# CHAPTER II
# LITERATURE REVIEW

## 2.1 Molecular Self-Assembly

Molecular self-assembly is a key concept in supramolecular chemistry since assembly of the molecules is directed through noncovalent interactions, i.e., hydrogen bonding, metal coordination, hydrophobic forces, van der Waals forces, π-π interactions, and/or electrostatic effects (Lehn 1988). Up to now, various self-assembly structures covering the rational, coherent approach to molecular associations, from the smallest to the largest, the organized phase, and to their designed manipulation have been reported: (1) the oligomolecular species from the intermolecular association of a few components (a receptor and its substrate(s)) based on the principles of molecular recognition, e.g., porphyrin(Yanagisawa et al. 2007) and deoxycholic acid. (Sada *et al.* 2001); (2) macrocyclic host molecules with nanochannel for guest species such as cyclodextrins, (Harada *et al.* 1993) calixarenes, (Corbellini *et al.* 2005) catenanes, (Livoreil *et al.* 1994) and rotaxanes (Bissell *et al.* 1994) (Figure 2.1); and (3) polymolecular entities, result from the spontaneous association of a large undefined number of components into a specific phase having more or less well-defined microscopic organization and macroscopic characteristic depending on its nature (such as films, layers, membranes, vesicles, micelles, mesomorphic phases, solid state structures, etc.) (Ariga *et al.* 2008). Polymers that form self assembly into ordered structures are mostly under amphiphilic structure. Amphiphilic structure consists of hydrophilic (water-loving) parts and hydrophobic (oil-loving) parts, which either existing as a core polymer with grafting chains or a copolymer with hydrophilic and hydrophobic parts. Amphiphilic polymers such as diblock or triblock copolymers, as well as graft copolymers with sufficiently long grafts and flexible backbones, have been known to self-assemble in the form of micelles when dissolved in selective solvents.

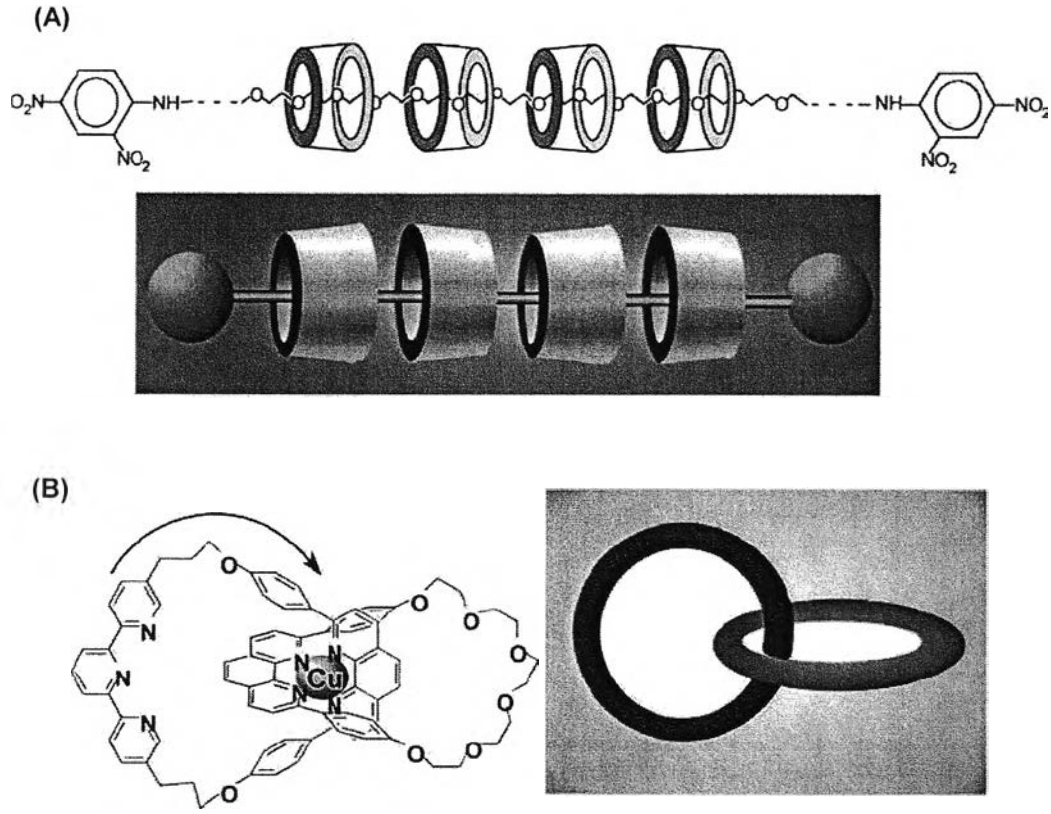


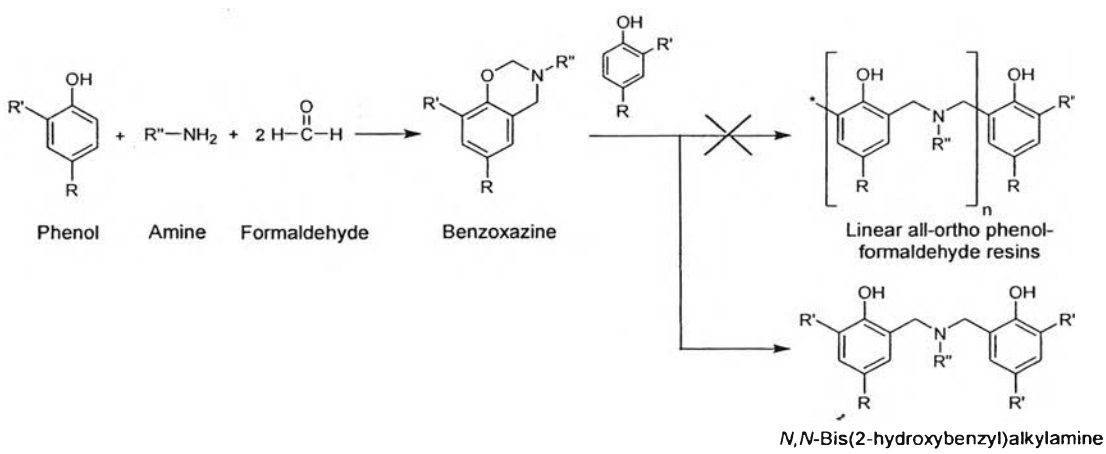

**Figure 2.1** Typical examples of (A) rotaxanes and (B) catenanes (Ariga *et al.* 2008).

A variety of applications can be obtained using molecular self-assembly concept ranging from the biological structures through complex organic syntheses to the design of new materials and electronics. Molecular self-assembly is crucial to the functions of cells. It is exhibited in the self-assembly of lipids to form the membrane, the formation of double helical DNA through hydrogen bonding of the individual strands, and the assembly of proteins to form quaternary structures (Atwood and Steed 2003). Molecular self-assembly is an important approach for fluorescence sensor. Systems of this kind include, e.g. polyaromatic molecules with protonated atoms in their backbone, that may work as fluorescent pH sensors (Van Duuren 1963). However selectivity and application flexibility are limited when using this molecules. A more sophisticated class of fluorescent sensors was developed with the concepts of supramolecular chemistry, capable of selectively sensing cations in solution (Pallavicini *et al.* 2008).

## 2.2 Inclusion Compounds Formed from N,N-bis(2-hydroxybenzyl)alkylamine Derivatives and Transition Metal Ions

Benzoxazine is a heterocyclic prepared from the Mannich reaction of phenol, formaldehyde, and amine derivatives. The ring opening reaction of benzoxazine proceeds easily under acid catalyst. In the past, our group declared that the ring opening of benzoxazine with the corresponding phenol is difficult to provide a linear polymer but quantitatively produces *N,N*-bis(2-hydroxybenzyl)alkylamine derivative possibly due to a single time of ring opening reaction (Scheme 2.1) (Laobuthee et al. 2003). Moreover our group also clarified the inclusion compound of *N,N*-bis(2-hydroxybenzyl)alkylamines with transition metals by using copper, cadmium and zinc as model ions (Phongtamrug et al. 2004). In solution, the host–guest ratios of *N,N*-bis(2-hydroxybenzyl)alkylamine derivatives with $CuCl_2$ were found to be 2:1. In the case of *N,N*-bis(2-hydroxy-5-methylbenzyl)methylamine shows a high ion extraction ability upto 80%.

**Scheme 2.1** Synthesis of *N,N*-bis(2-hydroxybenzyl)alkylamine



## 2.3 Basic Concept of Fluorescence

Fluorescence is a member of the ubiquitous luminescence family of processes in which susceptible molecules emit light from electronically excited states created by either a physical (absorption of light), mechanical (friction), or chemical mechanism. Generation of luminescence through excitation of a molecule by ultraviolet or visible light photons is a phenomenon termed "photoluminescence", which is formally divided into two categories, i.e., fluorescence and phosphorescence, depending upon the electronic configuration of the excited state and the emission pathway. Fluorescence is the property of some atoms and molecules to absorb light at a particular wavelength and to subsequently emit light of longer wavelength after a brief interval, termed the fluorescence lifetime. The process of phosphorescence occurs in a manner similar to fluorescence, but with a much longer excited state lifetime (Williams and Bridges 1964).

Fluorescence can be obtained from a highly conjugated polycyclic aromatic molecule that any of several energy levels in the ground state associates with a specific arrangement of electronic molecular orbitals. The electronic state of the molecule determines the distribution of negative charge and the overall molecular geometry. For any particular molecule, several different electronic states exist, depending on the total electron energy and the symmetry of various electron spin states. Each electronic state is further subdivided into a number of vibrational and rotational energy levels associated with the atomic nuclei and bonding orbitals. The ground state for most organic molecules is an electronic singlet in which all electrons are spin-paired (have opposite spins). At room temperature, very few molecules have enough internal energy to exist in any state other than the lowest vibrational level of the ground state, and thus, excitation processes usually originate from this energy level (Hof *et al.* 2005). The category of molecules results in fluorescence is known as fluorescent probes, fluorochromes, or simply dyes. Fluorochromes that are joined to macromolecules, such as nucleic acids, lipids, enzymes, or polymers) through adsorption or covalent bonds are termed fluorophores. In general, fluorophores are divided into two broad classes, termed intrinsic and extrinsic. Intrinsic fluorophores,

such as aromatic amino acids, neurotransmitters, porphyrins, and green fluorescent protein, are those that occur naturally. Extrinsic fluorophores are synthetic dyes or modified biochemicals that are added to a specimen to produce fluorescence with specific spectral properties.(Weiss 1999) Because of the tremendously sensitive emission profiles, spatial resolution, and high specificity, the technique is an important tool in analytical applications such as chemiluminescence in flow injection analysis system (Guilbault 1990).

## 2.4 Flow Injection Analysis

Flow injection analysis (FIA) is a simple, rapid, and versatile technique that is now firmly established, with widespread application in quantitative chemical analysis mainly in clinical and environmental analysis. Flow Injection Analysis (FIA) is an automated, continuous flow approach to perform chemical analysis, based on injecting a small, well-defined volume of sample into a continuously flowing carrier stream (Tyson 1985). In the simplest form of FIA the sample is injected into a continuous flow of reagent solution (carrier), dispersed, and transported to detector. Sample dispersion is controlled through the suitable choice of the injected sample volume, flow rate of carrier, length of the reaction coil, and diameter of the tube. A schematic diagram of the basic FI system is shown in Figure 2.2. In dealing with conventional spectrometry detectors, one of the most clear integrating effects is achieved when chemiluminescence reactions are involved, as the sample-reagent mixture reaches the luminescence detector in a time as short as necessary for proper detection of the transient emission from the deactivation of the product (Valcárcel *et al.* 1989). Thus, the method for the simultaneous determination of nitrate and nitrite in water in gas phase (Aoki and Wakabayashi 1995), the photometric biosensor for determining the activity of urease (Lei *et al.* 1995) and the chemiluminescence sensor for the determination of vitamin B12 (Qin *et al.* 1997) are basic examples of the integrating effect achieved.

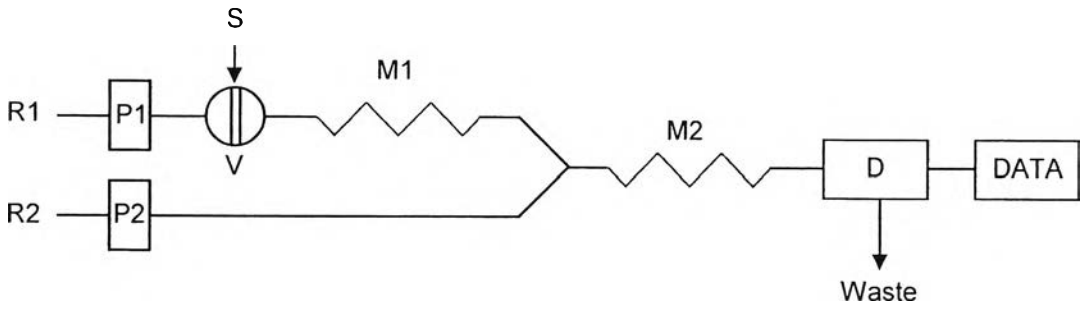



**Figure 2.2** Schematic diagram of the basic flow injection system (R1, R2: reagents, P1, P2: peristaltic pumps, V: injection valve, S: sample injection point, M1, M2: reaction coils, D: flow-through photometric detector).

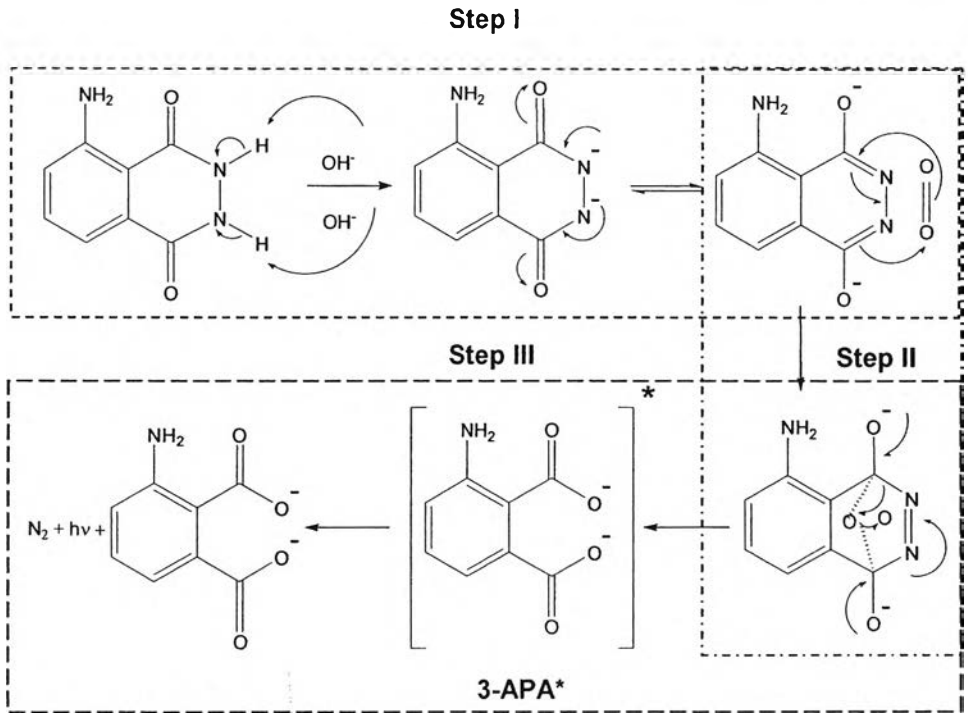
## 2.5 Chemiluminescence of Luminol and Related Compounds

Chemiluminescence (CL) is defined as the production of electromagnetic radiation (ultraviolet, visible or infrared) observed when a chemical reaction yields an electronically excited intermediate or product, which either luminesces or donates its energy to another molecule, which then luminesces. The chemiluminescence from synthetic compounds, including lophine derivatives, luminol-type compounds, acridinium esters, adamantyldioxetane compounds, peroxyoxalate esters and ruthenium complexes, has been reported. Among these chemiluminescent compounds, luminol derivatives have been well examined concerning their reaction mechanisms with oxidants and metal ion and thus have been widely used for many applications (Yamaguchi *et al.* 2002).

The chemiluminescence of luminol occurs in a basic solution to generate an energy-rich intermediate with subsequent light emission from 3-aminophthalate (3-APA*). To obtain chemiluminescence from luminol in an aqueous solution, an oxidizing reagent, e.g. hydrogen peroxide, is needed. The oxidization reaction of luminol is catalyzed by metal ions such as Co(II), Cu(II), Fe(III), etc., or by enzymes such as horseradish peroxidase, microperoxidase and so on. A sensible mechanism of the reaction is shown in Scheme 2.2. The base condition initiates the remove of protons from nitrogen atoms to be negatively charge ones which move onto the

carbonyl oxygen to form an enolate (Step I). In the next step, oxygen atoms perform cyclic addition to the two previous carbonyl carbons (Step II). Nitrogen is an excellent leaving group because its own bonds are so strong that the charges on the oxygens come back to form carboxylate anions by expelling nitrogen gas. This leaves 3-APA* (Step III) (García-Campaña and Baeyens 2000).

**Scheme 2.2** Chemiluminescence mechanism of luminol.




Many cyclic acylhydrazides originated in aromatic *o*-dicarboxylic acids show chemiluminescence, and substitution of the aromatic amino group has a great influence on the quantum yield of luminol derivatives. For example, 7-[*N*-(4-aminobutyl)-*N*-ethyl]naphthalene-1,2-dicarboxylic acid hydrazide (Figure 2.3A), benzo[*ghi*] perylene-1,2-dicarboxylic acid hydrazide (Figure 2.3B), 4-(9-acridonyl-10-methylene) phthalhydrazide (Figure 2.3C), and 4-(5′,6′-dimethoxybenzothiazolyl) phthalhydrazide (Figure 2.3D) have been known as intense chemiluminogenic compounds based on their highly fluorescent structures, naphthalene,

benzo[*ghi*]perylene, *N*-methyl acridone, and 2-phenylbenzothiazole, respectively (Ishida *et al.* 1995).

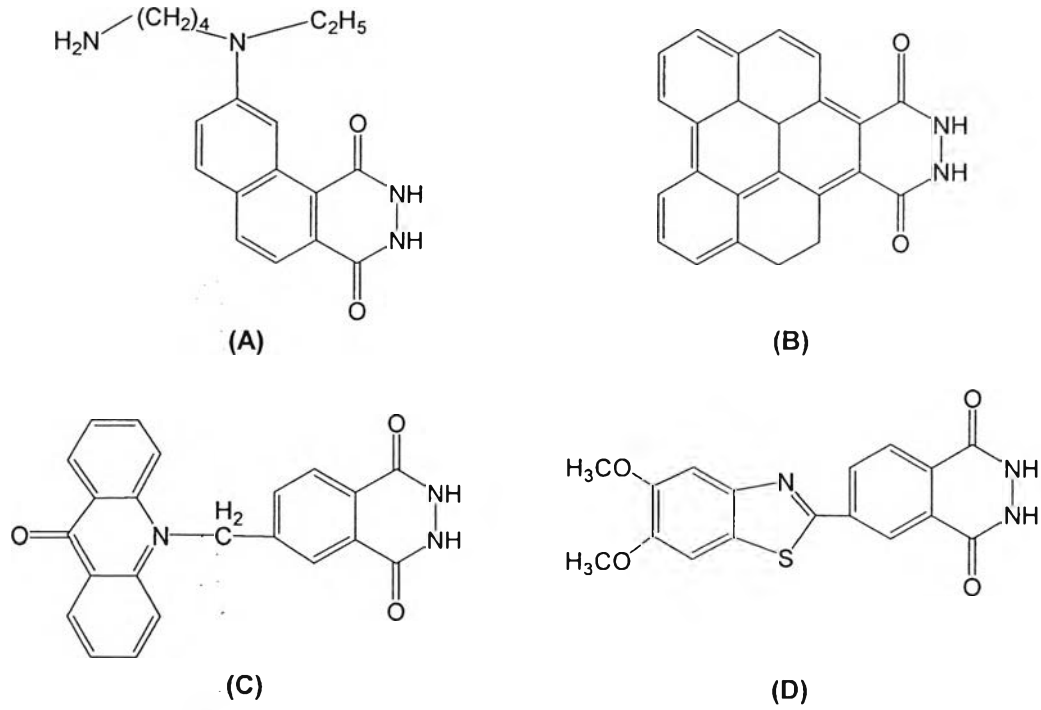



**Figure 2.3** Chemiluminogenic acylhydrazides. (A) 7-[*N*-(4-aminobutyl)-*N*-ethyl]naphthalene-1,2-dicarboxylic acid hydrazide; (B) benzo[*ghi*]perylene-1,2-dicarboxylic acid hydrazide; (C) 4-(9-acridonyl-10-methylene)phthalhydrazide; (D) 4-(5′,6′-dimethoxybenzothiazolyl)phthalhydrazide.

However, García-Campaña and Baeyens, (2000) reported some limitations have to be considered in CL analysis, such as the dependence of the CL emission on several environmental factors i.e., temperature, pH, ionic strength and solvent that hence must be controlled and the lack of selectivity because a CL reagent is not limited to just one unique analyte. This limitation, however, might be solved by supramolecular structure. The function of supramolecule is based on the molecular recognition, which host molecules selectively bind with guest species.

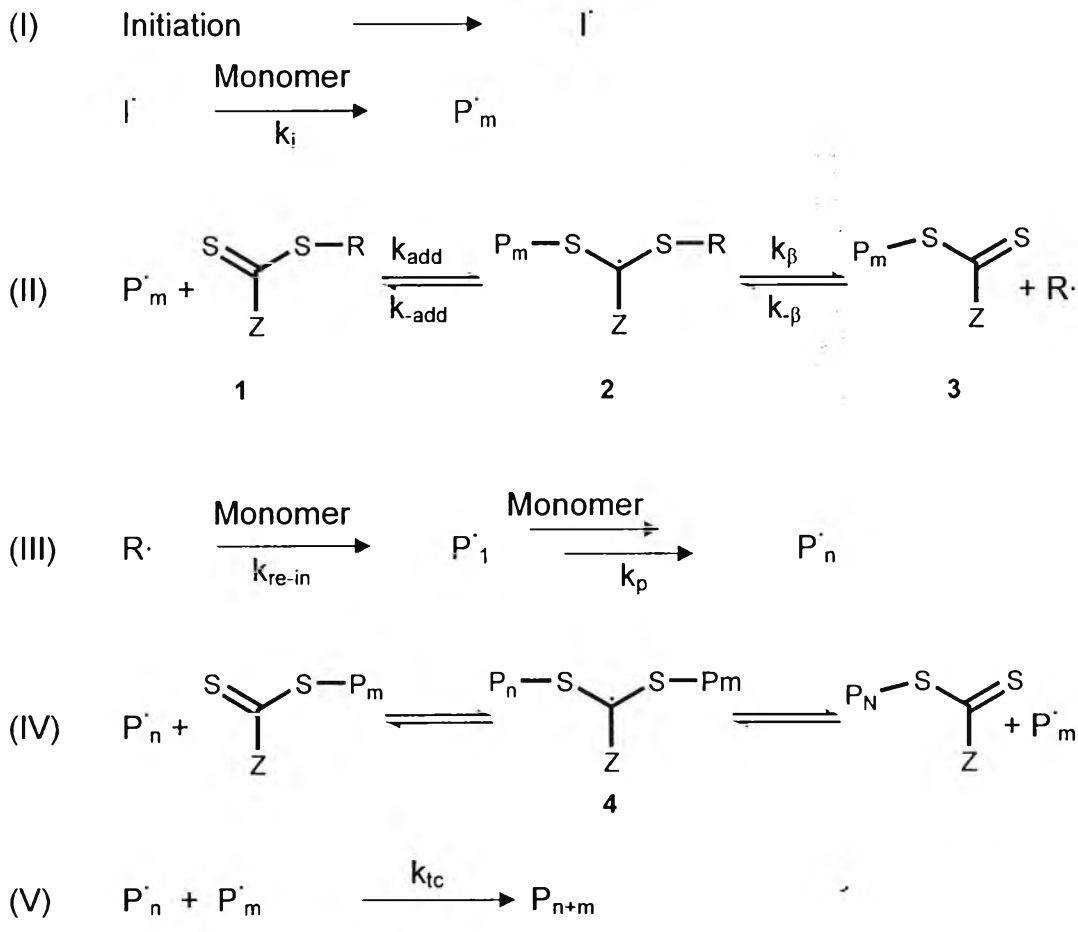
## 2.6 Reversible Addition–Fragmentation Chain Transfer (RAFT) Polymerization

Among the living radical polymerization techniques, reversible addition–fragmentation chain transfer (RAFT) polymerization is the most versatile processes in terms of the polymerization process (e.g., bulk, solution, and emulsion), (Moad *et al.* 2005) the variety of monomers (e.g., acrylamides, styrene, acrylic acid, 2-(dimethylamino)ethyl methacrylate) (Barner-Kowollik *et al.* 2003) for which polymerization can be controlled, tolerance to functionalities, and the range of polymeric architectures (e.g., linear, star, comb) (Perrier and Takolpuckdee 2005) that can be produced.

RAFT consists of the simple introduction of a small amount of dithioester of generic (Compound 1) (chain transfer agent (CTA); Scheme 2.2) in a conventional free-radical system (monomer + initiator). The transfer of the CTA between the growing radical chains which present at a very low concentration and the dormant polymeric chains which present at a higher concentration regulates the growth of the molecular weight and limits the termination. The mechanism of RAFT polymerization is depicted in Scheme 2.2 (Perrier and Takolpuckdee 2005). The radical species issued from the decomposition of the radical initiator reacts with the monomer resulting in growing polymer chains (Step I). The chains adding to the reactive C=S bond of the CTA (**1**) to form a radical intermediate, **2**, (Step II). The radical initiators may add directly onto the CTA, before reacting with any monomers. The fragmentation of the intermediate occurs reversibly either backwarding the initial growing chain (**1**) or forwarding the free re-initiating group (R) and a macro chain-transfer agent (macro-CTA; **3**). The R group can then re-initiate polymerization by reacting with the monomers to start a new radical polymer chain ($P^{\bullet}_1$), which will propagate to form $P^{\bullet}_n$ (Step III) or react back on the macro-CTA (**3**) (Step II). Once the initial CTA has been entirely consumed, the macro-CTA agent is solely present in the reaction medium and enters an equilibrium (Step IV). This equilibrium is considered the main equilibrium, and a rapid exchange between active and dormant (thiocarbonyl- thio capped) chains ensures equal probability for all chains to grow,

therefore leading to the production of polymers of narrow molecular weight distribution. Step V describes the unavoided terminations either combination or disproportionation. However, as the termination reactions are controlled to a minimum, the final product consists of a large majority of polymeric chains showing the re-initiating group (R) at one end and the thiocarbonyl-thio group at the other (Moad *et al.* 2005; Barner-Kowollik *et al.* 2003; Chiefari *et al.* 1998).

**Scheme 2.3 Mechanism of RAFT polymerization**



## 2.7 External Stimuli Responsive Micelles and Vesicles from Amphiphilic Block Copolymer Synthesized via RAFT Process

Several approaches can be considered to synthesize block copolymers. The most convenient consists in the successive polymerization of two or more monomers without purification steps of intermediate compounds (Matmour *et al.* 2006). However, this method is strongly limited to a few monomers, usually with the same chemical and physical properties (i.e. similar radical reactivity), which make them often inadequate for the material synthesis by self-assembly. (Quémener *et al.* 2006) RAFT polymerization is extremely versatile as most of the vinyl monomers can be polymerized under controlled/living conditions (Moad *et al.* 2005; Barner-Kowollik *et al.* 2003; Perrier and Takolpuckdee 2005; Chiefari *et al.* 1998).

Amphiphilic molecules consist of hydrophilic (water-loving) parts and hydrophobic (water-hating) parts, including also molecules with two different parts being both hydrophilic whereas one part can change to hydrophobic with external stimuli such as pH, temperature, and ionic strength. Amphiphilic polymers such as diblock or triblock copolymers, as well as graft copolymers with sufficiently long grafts and flexible backbones, have been known to self-assemble in the form of micelles or vesicles when dissolved in selective solvents. Driven by the need to develop technologically smart materials for use in various applications such as catalysis(Astruc and Chardac 2001; Jung *et al.* 2003), nanotechnology (Brunsveld *et al.* 2001), and drug delivery (Brondsted and Kopecek 1991), a number of stimuli-responsive polymers have been developed and extensively investigated. Of various polymeric systems, amphiphilic block copolymers with stimuli-responsive elements are of particular interest for two reasons: (i) they can self-assemble into various supramolecular structures and thus provide interiors that can noncovalently encapsulate guest molecules4 and (ii) the release of guest molecules can be triggered by external stimuli (Discher *et al.* 1999; Zhang and Eisenberg 1995; 1998). Self-assembly of amphiphilic molecules in aqueous media is of fundamental interest for applications in biotechnology and medicine, since most drug molecules are hydrophobic and therefore can be useful in drug delivery. A number of micellar

systems have been successfully developed so far. However, precisely switching on and off the release of the encapsulated guest molecules in response to environmental changes is still challenging. Toward this end, systems that respond to various stimuli such as light (Goodwin *et al.* 2005), temperature (Zhang *et al.* 2005), pH (Schilli *et al.* 2004), and redox potential (Ghosh *et al.* 2007) are becoming more prevalent for applications in biology (Adams *et al.* 2003), drug delivery (Brondsted and Kopecek 1991), recyclable catalysis (Bergbreiter *et al.* 1995), and separations (Li *et al.* 2004). Engineering new materials endowed with responsive properties for multiple stimuli can be highly beneficial to obtain more systematic release kinetics. These systems would provide a unique opportunity to fine-tune their response to each stimulus independently, as well as precisely regulate release profile during the combined effect of multiple stimuli.

## 2.8 Points of Research

As mentioned in 2.5, one of limitations of CL analysis is the lacking of selectivity including the simplification of FI system. On this viewpoint, the present project focuses on the molecular self-assembly of host-guest system to solve the problems. In Chapter III, we consider an introduction of the chemiluminescent molecules, luminol, onto *N,N*-bis(5-methyl-2-hydroxybenzyl)methylamine by diazotization reaction and we expected that this novel host compound might interact with transition metal such as Cu(II) to keep the solubility without adding the chelating agent to obtain the simple flow injection analysis system. The work focuses on the molecular design and simple synthesis pathway of the 5-((3-(((2-hydroxy-5-methylbenzyl)(methyl)amino) methyl)-2-hydroxy-5-methylphenyl) diazenyl)-2,3-dihydrophthalazine-1,4-dione compounds, host-guest complex with metal ion including the chemiluminescence properties.

During the past two decades, there have been numerous reports on stimuli-sensitive polymeric micellar systems. But a majority of them deal with response to single stimulus (Goodwin *et al.* 2005; Zhang *et al.* 2005; Ghosh *et al.* 2007). In nature however, the change in behavior of a macromolecule (i.e., proteins and nucleic

acids) is often a result of its response not to a single factor, but to a combination of environmental changes. To mimic this feature, formulation of materials which can sense specific changes and respond to multiple stimuli in a predictable manner would be important (Schilli *et al.* 2004). In Chapter IV, well-defined diblock copolymer poly(*N*-isopropyl acrylamide-*co*-*N*-vinylcarbazole)-*b*-poly(*2*-(dimethylamino)ethyl acrylate) (PNIPAAM-*co*-PNVC)-*b*-PDMAEA, containing a thermoresponsive PNIPAAM block, fluorescence tag NVC and pH-responsive PDMAEA block, was synthesized via reversible addition fragmentation chain transfer polymerization (RAFT). The block copolymers can self-assembly to form micelles in aqueous solutions in dependence of the pH and temperature with fluorescence properties resulting in multi-functional smart nanoparticles. The regulation of the micellar morphology is of great importance to obtain the desired functions and properties for a particular application. In Chapter V, the flexible controls of aggregation size and morphologies upon the pH and heating profile changing of (PNIPAAM-*b*-PDMAEA) were reported. The morphology changes from micelle to vesicles by decreasing pH from basic pH to acidic pH or the tuning the heating profile from slow to fast heating. Subsequently, Chapter VI involves the fluorescent nanoparticles from (PNIPAAM-*co*-PNVC)-*b*-PDMAEA. We can control morphology by changing the polymer composition, pH and temperature resulting in multi-stimuli responsive fluorescent nanoparticles. Chapter VII, the concept of mixed micelle from dissimilar copolymers: chitosan nanosphere and (PNIPAAM-*co*-PNVC)-*b*-PDMAEA block copolymer was reported. The sizes and behaviour of mixed micelle were control by pH and preparation method.