CHAPTER 1

Introduction

The advancement of technology has brought a significant benefit to human beings. Especially, in the area of material and medical sciences where a computational study could assist the analysis a system of interest and detection of a specific system. For example, the detection of toxic compound in food, the analysis of metal ions in human bodies, The design of probes and sensors for glucose detection and the development the fluorescent dyes for cancer cells. Therefore, they can be seen clearly that the advancement of technology is the important role for mankind.

1.1 Classical Detection [1-4]

Classical detection is the fundamental method in chemical analysis, which is the most important in scientific research and has been used by researcher even up to this date. In the field of analytical chemistry, the chemical analysis can be classified into two categories of chemical detection as qualitative detection and quantitative detection. The purpose of the qualitative detection is to determine or identify the compositions in an unknown sample. Many researches were carried out by separating the interesting component in a sample by qualitative detection such as precipitation method. While, the quantitative detection can separate compositions in unknown sample and determine the yielded products or amount of each present composition that could be recognized by their properties such as colors (chromatography), mass (mass spectroscopy), solubility (gravimetric technique), acidity and basicity of solution (titration technique, volumetric technique) [2, 4]. Although, these techniques are cheaper and easily available for laboratory and industry utilization, there are too many chemical reactions, which release many toxins and wastes to environment. In addition, the classical detection for these toxins and wastes consumes more time for analysis. Therefore, the new techniques for detection which are cheap, environmentally friendly, effective and consume less time for analysis have been discovered such as sensor.

1.2 Sensing Technology

One of the most attractive and effective techniques for chemical detection is a sensor. A sensor is a device that measures some physical or chemical properties of the interested system and converts it into a detectable signal of electronic output. The sensor is classified into three types; physical sensor, chemical sensor and biosensor [5].

1.2.1 Physical Sensors [6, 7]

A physical sensor is a device that provides information about a physical property of the system such as force, pressure, displacement, flow rate, thermal, electrical, and magnetic property [6]. Figure 1.1 illustrates the diagram of physical properties to electrical energy of physical sensors. They play an important role in daily life such as the development of industry, agriculture, military, aerospace and biomedical field. The example of physical sensor is speed sensor, which is controlled by monitoring for a deceleration of the wheel that is out of the ordinary.

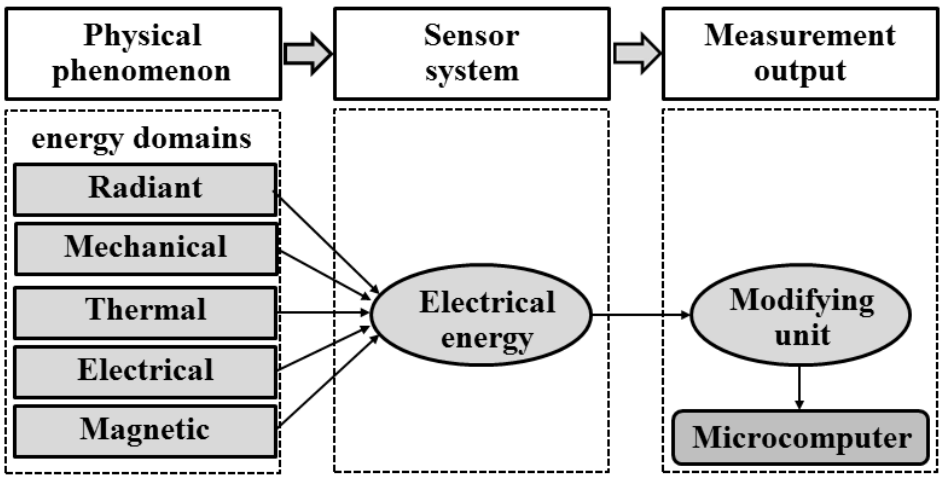


Figure 1.1 Physical properties to electrical energy of physical sensors.

1.2.2 Chemical Sensors [8]

The chemical sensors are used to detect chemical reagents such as specific components (ion and elements) of a considered system. They use fluorescence property of the fluorophore for detection, which is called a fluorescent sensor. On-Off sensor is the popular chemical sensor. When the analyte interacts with the fluorophore at binding

site, the signals can be detected and there are two possible “ON” or “OFF” signals as shown in Figure 1.2 (a) and (b), respectively.

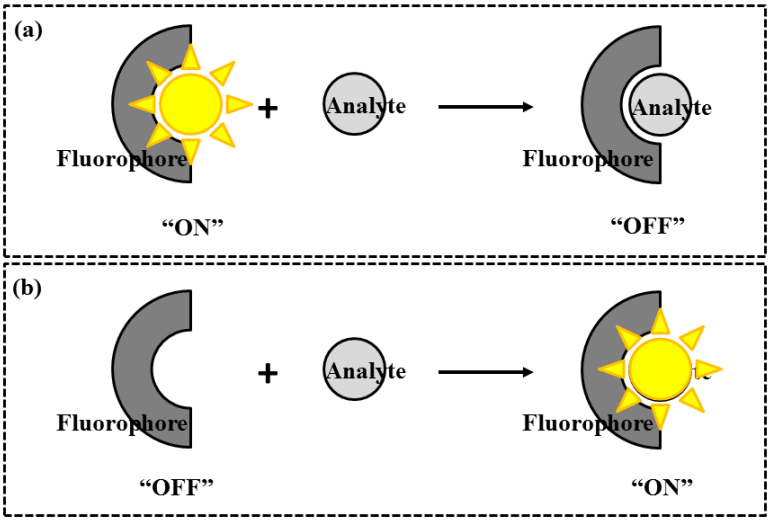


Figure 1.2 Principle of fluorescent sensor. (a) “OFF” and (b) “ON” signals of fluorescence.

A fluorophore is a part of a molecule which makes a molecule to be fluorescent. It contains π-conjugated and heterocyclic system. The common fluorophores used as fluorescent dyes such as rhodamine [9] , cyanine [10], coumarine [11, 12] and BODIPY [13, 14] have been utilized in many applications. The examples of fluorescent molecule which is applied as ON-OFF fluorescent dyes are exhibited in Figure 1.3.

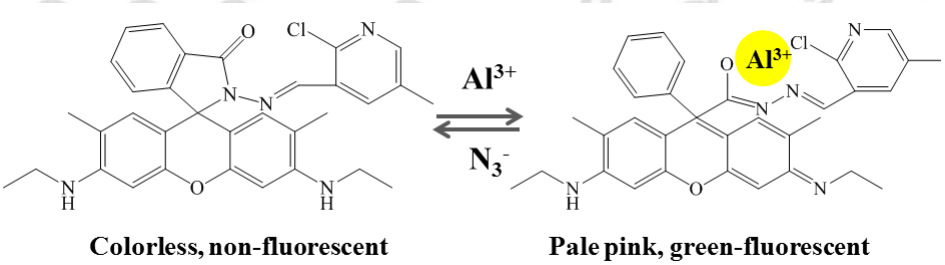


Figure 1.3 An ‘OFF–ON’ fluorescent chemosensor based on rhodamine6G-2-chloronicotinaldehyde for the detection of Al^{3+} ions [15].

1.2.3 Biosensors [16]

A biosensor mostly consists of two components (bioreceptor and transducer) which are displayed in Figure 1.4. Bioreceptor is a biomolecule which recognizes the target analyte. This recognition is then converted into a measurable signal by the transducer. Nowadays, the biosensor is the most attractive research topic for many chemists because they can be used in a wide range of applications, especially the biosensor in biological systems. The characteristic of a good biosensor includes good parameters such as sensitivity, selectivity, stability, low detection limit, long lifetime and reproductivity of the sensor output.

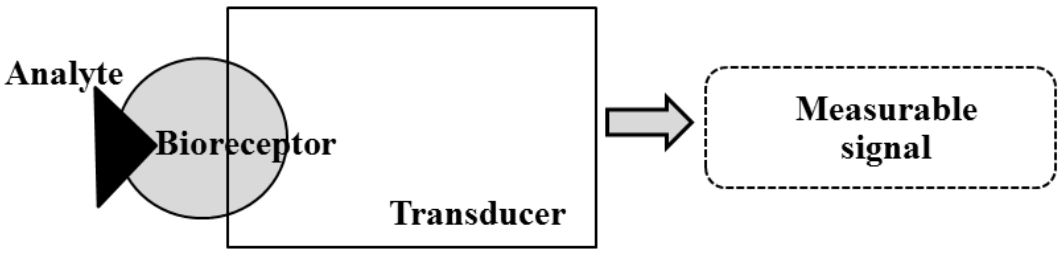


Figure 1.4 Schematic representation of component of biosensor.

As above mentioned, the application of biosensor is widely used in medical science. For example, the glucose concentration in blood can be measured directly by a biosensor made specifically for glucose measurement as shown in Figure 1.5(a). When the glucose reacts with glucose oxidase enzyme, which release two protons and two electrons. Then, the oxygen reacts with protons and electrons to produce hydrogen peroxide at platinum cathode. Therefore, we can detect the glucose concentration in blood by oxidation reaction of glucose oxidase, as shown in Figure 1.5(b) [17, 18].

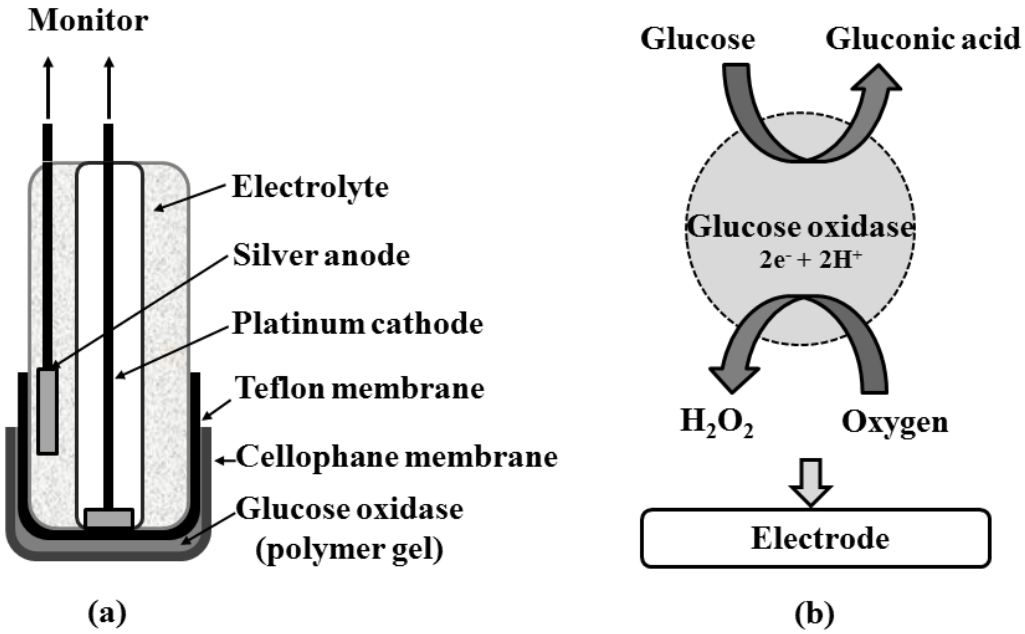


Figure 1.5 (a) The schematic representation of glucose sensors.
(b) the reaction mechanism of the glucose oxidase enzyme in glucose sensors.

1.3 Photoluminescence Concept

1.3.1 Principle of Photoluminescence [19, 20]

The sky is blue, roses are red and leaves are green. We see the world is colorful. These colors may be coming from natural or chemical process. The organic compounds possessing colors because of their ability to absorb and emit light in visible spectrum (400-700 nm), contain a conjugated system, where a resonance of electron can occur, which is called a chromophore. When a chromophore absorbs light and emits light of longer wavelength are called photoluminescence.

The Jablonski diagram (Figure 1.6) is basically an energy diagram to describe the photoluminescence process. When the fluorophores in ground state (S_0) is exposed to light they can absorb excitation energy and move to excited state (S_1). During excitation, they might lose energy by molecular collisions with solvent, vibrational relaxation, internal conversion and external conversion before relaxing to ground state (S_0) and emitting the photon as so called fluorescence. In addition, the gap between the maximum of the absorption and emission spectra is known as Stokes' shift. The large

Stokes' shift is one of the important factors of good fluorescent probes, because larger Stokes' shifts can eliminate spectral overlaps between absorption and emission, which increases stabilities and acceptable quantum yields. Other important required factors are low excitation energy and long maximum emission wavelength with high intensity. Some energy released from the excited-state which is not fluorescence is called non-radiative relaxation. However, a radiationless process involving a transition between two electronic states with difference spin multiplicity, or intersystem crossing (ISC), possibly occurs. The radiative decaying from an excited triplet state (T_1) back to ground state (S_0) is known as phosphorescence.

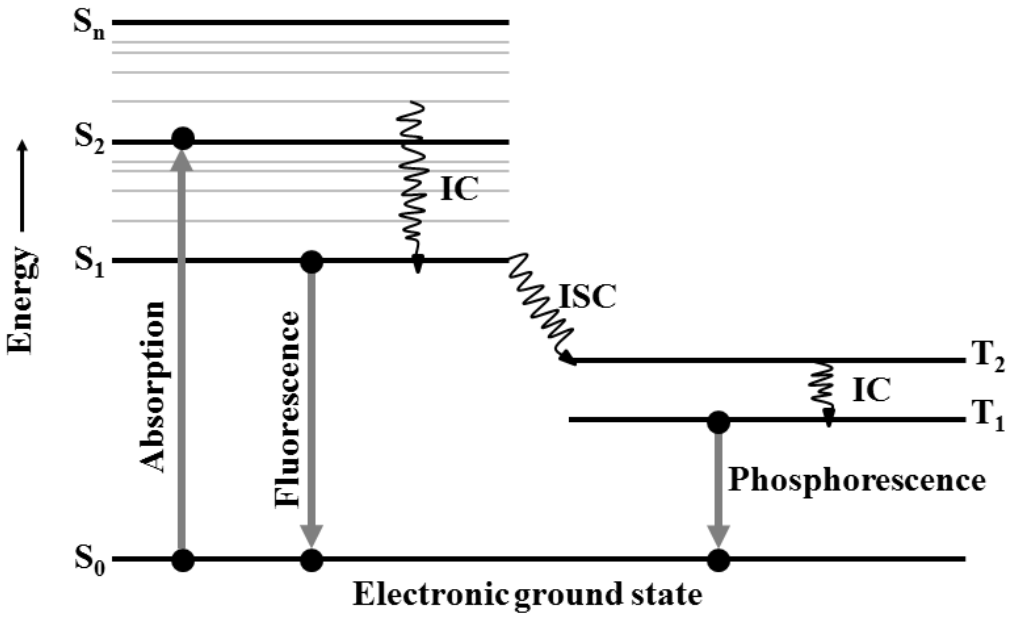


Figure 1.6 Jablonski diagram describing light absorption and emission processes.

1.3.2 Excited-state Intramolecular Proton Transfer [21, 22]

A molecule having the excited-state intramolecular proton transfer (ESIPT) which is one of important classes of fluorescent dyes has become an interesting topic of current study in many applications. In general, the ESIPT molecule requires the intrinsic hydrogen bond between proton donor and proton acceptor groups in the molecule. This process occurs from the redistribution of electronic charges between the proton donor and proton accepter groups upon photoexcitation in fluorescent molecule in which the

proton donor and proton acceptor moieties become more acidic and basic, respectively. As a result, an ultrafast proton transfer reaction from the proton donor to the proton acceptor takes place to give the keto tautomer and this keto will emit fluorescence before going back to its ground state, leading to the back proton transfer (forming the enol form) which is called four-level photocycle ESIPT as shown in Figure 1.7.

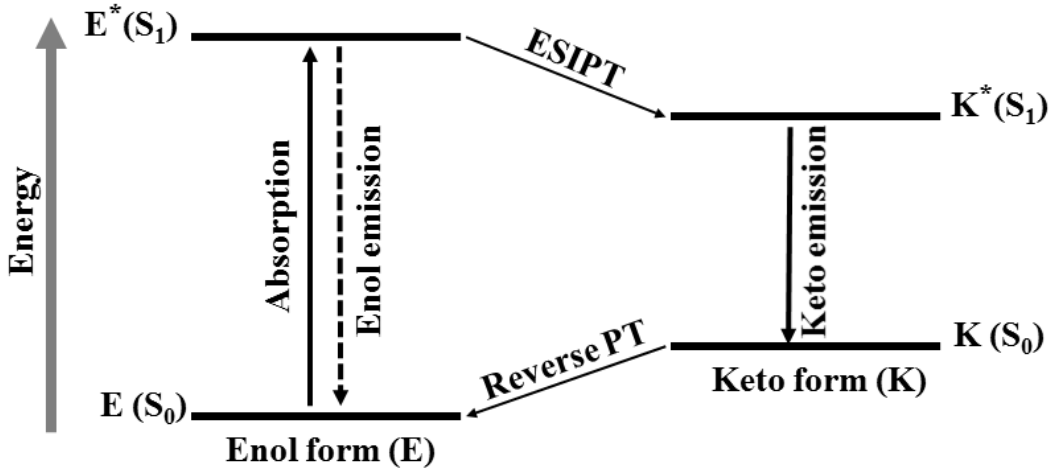


Figure 1.7 The photocycle of excited-state intramolecular proton transfer

In addition to the case of intracellular probe, large Stoke's shifts generally imply higher feasibility of near-infrared emission, of which penetration through tissues is maximized and absorbance by common cellular interferences (such as heme, water and lipids) is low [23].

1.3.3 Application and Instrumental Devices

Laser dyes

Laser dyes are organic fluorophores which absorb and emit photon when these molecules are dissolved in suitable solvent. These molecules absorbing photon at shorter wavelengths and emitting at longer wavelengths include coumarin [11, 12], fluorescein [24, 25] and rhodamine [9] anthracene [26], anthraquinone [27] and boron-dipyrromethene (BODIPY) [13, 14] which are the popular laser dyes. The molecular structures of organic fluorophore being applied in laser dyes are shown in Figure 1.8.

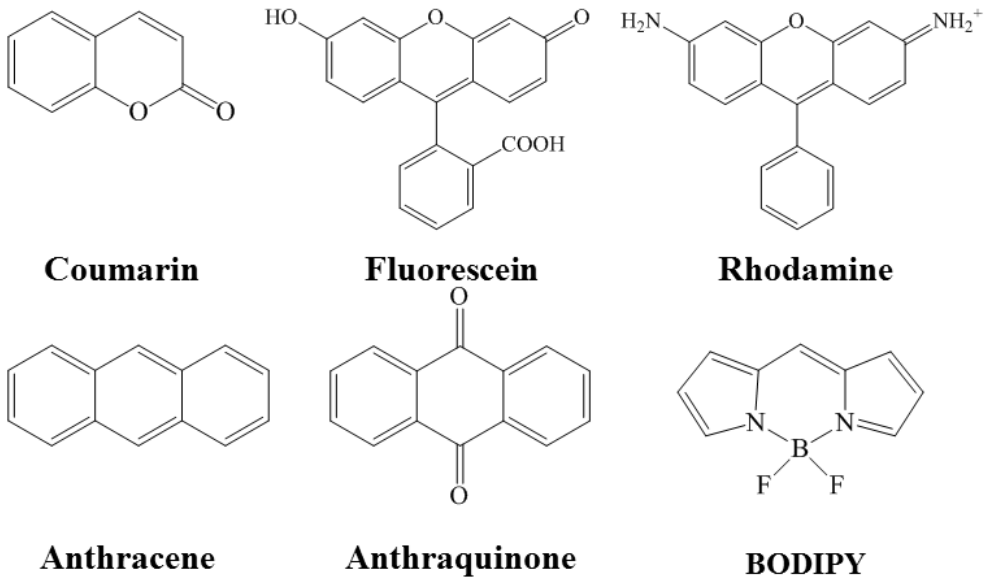


Figure 1.8 The molecular structure of some fluorophores.

Fluorescent probes [28]

Fluorescent probes are device that absorb and emit specific wavelength. They are mostly used to study in biological systems including biothiols (Figure 1.9a) [29] (for example cysteine, homocysteine and glutathione), reactive oxygen and nitrogen species (Figure 1.9b) [30], metal ions such as Zn^{2+}(Figure 1.9c) [31, 32], Hg^{2+} [33], Cu^{2+} [34] and Au^{3+} [35], and anions such as cyanide ion [36]. Some examples of fluorescent probes are shown in Figure 1.9.

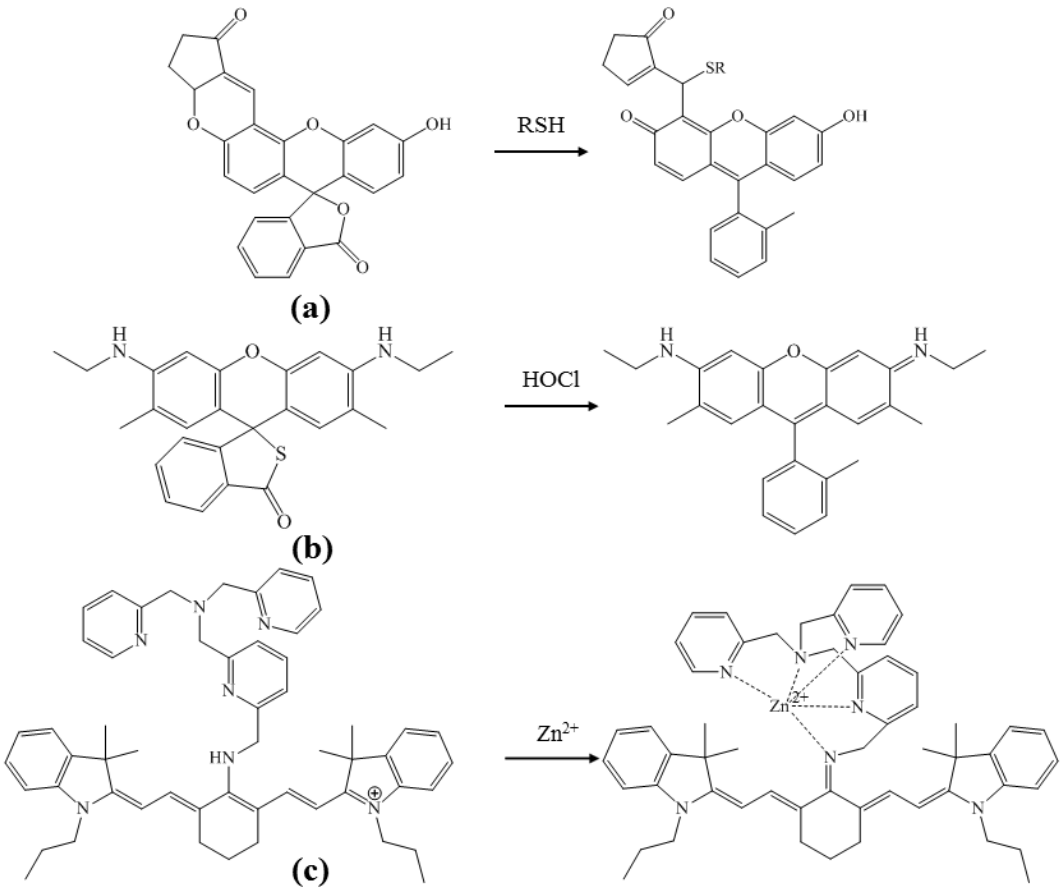


Figure 1.9 Some molecules as fluorescent probes for the detection of biothiols (a), reactive oxygen species (b) and metal ions such as Zn^{2+} (c).

Light-emitting diode devices [37]

The success of light-emitting diode (LED) application has opened the door to modern technology, especially, in the field of physical devices, architecture and materials. They are commercial products for significant fraction of the high-end smartphone. LED is a semiconductor device that emits visible light when electric current passes through it. The emitted light from an LED is caused by the injected electrons from the cathode and holes (proton) from the anode. The injected electrons and holes combine to the form excitations, which then decay to produce emitted light. The structure and working principle of LED are displayed in Figure 1.10.

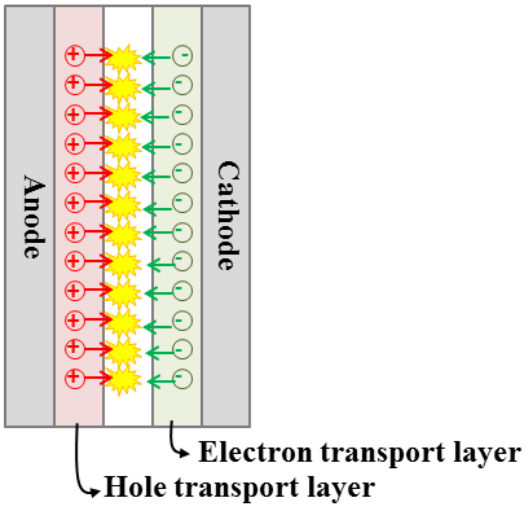


Figure 1.10 The structure of Light Emitting Diode (OLED).

1.4 Derivatives of 2-(2′-Hydroxyphenyl)benzimidazole)

2-(2′-Hydroxyphenyl)benzimidazole); HBI has attracted considerable interest of researchers around the world because it can be used in a wide range of applications [38] including antibiotics agents [39], laser dyes [40, 41], plastic scintillation [42] and optical materials [43]. It can also be developed as fluorescent probes for metal ions such as Zn^{2+}, Cu^{2+}, Al^{3+}, Ca^{2+}, Mg^{2+} and Ni^{2+} [44-46] as well as biomolecules in biological systems [47, 48]. The first study of benzimidazole began with the Van Leusen imidazole synthesis which allowed the preparation of imidazoles from aldimines by reaction with tosylmethyl isocyanide[49]. The reaction mechanism of Van Leusen imidazole synthesis is illustrated in Figure 1.11.

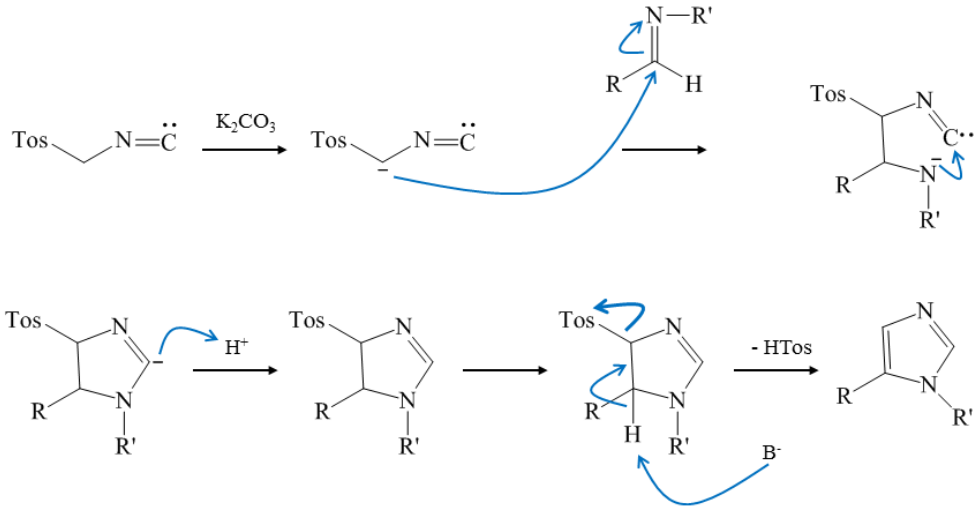


Figure 1.11 Reaction mechanism of Van Leusen imidazole synthesis.

Then, benzimidazole is synthesized by using a simple chemical reaction such as the reaction of o-arylenediamines with derivatives of carbonyl compound and miscellaneous compound [50] (Figure 1.12).

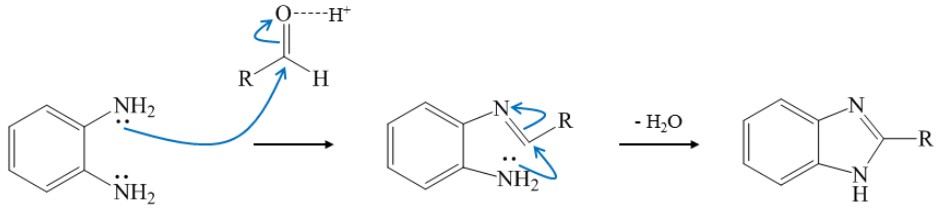


Figure 1.12 Reaction mechanism of benzimidazole synthesis.

One of the most widely studied molecule of benzimidazole derivative is 2-2′-hydroxyphenyl)benzimidazole): HBI that can be synthesized by the reflux reaction of o-phenylenediamine and o-hydroxybenzoic acid in phosphoric media [51, 52].

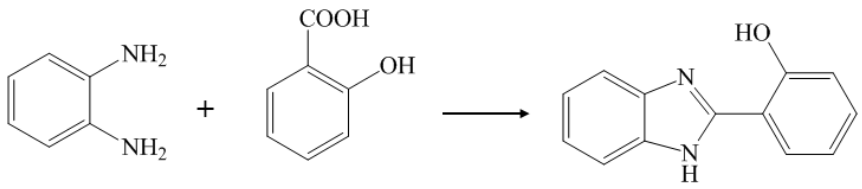


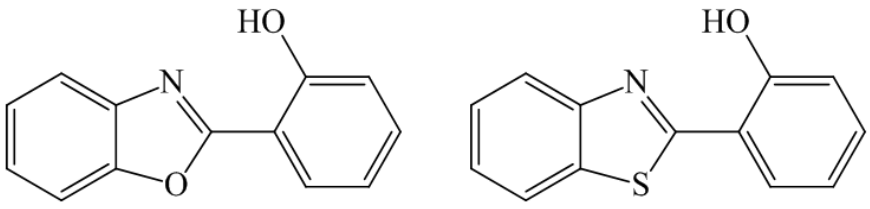
Figure 1.13 Reaction of 2-2′-Hydroxyphenyl)benzimidazole (HBI) synthesis.

The importance of HBI has been gotten more and more attention recently, because it can undergo ESIPT process. The first observation on ESIPT of HBI started in 1986 by Sinha *et al.*, in which its absorption and fluorescence spectra were observed in different solvents. When the polarity of solvent was increased, the maximum wavelength of emission was found to be blue shifted because the hydrogen bonding in polar solvent was stronger than that of non-polar solvent [53]. In 1993, Douhal *et al.* reported the absorption and emission spectra of HBI and its derivatives in different solvents [54]. Then, Mosquera *et al.* studied ESIPT behavior of the new derivative of HBI in water and ethanol and found that the ESIPT can occur in both solvents [55]. Prieto *et al.* studies the ESIPT process of 1-methyl-2-(2'-hydroxyphynyl)benzimidazole (MeHBI) and suggested that the steric hindrance of the methyl group of MeHBI caused non- planarity of MeHBI in the ground state [56]. Chipem *et al.* was the first group to study nitrogen substituent effects of HBI and reported the fluorescence spectra of tautomer in the ground state of HBI derivatives in different solvents [57]. In addition, they studied the temperature dependent effect on dual fluorescence of HBI and showed that with increasing of temperature, the *trans*-enol form increased while the *cis*-enol form decreased [58]. Then, they studied the ESIPT of the HBI and its nitrogen substituted complexes with bovine serum albumin (BSA) and found that the fluorescence of both normal and tautomer band increased substantially in BSA [48]. Fluorescence sensing abilities of its nitrogen substituted analogues of HBI derivatives doped with metal ion were studied in recent years, slight changes have been observed upon binding with transition metal ion [59].

Many computational studies on fluorescence properties of benzimidazole derivatives have been reported by employing the Gaussian-09 program using the density functional theory (DFT) and time-dependent density functional theory (TD-DFT). In 1992, Das *et al.* studied the excited-state intramolecular proton transfer (ESIPT) and rotamerism of 2-(2'-hydroxyphynyl)benzimidazole by the semiempirical methods [60]. The distinct excitation and emission spectra with large Stokes-shifted emission were reported. Then, Brauer *et al.* studied the ground state and excited state proton transfer of 4,5-dimethyl-2-(2'-hydroxyphenyl)imidazole in solution by B3LYP/6-311+G(d,p) level of theory combined with semiempirical calculation for salvation model [61]. They

revealed that in the excited state only the *cis*-enol form underwent the fast ESIPT to yield the keto tautomer while the *trans*-enol form did not give the ESIPT process. Chipem *et al.*[62] studied the effect of nitrogen substitution in benzene ring of HBI and the rotamerism effect for excited state proton transfer process by using DFT and TD-DFT. They found that the influence of nitrogen substitution on photophysics of HBI depended on number and position of nitrogen atom. The results of fluorescence spectra agreed well with the experimental data. It can be seen that, the effect of nitrogen atom substitution on spectral characteristics of HBI and its derivatives is important for the photophysics and photochemistry. In the same way, Tsai *et al.*[63] studied the excited state potential energy surfaces (PESs) and intramolecular charge transfer (ICT) of HBI and its amino derivatives by TD-DFT with PBE0 calculation. They showed that by using the TD-DFT the understanding of excited-state reaction mechanisms in terms of ESIPT and ICT can be provided. Lastly, Roohi*et al.* [64] studied intramolecular photoinduced proton of HBI using TD-DFT method with the PBE1PBE1/ 6-311+ + G(2d,2p) level of theory. The calculated results on absorption and emission spectra were found to be in good agreement with the experimental data.

Furthermore, the ESIPT reactions of HBI derivatives including 2-(2′-hydroxyphenyl) benzoxazole) ; HBO (Figure 1. 14(a)) and 2-(2′-hydroxyphenyl)benzothiazole); HBT (Figure 1.14(b)) were studied.



(a) (b)

Figure 1.14 The molecular structures of (a) 2-(2′-hydroxyphenyl)benzoxazole); HBO and (b) 2-(2′-hydroxyphenyl)benzothiazole); HBT

The ESIPT reactions of HBO and its derivatives were studied by Heller *et al.*[65] and their quantum efficiencies, excitation and fluorescence spectra were also reported. Woolfe *et al.*[66] studied the role of various anti-HBO and keto tautomer of HBO using

time-resolved emission spectroscopy. Houari *et al.* [67] investigated the ESIPT of substituted HBO with *ab initio* method. Their calculated data were found to be in agreement with the experimental data. ESIPT reaction of HBT tautomer was studied by Elsaesser *et al*. [68] and they reported the anion HBT formed in polar solvents containing hydroxide ions or other hydrogen-bonding compounds. Barbatti *et al.* [69] studied the ESIPT of HBT in the gas phase and non-polar solvent. They found the significant effect of solvent on the proton transfer time and its mechanism. Furthermore, Kungwan *et al.* [70,132] studied the molecular dynamics on the ESIPT of HBO and HBT with small cluster of water. They reported that there are two possible pathways through hydrogen bond network namely intramolecular and intermolecular proton transfers.

1.5 Derivatives of 2-(2′-Aminophenyl)benzothiazole)

Intracellular sulfur-containing biomolecules are involved in several key processes in living organism, including glutathione (GSH), cysteine (Cys) and homocysteine (Hcy) [23, 71]. The recent studies also discovered the toxic hydrogen sulfide (H_2S) to be regulatably generated in cells and function as a gasotransmitter next to nitric oxide (NO) and carbonmonoxide (CO) [72]. To date, it has been known to work as a neuromodulator in the brain and a tension reducer in blood vessels [72]. In mammals, H_2S is so far found to be released from lungs, liver, kidney, pancreas, heart and brain via the breakdown of Cys and Hcy [73-77]. Imbalance of H_2S quantity in cells could lead to serious physiological malfunctions such as Alzheimer's disease, impaired cognitive ability, gastric mucosal injury and hypertension [23, 78]. Therefore, the development of H_2S monitoring methods is considered highly important in tackling with these diseases.

Consequently, fluorescent probes provide an efficient approach for the H_2S detection because of their high sensitivity, non-destructivity and permeability through cells. Several strategies for probing intracellular H_2S have been explored, such as copper sulfide precipitation, nucleophilic reactions of H_2S, oxidation of selenoxides and reductions of azido or nitro groups [23, 79]. The last strategy is currently the most actively studied and will be the main interest of this research. Oxidized nitrogen species

(e.g. azido and nitro groups) are readily reduced by H_2S and form amines much faster than GSH and other thiols, signifying the high selectivity for H_2S detection (Figure 1.15).

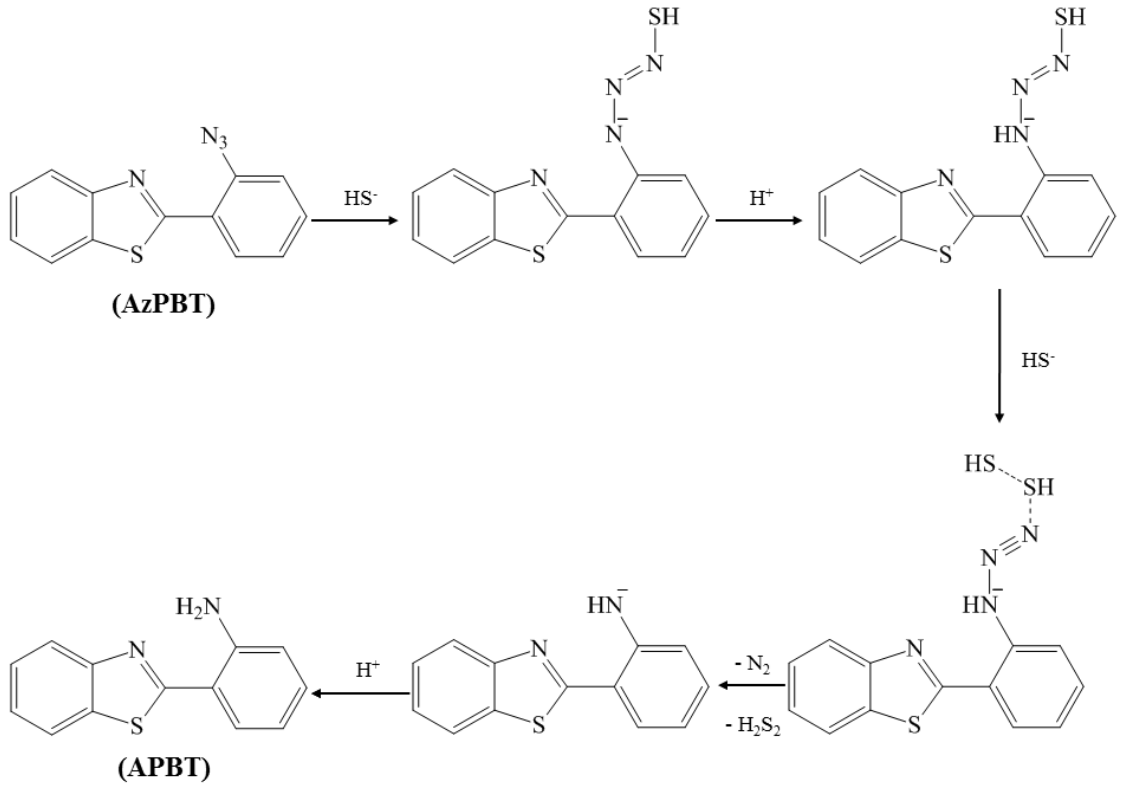


Figure 1.15 The reaction mechanism of azido to amine of APBT proposed by quantum mechanical calculations [23, 79].

The first application of azido-to-amine conversion strategy started in 2011 when Chang *et al.* developed a series of fluorophores on azido derivatives of Rhodamine 110. These probes were first applied to H_2S sensing in human embryonic kidney 293T (HEK293T) cells [54-55] . Afterwards, there have been many reports on the same strategy towards monitoring H_2S. Most of these probes rely on the introduction of azido groups which are well-known as ICT fluorescent molecules [23, 80-85].In 2014, Zhang *et al.* reported 2-(2'-azidophenyl) benzathiazole (AzPBT) (Figure 1. 15) as a very efficient probe for H_2S. AzPBT can be reduced to its amino derivative called 2-(2'-aminophenyl) benzathiazole (APBT) which is a bright fluorescent dye expressing a fluorescence with high quantum yield [86] . Furthermore, primary amino groups

attached to aromatic systems are known to be a strong electron donor and promote electron redistributions, which then lead to intense fluorescence by either internal charge-transfer (ICT) or ESIPT mechanisms. This property promises emissions with large Stoke's shifts and, hopefully, near-infrared emissions.

Chang *et al.* developed a series of fluorophores based on azido derivatives of Rhodamine 110. The probes were first applied to H_2S sensing in HEK293T cells [79, 87] and later improved for higher selectivity and longer *in vivo* lifetime [23, 88]. The ace of the series has the detection limit as low as 500 nM, and is useful in the investigation of endogenous H_2S pathways [87]. Afterwards, the bloom of reports on the same strategy towards monitoring H_2S then commenced. Most of these rely on the introduction of azido groups to well-known ICT fluorescent molecules and their derivatives as templates, such as luminol, naphthalimide, coumarin, cyanine, aryl-benzathiazoles, cresyl violet, etc. [23, 80-85]. Besides azides, a number of probes adopting nitro-to-amine strategies were also developed. Studies on nitro-functionalised fluorophores, such as heptamethinecyanines, coumarin and naphthalimides have recently been published [23, 71, 89, 90].

1.6 Research Objectives

The goal of this research is to study the photophysical and photochemical properties of HBX and APBT derivatives by using computation calculations. The effect of heteroatom substitution on spectral characteristics of HBX derivatives will be studied. In addition, the substitution effects by electron donating and withdrawing groups on absorption and emission spectra of APBT derivatives will be investigated. The potential candidates of new fluorescent dyes with larger Stokes' shift, low excitation energy and longer maximum emission wavelength with high intensity will be screened.

In the first section, we study the effect of heteroatom substitution on spectral characteristics of HBX derivatives and investigate the chances of ESIPT. We study these derivatives using DFT and TD-DFT calculations. The candidates of new fluorescent dyes with larger Stokes' shift, low excitation energy and longer maximum

emission wavelength with high intensity and the chances of ESIPT will be discussed in Chapter 3.

In the second section (Chapter 4) , we design the APBT derivatives to obtain desirable photophysical and photochemical properties for the fluorescence applications. We study substituent effect on photophysical and photochemical properties of these derivatives using DFT and TD-DFT calculations to explain spectral shift corresponding to HOMO and LUMO energy gaps of APBT derivatives. Moreover, the selected derivatives with more red-shifted and large Stokes' shift will be further investigated for the chances of ESIPT.

The modification of these compounds becomes a challenge for researchers around the world. By modification of their backbone, the desirable optical tuning can be achieved, however the process of synthesis takes time and money. Therefore, the computational chemistry which nowadays has become an effective tool for screening the candidates before real synthesis in the laboratory is employed in this work. The obtained information particular on photophysics and photochemistry of new designed fluorescent dyes will be very useful as guidance for organic chemists who are interested in developing the novel and effective fluorescent dyes.