CHAPTER I

INTRODUCTION

1.1 Fluorescent chemosensor

Nowadays, fluorescent chemosensors play an important role in detection method in chemical, biological, and environment fields because they have several advantages like high selectivity, high sensitivity and more simplicity. Most of fluorescent sensors are composed of two main component: first is a receptor unit for selective binding of the substrate and second is a fluorophore unit that provides the means of signaling this bonding, whether by fluorescence quenching or enhancement (**Figure 1.1**) [1].

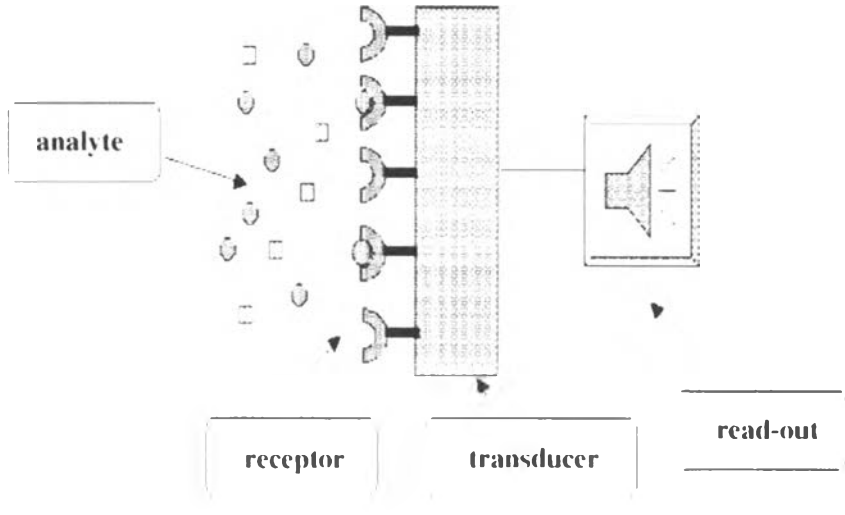

Figure 1.1 Basic principle of chemosensors

1.2 Fluorescence

The fluorescence phenomenon is a light emission process of aromatic compounds occurring after the molecules absorb energy in the form of lights. The process can be described by Jablonski diagram (**Figure 1.2**) [2]. When a molecule absorbs energy, its energy level will increase to a level which is called "excited state". The excited molecule is unstable, consequently, it will release some energy in other forms such as thermal (heat) or kinetic energy (molecular rotation and vibration) which are non-radiative processes. After these processes, the molecule resides in the lowest excited energy level that is called "semi-stable state" or "locally excited state".

Eventually, it will return to the ground state and release the last portion of energy in the form of fluorescence light.

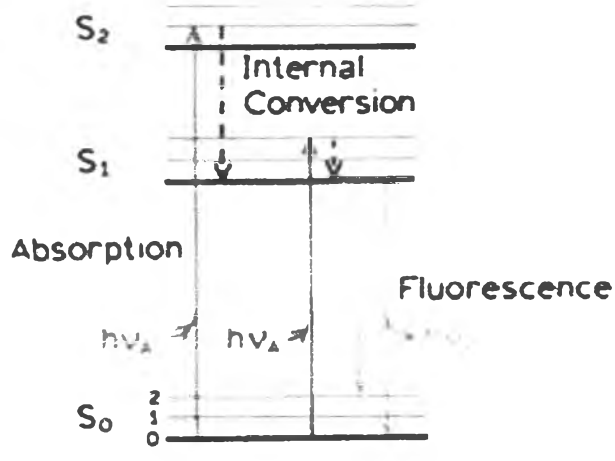

Figure 1.2 Jablonski Energy Diagram

1.3 Fluorescence quenching

One of the most common changes in fluorescent signal observed in fluorescent chemosensors is called "fluorescent quenching". There are several processes that could cause fluorescent quenching including photochemical reaction, molecular aggregation (self-quenching), electron transfer (ICT and PET), and energy transfer (collisional and static) [3]. There are two basic types of the quenching processes; one useful type of quenching is due to collisions between quencher and fluorophores, and is called **collisional or dynamic quenching**. The dynamic quencher provides a non-radiative pathway for loss of the excited state energy (**Figure 1.3**).

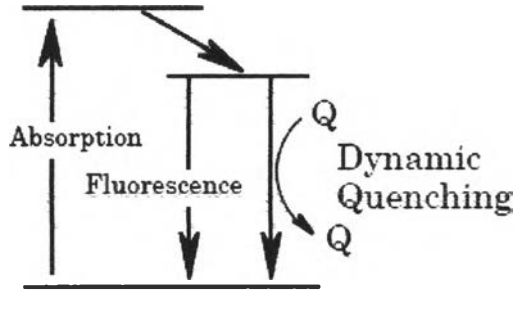

Figure 1.3 Diagram explaning dynamic or collisional quenching

The second type of quenching is **static quenching** which is the quenchers forms a non-fluorescence complex with fluorophores

$$\text{Fluor} + \text{Q} \rightleftharpoons \text{Fluor}\bullet\text{Q}$$
(Quenched)

Both types of quenching require direct contact between the fluorophore and the quencher. For dynamic quenching, the result of this contact is loss of the fluorescence pathway for return to the ground state.

In the case of dynamic quenching, contact must occur while the fluorophore is in excited state. Dynamic quenching is exhibits a concentration-dependence that is described by the Stern-Volmer equation:

$$\frac{F_0}{F} = 1 + k_q\tau_0[Q] = 1 + K_D[Q]$$

where τ_0 is the lifetime of the fluorescent state in the absence of the quenching agent. If the quenching is not known to be due to dynamic quenching, K_D is replaced by K_{SV}.

For dynamic quenching,

$$\frac{F_0}{F} = \frac{\tau_0}{\tau}$$

because the quencher decreases the lifetime of the excited state. Dynamic quenching increases with temperature, because temperature increases diffusion rates.

Static quenching is the result of the formation of a non-fluorescent complex between the quencher and the fluorophore. The association constant for the quencher fluorophore complex describes the effectiveness of a static quencher:

$$K_S = \frac{[FQ]}{[F][Q]}$$

where [FQ] is the complex concentration, and [F] and [Q] are the concentrations of free quencher and free fluorophore, respectively. Because the total fluorophore concentration, $[F]_0 = [F] + [FQ]$,

$$K_S = \frac{[F]_0 - [F]}{[F][Q]}$$

which rearranges to:

$$K_S = \frac{[F]_0}{[F][Q]} - \frac{1}{[Q]}$$

and therefore:

$$\frac{[F]_0}{[F]} = 1 + K_S [Q]$$

If you assume that the all of the decrease in observed fluorescence is due to complex formation, the equation becomes:

$$\frac{F_0}{F} = 1 + K_S [Q]$$

which is identical in form to the Stern-Volmer equation for dynamic quenching. Static and dynamic quenching can be distinguished by lifetime measurements because dynamic quenching reduces the apparent fluorescent lifetime, while static quenching merely reduces the apparent concentration of the fluorophore. Alternatively, temperature effects can be used to distinguish the two forms of quenching. Diffusion rates, and therefore dynamic quenching rates, increase with higher temperature. In contrast, complex formation strength tends to be inversely proportional to temperature, and therefore static quenching tends to be higher at lower temperatures (**Figure 1.4**).

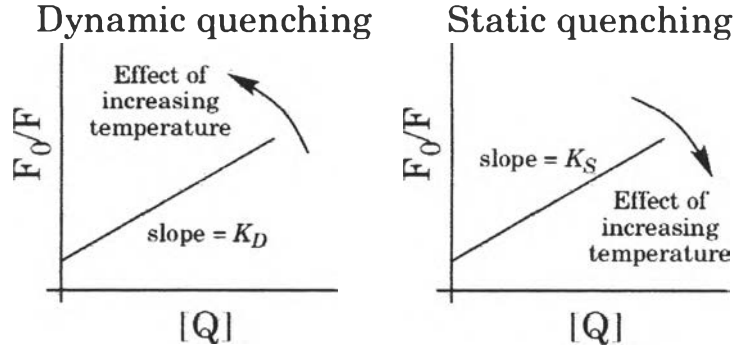

Figure 1.4 Effect of temperature on quenching mechanism

1.4 Internal charge transfer states

In some cases, the molecule in semi-stable or locally excited state can releases energy without emission through other pathways. For example, the good electron-donating and electron-withdrawing groups will cause an electron delocalization within molecule upon excitation, which is called internal charge transfer (ICT) process. In the ICT state, the molecule will have a different geometry and lower energy level

compared to the LE state. As the result, the energy released from ICT state to the ground state may not be radiative and the molecule with ICT generally exhibits low quantum efficiency (**Figure 1.5**) [4-6].

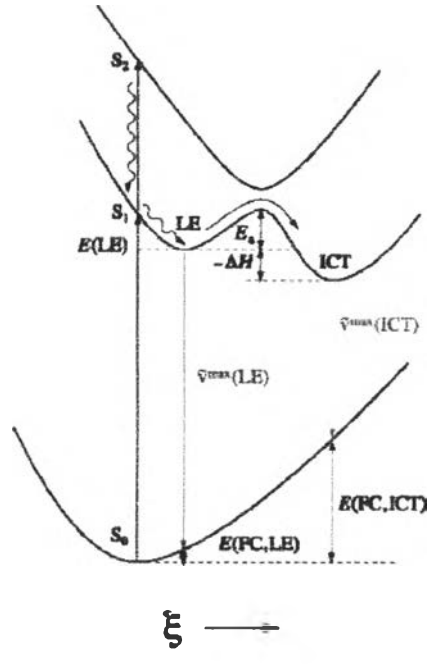

Figure 1.5 Potential energy surfaces of the ground state (S0) is excited to and S1 or S2 and then relaxed to LE, and ICT (FC = Franck-Condon).

1.5 Fluorene-based derivatives as fluorescent sensors

Jiang and co-workers, in 2008, synthesized fluorescent sensors from fluorene derivative (**Figure 1.6**) which had ethoxyethoxy groups as peripheral groups. It exhibited fluorescence quenched by Fe^{3+} and Cu^{2+} in acetonitrile solution [7].

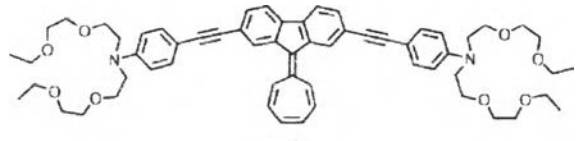

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Figure 1.6 Structure of compound had ethoxyethoxy groups as peripherals group

In 2010, Zhu and co-workers synthesized fluorene derivatives which had the difference of substituted of 9-position of fluorene (**Figure 1.7**). Both compounds showed high selectivity and sensitivity towards Hg^{2+} resulting in increasing of fluorescence intensity as well as colored-changing of green to be bright blue. The

coordination between Hg^{2+} and nitrogen atoms weakened the donating ability of nitrogen atoms. Even if green emission was suppressed, it was able to be recovered by addition EDTA [8].

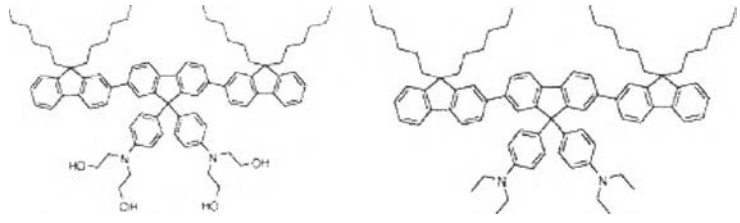

Figure 1.7 Structure of both compounds from fluorene derivative

In 2008, Xu and co-workers reported a highly reversible pH sensor from polyfluorene derivative which contained amino and carboxylic acid groups in each repeating unit (**Figure 1.8**). Fluorescence of polymer went down due to the decreasing of pH diminishing from 12 to 3. As pH became lower, polymer was going to get cation which resulted in aggregation together with fluorescence quenching. On the other side, the anionic ions showed the opposite result [9].

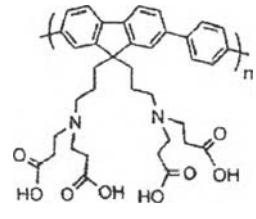

Figure 1.8 Structure of polyfluorene derivative compound

1.6 Salicylic acid as receptor unit for detection of metal ions

Sundari and co-workers, in 2005, had studied immobilized salicylic acid on XAD-2 in order to detect Cu^{2+}. It was discovered that salicylic acid responded to Cu^{2+} and the limit of detection equal to 0.5 mM. The structure of the complex between Cu^{2+} and salicylic acid is showed in **Figure 1.9**. The molecule of Cu^{2+} could be bonded with two molecules of salicylic acid. In addition, EDTA caused dissociation between Cu^{2+} and salicylic acid [10].

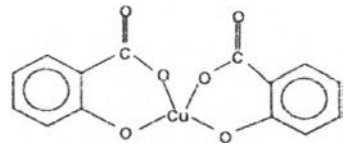

Figure 1.9 Proposed structure of copper (II)-salicylate complex

Sirilaksanapong and co-workers, in 2012, synthesized novel molecule which had 1,3,5-triphenylbenzene as a core structure and salicylic acid as a peripheral group (**Figure 1.10**). It was tested with 19 types of metal ions. The results demonstrated that it had the selectivity with Cu^{2+} so as to impede fluorescence quenching. K_{sv} and LOD values were found to be equal to $1.62x10^{6}$ M^{-1} and $1.03x10^{-7}$, respectively. Furthermore, Triton X-100, which was well-known surfactant could enhance fluorescence intensity as well as K_{sv} which was $1.5x10^{7}$ M^{-1} [11].

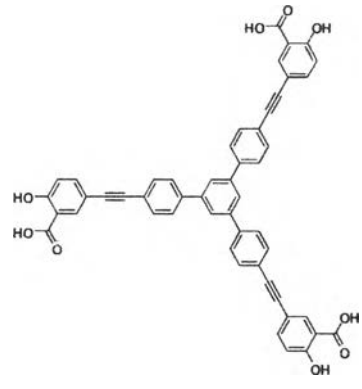

Figure 1.10 Structure of 1,3,5-triphenylbenzene as core and salicylic acid as peripheral group

In 2013, Attapornpitak and co-workers designed and synthesized water-soluble fluorescent sensor with n-phenylcabazole as a core unit and salicylic acid as a receptor unit (**Figure 1.11**). It exhibited high selectivity to Cu^{2+} in phosphate buffer pH 8 solution. The quenching of compound was unique resulting from synergetic works between multiple complexation of Cu^{2+} with compound induced superquenching and ICT process [12].

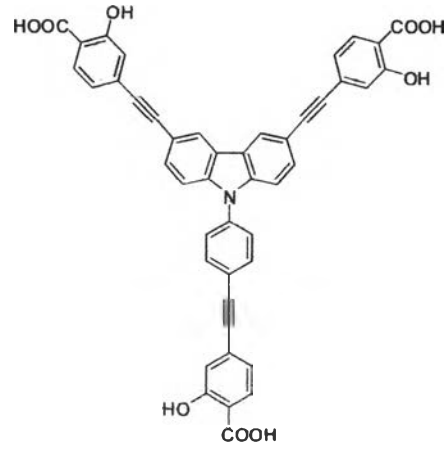

Figure 1.11 Structure of n-phenylcabazole as core unit and salicylic acid as receptor

In same year, Kimpitak and co-workers synthesized new molecule in which phenylene-ethylene served as a core and salicylic acid as an ended-group (**Figure 1.12**). When it was tested with various types of metal ions , the selectivity with Cu^{2+} had been discovered with $5.79x10^{6}$ M^{-1} of K_{sv} value. Additionally, it was utilized as detector on paper in order to detect Cu^{2+} by naked-eyes [13].

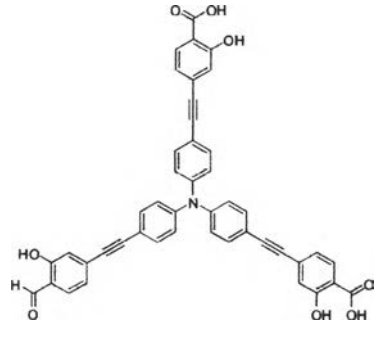

Figure 1.12 Structure of phenylene-ethylene as core and salicylic acid as ended-group

1.7 Objectives of this research

This research involved design, synthesis and characterization of novel fluorophores from fluorene derivatives (**Figure 1.13**). These compounds contain salicylic acid group as peripheral group. Photophysical properties and their applications for metal detection in aqueous media were investigated.

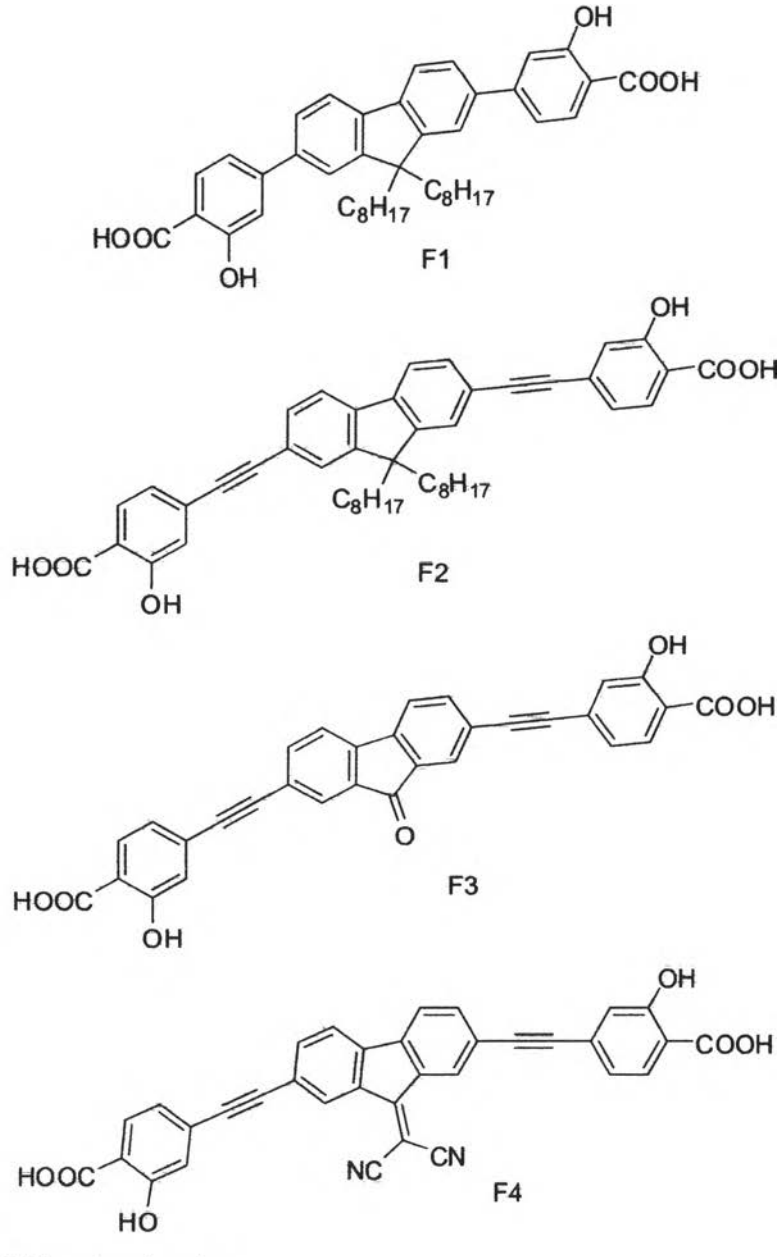

Figure 1.13 Target molecules