CHAPTER I

INTRODUCTION

1.1 A Brief History of Imprinting

The modern ideas about imprinting concept began when Wulff and coworkers [1] were among the first group to prepare molecularly imprinting polymers (MIPs) in 1972. They reported a covalent approach to prepare molecularly imprinted organic polymers for discriminating enantiomers of glyceric acid. Subsequently, in 1981 Mosbach and coworkers [2] reported that they had successfully prepared MIPs using non-covalent interactions. They showed that the covalent linkage between functional monomer and template are not necessarily required for molecular imprinting.

However, it is generally accepted that there are pros and cons to both approaches. In 1995, Whitcombe and coworkers [3] reported an intermediate approach that appeared to combine the advantages of both approaches. This approach relies on covalent interaction during the polymerization step but non-covalent interactions during rebinding procedure.

1.2 General Principle of Molecularly Imprinting Technique

Molecular imprinting polymers (MIPs) are attractive synthetic approach for preparing the highly cross-linked polymer matrix with specific recognition sites for specific detection of selected template molecules. MIPs are prepared using functional monomers that assemble around the template. Subsequently, interaction between functional groups on both the template and monomer is polymerized to form an imprinted polymer matrix. Then the template molecule is removed from the polymer matrix under certain conditions, leaving behind a cavity complementary in size and shape to the template. The obtained cavity can work as a selective binding site for a specific template molecule (Figure 1.1) [4].
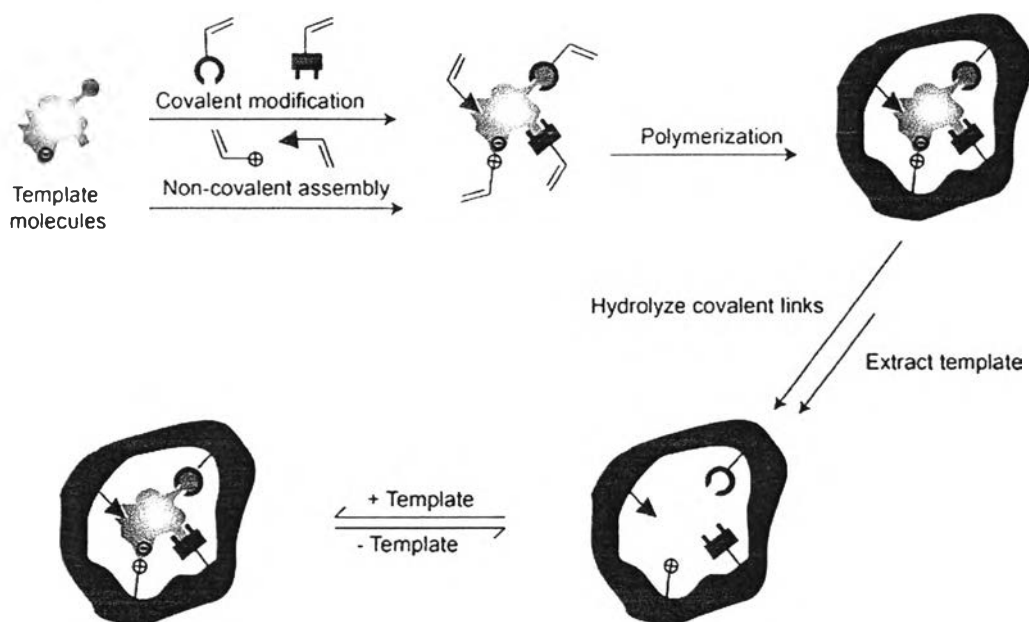
Figure 1.1 Schematic representation of imprinting process

The particular features have made MIPs the target of intense investigation [5]:

- Their high selectivity, which are similar to those of analyzed template molecules.

- The simplicity of their preparation and the ease of adaptation to different practical applications.

MIPs can be classified into three strategies, covalent bond (including metal-coordination), semi-covalent, and non-covalent interaction depending on the types of interactions between functional monomers and template molecules. However, nowadays the non-covalent imprinting approach has been used more extensively in MIPs synthesis for these reasons [6]:

- The interaction between monomers and templates are easily obtained when all compounds are mixed in polymerization step.

- Removal procedure of the templates is generally easier, usually accomplished by exhaustive extraction.

- A greater variety of functionality can be introduced into the MIPs binding site using non-covalent approach.

1.3 The Covalent Imprinting Approach

Covalent interactions were the first interactions to be used in the molecular imprinting approach. Functional monomer units and the templates are attached by covalent bonds to form a template-monomer by chemical step independent of polymer formation. Co-polymerization of this template-monomer with additional polymer-forming components, including a high proportion of cross-linker, in a porogenic solvent, results in a polymer which has template covalently bound within the polymer structure. After polymerization step, the covalent linkage is cleaved and the template is removed from the imprinted polymer matrix. Subsequently, rebinding procedure will both involve chemical reactions and the rebound template will be indistinguishable from template immediately following polymerization. The classical methods of covalent imprinting involve readily reversible condensation reactions. Boronic acid esters, ketals/acetals, and Schiff's bases formation are presented for very suitable covalent interactions [7, 8].

The boronic acids are used as binding sites. This group is very suitable for covalent binding with diol-containing templates, relatively stable boronic esters are formed. Its formation and dissociation are fast and easy. Boronic acids have five-membered cyclic structures, which are rigid enough to fix the covalent linkages for a desired conformation. After the polymerization, these linkages are cleaved by hydrolysis, and the boronic groups in the linkages are arranged suitably for guest binding. Thus, the boronic esters approach is undoubtedly the most successful of the reversible covalent methods, not least because it is applicable to the imprinting of carbohydrate derivatives. Template molecules imprinted using this approach such as glyceric acid [1], sialic acid [9], castasterone [10], and nucleotide [11]. Moreover, the boronic esters have also been incorporated in MIPs for fluorescent sensing [12] and functionalized polyaniline coatings for microtitra plates [13].

Ketals (or acetals) formation between a diol and a carbonyl compound has also been employed in covalent molecular imprinting. Mono- and di-ketone which used for template molecules have been extensively studied by Shea and coworkers [14] who synthesized a polymerizable diol as a binding site. They reported the results of an investigation of how molecular recognition is influenced by the initial

positioning of the functional groups at the binding site. A series of cyclic hemi-acetals has also been investigated for their potential as bind groups for monoalcohol.

The suitability of Schiff's bases for covalent binding was also extensively investigated. Schiff's bases involve the condensation of a primary imine and a carbonyl compound (usually an aldehyde). It is therefore potentially a useful method for the imprinting of either amine or aldehyde-bearing templates. Similarly, aldehyde-containing binding sites have been successfully applied for the imprinting with amino acid derivatives but the resultant enantioselective polymers is generally too slow for use in chromatographic separations [15, 16].

1.4 The Non-covalent Imprinting Approach

The synthesis of non-covalent imprinting approach requires a fundamental force that leads to self-assembly of the template-monomer complex. Non-covalent imprinting uses the typical forces of attraction between molecules such as hydrogen bonding, ionic interactions, electrostatic interactions, van der Waals forces, and hydrophobic forces to generate formation of template and functional monomers in solution [8, 17]. Unlike those used in covalent methods of imprinting, the imprinted polymer was obtained in situ simply by mixing the functional monomers and template into polymerization mixtures. The obtained non-covalent imprinting was spontaneously formed. The covalent linkage between functional monomers and template are not necessarily required prior to polymerization for this approach. Furthermore, after the polymerization, the template was easily removed by simple extracting the polymer with appropriate solvents. Because the preparation of non-covalent imprinting polymers is relatively straightforward and requires few simple syntheses, non-covalent molecularly imprinting has been widely attempted. The factors that can be manipulated in the design of an imprinting approach based on non-covalent interactions will be discussed in the following detail.

1.4.1 Functional monomers

A wide variety of commercially available functional monomers enable imprinters to take advantage of many different types of intermolecular interactions as shown in **Figure 1.2** [8].

Methacrylic acid
(MAA)

Trifluoromethacrylic acid
(TFMAA)

4-Vinylimidazole

Acrylamide

Methylmethacrylate
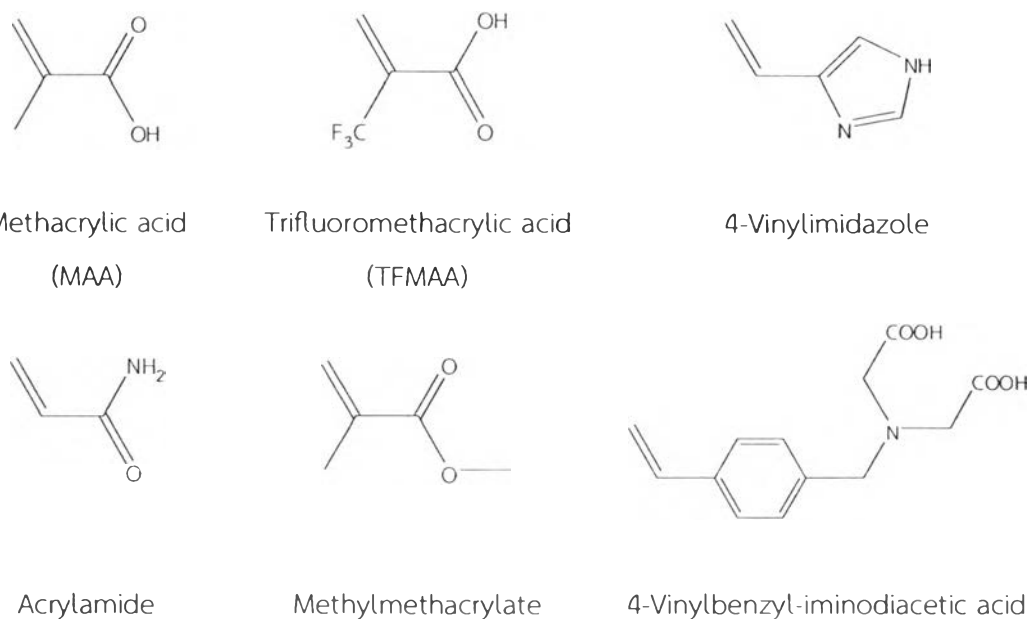
4-Vinylbenzyl-iminodiacetic acid

Figure 1.2 Some functional monomers used in non-covalent imprinting approach

Because the self-assembly process is governed by attractive interactions between the monomer and template, the choice of appropriate functional monomers is critical for achieving good imprints. Generally, to choose monomer with functional groups that complement those found on the template molecule. For example, if the template contains carboxylic or sulfonic acid groups, might select a functional monomer that contains an amine group, which can form strong ionic interactions with the template. When choosing the functional monomer, should consider the chemical environment and application in which the MIPs are to be used.

Methacrylic acid (MAA) [18] has been utilized as the most common functional monomer for a variety of template molecules such as peptides, nucleotides, drugs, herbicides, biologically active substance, and environmental contaminants. In addition to the strong ionic interactions that MAA can form with basic functional

group on template molecules, the carboxyl group on this monomer is an excellent hydrogen bond donor and acceptor. Frequently, trifluoromethacrylic acid (TFMAA) is used instead of MAA because the former is a stronger acid due to its electronegative fluorines than the latter, so that TFMAA is capable of forming stronger ionic interactions. Basic functional monomers, vinylimidazole, can participate in the formation of hydrogen bonds and can also form ionic interactions with acidic template molecules. Metal chelating monomer such as vinylbenzyl-iminodiacetic acid is also frequently used in molecular imprinting. For some applications, it is advantageous to use neutral monomers such as methylmethacrylate and acrylamide.

1.4.2 Cross-linkers

The main purpose of cross-linking monomer is to rigidly fix the functional monomers in place to produce stable binding cavities. This generally requires that the cross-linker must be present in very high proportions in the final polymer network. Some of the most common cross-linkers are shown in **Figure 1.3** [19]. For molecular imprinting in organic solvents, ethyleneglycol dimethacrylate (EDMA) and divinylbenzene (DVB) are often used. The used cross-linkers are also dependent upon the template molecules and solvent in the reaction mixtures.
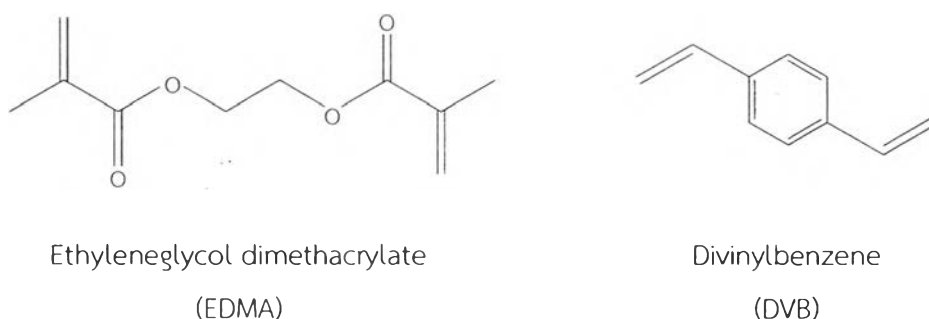

Ethyleneglycol dimethacrylate   Divinylbenzene
              (EDMA)   (DVB)

Figure 1.3 Some common cross-likers used in non-covalent

1.4.3 Solvents

The choice of solvent is critical for achieving good imprints and for successful rebinding results. It must use a solvent that dissolves all of the components of the pre-polymerization mixture and allowed for template-monomer interaction. Choice of solvent is dependent on the kind of imprinting approach. In covalent imprinting, many kinds of solvents are employable as long as they satisfactorily dissolve all the

components. In non-covalent imprinting, the choice of solvent is more critical to the promotion of the formation of non-covalent interaction between the functional monomer and template and thus to enhancement of the imprinting efficiency [8, 19].

In 1993, Mosbach and coworkers [20] reported the synthesis of an imprinted polymer, a method for creating selective recognition sites in synthetic polymers, to prepare polymers that mimic antibody combining sites. Molecular imprints made against the theophylline, which is used in the treatment of asthma. This research drew considerable attention to the field of molecular imprinting because it was the first to show that the MIPs and antibody-based assays exhibit similar selectivity, and both can discriminate between theophylline and structurally related compounds. An equilibrium binding assay is described which uses radiolabeled theophylline as a marker. Data are presented for which non-radiolabeled theophylline, caffeine, and theobromine are used in competitive binding assays. These assays provide valuable information about the capacity and selectivity of the MIPs.

In general, the non-covalent imprinting is easier to achieve and applicable to a wider several of template molecules than covalent imprinting approach. Compared to both approaches, during polymerization process, the covalent template-monomer linkage is unnecessary and the condition of polymerization process must be carefully chosen to maximize the formation of non-covalent interaction in the mixture. Furthermore, template molecules are easily removed from the polymer matrix using exhaustive extraction with appropriate solvents because of its weakly bound by non-covalent interactions. On the contrary, for covalent imprinting, the interaction between template-monomer linkages needs to be stable and specific geometric. The imprinting effect is in some case diminished in removal procedure, which requires rather severe conditions for cleavage of covalent linkages. However, it is necessary to choose the imprinting method according to the desire selectivity for analyzed template molecules.

1.5 The Semi-covalent Imprinting Approach

The semi-covalent approach attempts to combine the advantages of both methods by the polymerization of a template, covalently bound to a functional monomer by a cleavable linkage. Template removal, typically by hydrolysis, leaves imprint bearing functional groups, which are capable of interacting with in template in a non-covalent sense in the rebinding procedure. In other words, the imprinting process is covalent, but the rebinding procedure is non-covalent interaction. The semi-covalent approach was obtained by direct polymerization of template and functional monomers by an ester or amide linkage. Some of the limitations of the semi-covalent imprinting approach can be overcome by the use of a linker group between the template part and the functional monomer, which will be lost in template removal procedure. This linker group has the dual role of attaching the template to the functional monomer during polymer formation and acing as a spacer between the template and polymer-bound functionality to prevent steric crowding in the non-covalent rebinding. The spacer must be easily removed with template. It can be termed a sacrificial spacer [8].

The carbonyl group of carbonate ester was the first sacrificial spacer group to be used in the imprinting of cholesterol [3]. In this example, 4-vinylphenyl carbonate ester was used as functional monomer and co-polymerized with 3,4-ethylenedioxy-N-methylamphetamine (EDMA) in porogenic solvent. The template was attached covalently to the resultant polymer. Hydrolysis of the resultant polymers with NaOH in methanol and removed the cholesterol, as a template, with simultaneous loss of the spacer group as CO_2. Non-covalent rebinding of cholesterol in non-polar solvents was shown to occur by hydrogen bonding between the 4-vinylphenol residue of the polymer sites and the hydroxyl group of cholesterol.

1.6 Applications of Molecularly Imprinted Polymers (MIPs)

The molecularly imprinted polymer techniques have been applied in recognition and sensor applications including:

- Chromatographic Techniques:

Molecular imprinted polymeric materials have been used as stationary phase for separations by high performance liquid chromatography (HPLC). Analyzed template molecules with closely related structures can be separated such as different steroids, various herbicides, and underivatized amino acids.

- Solid-phase extraction and by-product removal:

MIPs were packed into solid-phase extraction (SPE) column as sorbents for separation and purification of analyzed molecules from complex matrices, which has been gained considerable interest in clinical, environmental, and food analysis.

- Enzyme mimics and controlled drug delivery:

MIPs were originally designed as artificial enzymes and artificial antibodies. Such possible medical applications include controlled release drugs, drug monitoring devices, and biological receptor mimetic.

- Chemical and bio-sensors:

The applications of MIPs-based on sensors are capable to recognizing and binding site target molecules with similar specificity and selectivity to their natural analogues. There is now a strong development towards the use of MIPs as the recognition element in sensors, for example, mass-sensitive transducers and electrochemical transducers.

1.7 Polythiophene

Polythiophene was a type of conducting polymers (CPs) that attracted much attention because of its high environmental stability in neutral form, its structural versatility, and ease of synthesis. Initially, polythiophene was prepared by electrochemical polymerization of thiophene monomers [21]. Since a polymer film was produced on the anode surface during electropolymerization, the obtained polymers are not easily processable after polymers were formed. The yields of polymers prepared from this method are moderate to low, and the polymers often do not have a well-defined structure.

In contrast to electrochemical polymerization, the yields of polythiophenes prepared from the oxidative polymerization with iron (III) chloride are relatively high. Moreover, the molecular weight of polymer synthesized by this method is sufficiently high for a film to be cast. Some polymers obtained are soluble in common organic solvents and their films can be formed by simply casting its solution on a solid substrate. Many poly(3-alkylthiophenes) are commercially prepared by this method.

Polymerization using a metal-catalyzed cross-coupling technique has been extensively investigated [22, 23]. The reaction is supposed to proceed by an oxidative addition of an organic halide with a metal catalyst and then transmetallation between the catalyst complex and a reactive Grignard or other organometallic reagent (or disproportionation) generates a diorganometallic complex. The last step involves reductive elimination of the coupled product with regeneration of the metal catalyst. Numerous organometallic species (including organomagnesium, organozinc, organoboron, organoaluminum, and organotin) have been demonstrated to be used in cross-coupling reactions with organic halides.

1.8 Solid State Polymerization of PEDOT

Poly(3,4-ethylenedioxythiophene) (PEDOT) is one of the most industrially important conjugated polymers because of its excellent electronic properties and high stability. Due to a stereoregularity of the only possible polymer structure, it has a very high conductivity (up to 550 S/cm in the electrochemical doped state). The remarkable stability of PEDOT in its doped state, compared with other CPs, allows a

number of potential applications [24]. Prepared by oxidative chemical and electrochemical polymerization method, PEDOT is found relatively insoluble in conventional organic solvents leading to the difficulties in fabrication process. Nevertheless, there is an anticipation that the increase of the alkyl chain length in the alkoxy group could lead to a soluble polymers in organic solvents.

Polymerization of PEDOT by traditional oxidative polymerization of 3,4-ethylene-dioxythiophene (EDOT) with $FeCl_3$ in organic solvents gives an insoluble blue-black polymer powder. The limitations of traditional polymerization methods can be a serious problem for PEDOT applications as well as for in-depth investigation of molecular order in these CPs. It is generally not possible to obtain a well-defined polymer structure, unless the synthesis of CPs is carried out via pure chemical polymerization routes, without adding any catalysts. A possible solution for this lies in a solid state polymerization (SSP) of a structurally pre-organized crystalline monomer.

The advantages of SSP including low operating temperatures, which restrain side reactions and thermal degradation of production, while requiring inexpensive equipment, and uncomplicated and environmentally sound procedures. However, at SSP low temperatures, rate of the reactions are slow compared to polymerization in the melt phase because of the reduce mobility of the reacting species, and the slow diffusion of the by-products.

Meng and coworkers [25, 26] discovered the SSP of 2,5-dibromo-3,4-ethylenedioxythiophene (DBEDOT) by chance as a result of prolonged storage (2 years) at room temperature or heated (50-80 °C) of the monomer **(Figure 1.4)**. The colorless crystalline DBEDOT transformed into a black blue material while retaining the morphology. Surprisingly, the conductivity of this decomposition product appeared to be very high (up to 80 S/cm) for an organic solid. Indeed, the most likely explanation for the observed transformation was polymerization with formation of bromine-doped PEDOT.

Figure 1.4 Solid state polymerization of DBEDOT

The highest conductivity belongs to the SSP-PEDOT polymer prepared at lowest temperature and longest reaction time, which may reflect achievement of a higher degree of order. Indeed, heating above the monomer's melting point results in dramatically reduced conductivity (0.1 S/cm), which rises up to 5.8 S/cm after doping with iodine, approaching the value of a $FeCl_3$-synthesized PEDOT (7.6 S/cm). Not very significant, but certain increase in conductivity of SSP-PEDOT (about 2 times) was found on exposing a sample to iodine vapor.

As a unique derivative of polythiophene, PEDOT possesses several advantageous properties compared with unsubstituted polythiophene and most other polythiophene derivatives. PEDOT had received considerable interests attributable to its low band gap, high electrical conductivity, good stability, and excellent optical transparency in the visible region. The ether groups at β,β'-positions of thiophene ring in PEDOT prevented the formation of α·β· linkages defect during polymerization. Thus derivatization of thiophene ring by other substituents such as alkoxy or polyether groups at the β positions could lead to higher solubility and improved physical and chemical properties.

The SSP of their new structurally pre-organized crystalline monomers could also result in another well-defined polymer structures with high conductivity. Moreover, this method was uncomplicated, less side reactions and environmentally sound procedures.

Based on the research as listed above, it seems that the shortage of suitable monomers and difficulty of making uniform conductive film further restricts SSP's wide applications. In 2013, Yin and coworkers [27] have found that PEDOT was

prepared by acid-assisted polycondensation based on 2-bromo-3,4-etylenedioxy thiophene (BEDOT) **(Figure 1.5)**. Under the exposure to ambient atmosphere, the formed PEDOT polymer is in doped state to some extent, showing poor conductivity of lower at 10^{-6} S/cm while improved to 0.3 S/cm along with further iodine doping. Such finding provides another alternative for the synthesis of conjugated polymers through simple acid-assisted polycondensation.
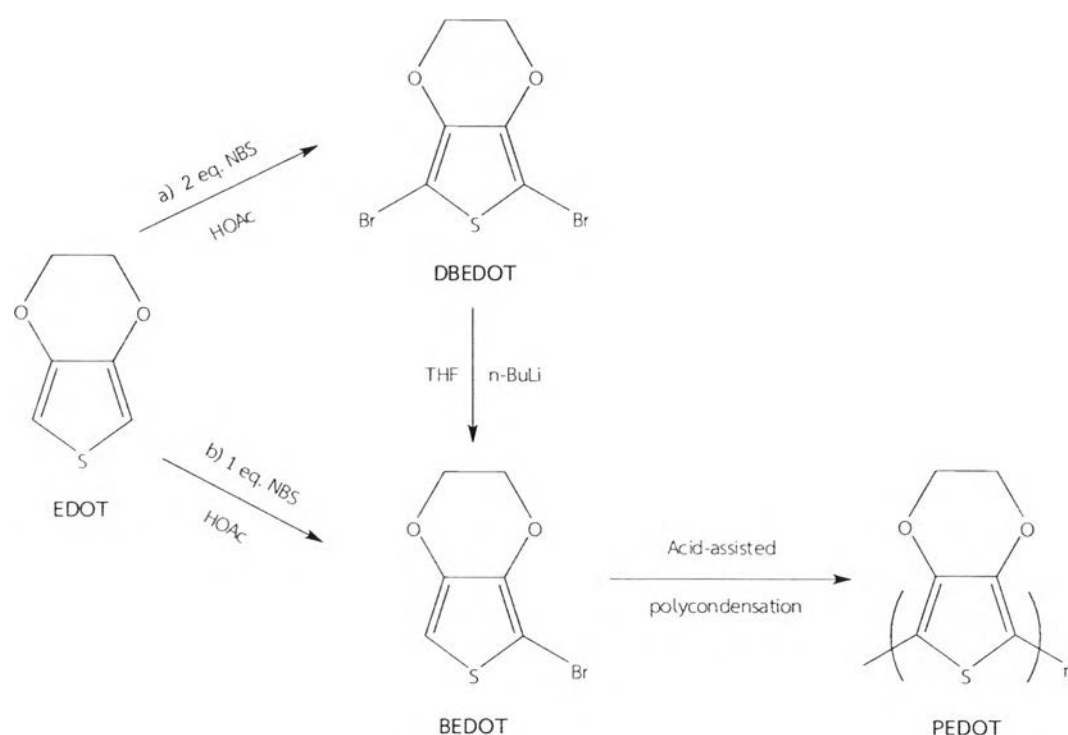

Figure 1.5 Synthesis of PEDOT by acid-assisted polycondensation

1.9 Literature Reviews

Kubo and coworkers [28] have designed 2-acrylamidoquinoline as a fluorescent functional monomer upon imprinting the cyclobarbital molecules. A cyclobarbital-selective MIP was polymerized using self-assembly of a template molecules with functional monomers followed by co-polymerization with a cross-linker. The resultant imprinted polymer exhibited an enhancement in fluorescence intensity when target molecules were bound. This result showed that the fluorescent

responsive imprinting method could be developed for quantification of non-fluorescent compounds.

Pardieu and coworkers [29] have developed an electrochemical sensor based on molecularly imprinted conducting polymer (MICP), which enabled the recognition of pesticide molecules, atrazine. The poly(3,4-ethylenedioxythiophene-co-thiophene-acetic acid), poly(EDOT-co-AAT), has been synthesized onto a platinum electrode by electrochemical polymerization in the presence of atrazine. In their studies, AAT monomers can interact with atrazine by hydrogen bonds which EDOT monomers polymerized to stable and homogeneous film. The obtained sensing MICP is highly specific towards newly added atrazine. The functional recognition sites were modified during the electropolymerization and quantitatively analyzed by the variation of the cyclic voltammogram of MICP.

Ho and coworkers [30] have modified PEDOT to immobilize the MIP particles onto the indium tin oxide (ITO) glass as a MIP/PEDOT modified electrode for an amperometric detection of morphine. The MIP prepared by precipitation polymerization gave uniform particles. In the rebinding experiment of a solution containing morphine, the maximum number of binding sites for MIP and NIP were 1.86 and 1.78 μmol (based on 10 mg particles), respectively. The sensitivity for the MIP/PEDOT-modified electrode was 41.63 $\mu A/cm^2$ mM, which was more sensitive than NIP/PEDOT and pure PEDOT electrode.

Bunte and coworkers [31] have developed the specific sensor coating materials based on MIPs by suspension polymerization as well as thin MIP coatings by direct surface polymerization on quartz crystal microbalances (QCM). The thin MIP coatings directly synthesized on the QCMs showed thicknesses of 20 to up to 500 nm. Performance tests of the 2,4,6-trinitrotoluene (TNT)-imprinted polymer beads showed that acrylamide (AA) and also methacrylic acid (MAA) possessed an enhanced adsorption tendency for gaseous TNT. An adsorption of 2,4-dinitrotoluene (2,4-DNT) by these MIPs was not detected. Using 2,4-DNT as template and methacrylamide (MAAM), a positive imprinting effect for gaseous 2,4-DNT was achieved with no measurable cross-sensitivity for 2,4,6-TNT.

Alizadeh and coworkers [32] have successfully prepared the high selective and sensitive electrochemical sensor for TNT determination. The MIPs having recognition sites for TNT was used as a recognition element, in the carbon paste electrode, using MAA and ethyleneglycol dimethacrylate (EDMA) as a functional monomer and cross-linker, respectively. The optimized sensor was used for TNT determination in water and soil samples.

Xu and coworkers [33] have designed the MIPs with trinitrophenol (TNP) as a dummy template molecule capped with CdTe quantum dots (QDs) using 3-aminopropyl-triethoxy silane (APTES) and tetraethoxysilane (TEOS) as the functional monomer and the cross linker, respectively, for the preparation of a high-affinity DMIP@QDs by a sol–gel process for the recognition and sensing of TNT. The developed DMIP@QDs could highly selectively rebind TNT and quickly quench the fluorescence of the QDs. The obtained result was successfully applied to develop sensors for the rapid recognition and determination of hazardous materials from complex matrices.

CPs have found wide applications not only for their electrical properties but also as sufficient electrochemical sensors. CPs can be chemically functionalized with various chemical groups, which can be chosen as tags for their ability to recognize biological or chemical target molecules. The key should be to build highly specific recognition sites in the CPs, which will both increase the selectivity and improve the sensitivity of the recognition process.

The molecular imprinting technology can be applied to generate the polymer matrix which determined molecular recognition properties. MIPs are obtained through polymerization in the presence of a template molecule. The SSP could also result in another well-defined polymer structures without adding any catalysts. Hence, highly specific cavities are created into the polymeric matrix. After target removal, MIPs demonstrate interesting recognition properties towards the template, originating from shape and chemical functionality considerations in the cavities present in the polymer matrix. Thus, the objective of high specificity together with high sensitivity can be realized by combining the concept of MIPs with the use of CPs.

1.10 Objectives

The goal of this research utilized the facile SSP of DBEDOT monomers surrounding selected template molecules to give an unprecedented imprinted PEDOT. The resulting molecularly imprinting conjugated polymers would carry selective and specific cavities towards the templates molecules, in which the binding property of the imprinted polymer was monitored by the altered concentrations of the template solution.