CHAPTER 1

INTRODUCTION

1.1 General Introduction

Molecules that relate to each other like a pair of hands are called chiral molecules. Molecules that are mirror images of each other are also called enantiomer. These molecules generally have a tetrahedral carbon atom with different substituents. Diastereoisomers or diastereomers are basically stereoisomers with two or more centers of asymmetry that are not enantiomers of each other. A molecule can have only one enantiomer; however, it can have several diastereomers or none. The differences in the physiologic properties between enantiomers of racemic drugs have not been fully examined in many cases, mainly because of difficulties in obtaining both enantiomers in optically pure forms. It is entirely possible that a patient may be taking a useless, or even undesirable, drug when ingesting a racemic mixture, since some enantiomers exhibit different pharmacologic activities. To ensure the safety and effectiveness of currently used and newly developing drugs, it is important to isolate and examine the enantiomers separately. Accurate assessment of the isomeric purity of substances is critical, since isomeric impurities may have unwanted toxicologic, pharmacologic, or other effects (Ahuja, 1997). Most methods employed for the separation of enantiomer pure compounds, such as stereoselective asymmetric synthesis and enantiomeric separation are based on the molecular recognition materials. For example, the use of membrane technology for chiral separation offers several advantages over the method, such as low cost, simple set up and relatively inexpensive technology (Duan *et al.,* 1998).

1.2 Literature Review

1.2.1 Molecular recognition

Specific molecular recognition is a fundamental requirement of living systems and, through millions of years and countless rounds of evolutionary optimization, "biology" has become a master of the art. At the cellular and sub-cellular level the fundamental processes of life, information transfer and reaction catalysis, rely on the specific interaction of low molecular weight molecules with macromolecular "hosts." In the majority of such events the macromolecule is a protein. Processes as diverse as neural transmittance, respiration, immune defence, cellular differentiation and nutrition all rely on the basic principle of specific molecular recognition (Sellergren *et al.,* 2005).

In general molecular recognition can be viewed as the ability of a certain biomolecule, also referred to as the recognition agent or recognition molecule in this article, to interact preferentially with a particular target molecule although a vast variety of different but structurally similar rival molecules are present. Recognition processes are governed by the interplay of noncovalent interactions of comparable strengths such as ionic binding, the van der Waals interaction, the formation of hydrogen bonds and hydrophobicity. The noncovalent interactions between the residues of the molecules lead to the formation of a complex where the two molecules form a mutual interface consisting of one or more patches on their surfaces. The knowledge of these principles is a necessary input for the design of synthetic heteropolymers with molecular recognition ability (Behringer *et al.,* 2007).

1.2.2 Molecular imprinted polymer (MIP)

1.2.2.1 Introduction of MIP

Molecularly imprinted polymer (MIP) is synthetic polymer which can recognize its template molecule and can have cross-reactivity with the compound structurally related to template that serves as a mould for the several template complementary binding sites (Sellergren *et al.,* 2005). Molecular recognition of MIPs can be explained on the basis of lock and key model.

The key is template and the lock is polymer which recognize to its template. Molecular recognition of MIPs will be derived by interaction between the MIP and template by covalent bond or non-covalent bond, the non-covalent bond such as electrostatic attraction, hydrophobic interaction, ligand-metal interaction, hydrogen bond and van der Waal force approach has been used more extensively for three reasons (Figure 1.1). The three reasons are non-covalent methodology of MIP and template, *i.e.,* (1) easier when all components are mixed in solution, (2) interaction between MIP and template with non-covalent bond can be easier remove and (3) a greater variety of functionality can be introduced into the MIP binding site (Spivak, 2005).

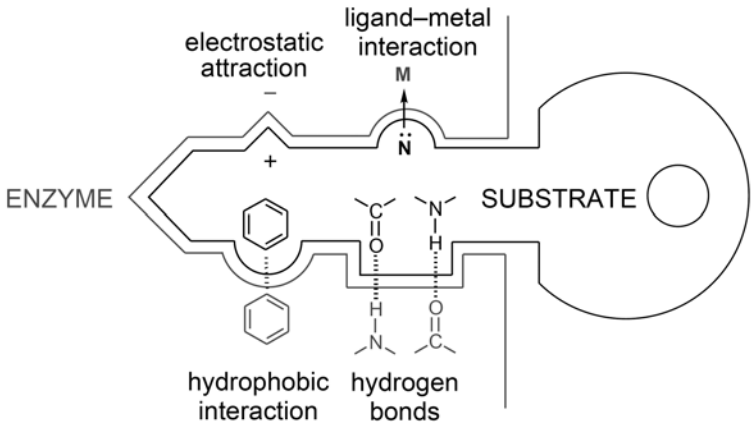

Figure 1.1 Lock and Key Theory of non-covalent bond for MIP (modify from Braden *et al.,* 1995)

MIP which contains a heterogeneous distribution of binding site that range in affinity and selectivity (Rushton *et al*., 2005) display the stability and robustness of synthetic polymer.

1.2.2.2 Chemical for MIP

The print molecule is the target compound. Small molecule such as drug, chemical or pesticide has been used as print molecule in imprinting polymerization. For large molecules for example enzyme, protein, nucleic acid which is more rigid structure can increase the number and strength of the interactions between complementary functionalities in the template and the polymer, hence the increased affinity of the MIP product (Ahmed *et al.,* 2006).

Criteria to consider when selecting a candidate molecule as a print molecule are its cost, its availability and, of course, its chemical functionalities defining its ability to strongly interact with monomers. In non-covalent imprinting, the interactions involved are weak therefore the candidate template should possess multiple functional sites in order to increase the strength of the template-functional monomers assemblies. Usually, the amount of template needed for the synthesis is high (often 1 mmol). The porogen solvent is one of the most important factors determining effective molecular recognition because the accuracy of the assembly between the template and the monomer is related to the physical and chemical characteristics of the solvent. Thus, polar interactions such as hydrogen bonds and electrostatic interactions can strongly take place in these organic media (Pichon, 2007). The bead particles made using chloroform as porogenic solvent were lower and had little rougher surface morphology and low "debris" on their surface (Mayes *et al.,* 1996).

The common used functional monomers for MIP product are methacrylic acid (MAA), 4-vinylpyridine (4-VP), acrylamide and acrylic acid, etc. In this non-covalent, the nature of the pre-polymerization complexes present is far from simple and a number of interactions need to be taken into account. It is important to bind the template with the monomer in complementary pattern, such as H-bond, to maximize complexation. Methacrylic acid has been by far the most successful. This probably stems from the fact that they have relatively few bonds with rotational degrees of freedom and their ability to interact in various ways with template: as H-bond donors, H-bond acceptors and through formal ion-pair formation, as well as weaker dipole–dipole interactions, etc. From the basic monomer, these have been effective in a few cases, but in general yield less satisfactory results than acidic monomers, possibly due to their much greater chain flexibility and the fact that the amine functionality is more remote from the polymer backbone (Mayes *et al.,* 2005).

For cross linking monomer; *p*-divinylbenzene, ethylene glycol dimethacrylate (EDMA), trimethylolpropane trimethacrylate, trimethylolpropane trimethacrylate (TRIM), etc have been widely used in the synthesis of MIP membrane. A criterion for cross-linker is its ability to control the morphology of polymer matrix. The cross-linker can stabilize the binding and physical property of polymer matrix. Enhanced performance in chiral recognition has also been

found in polymer prepared with novel difunctional cross-linkers, which superficially resemble EDMA (Mayes *et al.,* 2005).

For initiator, oil-soluble initiator (OSI) such as 2,2'-azobis izobutyronotrile (AIBN), dibenzoyl peroxide (DBP), etc. are used as initiator for polymerization. In this case, the decomposition of initiator leads to the formation of hydrophobic primary, secondary or tertiary radical able to enter or exit from polymerization mixture in the process but the polymerization by the oil-soluble initiators is slowly initiated than with water-soluble initiator (WSI; such as SO^{-}_{4}). The decomposition of initiator by thermal (60^{o}C) is easy to obtain radical but difficult control and generation by UV (366 nm) is easy control and high accuracy but depend on effective of UV lamp (Capek, 2001).

1.2.2.3 Overview of assembly of MIP

MIPs are produced by arranging of functional monomer around a ligand and it will form a pre-polymerization complex with the template by non-covalent or covalent bonding interaction. The complexes formed are subsequently co-polymerized with a suitable cross-linking monomer and the imprint molecules will be removed from the polymer to yield recognition site specific to the original template. The quality of the binding site of MIP is depended on the interaction between template and functional monomer molecules in terms of thermodynamics (Figure 1.2) (Nicholls *et al.,* 2001).

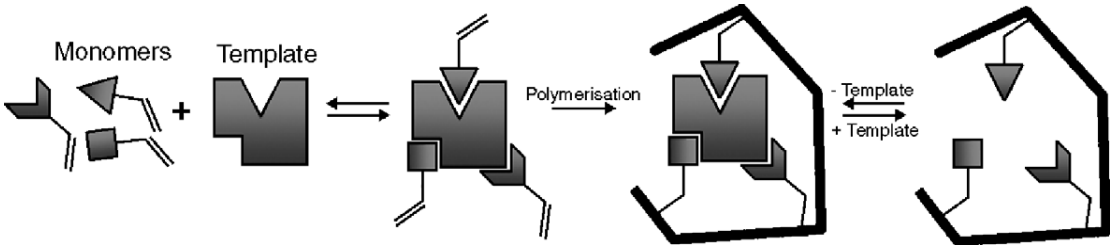

Figure 1.2 Schematic illustration of preparation of MIP (Behringer *et al.,* 2007)

1.2.3 Polymerization for synthesis of MIP and general characterization

For polymerization reaction, it can be categorized into two types, chain polymerization and step polymerization. The popular polymerization for molecularly imprinted

polymer is chain polymerization due to the high-molecular-weight polymer is formed immediately and molecular weight of the polymer is unchanged during the polymerization (Odian, 2004). For chain polymerization, a radical, anionic and cationic reactive center adds monomer unit can grow rapidly to a long size. MIP polymerization is popular used in radical polymerization because it can be attributed for a wide range of functional groups and template structures and the free radicals generated during the chain polymerization do not interfere with the intermolecular interactions which critical for the non-covalent imprinting system (Boonpangrak *et al.,* 2006). Cationic polymerization is little used because rate constants of propagation will be high even at low temperatures which be control of the reaction difficult, side reactions can take place and the polymerization are sensitive to impurities (Goethals *et al*., 2007) and anionic polymerization is necessary to monitor the monomer conversion during the polymerization process (Guadarram *et al.,* 2007).

In this work, the MIP was synthesized by suspension polymerization and grafting method. Suspension polymerization that can be initiated by radicals is preferred whenever it is desired to produce small spherical beads. This method is also called bead polymerization. Bead diameters range from 5 μm up to 1 cm. The organic phase, containing a single monomer (or mixed monomers) and a radical initiator, which is soluble in the monomer, is progressively add to a reaction vessel. The impeller breaks this phase into drops, in which monomer is converted to polymer by the radicals generated within. The size of particles involved mean that the controlling physical phenomena are different. As an example, consider particle breakage and coalescence: these mechanisms are greatly affected by the fluid dynamics in a stirred vessel (Rempp *et al.,* 1991 and Matyjaszewski *et al.,* 2002). Grafting method are those which involve polymerization of monomer B starting from sites created on the polymer A. Free radical sites can be formed on a polymer chain upon using chemical. This method is performed on a polymer swollen by a monomer, grafting generally results. This method of grafting has found a number of applications to modify polymers for special use, to render them hydrophilic, to increase their swellability or their biocompatibility. Chemical initiation has also been attempted. Here a polymer backbone is made, that contains some cleavable bonds. Upon heating this polymer in the presence of a second monomer, initiation takes place. But only half of the chains formed are grafted (Rempp *et al.,* 1991).

The properties of a polymer fall into two general classes. The first is material properties are mainly related to the nature of the polymer itself such as crystalline and amorphous, size, shape, porosity thermal properties, etc. and the second is specimen properties are primarily consequences of the size, shape and layout of the finished specimens prepared from that polymer, and the process used to prepare these specimens such as mechanical properties i.e., tensile strength etc. (Bicerano, 2002).

In the part of polymer bead, particle behavior is directly related to particle properties, including size, size distribution, density, shape. Particle behavior within these systems is greatly dependent on the forces between particles and the effect of external forces on particles. Fundamental particle properties, such as particle size, shape, surface roughness and surface area have significant effects on particle behavior. Fundamental properties are inherent attributes relating to individual particle or particle populations such as size, shape, porosity and surface area. Derived properties depend upon the fundamental properties and may be affected by environmental conditions. The size of a sphere is uniquely defined by its diameter. The equivalent spherical diameter relates the size of an irregularly shaped particle to the diameter of a sphere having the same size-dependent physical property. A population of pharmaceutical particles rarely contains uniformly sized (monodisperse) particles. Particle size is a very important parameter for predicting the behavior systems (Crowder *et al.,* 2003). Microparticles are of great interest in drug delivery. It appears that nature, in making the biological systems, has extensively used micrometer scale. If one has to go hand in hand with nature in controlling the activity one need to use the same scale (Gupta *et al.,* 2006).

1.2.4 Incorporation MIP and membrane base (MIP membrane)

1.2.4.1 Introduction of MIP membrane

MIP have application as separation for chromatography, solid phase extraction, nano reactors for synthesis of new enzyme inhibitor, artificial receptors for drug assay, biological receptor mimic, recognition elements for biosensor and drug delivery, as illustrated in Figure 1.3 (Lorenzo *et al.,* 2004 and Nantasenamat *et al.,* 2007). The method of development membranes

with controlled specificity for individual compounds has attracted considerable attention. So integration of MIP with membrane or called MIP membrane can synergist the merit of the either.

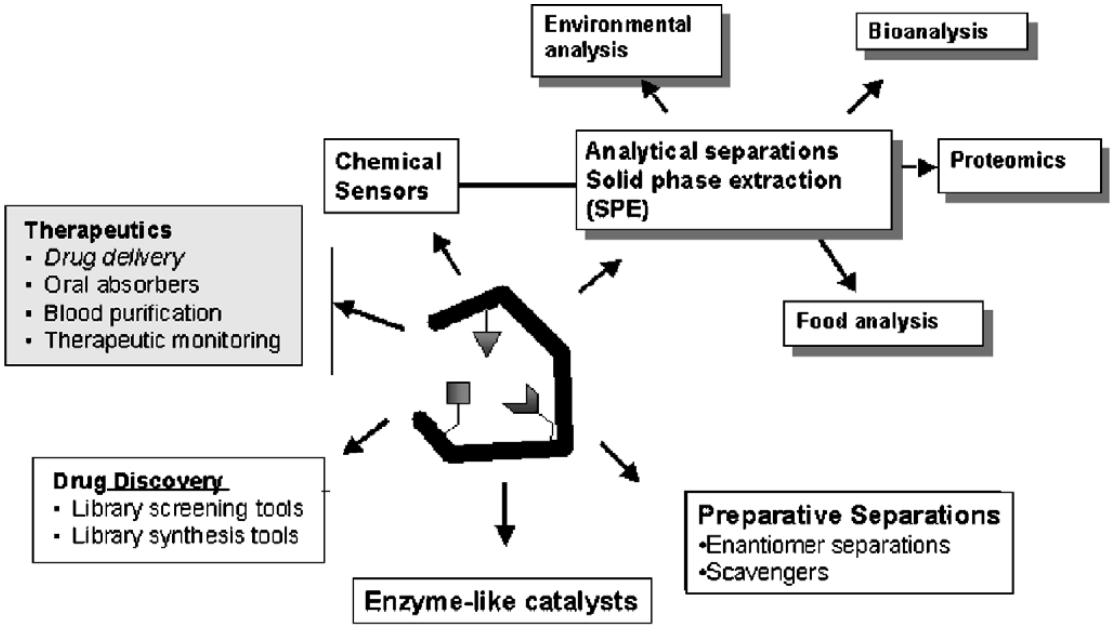

Figure 1.3 The application of MIP (Behringer *et al.,* 2007)

MIP membrane is a membrane containing molecular recognition that is created by molecular imprinting technology (Figure 1.4). Several advantages of MIP membrane include: the several preparation techniques, the flexible sheet can be obtained in case of MIP membrane and MIPs membrane have specific permeability and separation for template or ligands. Also, the use of MIP membrane as affinity phase benefits for affinity separation and membrane sensors. MIP membranes are mainly used in affinity separation. This will be advantageous for water purification, waste-material treatment or in chemical, pharmaceutical and biotechnology industry, the use of MIP membrane is beneficial for separation of drugs and chiral drugs from biological and environmental samples. Also, MIP membrane can be used in solid phase extraction for clean-up sample, preconcentration or pretreatment sample before analysis of target. Application in MIP membrane with special functions, MIP membrane can be applied to use in controlled drug delivery or the use of MIPs to alter the surface biocompatibility of medical devices (Hilal *et al.,* 2003).

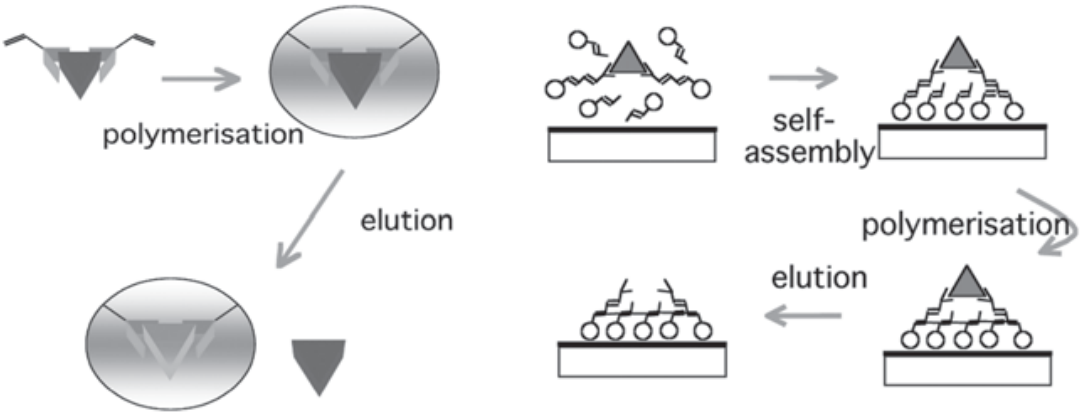

Figure 1.4 Overview of MIP assemblies as MIP membrane (Piletsky *et al.,* 2006)

MIP membrane can be identified into two groups. First is a simple MIP membrane which is a supported membrane containing a ready-made MIP. This type of MIP membrane gives very low permeability of the compound. The second is a MIP integrated with other matrix. For this work, the composite MIP cellulose membrane would be focused due to its high permeability and stability (Ubricht, 2004).

Composite MIP cellulose membrane can be classified according to some criteria, *i.e.,* (1) membrane material e.g. organic polymers, inorganic materials, (2) membrane cross-section morphology e.g. symmetric, asymmetric, (3) preparation method e.g. phase inversion of polymer, thin layer composite etc. and (4) membrane shape e.g. flat-sheet, hollow fiber.

Composite MIP cellulose membrane requires membrane base either as symmetric or asymmetric macroporous membrane. Symmetric macroporous membranes are commonly used in microfiltration process (Figure 1.5) and asymmetric microporous membrane is suitable for ultrafiltration use of MIP membrane (Figure 1.6) (Ubricht, 2004).

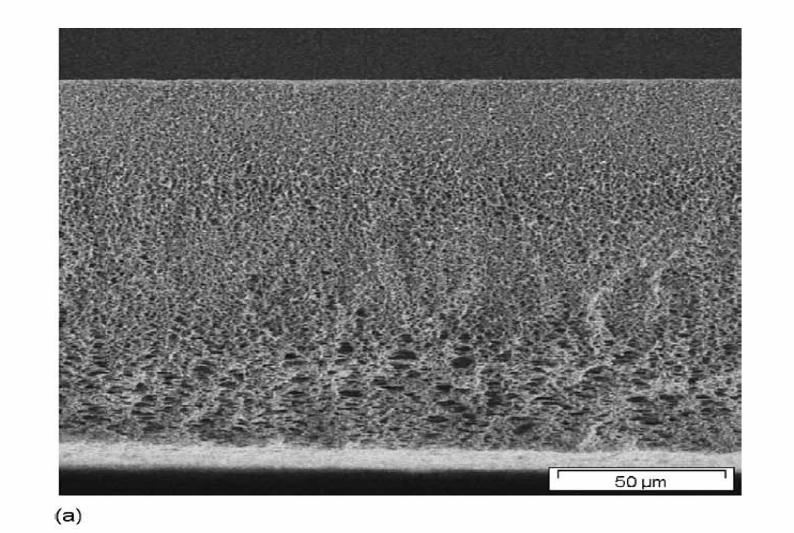

Figure 1.5 Symmetric macroporous membrane for microfiltration or as base material for membrane adsorption (Ubricht, 2004)

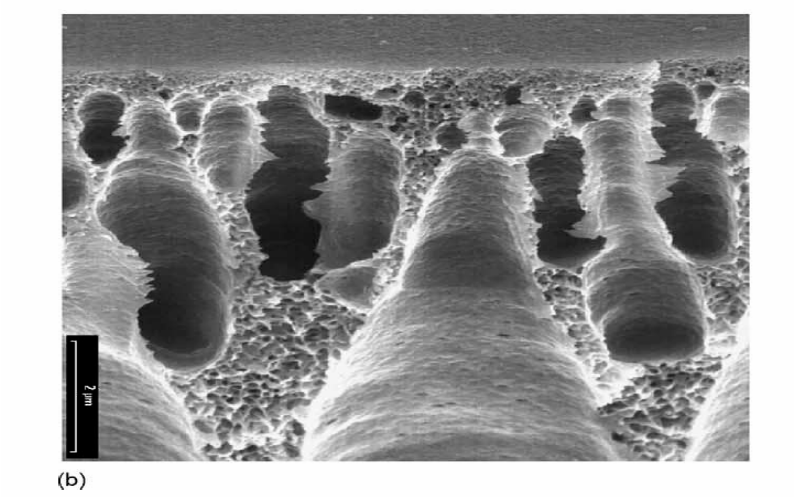

Figure 1.6 Asymmetric microporous membrane for ultrafiltration (Ubricht, 2004)

1.2.4.2 The membrane base

A number of polymeric membranes are used as a membrane base of MIP composite membrane including cellulose acetate, polyacrylonitrile and polyvinyl alcohol. For this research, bacterially derived cellulose membrane was chosen to be membrane base due to the fact that it has high resistance to corrosive chemical, biodegradable, ecofriendly and the porosity of membrane can be suitably tailored by the molecular recognition material. The application of

bacterial cellulose membrane is a medical application such as wound-dressing, temporary skin for patient burns (Figure 1.7) (Dubey *et al.,* 2002 and Sokolnicki *et al.,* 2006).

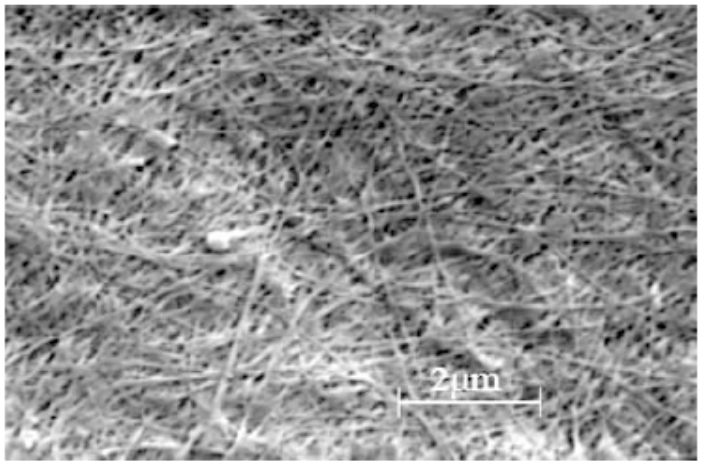

Figure 1.7 Bacterial cellulose membrane (Zhang *et al.,* 2005)

Bacterial cellulose membrane, as a hydrate membrane, is used as a membrane base for preparing MIP composite membrane. Bacterial cellulose membrane is β-1, 4 linked glucose polymer and reactive group are $–CH_2$-OH in cellulose membrane and it is asymmetric microporous membrane type. Bacterial cellulose membrane can be produced by the cultivation of bacteria, especially *Acetobacter xylinum* species which is a rod shape and aerobic gram negative. Bacterial cellulose membranes have a hydrophilic surface and abundant reactive hydroxyl group, as well as a low, non-specific adsorption (Zou *et al.,* 2001). Cellulose membrane consists of amorphous and crystalline regions together. The degree of crystallinity depends on the origin and chemical treatment of material. So native cellulose have two of more crystalline forms but mainly comprising 1α and 1β phase which 1α form is metastable and 1β form is stable form so native and derivatized cellulose membranes are soluble in some strong acids (Georage *et al.,* 2005) and bacterial cellulose membranes have excellent mechanical strength and high-surface area when compared with cellulose membrane derived from plant because of the highly crystalline structure and lower fiber diameter and property of bacterial cellulose membrane, selectivity, solubility, degree of sorption, permeate flux, can be controlled by concentration of organic in the feed (Pandey *et al*., 2005 and Sokolniki *et al.,* 2006).

1.2.4.3 Preparation of composite MIP membranes

Several methods have been applied for manufacturing of composite MIP cellulose membrane such as in situ polymerization, phase inversion and surface grafting.

1.2.4.3.1 In situ polymerization

For in situ polymerization technique, the functional monomer, cross linker and template are mixed together in the suitable solvent and the initiator is added. After polymerization by using UV or heat, the membrane will be formed, then remove the template to obtain the binding site inside the membrane. Composite MIP cellulose membrane which prepared from in situ polymerization is a brittle membrane by thermally initiated cross-linking copolymerization (Piletsky *et al.,* 2006).

1.2.4.3.2 Phase inversion

Dry phase inversion, template, polymer and solvent are mixed. Then the solvent is evaporated and the template is removed consequently. In this case binding site will be obtained inside the membrane. The preparation of MIP membrane by wet phase inversion can be carried out by mixing monomer, template, solvent together and binding sites are formed from the precipitation or the interaction of polymer in the water bath with subsequent removal of the template. The permeability of composite MIP cellulose membrane prepared from phase inversion was much higher as compared with the control membrane (Piletsky *et al.,* 2006). Since dissolving of cellulose is very difficult due to H-bond and partially crystalline structure to cellulose, the cellulose material has been converted to cellulose derivative with a strong acid or basic solvent before regenerate materials, which give arise a hazard by-product such as CS_2, H_2S, heavy metal. The *N*-methylmorpholine-*N*-oxide (NMMO) was chosen as solvent for dissolving the cellulose since it is a relatively simple, resource preserving and environmentally friendly method to produce regenerated cellulose fiber. The regenerate of cellulose membrane by phase inversion using NMMO solvent involves the following explanation. Cellulose, as any solid, can only be

dissolved in a solvent if the intermolecular attractive forces between differing species, here cellulose, NMMO and water, are greater than the attraction within each single species. Moreover, it has to be considered that with increasing temperature all interactions are reduced due to the increase in Braunian molecular motion. The rupture of the cellulose hydrogen bond system and the donator-acceptor interaction between the N→O dipole and the hydroxyl groups of the cellulose lead to a modified salvation of cellulose molecules in the NMMO-water system. After the chain orientation, the diffusion-driven precipitation of the cellulose in the coagulation bath was obtained. The exchange of solvent against non-solvent in the spinning ray leads to a desolvation of the cellulose membrane and to a re-formation of intra and intermolecular hydrogen bonds. Polar liquid, like water, alcohol and other miscible with NMMO, cause the removal of the solvent. Through the interaction with the precipitant medium water, desolvation occurs, i.e. the interaction between NMMO and cellulose is diminished and the oriented cellulose molecules predominantly interact among each other. During the coagulation of the cellulose solution, the NMMO molecules attract the precipitant molecules and the desolvation of the cellulose molecule proceeds. These processes lead finally to the formation of a highly swollen gel in fibrillar (Fink *et al.,* 2001).

1.2.4.3.3 Grafting

The preparation of composite MIP cellulose membrane by surface grafting method, MIP synthesis mixture is polymerized in mm-thick glass filters to fill their pores. Afterwards, reaction mixture is casted into the pores of asymmetric microfiltration membrane and a cross-linking copolymerization of a functional is then performed. The MIP layers on the support membrane have two functions. The first is minimizing non-specific binding. The second, MIP membrane has specific binding network into a porous of membrane support. The latter membrane has high template specific (Piletsky *et al.,* 2006).

1.2.4.4 Drug delivery by composite MIP membrane

1.2.4.4.1 Introduction

Free diffusion or passive transport of substances through membrane is a process of considerable importance in the pharmaceutical sciences. Transport processes across the membrane are the result of a driving force, which is typically associated with a gradient of concentration, etc. The ability of a number to effect separation of mixtures is determined by two parameters, its permeability and selectivity. The permeability is defined as the flux (molar or volumetric flow per unit membrane area) through the membrane scaled with respect to the membrane thickness and driving force. The second important parameter is the membrane selectivity, defined as the ratio of the individual permeabilities for the two species (Marcano *et al.,* 2002). Diffusion is defined as a process of mass transfer of individual molecules of a substance, brought about by random molecular motion and associated with a concentration gradient. The mass transfers through membranes vary, depending on many factors like the membrane structure, the specific interactions between the membrane and the fluid, and the overall operating condition. The passage of matter through a barrier may occur by simple molecular permeation or by movement through pores and channels. A second process may involve passage of a substance through solvent-filled pores of a membrane and is influenced by the relative sizes of the penetrating molecules and the diameter of the pores. Dialysis defines as a separation process based on unequal rates of passage of solutes and solvent through microporous membranes, carrier out in batch or continuous mode. The dialysis is the process of separating molecules in solution by the difference in their rates of diffusion through a semipermeable membrane. Dialysis is a common laboratory technique, and operates on the same principle as medical dialysis (Blowey *et al.,* 2005). At steady-state diffusion, Fick's First Law (equation 1) is used for explanation.

$$J = \frac{dM}{S * dt} \qquad \text{equation (1)}$$

where the amount *M* of material flowing through a unit cross-section, *S*, of a barrier in unit time, *t*, is known as the flux, *J*. The flux in turn is proportional to the concentration gradient, *dC/dx* :

$$J = -D\frac{dC}{dx} \qquad \text{equation (2)}$$

where *D* is the diffusion coefficient of a penetrant in cm^2/sec, *C* its concentration in g/cm^3, and x the distance in cm of movement perpendicular to the surface of the barrier. That is to say, diffusion occurs in the direction of decreasing concentration of diffusion coefficient; thus, the flux is always a positive quantity (Martin, 1993).

1.2.4.4.2 Facilitate and retard mechanism

For the appearance of transport of MIP composite membranes, two types of transport and separation, *i.e.,* facilitated template transport and retard template transport are displayed. Retardation of template transport occurs by the adsorption of MIP binding sites of membrane and template and facilitation for other molecule. Facilitation of template transport occurs by interaction between template and binding sites in MIP membrane so the template is selective for transport (Ubricht, 2004).

Gate effect and tarzan–swing mechanism are classified in facilitated template transport. Regarding to gate effect, the template binding with recognition site causing shrinking or swelling in MIP polymer and induces opening of “gates” for transport of molecules and ion through the MIP membrane by only template molecules and molecules with smaller size than template can pass through the gate (Piletsky *et al.,* 1999) and tarzan-swing mechanism, which the solute molecule passes from the first fix MIP site to the next by complexation and decomplexation reaction step by step through the membrane (Hadik *et al.,* 2005).

1.2.5 Confocal laser scanning microscope (CLSM)

1.2.5.1 Principle and operation

A confocal microscope is a tremendous advance in microscopy that uses laser technology to optimize light microscope optics. Light from a laser is focused onto a pinhole. The pinhole is imaged onto the object eye and objective lens. Reflected light and fluorescence from the object is imaged by the same objective lens onto a detection pinhole via a beamsplitter. A photomultiplier-detector records the light transmitted through the pinhole. In this way, the signals from outside the plane of focus, are removed by a pinhole detector, which further reduces out-of-focus information. An image of the object is obtained by scanning the light beam in x/y-directions, in raster-like manner. Finally, a 3D-image is obtained by scanning the object in z-direction and stacking the images obtained for each z-value (Figure 1.8) (Lai *et al.,* 2007).

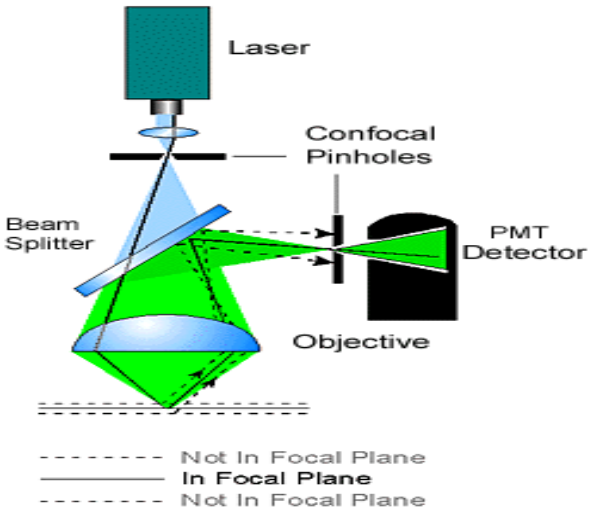

Figure 1.8 Confocal laser scanning microscope model (www.hi.helsinki.fi/amu)

The most important advantage of the confocal laser scanning microscope (CLSM) compared to conventional optical microscopes is the optical sectioning effect which significantly improves the 3D-imaging of thick, transparent specimens. The CLSM has better transverse resolution than conventional optical microscope and that specimens can be optically sectioned not only in the xy plane, but also vertically in the xz or yz or xyz plane. The main

limitation of CLSM are exposure to the high intensity laser illumination can be destructive to tissue, autofluorescence of biological sample, sample induces spherical aberration when focusing through an interface between materials of different refractive indexes and the speed for image is limited by the frequency line scanner. The 3D-imaging properties of the CLSM makes it ideally suited for studying topography, internal structure, and fluorescence properties of biological objects and materials with dimensions in the sub-mm to mm range (Alvarez-Roman *et al.,* 2004).

1.2.5.2 Application

Typical examples of such applications have more advantage, *i.e.,* (1) studying layers of glue, paint, and lacquer or surfaces, (2) measuring thickness of oxides on metals and semiconductors, (3) measuring topography on e.g. highly reflective, very rough metal surfaces, studying structure of fiber reinforced composites and epoxy, (4) examining morphology of biological cells e.g. tissue block, small organ, embryo etc., (5) delivery measurement via skin, nasal respiratory, ocular tissue etc (Alvarez-Roman *et al.,* 2004). The unique composition of stratum corneum (SC) gives the skin its remarkable barrier properties, hindering the penetration of highly polar substance and macromolecules. The SC structure can be illustrated as a brick (hexagonal and pentagonal corneocytes) and mortar (intercellular lipids) model (Hadgraft *et al.,* 1989). The only continuous region in the SC is the intercellular lipid domain. So, the applications of CLSM to measure the penetration via skin barrier are obtained (Godin *et al.,* 2004 and Maestrelli *et al.,* 2005).

1.3 The rationale and aims of this project

1.3.1 The template

β-adrenoceptor blockers appear to be of particular interest since they all posses an asymmetrical carbon atom in their propanolamine or ethanolamine side chain. Propranolol hydrochloride is a non selective β-blocking agent. It can be used for cardiovascular problems and hypertension, it is commercially available as a racemic mixture. The racemic drug is *S, R*-

propranolol hydrochloride, which the *S*-isomer shows for 100-130 times more blocking activity than the *R*-isomer and recent human studies have confirmed that only *S*-propranolol cause clinically relevant to β-adrenoceptor blockade whereas the corresponding *R*-propranolol does not contribute to this effect. In addition, the *R*- and *S*-propranolol have different pharmacokinetic properties (Stoschitzky *et al.,* 1996). Propranolol hydrochloride undertakes hepatic first-pass metabolism when administered orally, showing a systemic bioavailability of between 15-23% (Gumi *et al.,* 2005). It has a short elimination half-life of about 3 hours, which renders it suitable as candidate drug to be delivered transdermally at a controlled rate and be subjected to enantioselective separation by affinity membrane. For this research, propranolol enantiomer was used as print molecule.

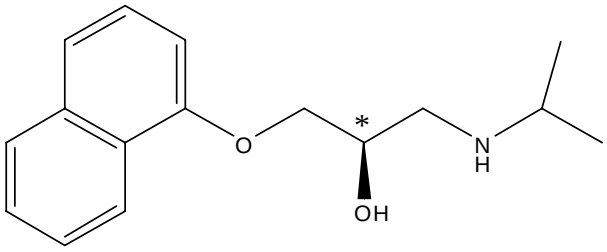

Figure 1.9 Structure of propranolol

1.3.2 The transdermal drug delivery application

Tighter control of drug input into the body in both quantitative and temporal senses is crucial, and the fabrication of delivery systems must respond to this demand for increase sophistication. Criteria to consider when selecting a candidate molecule as drug for TDDs are their melting point lowers 200^{o}C, molecular weight lower 500 Da and daily dose lower 10 mg, aqueous solubility higher 0.1 mg/ml and the partition coefficient (log *P*) are -2 to 3 (Wokovich *et al.,* 2006). Transdermal delivery has become an important means of drug administration from skin to systemic circulation. Many studies have been conducted into the measurement of penetration of drugs across excised skin sample. The major points relevant to transdermal drug delivery can be summarized. The idea membrane to be employed is human epidermis or a dermatomed skin

section no thicker than 0.5 mm. The sample can be taken from any specified site, which has been rationalized according to the intended site of application. Fresh skin should be used whenever possible; if the skin has been frozen and stored, the permeability characteristics should be checked using a standard penetrant (e.g., tritiated water). The skin should be mounted in a cell manufactured from a non-reactive material which will not absorb or adsorb significant amounts of the permeant. The drug should be prevented from volatilizing. The receptor phase volume should be such that it acts as a sink but is small enough to facilitate drug analysis. It should be well mixed and temperature controlled. The receptor fluid is ideally saline buffered to pH 7.4. A different pH can be used if it is justified in terms of the physicochemical properties of the drug. The concentration of the penetrant that builds up in the receptor phase should not exceed 15% of its saturated solubility. It may be necessary, for hydrophobic drugs to use non physiological receptor media. Thermostatting the receptor compartment should produce a skin surface temperature of 32±1^{o}C (Hadgraft *et al.,* 1989). In presenting the data, the kinetic analysis should include the lag time and the steady-state permeation rate. The point of interest is known as the lag time, t_L. This is the time required for a penetrant to establish a uniform concentration gradient within the membrane separation the donor from the receptor compartments. The lag time, t_L (time), is given by equation 3.

$$t_L = \frac{h^2}{6D} \qquad \text{equation (3)}$$

in which D is the diffusion coefficient of a penetrant in cm^2/sec and h is thickness in cm. In the case of time lag, the straight line of profile between cumulative amount and time to cross time axis may be represented the lag time (Martin, 1993). Finally, the delivery system will be designed to generate the required concentration vs. time profile. If this involves sustained, zero-order delivery should be produced over the time span of application.

Transdermal drug delivery systems offer many advantages, including improved systemic bioavailability of active pharmaceutical ingredients, lower administration frequency, longer duration of therapeutic action, reduction of side effects and steady drug delivery profile, etc. but small molecule and log P to be appropriate are the limitation of TDDs. In recent years, there has been an increased interest in controlled transdermal drug delivery systems, as a new

approach to drug administration (Zhan *et al*., 2006) *i.e.,* the use of polymeric materials such as chitosan, polymethyl methacrylate, cellulose ether and polyvinyl pyrrolidone (Valenta *et al.,* 2004 and Obara *et al*., 2005). But, the main problem of transdermal drug delivery is stratum corneum layers of skin, as rate limiting step. The stratum corneum is actually a compact and highly organized protective covering, as is revealed in unfixed sections of epidermis and lipids, especially polar lipid, played a critical role in the barrier. These intercellular lipid sheets would seem to provide the barrier to water loss through the skin as well as to limit the penetration of water–soluble agents from the environment. Drug penetration is limited so three principle stages of transdermal drug delivery for through skin are *i.e.,* (1) delivery of the molecule to the skin surface, (2) passage of the molecule through the skin, (3) delivery of the molecule into the body (Hadgraft *et al.,* 1989).

1.3.3 Research study

In a previous study, cellulose and its derivatives have proven stereoselectivity to propranolol both in adsorption chromatography and in column chromatography (Heard *et al.,* 1996) was used an excipient for formulation (Siepmann *et al*., 2004). Development of cellulose membrane for chemical affinity was interested which cellulose membranes for affinity separation or called “affinity membrane” was prepared from biological receptor such as antibody cells interact on the surface membrane (Ahmed *et al.,* 2006). The biological receptor had low stability and high cost. The molecularly imprinted polymer, which is an artificial receptor but mimics the biological receptor, was suitably used as a recognition material for the affinity membrane. For this work, two types of the composite MIP membranes were prepared; *S*-propranolol molecularly imprinted polymer particulate with or without racemic propranolol embedded into bacterial cellulose membrane using phase inversion method and bacterial cellulose membrane grafted with molecularly imprinted polymer using pore functionalization method were brought to investigate in enantioselectively-controlled release property. Thus, the objective of this study included:

1. To prepare the composite MIP cellulose membranes as drug delivery system of S-propranolol
2. To verify the parameters that effect to enantioselectivity of delivery of chiral drug by composite MIP cellulose membrane

3. To investigate inside the mechanism of enantioselective penetration for composite MIP cellulose membrane by using CLSM