosensor for detection of human IgG. In this present study, the PP3C film was successfully prepared by electropolymerization, which was used for the detection of human IgG. The optical properties of PP3C film, kinetic property during electropolymerization of PP3C and the utility of PP3C-based immunosensor for detection of human IgG was in situ monitored by EC-SPR and EC-TSPR. The response of the surface plasmon optical techniques was exhibited in term of reflectivity and transmission intensity for EC-SPR and EC-TSPR measurement, respectively.

Firstly, PP3C was fabricated by electropolymerization on the gold surface from a 0.1 M pyrrole-3-carboxylic acid (P3C) monomer in 0.5 M H₂SO₄ solution by cyclic voltammetry (CV) with a potential range from 0.0 to 1.0 V at a scan rate of 20 mV/s with Ag/AgCl as a reference electrode and Pt wire as a counter electrode. From
the CV trace, the anodic scan and the cathodic scan was obtained at about 0.5 V and 0.2 V, respectively. The dramatic increase after the anodic scan, suggesting that the electrooxidation of the monomer precursor to form PP3C film on the gold substrate. On the reversal potential scan the current slightly decreased during the cathodic scan, demonstrated that the dedoping process of the deposited film. The corresponding relationship of the kinetic data during by electropolymerization of P3C was monitored in situ by EC-SPR and EC-TSPR measurement. It was found that the SPR reflectivity and transmission intensity response changed during the polymerization and doping-dedoping processes increased with the number of cycles, indicating the formation and increasing of the thickness of PP3C deposited on the gold substrate. The thickness of the polymer film was about 16 nm.

Next, the UV-vis spectroscopy was performed to characterize the property of PP3C film in PBS at several constant applied potentials. The absorbance in the visible region of PP3C film increased with decreasing, indicating that the $\pi-\pi^*$ transition due to the dedoping of the PP3C film. Moreover, the potentiostatic measurement of PP3C film in neutral PBS solution at several constant applied potentials was also measured by EC-SPR and EC-TSPR. For SPR measurement, the SPR dip angle increased with increasing the constant applied potential. Also, the TSPR spectra changed following the constant applied potential, the TSPR spectra decreased with increasing to the constant applied potential. This is caused by the change of the PP3C thickness and dielectric constant due to the applying potential. It should also be noted that, the PP3C shows electroactivity in neutral PBS solution and can be used to control the topology of PP3C, which plays an important role in an effective immobilization in the PP3C-based immunosensor system by control the space for binding site in polymer chain.
Then, the PP3C-based immunosensor was successfully fabricated for the detection of human IgG. The immobilization of anti-human IgG and its interaction with human-IgG were investigated in situ by EC-SPR and EC-TSPR technique. The SPR reflectivity change increased following the injection of human IgG on the functionalized surface, indicating the binding of human IgG to anti-human IgG. Moreover, the SPR response during the binding process of anti IgG-human IgG on PP3C film shows a higher efficiency than that of the standard MUA system. The detection of human IgG-based PP3C immunosensor can be improved by controlling potential. The applied potentials of 0.3 and 0.6 V exhibited the higher amount of human IgG binding. It was found that at an applied potential of 0.6 V showed the highest adsorption capacity and degree of heterogeneity, which corresponded to the observations of roughness in AFM images. Therefore, it can be concluded that electrochemically controlled PP3C-based SPR sensor is a sensitive tool for enhancing the binding of biological molecules.

Finally, the performance, sensitivity, and utility of EC-TSPR spectroscopy based upon a simple experimental set up, inexpensive diffraction grating substrate and sensitive platform was represented a novel technique to detect the immunoreactions between anti IgG and human IgG. The real-time kinetic curve during the construction of PP3C-based immunosensor was monitored by TSPR. The TSPR intensity change during the construction of PP3C-based immunosensor was observed following the surface modifying process. Furthermore, PP3C-based TSPR sensor was used for the detection of different concentration of human IgG at several constant applied potential. The binding process of the electrochemically controlled system was better than that of a standard MUA system. Enhanced binding of human IgG on
electrochemically controlled PP3C film could be created by morphological changes at constant applied potentials, swelling/shrinking, and open/closed spaces of the binding site inside the polymer chain. The detection limit of the PP3C-based TSPR sensor is 1 ng/mL of human IgG. We conclude that this technique is comparable or even better than that of EC-SPR technique.

Hence, this result clearly demonstrated that an electrochemically controlled PP3C-based surface plasmon optical technique is a sensitive tool to enhance the binding of biological molecule and could be applied to the study of biomolecular interactions in various systems.

**Suggestion for future works**

1. PP3C-based surface plasmon optical sensor will be fabricated for analysis of a variety of biomolecular interactions.

2. The surface plasmon optical techniques will be investigated for study the properties of the other kind of CPs.

3. The other kind of CPs-based surface plasmon optical sensor will be fabricated for detection of human IgG.
CURRICULUM VITAE

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Working experiences

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Publications and Presentations

Journal Article


**Conference/Presentation**


3. Janmanee R., Pirakitikulr P., Liewhiran C., Phanichphant S. Influence of the *Broussonetia papyrifera* (L.) Vent pulp to control the size of SnO$_2$ nanoparticles by precipitation coupling with thermal decomposition methods., Oral Presentation, The 25$^{	ext{nd}}$ Annual


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Award

- “The excellent poster presentations award”, Commission on Higher Education Congress IV University Staff Development Consortium (CHE-USDC Congress IV, 14–16 August 2011, The Zign Hotel, Pattaya, Thailand.

- “The excellent poster presentations award”, KJF International Conference on Organic Materials for Electronics and Photonics (KJF) 2012, 29 August–1 September 2012, Sakura Hall, Tohoku University, Sendai, Miyagi, Japan.