MOLECULAR IMPRINTING OF HYDROGELS:
IMPROVED DRUG DELIVERY VEHICLES

By

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ABSTRACT

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The present study aimed to characterize and evaluate the ability of two molecularly imprinted hydrogel polymers to uptake drug template molecules from solution. A copolymer of methyl methacrylate (MMA) and N,N-dimethyl acrylamide (DMAA), and a homopolymer of 2-hydroxyethyl methacrylate (HEMA) were synthesized. Both polymer types were either molecularly imprinted (MIP) with a drug template molecule (propranolol, naproxen or timolol) or prepared without a template (non-imprinted polymer, NIP). The polymers were characterized by water content, FTIR, DSC, XRD, and SEM. With the exception of the SEM data, no differences between MIPs and NIPs were noted. Polymers were used in template re-uptake studies and their isotherms fit to Langmuir and Freundlich models. Based on the results, we conclude that the MMA-DMAA ProMIP was most successful at rebinding propranolol compared to the corresponding NIP. By changing the composition of the polymer backbone the drug uptake ability of the polymer changes drastically.

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Pharmaceutical formulations must be able to achieve several, sometimes conflicting, outcomes. They must be able to deliver a specific dose of drug for a specific time period in a safe way that is also compatible with patient convenience. It is not trivial to design these multiple features into a given drug formulation. As a result, the pharmaceutical industry spends a great deal of effort on innovating drug delivery systems.¹⁻⁵

There are numerous drug delivery modes currently in use. These include, for example, oral (tablets or enteric coatings), intravenous, intramuscular or transdermal (patches and creams) delivery. Although widely used, each of these methods has limitations. Oral administration implies a drug will pass through different pH conditions between the time of ingestion and absorption into the circulatory system. These conditions often result in the drug being metabolized prematurely such that a much smaller amount of active drug than that ingested reaches the tissue of interest. Intravenous or intramuscular administration is inconvenient as patients typically require assistance from health professionals, most often in a clinical setting. Even in the case of diabetics who become adept at administering insulin, the daily injection is inconvenient and unpleasant. Transdermal administration is less common because there are only a few drugs that can be prepared in this form.

Of the various drug delivery modes, and in spite of its limitations, oral administration is generally viewed as most convenient by patients.⁶ In recent years

attention has turned to the use of “smart” polymer systems for oral drug delivery. Controlled drug release can be achieved by choice of a suitable drug delivery system, such as smart polymers.^{4, 7, 8} This trend has been driven by factors including the recognition that controlled release of a drug over an extended time period provides a more suitable plasma level of drug than the “peak-and-plummet” levels observed with multiple doses of drug from fast release formulations.^{6, 9}

One category of smart polymer that has attracted significant interest in recent years is the stimulated release polymers.^{1, 3-5, 7, 8, 10} These polymers are stimulated to release their drug load by factors such as moisture content, temperature or pH. For example, temperature dependent smart polymers swell to different degrees at different temperatures. At lower temperatures (15-30°C) the polymer has a relatively large swelling ratio, while at higher temperatures (>30°C) the swelling ratio is greatly decreased.^{3, 11-13} Polymers designed for stimulated release with good temperature sensitivity would qualify for a potential application in controlled drug release.^{3, 11}

With respect to pH, an increase in the ionic content of certain gels increases their hydrophilicity leading to faster swelling and a higher equilibrium degree of swelling.⁷ Anionic hydrogels, which contain carboxylic acid groups, swell at a pH higher than the gel pK_a because of ionization within the network. The opposite behaviour occurs for cationic gels that often contain amino groups.^{7, 14, 15} This characteristic has lead to the development of hydrogels for insulin release because glucose oxidase (sensor) produces gluconic acid which lowers the pH of the medium which results in release of insulin due to significant changes in hydrogel swelling.^{7, 14, 15}

In order for smart polymers to be useful in drug delivery, it is also important that they can be loaded with a drug of interest. The smart polymer systems mentioned above are ones that have been prepared using the concept of molecular imprinting. Molecular imprinting is the process of polymerizing in the presence of a template molecule (i.e., the drug of interest). When the template molecule is removed after polymerization, the imprinted polymer will have cavities that are the size and shape of the template/drug molecule and which have functional groups arranged in such a way as to complement functional groups on the template/drug molecule. This permits better loading of the drug into the polymer than would be possible if the loading were only determined by non-specific drug-polymer interactions only.^{3, 4, 10} By combining imprinting with stimulated release due to the swelling differences under different conditions (low/high temperatures, low/high pH, changes in moisture level) there exists the potential for both high drug uptake and subsequent release.

By varying the amount of cross-linker used during polymerization, molecularly imprinted polymers (MIPs) will have different characteristics. With high amounts of cross-linker the polymers will be very rigid, resulting in high drug uptake but very slow drug release, or with low cross-linker levels, the polymers will be very flexible and result in moderate to low uptake with very quick release. A balance of these competing behaviours has been a subject of much recent interest in the field of MIPs for drug delivery.^{1, 3, 4, 16} One approach has been the use of hydrogels.

As mentioned above, another trigger for stimulated release of drugs from molecular imprinted polymers (MIPs) is moisture. These polymers are called hydrogels because they are insoluble in aqueous solutions but swell when exposed to water and

shrink when they are dried. Imprinted hydrogels are able to uptake large quantities of drug but are able to undergo faster yet controlled release compared to highly rigid polymers. Hydrogels have been used to regulate drug release in reservoir-based, controlled release systems or as carriers in swellable and swelling-controlled release devices.^{4, 7, 17} Due to their significant water content, hydrogels also possess a degree of flexibility very similar to natural tissue, which minimizes potential irritation to surrounding membranes and tissues.⁷ The hydrophilic and hydrophobic balance of a gel carrier can be altered, providing tuneable contributions that present different solvent diffusion characteristics, which in turn influence the diffusive release of drug contained within the gel matrix.⁷

Polymers for drug delivery are required to have a high chemical and mechanical stability to withstand enzymatic and chemical attack along with shear stress once ingested into the body.^{3, 18} Not only can molecularly imprinted polymers be used for drug delivery, but also in chemical separations, solid phase extraction, sensing, the removal of undesirable compounds from foods or fluids and as screening tools in drug discovery.^{1, 11, 19}

The main focus of this research is (i) to determine how molecular imprinting affects polymer properties, (ii) to investigate differences in drug uptake by imprinted vs. non-imprinted polymers, (iii) to investigate the specificity of template uptake, and (iv) to study the release of template drugs from partially cross-linked hydrogels. Two polymer networks will be used. The first is a copolymer of methyl methacrylate and N,N-dimethyl acrylamide and the second, a homopolymer of 2-hydroxyethyl methacrylate. Both networks have the same functional monomer (i.e., the polymer component that

interacts specifically with the template/drug molecule in the molecular imprinting process) and cross-linker, in the same quantities, but different backbone monomers (i.e., the components that define the polymer network). Each polymer type will be imprinted with one of two drug molecules, propranolol or naproxen, and as a negative control, a non-imprinted polymer will also be synthesized. Propranolol and naproxen are both small molecule fluorescent templates which are pharmaceutical drugs.⁶ By incorporating templates within these polymer networks we hope to create an effective material for drug uptake/delivery. Polymer properties will be investigated with analytical techniques including Fourier Transform InfraRed Spectroscopy (FTIR), Differential Scanning Calorimetry (DSC), Powder X-Ray Diffraction Spectroscopy (XRD), Gel Permeation Chromatography (GPC) and Scanning Electron Microscopy (SEM). Water content, refractive index, and drug uptake will also be measured for the various polymer/drug combinations. Through this extensive characterization we hope to determine the properties that are unique to each type of polymer, the structural differences between imprinted and non-imprinted polymers, and to identify the properties that make them suitable for drug uptake, and controlled drug release.

For our templates, we are interested in the non-selective β -adrenergic receptor blocker, propranolol, which is administered orally, and prescribed to patients with hypertension and myocardial infarctions. The second drug of interest is also administered orally, but is a non-steroidal anti-inflammatory drug (NSAID), naproxen. This substance is commonly used to treat inflammation and pain relief especially that associated with arthritis. The goal is to encapsulate the pharmaceutical product, either propranolol or naproxen, in a polymeric drug delivery vehicle which will be able to avoid

first-pass metabolism (*vide infra*) to a point which would increase the bioavailability of orally ingested drugs.

Previous groups have integrated synthetic hydrogels into contact lenses for the delivery of a glaucoma treating drug, timolol.²⁰⁻²² That work used the same polymer types as the current study. For this reason, and because timolol and propranolol have certain structural similarities, we are also interested in timolol as a comparator template.

Ultimately, we would like to be able to develop a partially cross-linked molecularly imprinted polymer which can provide the selectivity and high drug loading of rigid molecular imprinted polymers with the release ability of more flexible polymers such as hydrogels. This polymer would then be able to not only load the templated drug of interest, but also release it under controlled conditions at the desired body tissue. All of these factors would allow for a drug delivery vehicle that can undergo controlled release at specific tissues, thereby decreasing the dosage and number of instances of drug administration thereby increasing patient compliance.

1.2 Drug Delivery

Drug delivery has been described as the application of chemical and biological principles to control the *in vivo* location of drug molecules for clinical benefit.²³ Drug delivery involves the administration of a pharmaceutical product to the body as well as the details of processes that influence the distribution of the drug after administration (i.e., adsorption and metabolism). The administration can be achieved through a number of modes including enteral, parenteral, transdermal, or topical,^{6, 24, 25} depending on the specific characteristics of the drug and its mode of action. However, drugs delivered through these routes of administration may undergo a degree of degradation (i.e., first pass metabolism), and thus, not deliver their full effect once they have reached the target tissue.²³ Further, some modes (e.g., injection) are unpleasant and negatively impact patient compliance.

Drug delivery is becoming an increasingly demanding field for three main reasons: (i) the emergence of more challenging low-molecular weight molecules and biomacromolecules with either poor aqueous solubility, poor tissue permeation, or both; (ii) the increased use of biological materials with poorly understood physical properties; and (iii) the realization that if the portion of the dose responsible for adverse events could be directed away from sites where they originate, toxic side effects would become less frequent.²³

1.2.1 Drug Administration

³¹ Drugs can be delivered by many routes. Oral administration is the most common mode being responsible for about 70% of all forms of drug therapy.²³⁻²⁷ There are several

oral formulations that a drug can be packaged in, including tablets, enteric coatings, and sustained release preparations.^{9, 26} Tablets are mixtures of a drug with binders and fillers, all of which have been compressed together.^{25, 27} Tablets that are made by different manufacturers can differ in their rates of disintegration and dissolution, resulting in differences in bioavailability.⁹ The major advantage of tablets is that a dosage form can be mass produced²⁵ simply and quickly making the manufacturing cost much lower than other dosage forms.²⁶

Enteric-coatings are outer layer materials designed to dissolve in the intestine but not in the stomach. The coating protects drugs from the harsh acidic conditions of the stomach and won't dissolve until reaching the alkaline conditions of the intestine.²⁵⁻²⁷ Enteric coatings also protect the stomach from the irritant effects of certain drugs.²⁶ However, it is possible that enteric-coated drugs may pass through the gastrointestinal tract without being absorbed at all. This is because gastric emptying time can vary from minutes up to 12 hours, and enteric-coated preparations cannot be absorbed until they leave the stomach. Also, variations in gastric coatings sometimes cause them to fail to dissolve.⁹

Sustained-release formulations are capsules filled with tiny drug-filled spheres, which dissolve at variable rates.^{9, 26} Since some spheres dissolve more slowly than others, the drug is released steadily throughout the day. The primary advantage of sustained-release preparations is that they allow for a reduction in the number of daily doses, thereby increasing patient compliance.²⁶ These formulations have the additional advantage of producing relatively steady drug plasma levels over an extended time.²⁶

The major disadvantages of sustained-release formulations are high cost of manufacture and the potential for variable absorption.^{9, 26}

The advantages behind encapsulating pharmaceutical drug compounds within the biodegradable polymeric systems of this study are that the drug will undergo controlled release in the desired conditions at the specific tissue of interest.

1.2.2 Drug Absorption

Pharmacokinetics is the science of the relationship between the dose of a particular drug and the concentration of that drug in the body over time.^{6, 28} The detailed pharmacokinetics for a given drug are determined by rates of drug absorption, drug distribution and drug elimination. Drug absorption involves the passage of a drug from its site of administration into the circulation.^{6, 29} The method by which the drug is administered has a large effect on how it is absorbed by the body. Thus, pharmacokinetic considerations play a large part in the early stages of drug delivery vehicle design.⁶

Drugs can be absorbed both passively and actively across a biologic barrier (membrane) and into the circulation. The majority are absorbed by passive diffusion, and this rate is proportional to the drug concentration gradient at the particular absorption site.^{6, 29, 30} Some drugs are weak acids or bases that exist in both ionized and un-ionized forms in the body. The un-ionized forms of these drugs are highly soluble in membrane lipids and are readily transferred across cell membranes while the ionized forms are not.²⁸ Rates of drug absorption, distribution, or elimination are influenced by the ratio of the ionized to un-ionized forms at a particular site within the body.⁶

In this study, propranolol and naproxen are a weak base and weak acid, respectively. The protonated form of a weak acid is un-ionized whereas the protonated form of a weak base is ionized. At a pH equal to the pK_a , equal amounts of the protonated and non-protonated forms are present. If the pH is less than the pK_a , the protonated form predominates. If the pH is greater than the pK_a , the non-protonated form predominates. In the stomach, with a pH of 1, weak acids and bases are both protonated, but the acid will be uncharged, while the base will possess a +1 charge. This means that weak acids are more readily absorbed from the stomach than are weak bases. In the intestines, with a pH of 6-7, weak bases are also mostly ionized, but much less than in the stomach, and therefore weak bases are absorbed more readily from the intestines than from the stomach.^{6, 28} However, it is desired that both propranolol and naproxen be absorbed by the intestines as to bypass biotransformation and maximize the bioavailability of these drugs. Encapsulation of these drug compounds in a polymeric vehicle would limit the possibility of absorption in the stomach.

1.2.3 Drug Distribution

Drugs are distributed to organs and tissues of the body via the circulation system. Drugs with sufficient lipid solubility that diffuse, or are actively transported into hepatic cells may undergo enzymatic biotransformation.^{6, 29} This biotransformation (first-pass metabolism) is the process that we hope to avoid. By eliminating this enzymatic metabolism step, which occurs in the liver, the portion of active drug in the body will remain higher, allowing for more of the desired drug product to reach the tissues and have a longer lasting effect within the body.

In the intestines drugs are transported from the blood to the organs of interest, by a glycoprotein (P-glycoprotein, Pgp) and other carrier proteins. Pgp regulates the distribution and bioavailability of drugs. An increased amount of Pgp in the intestines will decrease the absorption of drugs. These proteins also carry the unused or metabolized drugs back into the bloodstream (away from the tissue of interest) which will then secrete various drugs into the intestinal tract, which also serves as a detoxifying mechanism.^{6, 28, 30}

The factors that affect drug distribution include organ blood flow, plasma protein binding, molecular size and lipid solubility.²⁸ The rate at which a drug is distributed to various organs after a drug dose is administered mainly depends on the cardiac output received by those organs. Drugs are rapidly distributed to highly perfused tissues (brain, heart, liver and kidney) and more slowly to less highly perfused tissues (skeletal muscle and skin).²⁹ Almost all drugs are reversibly bound to plasma proteins and the extent of binding depends on the affinity of a particular drug for protein-binding sites. As free drug diffuses into interstitial fluid and cells, drug molecules dissociate from the binding protein to maintain the equilibrium between free drug and bound drug.⁶ This allows for a certain concentration of the drug to be working while the remaining dose remains in the area surrounding the tissue until it is needed.

1.2.4 Drug Metabolism/Biotransformation

Drug biotransformation and excretion are the two processes responsible for the decrease in the plasma drug concentration over time within the body. Drug biotransformation, or drug metabolism, is the enzyme-catalyzed conversion of drugs to

their metabolites.^{6, 28, 29} Most drug biotransformation takes place in the liver, but there are drug-metabolizing enzymes found in many other tissues as well.²⁹ The biologic purpose of biotransformation enzymes is to inactivate drugs and other foreign compounds to detoxify the body.^{6, 28} Drug metabolites are usually more water-soluble than the drug molecule itself, and are more readily excreted by the kidneys. Drug metabolites can be active or inactive, depending on the drug.

Drugs that are absorbed from the gastrointestinal tract reach the liver through the hepatic portal vein before entering the systemic circulation.^{6, 28, 29} Many drugs are converted to inactive metabolites during their first pass through the gut wall and liver, resulting in low bioavailability after oral administration. This phenomenon is called the first-pass effect.^{6, 29} When a drug is administered orally, it is likely that less than 50% will remain in its active form after the first pass metabolism process.⁶ Drugs administered by the sublingual or rectal route undergo less first-pass metabolism and may have a higher degree of bioavailability than do drugs administered by the oral route.⁶

Drug biotransformation can be divided into two phases, each composed of unique biotransformation reactions. Phase I reactions create or reveal a chemical group that is later required for a Phase II reaction. Although Phase I metabolites often have pharmacologic activity, most Phase II metabolites do not.⁶ Phase I biotransformations include oxidative, hydrolytic and reductive reactions.^{6, 28} Oxidative reactions are the most common type of Phase I biotransformation. They are catalyzed by enzymes isolated in the liver and by cytoplasmic enzymes.⁶ Examples of oxidative reactions are those that occur with β -blockers such as propranolol and with NSAIDS like naproxen.⁶

The microsomal cytochrome P450 monooxygenase system is a family of liver enzymes that catalyzes the biotransformation of drugs with a wide range of chemical structures.²⁸ The role of the P450 family is to metabolize specific drugs; however, many drugs alter drug biotransformation by inhibiting or inducing cytochrome P450 enzymes.³⁰ Drug interactions can thus occur when such substances are co-administered with other drugs that are metabolized by cytochrome P450.⁶ Drug delivery systems which limit biotransformation might also have benefits for avoiding such multi-drug interactions.

Phase II biotransformation involves drug molecules which undergo conjugation reactions with an endogenous substance.²⁸ Conjugation enzymes, which are present in the liver and other tissues, serve to conjugate various drugs with the endogenous substance to form water-soluble metabolites that are more easily excreted. Most conjugated drug metabolites are pharmacologically inactive.⁶

1.2.5 Drug Excretion

Drug excretion occurs through the urine, either as a drug compound or as a drug metabolite/conjugate.²⁸ Drugs, which are filtered by the kidneys in the same manner as physiologic substances, undergo the processes of glomerular filtration, active tubular secretion, and passive tubular reabsorption.^{6, 28} The amount of drug excreted is the sum of the amounts filtered and secreted minus the amount reabsorbed.

In pharmacokinetics, most drugs exhibit first-order excretion kinetics, in which the rate of drug elimination is proportional to the plasma drug concentration.⁶ For drugs that exhibit first-order kinetics, the plasma drug concentration can be determined from the drug dosage and its rate of clearance from the body. It is possible to use pharmacokinetic

expressions to determine and adjust the drug dosage to achieve desired therapeutic effects because the plasma drug concentration is often correlated with the magnitude of a drug's effect.⁶ For first-order kinetics, a drug's rate of elimination is equal to the plasma drug concentration multiplied by the drug clearance. The elimination rate declines as the plasma concentration declines. Clearance is defined as the volume of body fluid from which a drug is removed per unit of time.⁶ The elimination half-life is the time required to eliminate half of the amount of a drug in the body or to reduce the plasma drug concentration by 50%.

By encapsulating pharmaceutical products within a MIP the drug level is maintained in the therapeutic range for a longer period of time because of the slower release into the body, thereby lowering the excretion rate.

1.2.6 Bioavailability

Bioavailability is defined as the fraction of the administered dose of a drug that reaches the systemic circulation in an active form.^{6,31} The bioavailability of orally administered drugs is of particular concern because it can be reduced by many pharmaceutical and biologic factors. Pharmaceutical factors include the rate and extent of tablet disintegration or drug dissolution. Biologic factors include the effects of food, which can sequester or inactivate a drug; the effects of gastric acid, which can also inactivate a drug; and the effects of gut and liver enzymes, which can biotransform a drug during its absorption and first pass through the liver.^{6,9}

Effective drug delivery to the site of action is dependent on several factors that influence the pharmacokinetics and pharmacodynamics of drugs.³¹ The processes

involved in the delivery of a drug from its site of administration to its site of action can be viewed by considering each barrier along the delivery path as a “location” of possible variability in drug response.³¹ Each barrier represents one or more mechanism(s) that may be affecting the rate and extent of drug that reaches the site of action. The locations in the following figure (Figure 1.1) are representative examples for oral administration.³¹

Typically, when drugs are administered they follow a pattern called single-dose kinetics. After a single dose of drug is administered, the plasma concentration increases as the drug is absorbed, reaches a peak as absorption is completed and then declines as the drug is eliminated. When a patient takes repeated doses of a drug, the process is more complex and results in drug accumulation in the body until a steady level has been achieved. When the amount of drug eliminated between doses equals the dose administered, average drug levels will remain constant and a plateau will have been reached. Single-dose and multi-dose kinetics are compared in Figure 1.2.

Figure 1.1: Schematic representation of some of the barriers faced by orally administered compounds before reaching the site of action. These barriers must be passed in order for the drug to reach the systemic circulation and contribute to the final bioavailability of the compound. Barriers that must be overcome after the drug leaves the bloodstream to reach the site of action are related to drug targeting. The overall consideration of barriers from the site of administration to the site of action is related to targeted bioavailability.³¹

Figure 1.2: Comparison of (a) single-dose and (b) multi-dose kinetics. The arrows represent the frequency at which a drug dose is administered. Single-dose kinetics results in one slow entry and exit of the therapeutic level for the drug, where multi-dose kinetics allows for a longer duration within the therapeutic range; however there are slight peak-and-plummet regions at the times when the dose is administered.^{6,9}

1.2.7 Pharmacodynamics

Pharmacodynamics is concerned with the detailed mechanisms of action by which drugs produce their biochemical and physiologic effects.⁶ It is also concerned with the dose response relationship, which is the relationship between drug concentration and the magnitude of drug effect. Pharmacodynamics provides a scientific basis for the selection and use of drugs to counteract specific pathophysiologic mechanisms in particular diseases. Drugs produce their effects by interacting with specific cell molecules called receptors. Most ligands (drugs) bind to protein receptors which, for the most part, are located on the cell membranes.

Drug receptors are classified according to drug specificity, tissue location, and by their molecular structure. For example, propranolol, a β -adrenergic blocker, uses adrenergic receptors whereas naproxen, an NSAID, will use the cyclooxygenase enzyme as its receptor.⁶ In many cases, each type of receptor corresponds to a single, unique gene with subtypes arising from different transcripts of the same gene.

The ability of a drug to initiate a cellular effect is called intrinsic activity or efficacy. Efficacy is the ability of a ligand to stimulate the receptor and start the signal transduction pathway.⁶ Drugs that have both receptor affinity and efficacy are called agonists, whereas drugs that have receptor affinity but lack efficacy are called antagonists. Both agonists and antagonists have common components sufficient for receptor affinity, but only agonists have the structure required for efficacy. Antagonists can prevent the action of agonists and inverse agonists by occupying receptor-binding sites.

The maximal response produced by a drug is also known as its efficacy. A full agonist has maximal efficacy, whereas a partial agonists has less than maximal efficacy. The flowchart in Figure 1.3 shows all of the different stages relevant to drug delivery, from drug administration to its pharmacokinetic and pharmacodynamic behaviours.

1.2.8 Optimizing Drug Delivery: Smart Polymers

Efforts are being made in the areas of controlled and targeted drug delivery to bypass unwanted drug metabolism and to have drug release occur only at the tissue of interest.^{2, 3, 7, 8, 11, 19-22, 32, 33} The goal is to optimize bioavailability and efficiency when and where they are needed. Encapsulating a drug within a smart polymeric delivery system would allow control over the release conditions of the drug.^{5, 18} Safety and efficacy of drug delivery is maximized by regulating the rate of release (controlled release) and/or by targeting the drug to a specific site (targeted release).^{3, 18} To be considered efficacious, the drug delivery system should provide the desired rate of delivery of the therapeutic dose to the appropriate tissue in the body. These two factors would prolong the duration of pharmacological action, optimize bioavailability, reduce adverse effects, minimize the dosing frequency and enhance patient compliance.^{3, 5, 18}

1.3 Molecular Imprinting of Polymers

Molecular imprinting is a synthetic approach that mimics nature by forming substrate-selective recognition sites in a matrix. It is a technique that involves creating cavities within polymeric systems that reflect the structure (size, shape, and spatial distribution of functional groups) of a template molecule. These cavities are formed by backbone and functional monomers cross-linking in the presence of the template molecule, followed by polymerization.^{1-4, 7, 16, 19-21, 32, 34-44} The resulting cavities will have a size and shape that is complementary to the template and possess functional groups in place to interact with the functional groups of the molecule of interest (template). In principle, this method would provide highly specific uptake of the template molecule by an appropriately imprinted polymer.

Molecular imprinting occurs by allowing the template molecule to form interactions or bonds in solution with one or more types of functional groups in a pre-organizing step. This is followed by the locking-in of these interactions/bonds to yield a polymer matrix with recognition sites selective for the specific template.^{5, 8, 10, 11, 33, 45-56} The concept is illustrated schematically in Figure 1.4. The concept of molecular imprinting is based on the “lock and key” idea that depicts the way a substrate interacts with an enzyme. This molecular key may be any substance ranging from small molecules (drug substances, amino acids, steroid hormones, or metal ions) to larger molecules (peptides or proteins).^{1-4, 7, 16, 19-21, 32, 34-44} However, the difficulty of molecular imprinting generally increases with the size of the selected template molecule, as more interactions are required to have an effective interaction between the template and the functional monomer(s).

Molecularly imprinted polymers are able to withstand various external degrading factors (e.g., enzymatic digestion) due to their physical and chemical properties.^{5, 8, 10, 11, 33, 45-56} Thus, molecularly imprinted polymers are remarkably stable against mechanical stress, elevated temperatures, high pressures, as well as resistant against treatment with acid or base and stable in a wide range of solvents. Polymers can be stored for several years at ambient temperatures without any apparent reduction in performance. Also, the polymers can be used repeatedly, in excess of 100 times during periods of years without loss of their “molecular memory”.⁵⁵

Figure 1.4: Schematic description of the molecular imprinting process. Functional monomers interact with the template and form a pre-organized complex. Backbone monomers and cross-linker are added and polymerization occurs. Template molecules are washed away leaving the resulting polymer with cavities of the size and shape of the template molecule.¹

1.3.1 Types of Molecular Imprinting

There are two types of molecular imprinting, covalent and non-covalent.⁵⁵ The difference between the two methods is dependent upon the nature of the adducts formed between the functional monomer and template.⁵⁵ Both methods have advantages and disadvantages.

Wulff and colleagues first developed the covalent approach to molecular imprinting in 1977.^{46, 48, 56-58} In this approach the template and the monomer undergo reversible covalent binding before the supramolecule is polymerized keeping the covalent bond intact. After this the bond is cleaved to remove the template.^{46, 48}

Non-covalent molecular imprinting connects the functional monomer with template via weak intra molecular forces such as hydrogen bonding, van der Waals forces, π -stacking, and electrostatic interactions.^{46, 48, 57} All components are added to the reaction mixture, which is then polymerized under a variety of conditions. Washing with appropriate solvents removes the template, and the polymer remains intact with cavities resembling the template molecule. The only bonding that the template is involved in is non-covalent. This method was first performed by a group lead by Mosbach.^{48, 57} Both the covalent and non-covalent approaches demonstrate the concept of molecular imprinting to the same extent, and provide options depending on the polymerization system.

1.3.2 Comparing the Two Approaches

The advantages of the covalent method include a clear cut imprinting process in which the stoichiometry between functional monomer and template is known, and,

because a stable conjugate is formed as a preliminary step, a larger variety of polymerization conditions become available.^{46, 58} The process of conjugate formation before polymerization tends to produce faster polymerization kinetics when compared to template/monomer binding. However, the disadvantage of covalent imprinting is that in order to break the conjugate bond and remove the template molecules, rigorous conditions are often required, which could disrupt the imprinting effect. There may also be added difficulty, cost and time involved for conjugate synthesis.^{46, 48, 58} Due to the covalent linkage formation upon rebinding of the template, this bond must be re-broken in order for the template molecule to be released. Thus, the release of the guest molecule is considerably slower than with non-covalent systems. In principle, the covalent method should provide more homogeneous binding sites; however, the rebinding is slower due to the formation of the covalent bond between template and MIP.^{46, 57}

The advantage of the non-covalent imprinting method is that it is simpler and requires no synthesis prior to polymerization. Additionally, the template is easily removed from the polymer under very mild conditions since the template is only weakly bound to the polymer matrix. Disruption of the imprinting site is rare, and guest binding and guest release occurs relatively quickly.^{46, 57} The major disadvantage to non-covalent bonding is that excess monomer is required to drive the polymerization reaction, (a 4:1 concentration ratio of functional monomer to template).^{53,56} Also, there is the possibility of non-selective binding site development due to monomer excess. The non-covalent method is easier to prepare than a covalent one, although it may create non-specific binding sites due to the relatively weak interactions used in template binding.^{46, 58}

Molecular imprinting work has shifted towards the non-covalent method because of its

advantages in the area of drug delivery systems, in particular rapid release of drug without the requirement of involving bond-cleaving reagents.^{53, 56}

1.3.3 Host and Guest

Molecularly imprinted polymers can be prepared to recognize a large variety of target structures with affinities that resemble antigen-antibody interactions.³⁹ Initially, template molecules and functional monomers interact and form a template-monomer complex. These complexes are first allowed to self-assemble in solution via non-covalent interactions such as hydrogen bonds, dipole-dipole interactions, hydrophobic interactions, van der Waals forces, etc. Subsequently, the three dimensional architecture of the complexes is arrested by polymerization with a cross-linker.⁷ The number and type of complementarily interacting functional groups has been shown to be a major influence in the performance of the resultant polymers.^{1, 59, 60} The structure of the template molecule is also one of the key factors affecting the stability of the complex.^{45, 53, 61-65} Effective self-assembly of the functional monomer(s)-template complex is crucial for good imprinting efficacy.^{61, 62, 65} After polymerization is complete and all template molecules have been removed, the host, in this case the molecularly imprinted polymer, is said to be pre-organized which ensures that the host does not have to undergo significant conformational changes when it (re)binds the guest (template) molecule.^{53, 61} The host binds the guest to produce a host-guest complex.⁴ Pre-organization enhances the stability of the resulting host-guest complex due to its well-defined geometry, allowing for lock and key complexation.⁶⁰ This process is energetically favourable for the system because

there is a large enthalpy payoff once binding occurs with minimized entropic penalty for host conformational changes.^{59, 66}

Host pre-organization represents a major enhancement to the overall free energy of guest binding. In the absence of pre-organization the host-guest binding process may be divided into two stages; the first is an activation stage in which the host undergoes conformational readjustment in order to arrange its binding sites in the fashion most complementary to the guest and at the same time minimizes unfavourable interaction between one binding site and another on the host.⁶⁶⁻⁶⁸ This is energetically unfavourable, and because the host must remain in this binding conformation throughout the lifetime of the host-guest complex, this energy is never paid back. The second stage involves binding which is energetically favourable because of the enthalpically stabilizing attraction between mutually complementary binding sites of host and guest.^{59, 60, 66} The overall free energy of complexation represents the difference between the unfavourable reorganization energy and the favourable binding energy. If the host is pre-organized, this rearrangement energy is small.^{60, 66} The more rigid pre-organized hosts may find it difficult to adopt the transition state complexation and have slow, but strong binding and slow release.^{60, 66} The binding of templates with rigid pre-organized hosts is also very specific because the host is in a conformation exactly complimentary to the template. When a variety of guests are introduced to a rigid host, the uptake will be very selective for the imprinted molecule with some uptake of templates with similar size, shape and functionality.^{60, 66} A more flexible host can adjust for both complexation and decomplexation. The result is that uptake is faster, but the amount of uptake and selectivity are decreased.^{60, 66-68}

An example of a flexible host is the cyclodextrin family where the main feature is the hydrophobic cavity, which can bind a wide variety of hydrophobic guests. Adding functional groups to the hosts can increase the specificity of a flexible host. However, functionalizing will not increase the specificity to the same degree as that of a rigidly pre-organized host. A compromise between flexibility and rigidity allows for controlled uptake and release of the template along with maintenance of conformation in the absence of a template.⁶⁶⁻⁶⁸

1.4 Hydrogels

Hydrogels are polymers that are insoluble in water and have the ability to absorb significant amounts of water, giving them a unique property of swelling and shrinking under different moisture conditions.^{60, 66} This inherent flexibility of hydrogels is a property that gives them the potential to be useful for human drug delivery. Factors that can also trigger hydrogel swelling include pH, temperature and drug concentration.^{3, 18, 60, 66} As previously mentioned, temperature sensitive polymers can swell to different degrees with different temperatures, and increasing the ionic content of a gel can result in faster swelling.

Imprinting within hydrogel networks has been shown to be more complex than imprinting within rigid structures.^{1, 4, 69, 70} The success of imprinting depends on structural considerations and the flexibility of polymer chains.^{1, 69} It has been suggested that for effective recognition of the template, the hydrogel should have a similar volume in the swollen and collapsed states.⁴ The expansion of polymer chains when swollen increases the free volume available for template transport, but will likely decrease the effectiveness of the imprinting site. In gels where significant collapse of the structure can occur, template transport may be significantly decreased and the binding cavity may be significantly altered in a collapsed state.⁶⁹ Polymers which contain more hydrophilic moieties along the polymer backbone will tend to swell or expand more than gels containing hydrophobic groups.⁴

Another factor influencing imprinting is the number of template molecules in relation to the number of functional monomeric species. A large excess of functional monomer to template is typically used.⁴ One reason for the excess of functional

monomer to template is to utilize as many of the template functional groups as possible in the templating process and to create specificity within the polymer. This occurs by overloading the system with the component that will bind the template functional groups thereby allowing for increased interactions. The strength of the monomer-template interactions and the polymerization reaction are also important factors.⁴

By combining the binding specificity of molecular imprinting with the variable swelling of an insoluble gel, it may be possible to develop a viable drug delivery tool. By controlling the amount of swelling in the molecularly imprinted hydrogel system, we ultimately control the amount of drug that is released or absorbed, as well as the location for release from the hydrogel.⁴

1.5 The Experimental System

All components (structures and properties) used in the experimental system can be found in Tables 1.1 and 1.2.

1.5.1 Choice of Template

Initially, three commercial pharmaceuticals will be tested as templates: propranolol, naproxen and timolol. These drugs were chosen because they are commonly prescribed. There are problems with their current delivery modes in that propranolol and timolol are highly metabolized before reaching the tissues of interest, naproxen is very potent and there are problems with its safety and toxicity. All three drug templates are likely to interact with the functional monomer that has been chosen (*vide infra*), and all are fluorescent and thus easy to analyze.

1.5.1.1 Propranolol

Propranolol belongs to a class of substances known as the β -adrenoreceptor blocking drugs.⁴ It is the first non-selective β -blocker that has been approved for clinical use under the commercial name Inderal® and its formal chemical name is ($\pm$)-1-isopropylamino-3-(1-naphthyloxy)-2-propanol hydrochloride. It is administered orally to patients with acute myocardial infarction in attempt to slow heart rate or treat arrhythmias^{1,3} as well as a treatment for hypertension.^{6, 71} Propranolol is also undergoing investigational use for stage fright.⁶ β -blockers are antagonists of β -receptors and prevent binding of the endogenous agonists such as noradrenaline or adrenaline. Propranolol is very effective at lowering blood pressure in mild to moderate hypertension. In severe

hypertension, propranolol is especially useful in preventing the reflex tachycardia that often results from treatment with direct vasodilators. When propranolol is first administered to a hypertensive patient, blood pressure decreases primarily as a result of the decrease in cardiac output associated with bradycardia. With continued treatment, however, cardiac output returns toward normal, whereas blood pressure remains low, owing to the reduction of peripheral vascular resistance.⁷²

Propranolol has a 25% oral bioavailability, so only one quarter of the drug dose will reach the systemic circulation in its active form.⁹ Due to its lipid solubility, propranolol can easily cross cell membranes and is widely distributed to all tissues and organs, including the central nervous system.⁷¹ The drug's effectiveness can be limited by factors such as rate of tablet disintegration or drug dissolution, effects of food (which can sequester or inactivate the drug), gastric acid effects and the presence of gut and liver enzymes after first pass metabolism.⁶ Also, propranolol has an elimination half-life of 4 to 6 hours via hepatic excretion, so the drug level is not maintained in the body and may require many administration repeats.⁹

Establishing an effective propranolol dosage is difficult for two reasons. The first is that patients vary widely in their requirements for propranolol and the second is that there is a poor correlation between blood levels of propranolol and the response.⁶ An explanation for this is that responses to propranolol are dependent on the activity of the individual's sympathetic nervous system.⁶ If sympathetic activity is high, then the dose needed to reduce receptor activation will be high as well. On the other hand, if sympathetic activity is low, then low doses will be enough to produce an effective

receptor blockade.⁹ Accordingly, the dosage must be adjusted by monitoring the patient's response, and not by relying on dosing information in a drug reference.

Propranolol is a chiral molecule, with one chiral center. Both enantiomers have a similar potency for the membrane stabilizing action that is characteristic of antiarrhythmics but the β -blocking activity is 100 times more potent for (S)-propranolol than for (R)-propranolol.^{9, 71} Both the efficacy of the compound in treating hypertension as well as most of its toxic effects result from the beta blockade, and thus the (S)-propranolol isomer is of more interest. In the current study, a racemic mixture of propranolol is used for templating.

The pK_a of propranolol is 11.1, and thus it exists mainly in the protonated (cationic) form in the body.

Propranolol is potentially an effective templating molecule because it has three hydrogen bonding sites, and the more stable conformers exhibit one or two intramolecular hydrogen bonds.⁹ Propranolol has been known to template within a MMA-EGDMA type polymer that was used for drug extraction in aqueous media.⁷¹

1.5.1.2 Naproxen

Naproxen (also known as 6-methoxy- α -methyl-2-naphthelene acetic acid), is a member of the Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) category of drug compounds, but also has analgesic (pain relief) and antipyretic (body temperature control) properties.^{6, 9, 72-75} It is a prescription drug used in the treatment of pain and inflammation, infection, autoimmune disorders and joint degeneration.^{73, 74} It is sold under the common name of Aleve®. It works by inhibiting the cyclooxygenase enzymes

(COX-1 and COX-2), which are responsible for the formation of important biological mediators (prostanoids) in the body. Naproxen is one of the few pain relievers which causes reversible inhibition.

Naproxen is a carboxylic acid that can use the carboxylate group for metal-ligand interactions.⁷⁵ At neutral pH it exists mainly in the anionic form as its pK_a is 4.2.^{6, 76}

It is orally administered like propranolol, although it has a bioavailability of 95%, which means that most of the drug reaches the systemic circulation system in the active form. However, it is metabolized to inactive metabolites before renal excretion. Naproxen has a relatively long half-life (12-15 hours) compared to other NSAIDs, and thus is administered less frequently.⁷⁵ NSAIDs like naproxen can commonly lead to severe toxic side effects from over dose or chronic abuse.⁷⁵ It is common for a person to unknowingly take naproxen in excessive amounts by treating it like any other NSAID, in order to reduce or relieve daily aches and pains. In the case of naproxen, this causes a much higher amount of active metabolite to be present in the blood, potentially reaching the toxic level while not maintaining the desired therapeutic effect. Naproxen toxicity can lead to many adverse effects, some of which include an increased risk of myocardial infarction or vascular events. For reasons that are not well understood, individual patients may respond better to one agent than to another. Individual patients may tolerate one NSAID better than another, thus in order to optimize therapy for each individual, therapeutic trials with more than one NSAID may be required to identify the one that is both effective and safe for a given patient.⁶ For all of these reasons, a controlled release mechanism for naproxen could be safer for patients.

1.5.1.3 Timolol

Timolol is also a member of the β -adrenergic receptor antagonists, or β -blockers. It is similar to propranolol in that it is a non-selective β -blocker which means that in addition to blocking β_1 receptors in heart tissue, it also blocks β_2 receptors in smooth muscle, liver and other tissues.⁷⁶ In fact, there are several structural similarities between timolol and propranolol (See Table 1.1).

Timolol is used in this study because it is a small drug molecule that has been reported to template many polymer systems, including 1-(trimethylsiloxysilylpropyl)-methacrylate (SiMA)-DMAA,²⁰ N,N-diethyl acrylamide (DEAA),¹⁹⁻²¹ DMAA-tris(trimethylsiloxy)silylpropyl methacrylate (TRIS),¹⁹ as well as the two systems used in this study, MMA-DMAA²⁰ and HEMA.^{20, 32} There have been many different approaches to molecular imprinting with timolol; however the non-covalent method with MAA and EDGMA as the functional monomer and cross-linker, respectively, has been the most commonly used. All of these systems have shown that the timolol imprinted polymer was more successful at timolol uptake and release than the corresponding non-imprinted polymer.

Timolol is more commonly known as Timoptic® and its formal chemical name is (-)-1-(*tert*-butylamino)-3-[(4-morpholino-1,2,5-thiadiazol-3-yl)oxy]-2-propanol maleate. As a non-selective β -blocker, timolol blocks the effects of norepinephrine and other adrenergic agonists and it also exhibits intrinsic sympathomimetic activity. Like propranolol, timolol has very poor bioavailability when it is administered orally (just 50%), and an elimination half-life of 4-6 hours.⁶ It is partially lipid soluble, but has no membrane-stabilizing activity.⁶ Timolol is used to treat hypertension and myocardial

infarctions and to prevent migraine headaches. Timolol has also been used to treat glaucoma through ocular administration when in solution. Glaucoma is the term used to refer to a group of diseases characterized by visual field loss and optic nerve damage.⁹ It is the leading cause of preventable blindness in the United States.⁹ Many types of drugs can treat glaucoma, but the β -blockers are the first choice. When delivered directly to the eye the drug is absorbed through the cornea and penetrates to the ciliary body thereby reducing aqueous humour secretion and intraocular pressure.^{6, 9} However, when timolol is administered topically, a substantial amount of the solution is lost to the eye and undergoes systemic absorption via the nasal mucosa which may cause undesirable cardiovascular and respiratory side effects.⁷⁷ These side effects could include cardiac arrhythmias and severe bronchospasms, it could also lead to fainting, congestive heart failure, depression, and confusion. For this reason, a slow sustained delivery approach would offer advantages such as improved therapeutic efficacy and reduced systemic toxicity.⁷⁷

1.5.2 Functional Monomer and Other MIP Components

Two polymeric systems were chosen for this study: Methyl methacrylate (MMA) and N,N-dimethyl acrylamide (DMAA) or 2-hydroxyethyl methacrylate (HEMA) are the backbone monomers. Methacrylic acid (MAA) is the functional monomer and the cross-linker is ethylene glycol dimethacrylate (EGDMA) in both cases. A non-imprinted hydrogel will also be synthesized in the absence of the propranolol, naproxen or timolol template as a control material.^{1, 3, 7, 11, 19-22, 32, 33, 46} The choice of functional monomer is based on the ability of MAA to create good binding sites for a large variety of template

molecules containing hydrogen bonding and proton accepting functional groups, thus creating a mode of interaction within the polymerizable system.^{1, 8}

The role of the functional monomer is to interact with the template molecule. MAA is the most commonly used functional monomer due to its ability to participate in ion-ion, ion-dipole, and dipole-dipole interactions.⁷⁸ MAA also exhibits bioadhesive characteristics to facilitate drug targeting, especially through mucus membranes, for non-invasive drug administration.⁸ There have also been studies in which the ratio of functional monomer to template was varied and the optimal concentration ratio was determined to be 4:1.⁵³ Lastly, there have also been previous studies done by Hiratani et al. who successfully used MAA with a similar fluorescent small molecule, timolol, as their template. These studies provide support for the choice of MAA as the functional monomer for propranolol and naproxen.

The role of the cross-linking agent is to provide the structural scaffolding upon which the MIP is formed, and is a major fraction of the polymers weight (85-90%).⁷⁸ A high degree of cross-linking is necessary to achieve a structure that results in template specificity, but polymers with a high degree of specificity are very rigid structures that are difficult to manipulate. Although, polymers that are highly cross-linked may template very effectively, they also bind and release the template in a very slow manner. As cross-linking increases, so does the density of the polymer matrix, which increases the number of potential imprinted sites, although the ease at which the template can be removed decreases.⁷⁸ On the other hand, polymers that are weakly cross-linked do not have template specificity, but can bind and release a variety of templates with ease. The cross-linker chosen is one that is commonly used in hydrogel templating systems due to its high

degree of flexibility.⁵³ However, it should be noted that EGDMA may also be involved in non-specific binding. After polymerization it can participate in hydrogen bond interactions with templates.⁷⁹

The purpose of using different backbone monomers is to manipulate the material properties of the final polymer. That is, polymers prepared with HEMA are expected to be more flexible but undergo a lower degree of swelling²⁰ than those prepared with MMA-DMAA mixtures. Molecular imprinting is favoured by heteropolymer systems because one type of functional monomer does not provide optimal interactions between the polymer chains and the template.⁴ For this reason, the current research will investigate both the homopolymer and heteropolymer case for molecular imprinting. Neither polymer type is influenced greatly by temperature changes although the swelling of MMA-DMAA is more temperature sensitive than that of HEMA. The MMA-DMAA polymer is also reported to have a greater overall imprinting effect, or the greater relative increase in overall affinity, compared to that of its non-imprinted polymer and compared to HEMA.²⁰ With respect to release profiles of the rebound template, the MMA-DMAA profile will release its entire imprint in roughly 3 hours based on experiments with timolol, while the HEMA polymer has a slower release profile with complete template removal taking approximately 9 hours.²⁰

Polymer synthesis is achieved through free radical polymerization starting with the photoinitiator, Darocur 1173. Free radical polymerization is the most widely used process for polymer synthesis.⁸⁰ It is much less sensitive to impurities within the monomers and it is much faster than other polymerization methods.⁸⁰

Darocur 1173 (also known as 2-hydroxy-2-methyl-1-phenyl-1-propanone) belongs to the class of α -hydroxyketones and is a clear, light yellow liquid, used for surface curing.⁸¹ When added to the reaction mixture in a volume of 0.04% v/v, it immediately catalyzes the photochemical reaction upon irradiation with UV photons.⁸¹ It is a highly versatile efficient liquid photoinitiator, which is used to initiate the photopolymerization of chemically unsaturated pre-polymers (acrylates) in combination with mono- or multi-functional monomers.⁸¹ The good solvency property of Darocur 1173 makes it ideal for polymer blends.⁸¹

Template binding properties are strongly dependent on the network structure. The network structure of free-radical polymers of multifunctional monomers depends upon monomer size, flexibility, functionality, the amount of solvent and concentration of monomers and initiators, initiation methods and initiation rate, as well as diffusion reaction constraints of propagating polymer chains.⁴ With respect to reaction initiation, reactions preformed by free-radical, UV-initiated imprinted polymerizations have been demonstrated to lead to better binding parameters compared to thermally initiated polymerizations due to lower temperatures of polymerization.^{4, 82, 83}

Table 1.1: Structures, molecular formulas, and molecular weights of drug templates.

Drug	Structure	Molecular Formula	Molecular Weight (g/mol)
Propranolol Hydrochloride		$C_{16}H_{21}NO_2 \cdot HCl$	295.80
Naproxen		$C_{14}H_{14}O_3$	230.26
Timolol Maleate		$C_{13}H_{24}N_4O_3S \cdot C_4H_4O_4$	432.49

Table 1.2: Structures, molecular formulas and weights of polymer components.

Component	Structure	Molecular Formula	Molecular Weight (g/mol)
MMA		C ₅ H ₈ O ₂	100.12
DMAA		C ₅ H ₉ NO	99.13
HEMA		C ₆ H ₁₀ O ₃	130.14
MAA		C ₄ H ₆ O ₂	86.06
EGDMA		C ₁₀ H ₁₄ O ₄	198.22
Darocur 1173		C ₁₀ H ₁₂ O ₂	164.20

1.6 Drug Uptake and Release Models

The uptake and release of template molecules is a key polymer property for drug delivery. In order for the polymer to be an effective drug delivery vehicle it needs to be able to uptake and release the template drug efficiently and selectively. Drug uptake is an important factor because a polymer needs to be able to absorb the drug of interest, through specific and non-specific binding, before it can be administered to patients for subsequent controlled drug release. Polymers that have been molecularly imprinted with the drug templates (propranolol, naproxen or timolol) are expected to be selective for those drugs. In the present study, cleaned, dried polymers were placed in contact with drug solutions at a range of drug concentrations between 5 and 100 μM , and allowed to equilibrate for 72 hours with agitation. The difference in drug concentration before and after polymer equilibration was calculated and the data were fit to both Langmuir and Freundlich isotherm models, to determine how well the polymers were able to rebind the drug of interest.

1.6.1 Binding Isotherms

Binding isotherms measure the concentration dependent adsorption behaviour of an adsorbent system.⁸⁴ Binding isotherms are most often plotted as the concentration (or mass or number of moles) of analyte bound to the solid phase (adsorbent material) vs. free analyte in solution at equilibrium. When the polymer is saturated and binding sites are filled, the observed concentration of unbound analyte will remain constant. The rebinding experiments in this study used a constant weight of polymer that had been equilibrated with analyte solutions of varying initial concentrations. After equilibration

with agitation for 72 hours the solid polymer was separated by centrifugation, and the concentration of free analyte remaining in solution measured by fluorescence spectroscopy. The corresponding bound concentration of analyte was calculated as the difference between the initial and final free concentrations.

Binding isotherms allow for the comparison of binding properties either graphically, or through the calculation of binding parameters from binding models.⁸⁴ Binding models can be classified as homogeneous or heterogeneous. Homogeneous binding models assume that there is only one type of binding site with a single set of binding parameters while heterogeneous binding models assume that there are two or more types of binding sites in the polymer, each with its own binding constant.

Binding models such as Langmuir and Freundlich, give particular mathematical relationships between the concentrations of bound and free analyte. These mathematical relationships have their basis in assumptions about the physical composition of a system, specifically the number of different types of binding sites in the polymer.⁸⁴

1.6.1.1 Langmuir

The Langmuir adsorption model is the most common model used to quantify the amount of analyte adsorbed on an adsorbent. It is a model for homogeneous binding sites where the analyte is presumed to bind at a series of distinct sites on the surface of the solid.^{84, 85} The Langmuir model assumes:

$$\text{Drug}_{\text{aq}} + \text{Surface Binding Sites} = \text{Adsorbed Drug} \quad (1)$$

$$1/q = 1/(q_{\text{max}} + K_L)1/C_{\text{eq}} + 1/q_{\text{max}} \quad (2)$$

Where the intercept is $1/q_{\max}$, and $q_{\max}$ is typically interpreted as being equal to $\Gamma_{\max}$, the total number of surface binding sites per mass of sorbent. The slope is equal to $1/(q_{\max} * K_L)$, where K_L is the Langmuir constant, which is interpreted as the equilibrium constant for the binding process. Here, we define $K_{id} \sim q_{\max} * K_L$ on a per kg basis as the solid-water distribution coefficient, which describes the relative concentration of bound sorbate to unbound sorbate in solution.⁸⁵ This value can be interpreted as a type of binding constant on a per mass basis for the polymer.

1.6.1.2 Freundlich

The Freundlich model assumes that there are multiple types of sorption sites acting in parallel, with each site type exhibiting different sorption free energies and total site abundance.^{84, 85} It is also more useful for heterogeneous binding systems.

The Freundlich model assumes:

$$\log q = n \log C_{eq} + \log K_F \quad (3)$$

Where q is the amount of analyte adsorbed in $\mu\text{mol/g}$ polymer, C_{eq} is the concentration of the analyte remaining in supernatant (i.e., the liquid in contact with the polymer) at equilibrium, K_F is the capacity factor and n is the slope of the Freundlich plot. These values are then used to calculate the K_{id} values where $K_{id} \sim K_F * C_{eq}^{n-1}$ on a per kg basis, (determined at a low value of C_{eq} , 5 μM in this study), or the solid-water distribution coefficient, which gives the relative amount of bound sorbate bound to unbound sorbate.

Not all imprinted polymers can be modeled using solely Langmuir or Freundlich isotherms because although both isotherms can be used to characterize MIPs, they are only accurate within limited concentration regions. The Langmuir isotherm best models

the saturation behaviour of a MIP, usually in the high concentration ranges; whereas, the Freundlich isotherm is limited to the lower sub-saturation concentration region of the binding isotherm.^{84, 85} As MIPs equilibrate with the drug solutions, the concentration window that is measured will begin to span both saturation and sub-saturation regions. To more accurately characterize a system, the isotherm would be ideally measured over both concentration regions. For this reason a Langmuir-Freundlich (LF) isotherm evolved, which is a heterogeneous binding model based upon a broad unimodal distribution.⁸⁴ This expression for the LF isotherm is:

$$B = \frac{N_T a F^m}{1 + a F^m} \quad (4)$$

Where B is the concentration of analyte bound to the solid phase, F is the concentration of free analyte in solution, N_T is the total number of binding sites, a is equal to $10^{(y\text{-intercept})}$, and m is the slope.⁸⁴ In practice, however, relatively few MIPs have been measured in a sufficiently wide concentration range to merit analysis by the LF isotherm. The majority of MIPs can be equally well modeled by the local Langmuir or Freundlich isotherms.⁸⁴

Binding isotherms are an important method to characterize the binding characteristics of MIPs and NIPs. In particular, binding isotherms allow for the quantification of binding properties such as the total number of binding sites and average binding affinity.⁸⁵ For these reasons, the measurement of binding properties of MIPs using binding isotherms has become crucial in the optimization and in the understanding of the mechanism of the imprinting process.

1.6.2 Template Release

Template release is the other important factor for application of molecularly imprinted polymers in drug delivery. Once the polymer has been loaded with the template drug, it is then administered orally to the body. While at 37°C in the body, the polymer drug complex will enter a variety of pH environments. These environments will cause the polymer drug complex to react in different ways, and shrink and swell to different degrees accordingly. Methacrylic acid is one of the most commonly used monomers for the fabrication of anionic pH-sensitive hydrogels.⁸ This is the functional monomer incorporated in both types of hydrogels used for this study.

There are three types of controlled drug release; these include diffusion, swelling, and chemically controlled drug release.⁸ Diffusion-controlled is the mechanism that is most applicable for describing drug release from hydrogels. It can be described by Fick's law of diffusion where the diffusion coefficient, either constant or variable, is used in modelling the diffusion controlled release.⁸ Fick's first law of diffusion through a membrane predicts a release rate as:

$$J = -D(\Delta c / \Delta \chi), \quad (5)$$

Where J is the rate of transport or diffusion flux, D is the diffusion coefficient, c is the concentration and χ is the distance.⁸ Here, the assumption is that steady-state diffusion occurs, and the flux of the diffusing species is proportional to the concentration gradient.⁸

Swelling-controlled release occurs when diffusion of drug is faster than hydrogel swelling.⁸ Finally, chemically controlled release is used to describe drug release which is determined by reactions that occur within a delivery matrix (e.g., acid catalyzed hydrolysis of the polymer framework).⁸

1.7 Project Hypotheses

The objectives of this study are: (i) to investigate whether molecularly imprinted hydrogels can be synthesized to find a useful compromise between the rigid highly cross-linked polymers with high selectivity and slow release, and the flexible low cross-linked polymers with low selectivity and quick release; (ii) to investigate whether there are differences in the structural characteristics between imprinted and non-imprinted polymers; (iii) to determine whether template uptake (rebinding) is superior for MIPs compared to NIPs; (iv) to determine whether MIPs demonstrate selectivity for rebinding the imprinted drug molecules (propranolol and naproxen and with a comparison to timolol); and (v) to examine the release properties of the drug template molecules from the MIPs and NIPs.

CHAPTER 2

EXPERIMENTAL

2.1 Materials

Drug templates ($\pm$)-propranolol hydrochloride (>98%), S-(+)-naproxen (>98%) and S-(-)-timolol maleate salt (>98%); polymer components methyl methacrylate (MMA), (99%, inhibited with 10-100 ppm monomethyl ether hydroquinone, MEHQ) N,N-dimethyl acrylamide (DMAA), (99%, stabilized with 500 ppm MEHQ), methacrylic acid (MAA), (99+%, stabilized with 250 ppm MEHQ), ethylene glycol dimethacrylate (EGDMA), (98%, inhibited with 100 ppm MEHQ), 2-hydroxyethyl methacrylate (HEMA), (98%, inhibited with 200 ppm MEHQ); and the analytical grade solvents: ethanol, acetone, phosphoric acid, dichloromethane (DCM), tetrahydrofuran (THF), dimethyl sulfoxide (DMSO), acetonitrile (MeCN), hexane, acetic acid and chloroform, were all purchased from Sigma Aldrich and used as received. The photoinitiator Darocur 1173 was donated by CIBA Chemicals and used as received. The 20 mL capacity aluminum dishes used as polymerization vessels and the salts used for buffer preparation (disodium hydrogen phosphate, Na_2HPO_4 (99%), sodium chloride, NaCl (99.9%) and sodium hydroxide, NaOH (98%)) were purchased from VWR. Potassium bromide, KBr , was purchased from Sigma Aldrich and used as received. Deuterium oxide, D_2O (99.9%) was purchased from Sigma Aldrich, and deuterated chloroform, CDCl_3 (99.8%) was purchased from Cambridge Isotope Laboratories Inc. Dialysis tubing (2000 MWCO) was obtained from Spectra/Por. Soxhlet thimbles were purchased from VWR. Solid calibration standards (samples of anthracene or naphthelene) from Starna Scientific were used to calibrate the fluorescence signal from the LS50B luminescence spectrometer.

2.2 Instrumentation

Photoinitiated polymerization was achieved with a Novocur UV curing system from EFOS. Spectral output of the source was monitored with a UV Power Puck from EIT borrowed from Chemque Inc., Mississauga, Ontario. UV/Vis absorption spectra were monitored on a Perkin Elmer Lambda 20 spectrophotometer and fluorescence spectra recorded with a Perkin Elmer LS50B luminescence spectrometer. FTIR spectra were determined with a Perkin Elmer Spectrum One instrument. The differential scanning calorimeter instrument used to determine T_g and other thermodynamic values was a Pyris Diamond DSC. Powder X-ray diffraction data was collected using a Siemens/Bruker AXS D5000 X-Ray Diffractometer Spectroscopy system housed and operated at the University of Toronto. Gel Permeation Chromatography was carried out on a Viscotek VE 2001 GPC Solvent/Sample Module and Scanning Electron Microscopy with a Jeol JSM-638OLV SEM. ^1H -NMR spectra were obtained from an Avance 400MHz NMR from Bruker. Polymer samples were ground using a tungsten/carbide ball mill (MM307) from Retsch GmbH. Refractive indices were determined at 21°C using the Sodium D line (589.3 nm) in a refractometer from Officine Galileo Di Milano (No. 45916).

2.3 Methods

2.3.1 Fluorescent Properties of Template Drugs

Fluorescence is a very sensitive analytical technique which can be employed to determine very low concentrations of fluorophore. The fluorescence technique can also be used with both liquid and solid samples, the latter typically measured on slides using the front face attachment of the fluorescence instrument.

To determine the excitation, emission and absorption properties of the template molecules, propranolol, and naproxen, 100 μM solutions of the template molecule in water were made and scanned with the UV/Vis Lambda 20 spectrophotometer and the LS50B luminescence spectrophotometer. Propranolol absorbs light at $\lambda_{\text{max}} = 285 \text{ nm}$ and emits light at a maximum intensity at 352 nm, while naproxen absorbs light at $\lambda_{\text{max}} = 255 \text{ nm}$ and emits light at a maximum intensity at 354 nm. For the fluorescence measurements the slit widths were typically 4.2 nm (excitation) and 5 nm (emission), and the scan speed was 100 nm/minute.

2.3.2 Hydrogel Preparation

Two types of hydrogels were prepared. The functional monomer, methacrylic acid (MAA) (0.401 mmol), and the cross-linking agent, ethylene glycol dimethacrylate (EGDMA) (0.583 mmol) were dissolved in 4 mL of the backbone monomers. The first type of hydrogel was prepared with 19.41 mmol of N,N-dimethyl acrylamide (DMAA) and 18.78 mmol of methyl methacrylate (MMA) as the backbone monomer components. The second type of hydrogel was prepared with 32.92 mmol of 2-hydroxyethyl methacrylate (HEMA) backbone monomer. In the absence of templates, these

preparations yielded the non-imprinted polymers. For imprinted polymers, 0.029 g of propranolol (9.80×10^{-5} mol) or 0.023 g of naproxen (9.99×10^{-5} mol) was dissolved in 4 mL of the monomer solution before the addition of Darocur 1173 (0.4% v/v).

For both imprinted and non-imprinted systems, the monomer solutions were poured into 20 mL aluminum dishes with a diameter of 4.4 cm and subjected to free radical polymerization that was initiated by UV irradiation through a Novocur curing system. Samples were subjected to an average UV dosage of $3.80 \text{ J/cm}^2 \pm 0.369$ (A) and $2.32 \text{ J/cm}^2 \pm 0.242$ (V) and irradiance of $3.05 \times 10^{-2} \text{ W/cm}^2 \pm 1.90 \times 10^{-3}$ (A) and $1.90 \times 10^{-2} \text{ W/cm}^2 \pm 1.90 \times 10^{-3}$ (V) for 2.5 minutes at a distance of 4 inches from the light source. Here UV(A) and UV(V) correspond to wavelengths in the ranges of 320-390 nm and 395-445 nm, respectively. These dose and irradiance measurements were measured with an EIT UV Power Puck.

2.3.3 Polymer Washing

Polymer washing was carried out as a procedure to remove the unreacted monomers and template from the polymer. This would ensure that the template has been removed from the imprinted cavities to maximize the uptake ability of the polymers. Several different approaches were evaluated for effectiveness.

2.3.3.1 Conventional Washing Method

After polymerization but before grinding, the gels were removed from the moulds and washed with deionized distilled water, then saline (0.9% NaCl) and finally deionized distilled water once more, all at room temperature. The washing process lasted a total of

7 days, each individual phase lasting a minimum of 2 days and changing the solutions 4 times/day. The absence of any unreacted monomers or template in the wash liquid was verified through the lack of UV absorption bands between 190 and 350 nm. The washing solutions were then measured for fluorescence between 300 nm and 450 nm as fluorescence is a more sensitive analytical method for detection of the templates. Fluorescence is between 100-1000 times more sensitive than absorbance measurements.^{86, 87} This method results in 40–90% of the template being removed from the polymer matrix.¹

2.3.3.2 Altering pH

Two buffers were prepared with 1 Litre 0.05 M solutions of Na_2HPO_4 and adding H_3PO_4 or NaOH drop wise for pH3 and pH12 respectively. Propranolol imprinted gels were washed in this buffer of pH3 and naproxen imprinted gels were washed at pH12. This ensured the drugs would be in their more soluble ionic forms and increasing the tendency for them to be removed from the hydrogel. This washing period also followed the scheme that was mentioned earlier, with buffer, 0.9% NaCl , and buffer again each for two days, changing the solution 4 times daily encompassing a total washing period of 7 days. The washings were tested on both the UV/Vis (between 190 and 350 nm) and fluorimeter (between 300 nm and 450 nm) to check for the presence of template molecules and unreacted monomers in the wash.

2.3.3.3 Dialysis

After synthesis, polymers were dried in a Fischer vacuum oven at 50°C for 24 hours, ground (see below) and placed in dialysis tubing with a molecular weight cut-off of 2000 Daltons. Samples were allowed to dialyse with stirring at room temperature in a 1:1 v/v mixture of ethanol and water for 48 hours, changing the outer solution every 12 hours. The outer washing solution was tested for remaining template molecules and unreacted monomers using the same methods as mentioned above.

2.3.3.4 Soxhlet

Freshly made polymer samples (the uncleaned reaction product) were placed in single thickness thimbles of a Soxhlet system and extracted for 5 days with refluxing ethanol, changing the washing solution every 2 days. The liquid in the round bottom flasks was measured with UV/Vis and fluorescence spectroscopy to check for unreacted monomers or template molecules.

2.3.4 Residual Fluorescence in Cleaned Polymers

For drug rebinding to imprinted polymers, it is important that the washing process remove essentially all the template. In our experiments, once the various polymers were cleaned via each of the methods listed above (conventional washing, at variable pH, dialysis or the Soxhlet method) they were ground, dried and adhered to glass microscope slides with double sided tape. The polymers were tested for fluorescence using the front face attachment of the fluorimeter with excitation and emission slits of 2.5 nm at a scan speed of 100 nm/minute. Propranolol imprinted polymers were excited at 285 nm, and

naproxen imprinted polymers were excited at 255 nm. Scans of the glass slide alone, the glass slide with double sided tape, and the non-imprinted polymers were performed as negative controls under the same scan conditions and for both fluorescent templates.

2.3.5 Hydrogel Characterization

2.3.5.1 Water Content (Q)

Gels were weighed after reaching equilibrium in distilled water in the swollen state and once they had been dried for 24 hours under vacuum at 50°C.

2.3.5.2 Polymer Grinding

The dried polymers were placed in the ball mill at 30 oscillations/second for 1 minute. This grinds the polymer to a consistent sized particle of roughly 100-500 μm .

2.3.5.3 Refractive Index (RI)

The RIs of the individual monomers and cross-linker were measured with the sodium D line of the refractometer at 21°C and compared to the literature values. The mixtures consisting of all the polymer components were also measured for the non-imprinted polymer as well as both of the molecularly imprinted polymers containing the desired amount of drug.

2.3.5.4 Fourier Transform InfraRed Spectroscopy (FTIR)

The ground polymers were incorporated into KBr pellets and scanned from 400 to 4000 cm^{-1} in the Spectrum One FTIR spectrometer. Approximately, 0.2 g of KBr was mixed with 1-2 mg of imprinted and non-imprinted polymers to obtain suitable pellets.

2.3.5.5 Differential Scanning Calorimetry (DSC)

DSC characterization was carried out on samples of freshly prepared gels with mass between 6 and 10 mg. DSC measurements were made on both washed and unwashed samples that had been dried and ground. Samples were put in sealed aluminum holders which were heated from 50 to 250°C, then cooled back to 50°C at a rate of 20°C/minute in the Pyris Diamond Differential Scanning Calorimeter. Nitrogen was used as a purge gas at a flow rate of 20 mL/minute. The instrument was calibrated with Indium (melting point 156.60°C and transition energy of 28.45 J/g). The glass transition onset temperature is reported as the value of T_g .

2.3.5.6 Gel Permeation Chromatography (GPC)

Polymer samples (non-imprinted) consisting of all components with the exception of the cross-linker were prepared using the photo-polymerization method. The MMA-DMAA polymer was dissolved in THF at a concentration of 3 mg/mL and 3 samples of 100, 80 and 60 μL were injected into the GPC at a flow rate of 1 mL/minute. The column temperature was 30°C and each sample was run for 40 minutes. The HEMA polymer did not dissolve in any organic solvent, (DCM, THF, chloroform, acetone, ethanol, MeCN, and DMSO) so DSC measurements were not obtained for this polymer system..

2.3.5.7 X-Ray Diffraction Spectroscopy (XRD)

Samples representing all polymer test conditions (MMA-DMAA and HEMA, imprinted and non-imprinted) were ground and packed into normal 35 x 16 x 1 mm sample holders. They were analyzed with a Siemens/Bruker AXS D5000 X-Ray Diffractometer Spectroscopy system at the University of Toronto. Using step mode for data collection, two scans were performed on each sample: first a fast routine scan between 3-35 2Θ followed by a more detailed slower scan between 2-40 2Θ . The spectra were analyzed and fit to a specific profile with Topas v. 2.1 profile fitting software.

2.3.5.8 Scanning Electron Microscopy (SEM)

Dried, ground samples of the polymers cleaned through Soxhlet extraction (MMA-DMAA ProMIP, NapMIP and NIP as well as HEMA ProMIP, NapMIP and NIP) were adhered to double-sided tape. The tape strips were then attached to the platform of the SEM which was in turn placed in the sample chamber. Low magnification (40X, 20 kV) images were obtained using non-conducting polymers. These provided an idea of general morphology and made it possible to identify particles for more detailed examination. At high magnification, charging effects were observed with the non-conducting samples. Samples of several microns in thickness were prepared and gold using a DESK IV gold coating device from Denton Vacuum, LLC. The gold atoms come from the gold foil on the top of the vacuum cylinder chamber at 80 mTorr for 60 seconds at a current of 30 mA. The gold coated samples were examined at a much higher magnification (10 000X and 10 kV) to try and identify morphological differences between imprinted and non-imprinted polymers.

2.3.6 Possible Side Reactions during Polymerization

Propranolol is a secondary amine and can therefore in principle undergo a Michael reaction with acrylamides such as DMAA.⁸⁸ A number of tests were done to determine if this side reaction might be taking place in our polymerization mixture. Propranolol MIP preparation mixtures were prepared as described above but without photoinitiator. They were subjected to the usual polymerizing conditions (UV irradiation). The resulting solutions were diluted with dichloromethane (DCM) and were spotted on silica plates. Samples were eluted with an 85:15 mixture of DCM: acetone through thin-layer chromatography (TLC) along with the individual components of the mixture to determine whether any covalent interactions were occurring during polymerization. Any indication of new products on the TLC would indicate the possibility of the drug reacting with the polymer components. The same procedure was used for naproxen monomer interactions but with 5:1:1.5 mixture of MeCN: MeOH: H₂O. Samples were visualized with a UV light and also stained with KMnO₄.

As verification, the proton NMR of the various components and mixtures in D₂O were also obtained. Samples of propranolol alone, and DMAA monomer alone were compared to a sample of propranolol and DMAA that had been mixed together in the same ratio as in MIP preparation was heated at 70°C (water bath) for 1 hour. These solutions were diluted in D₂O prior to obtaining the ¹H-NMR spectra.

2.3.7 Pre-Organization Tests

A number of tests were carried out to seek evidence for interaction/association of the template molecules and the functional monomer.

2.3.7.1 Absorbance Spectroscopic Titrations

Solutions of the drug templates propranolol and naproxen in chloroform (25, 50, 100 and 200 μM) were prepared. 3 mL of each solution were titrated with 3 μL aliquots of MAA and the absorbance recorded at 294 and 274 nm for propranolol and naproxen, respectively, after being allowed to equilibrate for 3 minutes. Samples of MAA in chloroform at the same concentration of the test samples were used as a reference solution prior to each run.

2.3.7.2 ^1H -NMR

Solutions of the drug template propranolol (25 mM) alone, MAA (100 mM) alone and with both combined in a monomer: template ratio of both 1:1 and 4:1 (at these same concentrations) were prepared in CDCl_3 and analyzed with 400 MHz ^1H -NMR spectrometer to determine complexation by observing the amino proton resonance at approximately 8 ppm.

2.3.8 Drug Loading

Dried, ground and washed polymers (0.050 g), either imprinted with the drug template (propranolol or naproxen) or non-imprinted, were suspended in 5 mL aqueous solutions containing a drug at a range of concentrations (5 – 100 μM). Samples were allowed to equilibrate at either 21°C or 37°C for 72 hours, while shaking to maintain particle suspension. After the equilibration period, samples of the uptake solutions were placed in the fluorescence spectrometer and excited at 285 and 255 nm for propranolol and naproxen, respectively. The slit widths were 4.2 nm (excitation) and 5 nm

(emission), and the scan speed was 100 nm/second. The emission intensities were recorded at 352 nm for propranolol, and 354 nm for naproxen and compared to the appropriate calibration curve (*vide infra*) to determine drug concentration. The fluorescence spectrometer was calibrated using an anthracene/naphthelene solid standard (Starna Scientific) excited at 360 nm with an excitation slit width of 5 nm and an emission slit of 12 nm.

The amount of drug that was absorbed was calculated by the difference between the initial concentration of the drug solution and final concentrations of the supernatant after equilibrium for 72 hours either at room temperature or at an elevated 37°C temperature, with vigorous shaking. This equilibrium concentration (C_{eq}) value was then converted into μmol of drug absorbed/g polymer.

2.3.8.1 Preparation of Fluorescence Calibration Curves

In this procedure, the fluorescence intensity of a number of standard solutions of the reference substance at concentrations ranging from 5 – 100 μM was measured on the LS50B spectrophotometer and a calibration curve was constructed. The concentration of the drug in the sample solution was read from the graph as the concentration corresponding to the observed fluorescence intensity of the solution. For example, the calibration curve of propranolol obtained at 352 nm was used to determine the amount of analyte rebound to the polymers at equilibrium.

2.3.9 Timolol Studies

Polymers imprinted with timolol were synthesized in the same manner as those with propranolol or naproxen. A total of 0.043 g of timolol was required in 4 mL of backbone monomers to make the final 25 mM concentration of the template. Timolol absorbs light at 295 nm, and emits light at a $\lambda_{\text{max}} = 412$ nm, which can be seen with a 100 μM solution. Timolol has a low fluorescence quantum yield compared to propranolol and naproxen, and thus the scan parameters were set to excitation and emission slit widths of 10 nm each. Even under these high light conditions, the observed timolol fluorescence was weak. For this reason UV/Vis absorbance was the method chosen to analyze timolol uptake.

2.3.9.1 Calibration Curve for Timolol

Timolol solutions ranging from 5 – 65 μM were made and their absorbance values determined with the UV/Vis spectrometer. The absorbance value was plotted against concentration, as per Beer's Law, to provide a calibration curve with an $R^2 = 0.9995$. This curve was then be used to determine the amount of bound and unbound timolol in the uptake studies with the different polymers.

2.3.9.2 Removal of Timolol Template

Timolol imprinted polymers were cleaned by the Soxhlet method with ethanol as previously described. Once cleaned, dried and ground, the polymers were tested for fluorescence by attaching them to glass slides and using the front face attachment of the LS50B spectrophotometer. The scan parameters involved excitation at 295 nm and an

emission range between 300 – 450 nm. The excitation and emission slit widths of 5 nm and a scan speed of 100 nm/minute. Absence of fluorescence indicated that the polymers were efficiently cleaned and ready for template loading.

2.3.9.3 Timolol Loading

Imprinted (timolol or propranolol) or non-imprinted, dried, ground and washed polymers (0.050 g), were placed in 5 mL aqueous solutions containing a drug at a range of concentrations (5 – 65 μ M). Samples were allowed to equilibrate at 37°C for 72 hours, while shaking to maintain particle suspension. After the equilibration period, samples of the uptake solutions were placed in the UV/Vis spectrometer and the absorbance measured at 295 nm. The equilibrium concentration of drug that was absorbed by the polymer was calculated as the difference between the initial concentration of the drug solution and final concentrations of the supernatant after equilibrium. This C_{eq} value was then converted into μ mol absorbed/g polymer by the same method as for propranolol loading. In this case, the timolol imprinted polymer is the imprint of interest, and the propranolol imprinted polymer is the inappropriate MIP used to test uptake selectivity.

CHAPTER 3

RESULTS

3.1 Optical Properties of Templates

Propranolol and naproxen are promising templates for molecular imprinting because of their ability to form multiple hydrogen bonds and have significant dipole-dipole interactions with the functional groups of the monomers used for polymerization. The drug templates are chemically stable under the polymer synthesis conditions used here and are not likely to undergo any degradation during synthesis, washing, or uptake processes. In addition to their common use as water-soluble, small molecule drugs, propranolol and naproxen were chosen as templates because they are a good comparison group to other small molecule templates as well as larger templates like cholesterol or peptides. Finally, both drugs are fluorescent so they are easy to assay.

Propranolol absorbs light at a wavelength of 285 nm and emits light at 352 nm whereas naproxen absorbs light at a wavelength of 255 nm and emits 354 nm in water (Figure 3.1 a, b).

Both drugs were readily dissolved in the solution of monomers, and their presence did not alter the refractive indices (Table 3.1b) of the reaction mixtures for the imprinted and non-imprinted polymers. The refractive indices (RI) were measured for the pre-polymer solution mixtures, omitting the photoinitiator to maintain the mixture in liquid form and allow for measurement. Measurements were also conducted for the individual monomer components to compare to the literature values (Table 3.1a). The RI values provide a measure of the purity of the monomers used. RI values were temperature corrected to 25°C with the following equation:

$$\eta_D^{25} = \eta_D^y - (25.0 - y)(0.00045) \quad (6)$$

Where η_D is the refractive index of the measured liquid and y is the temperature during measurement.

After polymerization the hydrogels remained optically clear and there were no visual differences between imprinted and non-imprinted polymers.

a)

b)

Figure 3.1: Absorbance and emission spectra for 100 μM drug solutions of propranolol and naproxen in water. (a) Absorption spectra. Slit width 2 nm, scan speed 240 nm/minute. (b) Emission spectra for propranolol excitation wavelength at 285 nm, naproxen excitation wavelength at 255 nm, with both scans having excitation and emission slit widths of 4.2 and 5 nm respectively, and scan speed 100 nm/minute.

Table 3.1: Refractive Indices for (a) individual polymer components and (b) polymer mixtures prior to polymerization. Values were temperature corrected to 25°C.

a)

Polymer Component	Literature Values	Measured Values
MMA	1.41195 ⁸⁹	1.4162
DMAA	1.47075 ⁸⁹	1.4733
HEMA	1.44925 ⁸⁹	1.4524
MAA	1.45175 ⁸⁹	1.4313
EGDMA	1.4532 ⁸⁹	1.4543

b)

Polymer Type	Refractive Index
MMA-DMAA	
NIP	1.4462
ProMIP	1.4486
NapMIP	1.4477
HEMA	
NIP	1.4519
ProMIP	1.4500
NapMIP	1.4514

3.2 Water Content

The hydrogels were tested for water content, which is an indication of the swelling ability when the dry polymers are placed in water. Water content was calculated with the following equation:

$$Q = (W_s - W_d) \times 100 / W_s \quad (7)$$

Where Q is the water content percentage, W_d is the dry weight measured after polymer synthesis and drying at 50°C overnight and, W_s is the swollen weight measured after polymers were washed to remove any unreacted products for approximately 7 days in water/saline/water solutions. Literature values represent water content data at 21°C.²⁰ The data are presented in Table 3.2. From this data it can be noted that the MMA-DMAA polymers swell twice as much as the HEMA polymers. There is no significant difference between imprinted and non-imprinted polymers within each group, with respect to water content. The values found in this study are representative of other similar polymers reported in the literature at 21°C.

Table 3.2: Water content measurements for MMA-DMAA and HEMA polymers.

Polymer Type	Water Content (Q) (%)
MMA-DMAA	
NIP	64.92 ± 2.77
ProMIP	66.91 ± 2.35
NapMIP	67.91 ± 1.96
Literature Value	64.00^{20}
HEMA	
NIP	34.92 ± 0.39
ProMIP	34.41 ± 0.50
NapMIP	34.11 ± 0.54
Literature Value	38.00^{20}

3.3 FTIR

The drug templates did not interfere with the polymerization process with respect to the functional groups observed with Fourier Transform InfraRed (FTIR) spectroscopy. Both imprinted and non imprinted polymers showed similar spectral features. FTIR scans are shown for the MMA-DMAA and HEMA polymers in Figure 3.2. Both types of polymers exhibit similar, but not identical, FTIR patterns. In particular the peak at 1640 cm^{-1} for the MMA-DMAA polymer is absent from HEMA. This peak position suggests that it represents a tertiary amide carbonyl stretch due to the tertiary amide of N,N-dimethyl acrylamide, a functional group not found in HEMA. The stretch at 1735 cm^{-1} is characteristic of ester carbonyls, a group found in both MMA as well as HEMA. The broadness of the band (hydroxyl stretch) at 3440 cm^{-1} indicates a strong hydrogen bonding capability through the hydroxyl groups of the polymers. Scans were run for all polymers, imprinted and non-imprinted. No differences were observed between the spectra for non-imprinted polymers compared to propranolol or naproxen imprinted polymers for either the MMA-DMAA or the HEMA systems, and for this reason the imprinted spectra are not shown in Figure 3.2.

Figure 3.2: Fourier Transform Infrared Spectra for non-imprinted MMA-DMAA and HEMA polymers. Samples were pressed into a KBr pellet and scanned from 4000 to 400 cm^{-1} .

3.4 DSC

Differential Scanning Calorimetry (DSC) experiments were run to determine the glass transition temperature (T_g) and the melting temperature (T_m) of the polymers. Samples were heated at 50°C for 30 minutes, and then raised to 250°C at a rate of 20°C/minute, where they were held for 5 minutes before being cooled back to 50°C at the same rate. The DSC was calibrated with an Indium sample for accuracy. The DSC data (Table 3.3) show that the glass transition temperatures for the MMA-DMAA and HEMA polymers average to 130°C and 96°C, respectively. There are no significant differences between the glass transition temperatures for the imprinted and non-imprinted polymers within each group. The experimental values for both the MMA-DMAA and HEMA polymers correspond to the literature values.²⁰

Melt transitions for these polymers do not occur, but at temperatures, above 275°C, they are found to simply degrade. This was determined through visual analysis following the phase changes of approximately one milligram of polymer upon heating from 20 to 300°C on a melting point apparatus.

Table 3.3: Glass transition temperatures (T_g) for each polymer type using Differential Scanning Calorimetry (DSC).

Polymer Type	T_g Onset ($^{\circ}\text{C}$)
MMA-DMAA	
NIP	128.51 ± 5.52
ProMIP	130.50 ± 2.96
NapMIP	132.65 ± 5.94
Literature Value	$108\text{-}137^{20}$
HEMA	
NIP	95.45 ± 1.84
ProMIP	96.56 ± 2.55
NapMIP	95.56 ± 3.03
Literature Value	102.00^{20}

3.5 XRD

Samples of both MMA-DMAA and HEMA polymers, imprinted and non-imprinted, were examined by powder X-Ray Diffraction spectroscopy (XRD). The data are presented as 2Θ values on the x-axis vs. intensity. The 2Θ value is converted into a d-spacing value using the Braggs law:⁹⁰

$$n\lambda = 2d \sin\Theta \quad (8)$$

Typical spectra are presented in Figure 3.3. Scans were run for all three polymers (Figures 3.3 a, b) of each type (i.e., NIP, ProMIP and NapMIP), and show that there are no differences in crystallinity between imprinted and non-imprinted polymers. These scans were then subjected to profile fitting which compares the experimental values with simulated values and provides an Rwp value, or weighted R-factor, indicating the goodness of the fit. A value less than 10 is considered to be an acceptable fit. The Rwp values are 3.5 and 3.8 were found for the MMA-DMAA and the HEMA polymers respectively. The profile fitting is seen in Figure 3.4 a, b. The 2Θ and d-spacing values obtained from the fits are presented in Table 3.4.

Figure 3.3: Powder X-Ray Diffraction spectra for (a) MMA-DMAA and (b) HEMA polymers.

Figure 3.4: Profile fitting for XRD spectra of (a) MMA-DMAA and (b) HEMA polymers using Topas 5.1 software.

Table 3.4: XRD data for both types of polymers, MMA-DMAA and HEMA, indicating the predominant 2Θ and d-spacing values. Scans were run from 2-37 2Θ and values were calculated to d-spacing values with $n\lambda = 2d \sin\Theta$.

Polymer Type	2Θ	d-spacing (Å)
MMA-DMAA		
Peak 1	11.55	7.5
Peak 2	22.84	3.94
Literature pMMA-DMAA	--	5.9 ⁹¹
Literature pMMA	14, 28 ⁹²	8, 3.2 ⁹²
Literature pDMAA	--	7.8, 5.0 ⁹³
HEMA		
Peak 1	3.87	22.8
Peak 2	8.50	9.7
Peak 3	12.89	6.8
Peak 4	18.13	5.01
Peak 5	30.48	2.98
Literature pHEMA	10, 18, 30, 42 ⁹⁴	5 ⁹⁵

3.6 SEM

Scanning Electron Microscopy (SEM) was performed to try to visualize any changes in polymer morphology that may occur as a result of the imprinting process. The 10 000X magnification images were gold coated for better resolution by avoiding charging effects. SEM images are shown in Figures 3.5 and 3.6 for MMA-DMAA and HEMA polymers, respectively. For the MMA-DMAA polymers, there is an apparent difference between the NIP and ProMIP in the high magnification (10 000X) image, however, the NapMIP is indistinguishable from the non-imprinted polymer. With respect to the HEMA polymers, all three 10 000X magnification images look very similar to each other and also to the NIP of the MMA-DMAA polymers.

a)

MMA-DMAA NIP

b)

MMA-DMAA ProMIP

c)

MMA-DMAA NapMIP

Figure 3.5: Scanning Electron Microscope images of MMA-DMAA polymers (a) NIP, (b) ProMIP and (c) NapMIP. The panels on the left are at 40X magnification, and those on right at 10 000X magnification (with gold coating). Note the presence of pores in the right panel of (b).

a)

HEMA NIP

b)

HEMA ProMIP

c)

HEMA NapMIP

Figure 3.6: Scanning Electron Microscope images of HEMA polymers (a) NIP, (b) ProMIP and (c) NapMIP. The panels on the left are at 40X magnification, and those on right at 10 000X magnification (with gold coating).

3.7 GPC

Gel Permeation Chromatography (GPC) was used to determine the molecular weight and poly dispersity indices (PDI) of the polymers. The only polymer that was soluble in an organic solvent was the MMA-DMAA polymer synthesized in the absence of cross-linker. A 3 mg/mL sample was dissolved in THF and three injection volumes were injected into the column. An absolute molecular weight determination of the resulting bimodal polymer was 47 503 and 30 392 Daltons and PDIs of 1.067 and 1.026 respectively. One possible explanation for the bimodal behaviour is that in the absence of cross-linker, the MMA and DMAA components may have polymerized separately, or at different rates leading to polymers with different molecular weights.

Table 3.5: Absolute molecular weight and PDI values for 3 separate injections of 3 mg/mL of MMA-DMAA polymer synthesized under the same conditions with the exception of cross-linker. The MMA-DMAA polymer with cross-linker, and the HEMA polymer with and without cross-linker would not dissolve in any organic solvent, so GPC data could not be obtained.

Polymer	Peak 1: M_w , Da (PDI)	Peak 2: M_w , Da (PDI)
MMA-DMAA (w/o cross linker)		
100 μL	47 298 (1.094)	33 056 (1.125)
80 μL	47 836 (1.062)	27 117 (0.897)
60 μL	41 376 (1.046)	31 003 (1.057)
Average	47 503 (1.067)	30 392 (1.026)
Standard Deviation	3 584 (0.024)	3 016 (0.117)

3.8 Polymer Washing

Imprinting effectiveness can be determined by assessment of the parameters of template binding affinity (i.e., the equilibrium association or dissociation constant between the ligand drug molecule and the polymer network), capacity of loading (i.e., the maximum ligand bound per mass or volume of polymer), and selectivity (i.e., the ability to differentiate between a specific template ligand and other molecules). Compared to highly cross-linked imprinted networks, weakly cross-linked gels will have most of the template removed before rebinding experiments. Proper template washing procedures and verification of template removal is very important in the analysis of rebinding parameters. Binding characterization in MIPs can be incorrect if post-polymerization washing of template is not verified to be effective.⁴

3.8.1 Conventional Washing Method

After the polymer synthesis with templates, the template and any unreacted products were washed away to yield, in principle, a polymer with cavities that are complementary in shape and size to the template. It is important to remove all the template so that during subsequent uptake (i.e., template rebinding) studies, the polymer can bind a maximum amount of drug from solution. The method that is used in numerous papers^{5, 20, 22, 78, 96-97} is to wash the polymers with distilled water, saline, and distilled water again, each for a period of 2 days, changing the washing solution every four hours. When this washing period is over the final washing solution is tested by UV/Vis spectroscopy to ensure that there is no absorbance at the specific wavelength of the template. This protocol was followed, but when the absorbance of the washing solution

indicated no template remaining, the fluorescence of the washings was monitored on the fluorimeter, which revealed a fluorescent signal from the template.

3.8.2 Altering pH

The washing method was modified and the polymers were washed in a solution of pH3 or pH12 (depending on the drug template) to ensure that the drug would be fully ionized and dissolve in the aqueous washing solution more effectively. These washings still showed significant fluorescence even when collected after 10 – 15 days of washing.

3.8.3 Soxhlet Extraction

The polymers were extracted in a Soxhlet apparatus with refluxing ethanol. The Soxhlet treated polymers showed minimal template fluorescence. Sample polymers from the different washing techniques were dried and applied to a glass slide with double sided tape to check for fluorescence within the solid polymer network after cleaning. The results of the washing tests are presented in Figure 3.7. This study provided confidence that washing the polymers by Soxhlet extraction was the most effective method, and would be the washing method of choice from this point on.

Figure 3.7: Washing results for all (a) propranolol and (b) naproxen imprinted polymers using the water wash, pH-controlled and Soxhlet with ethanol extraction methods. The black solid line indicates a 100 μ M propranolol (a) or naproxen (b) solution in water. All of the other lines represent polymer samples adhered to a glass slide and tested for fluorescence with the front face attachment of the fluorimeter. Non-imprinted polymers were used as a negative control at the baseline.

Figure 3.8: (a) Propranolol in ethanol compared with a propranolol imprinted polymer and (b) naproxen in ethanol compared with a naproxen imprinted polymer.

3.9 Template Environment

Figure 3.8 shows a comparison of the fluorescence spectrum of either template in ethanol and the spectrum of that template within the polymer network. The spectrum for the propranolol imprinted polymer very closely resembles that of the spectrum for propranolol in ethanol, both of which vary in shape from the spectrum of propranolol in water, seen in Figure 3.7. This is an indication that propranolol might be useful as an environment-sensitive fluorescence probe. The naproxen spectra are all very similar to one another, in water, ethanol or within the polymer network.

3.10 Propranolol and Naproxen Uptake

After efficient polymer cleaning to remove all of the drug template molecules, the polymers were tested for rebinding of propranolol. 50 mg samples of ground ProMIP, NapMIP and NIP were each equilibrated, with agitation, in contact with a series of 5 mL propranolol solutions with concentrations ranging from 5 – 100 μM for 72 hours at both room temperature (21°C) and at an elevated temperature (37°C). The fluorescence intensity of the supernatant liquid at equilibrium was determined using a corresponding calibration curve. This intensity was then subtracted from the original fluorescence intensity of the corresponding propranolol solution. The room temperature binding isotherms (Figure 3.9) and the elevated temperature isotherms (Figure 3.10) show a difference in the rebinding of propranolol for the MMA-DMAA and HEMA polymers in panel a and b, respectively. For these isotherm plots, a guide line has been added as a visual aid and does not represent any sort of fit to the data. The data were fit to both the Langmuir (Figures 3.11 and 3.12) and Freundlich (Figures 3.13 and 3.14) binding models to determine the respective binding parameters.

The Langmuir modelling for the majority of the isotherm data resulted in negative values for Γ_{max} , as well as the Langmuir constant, K_L . These negative values indicate that the Langmuir model is inappropriate for these polymer systems, and the calculated K_{id} values inaccurate. In this case, it is more reasonable to use the Freundlich model. Using the Freundlich model, the ProMIP using MMA-DMAA as the polymer backbone was able to rebind propranolol 4-fold greater than the non-imprinted polymer and 5-fold more than inappropriate imprinted polymer (NapMIP) of the same type at room temperature. There do not appear to be any differences between the ProMIP, NapMIP or NIP when the

temperature is elevated to 37°C. Langmuir and Freundlich binding data for propranolol uptake have been summarized in Tables 3.6 and 3.7.

The HEMA polymers exhibit propranolol binding that is very similar among the three polymers tested. Statistically, there are no differences within this group at 21°C and very little difference at the elevated temperature of 37°C. Again, the Langmuir model was found to be inadequate, providing negative $\Gamma_{\max}$ and K_L values for the polymers tested. Hence, we must rely on the Freundlich model. Even when the temperature is raised to 37°C, the HEMA polymers have a lower tendency to uptake propranolol compared to the MMA-DMAA polymers, as demonstrated by the lower K_{id} values. The K_{id} values for the HEMA polymers remain in the low hundreds, where the K_{id} values for the MMA-DMAA polymers are in the low thousands.

The data show that propranolol successfully imprinted the polymer matrix and that there is a significant difference between MMA-DMAA polymers with respect to propranolol uptake, at least at 21°C.

Similar tests were run to test naproxen uptake by the same polymers. Uptake was performed under a controlled pH of 3 to maintain naproxen in its fully non-ionized neutral form at 37°C. These results (Figure 3.15, Tables 3.8 and 3.9) indicated that there were no differences in binding behaviour between the NapMIP and NIP.

The calibration curves (Figure 3.16 and 3.17) for propranolol and naproxen respectively are examples of the fluorescence intensity of the solutions used for the drug uptake experiments. It is not a linear relationship; however, the correlation coefficient is very high when it is fit to a second-order polynomial.

Figure 3.9: Isotherm for propranolol uptake of (a) MMA-DMAA and (b) HEMA polymers at room temperature (21°C).

Figure 3.10: Isotherm for propranolol uptake of (a) MMA-DMAA and (b) HEMA polymers at an elevated temperature (37°C).

Figure 3.11: Langmuir modelling for propranolol uptake of (a) MMA-DMAA and (b) HEMA polymers at room temperature (21°C).

Figure 3.12: Langmuir modelling for propranolol uptake of (a) MMA-DMAA and (b) HEMA polymers at an elevated temperature (37°C).

Figure 3.13: Freundlich modelling for propranolol uptake of (a) MMA-DMAA and (b) HEMA polymers at room temperature (21°C).

Figure 3.14: Freundlich modelling for propranolol uptake of (a) MMA-DMAA and (b) HEMA polymers at an elevated temperature (37°C).

Table 3.6: Binding constants for propranolol uptake with Langmuir model for (a) MMA-DMAA (21°C), (b) MMA-DMAA (37°C), (c) HEMA (21°C), and (d) HEMA (37°C).

a) $R^2 > 0.95$, $\alpha = 0.05$, p-value = 0.002

Polymer Type	$\Gamma_{\max}$, $\mu\text{mol/kg}$	K_L , L/ μmol	K_{id} , L/kg
NIP	49.90	12.15	243
ProMIP	49.19	92.07	1 871
NapMIP	-13.60	-2.095	154

b) $R^2 > 0.78$, $\alpha = 0.05$, p-value = 0.856

Polymer Type	$\Gamma_{\max}$, $\mu\text{mol/kg}$	K_L , L/ μmol	K_{id} , L/kg
NIP	-46.10	-52.90	1 147
ProMIP	-326.80	-162.08	495
NapMIP	-63.69	-72.40	1 136

c) $R^2 > 0.97$, $\alpha = 0.05$, p-value = 0.905

Polymer Type	$\Gamma_{\max}$, $\mu\text{mol/kg}$	K_L , L/ μmol	K_{id} , L/kg
NIP	155.50	96.34	619.5
ProMIP	35.40	15.30	432.3
NapMIP	257.73	332.0	1288

d) $R^2 > 0.81$, $\alpha = 0.05$, p-value = 0.838

Polymer Type	$\Gamma_{\max}$, $\mu\text{mol/kg}$	K_L , L/ μmol	K_{id} , L/kg
NIP	-80.13	-17.13	213.9
ProMIP	83.13	35.59	428.3
NapMIP	-515.46	-50.54	98.11

Table 3.7: Binding constants for propranolol uptake with Freundlich model for (a) MMA-DMAA (21°C), (b) MMA-DMAA (37°C), (c) HEMA (21°C), and (d) HEMA (37°C).

a) $R^2 > 0.93$, $\alpha = 0.05$, p-value = 0.002

Polymer Type	n	K_F , ($\mu\text{mol/g})(\mu\text{M})^{-n}$	n - 1	K_{id} , L/kg
NIP	0.938	0.276	-0.062	250
ProMIP	0.729	1.641	-0.271	1 060
NapMIP	1.0278	0.159	0.028	166

b) $R^2 > 0.86$, $\alpha = 0.05$, p-value = 0.856

Polymer Type	n	K_F , ($\mu\text{mol/g})(\mu\text{M})^{-n}$	n - 1	K_{id} , L/kg
NIP	0.9861	1.244	-0.0139	1 217
ProMIP	1.2839	0.7263	0.2839	1 146
NapMIP	0.8501	1.4679	-0.1499	1 153

c) $R^2 > 0.96$, $\alpha = 0.05$, p-value = 0.905

Polymer Type	n	K_F , ($\mu\text{mol/g})(\mu\text{M})^{-n}$	n - 1	K_{id} , L/kg
NIP	0.7202	0.6122	-0.2798	390
ProMIP	0.8210	0.5499	-0.1790	412
NapMIP	0.5282	0.7418	-0.4718	347

d) $R^2 > 0.88$, $\alpha = 0.05$, p-value = 0.838

Polymer Type	n	K_F , ($\mu\text{mol/g})(\mu\text{M})^{-n}$	n - 1	K_{id} , L/kg
NIP	1.0656	0.2289	0.0656	254
ProMIP	0.8252	0.5019	-0.1748	379
NapMIP	1.2531	0.1502	0.2531	226

3.11 Statistical Data Manipulation

Statistical data was generated with the single factor analysis of variance (ANOVA) test. P-values were determined at an α value of 0.05. Low p-values indicate that at least one group is different from the others, and suggests a statistical difference between imprinted and non-imprinted polymers. High p-values indicate that all groups are the same and there are no differences between imprinted and non-imprinted polymers. In this study, we consider a p-value greater than 0.85 as a high p-value indicating that there are no differences between polymers. This study used the μmol of analyte absorbed per g polymer (q) as the different populations for p-value calculations. The single factor ANOVA test involves one independent variable, in this case the population mean. The test makes the assumptions that the populations are normally distributed, the samples are independent and the variances equal.

Figure 3.15: Isotherm for naproxen uptake under controlled pH(3) of (a) MMA-DMAA and (b) HEMA polymers an elevated temperature (37°C).

Table 3.8: Binding constants for naproxen uptake under controlled pH(3) with Langmuir model for (a) MMA-DMAA (37°C), and (b) HEMA (37°C).

a) $R^2 > 0.59$, $\alpha = 0.05$, p-value = 0.931

Polymer Type	$\Gamma_{\max}$, $\mu\text{mol/kg}$	K_L , L/ μmol	K_{id} , L/kg
NIP	28.80	67.31	2337
NapMIP	23.30	60.14	2581
ProMIP	21.30	45.59	2140

b) $R^2 > 0.60$, $\alpha = 0.05$, p-value = 0.953

Polymer Type	$\Gamma_{\max}$, $\mu\text{mol/kg}$	K_L , L/ μmol	K_{id} , L/kg
NIP	28.30	56.51	1997
NapMIP	21.90	42.92	1960
ProMIP	25.50	49.70	1949

Table 3.9: Binding constants for naproxen uptake under controlled pH(3) with Freundlich model for (a) MMA-DMAA (37°C), and (b) HEMA (37°C).

a) $R^2 > 0.31$, $\alpha = 0.05$, p-value = 0.931

Polymer Type	n	K_F , $(\mu\text{mol/g})(\mu\text{M})^{-n}$	n - 1	K_{id} , L/kg
NIP	0.2401	3.492	-0.7599	734
NapMIP	0.3065	3.132	-0.6935	1026
ProMIP	0.3375	1.973	-0.6625	679

b) $R^2 > 0.35$, $\alpha = 0.05$, p-value = 0.953

Polymer Type	n	K_F , $(\mu\text{mol/g})(\mu\text{M})^{-n}$	n - 1	K_{id} , L/kg
NIP	0.2694	1.264	-0.7306	390
NapMIP	0.3555	1.401	-0.6445	497
ProMIP	0.3180	1.497	-0.6820	500

Figure 3.16: Propranolol calibration curve, solutions from 5 – 100 µM.

Figure 3.17: Naproxen calibration curve (pH3), solutions from 5 – 100 μM .

3.12 Possible Side Reactions

3.12.1 TLC

TLC experiments were performed to evaluate the possibility of the Michael reaction, and these were verified by ^1H -NMR. The TLC experiment involved spotting mixtures of propranolol alone, DMAA alone, and propranolol and DMAA together. All solutions were made by dissolving 100 μL of the components in 2 mL of DCM, subjected to 2.5 minutes of UV light exposure, spotted and eluted with an 85:15 mixture of DCM: acetone. The propranolol and DMAA spotted alone each showed one spot on the plate, whereas the mixture of propranolol and DMAA showed two separate spots corresponding to the two components in the mixture. The two spots indicate that there is not a Michael reaction occurring between propranolol and DMAA.

3.12.2 ^1H -NMR

The ^1H -NMR spectra in Figure 3.18 show that propranolol and DMAA remain as separate molecules within the reaction solution at 1 hour after exposure to 70°C . The reaction mixture shows a combination of peaks for every peak seen in the two individual spectra. There are no additional peaks seen in the combination spectra, and all the peaks can be accounted for from the ^1H -NMR scans of the two components on their own.

Both types of experiments indicated that a side reaction between propranolol and N,N-dimethyl acrylamide would not occur with UV light exposure (TLC) or heat (^1H -NMR) as the driving force.

Figure 3.18: ^1H -NMR of (a) DMAA, (b) propranolol and (c) propranolol + DMAA.

3.13 Pre-Organization

3.13.1 Titration Experiments

Titration experiments were performed to evaluate the complexation of the functional monomer with the template of choice. MAA, the functional monomer, was equilibrated with either propranolol or naproxen templates in chloroform to mimic the non-polar environment in which the polymers are synthesized. By adding small aliquots of monomer to the template solution, possible complexation was monitored by observing the absorbance of the solutions at 294 nm and 274 nm, a wavelength where propranolol and naproxen absorb, respectively, but MAA does not. Titration results seen in Figure 3.19 a and b indicate that the complexation is negligible through this type of measurement.

3.13.2 ^1H -NMR

^1H -NMR experiments were also conducted to determine whether pre-organization was occurring with propranolol and MAA before polymerization. We are most interested in the amine proton peak of propranolol at 8 ppm. Figure 3.20 shows the NMR spectra for 25 mM propranolol solution, 100 mM MAA solution, and the combination of 25 mM propranolol plus 100 mM MAA. These concentrations were used because they reflect the 4:1 ratio of functional monomer: template that has been proven most effective for templating. A 1:1 sample of functional monomer: template was also tested. These data indicate that there is a slight shift upfield of the peak corresponding to the amine proton which suggests that there is an interaction between propranolol and MAA before polymerization.

Figure 3.19: Template titrations for (a) propranolol and (b) naproxen with MAA as the functional monomer. Different total drug concentrations (as indicated in figure legend) were used to determine the different ratios.

MAA

Propranolol

MAA + Propranolol (1:1)

MAA + Propranolol (4:1)

Figure 3.20: ^1H -NMR of (a) MAA, (b) propranolol, (c) MAA + propranolol (1:1), and (d) MAA + propranolol (4:1).

3.14 Timolol Studies

Similar studies to those described above for propranolol and naproxen were performed for MMA-DMAA and HEMA polymers imprinted with timolol. The purpose of these studies was to compare our system of propranolol and naproxen imprinted polymers with polymers that have been imprinted with a molecule that has previously been reported to template very efficiently within these polymer types.

3.14.1 Optical Properties of Timolol

The absorption and emission spectra for timolol are shown in Figure 3.21. Timolol absorbs light at 295 nm and emits light at a $\lambda_{\text{max}} = 412$ nm. The absorbance scan was run with a slit width of 2 nm and scan speed of 240 nm/minute. From the slope of the Beer's Law plot in Figure 3.25, the absorption coefficient, ϵ , of timolol is $9\,700\text{ M}^{-1}\cdot\text{cm}^{-1}$. The fluorescence spectrum used a $100\text{ }\mu\text{M}$ solution of timolol in water, excited at 295 nm, excitation and emission slit widths of 10 nm, and a scan speed of 100 nm/minute.

3.14.2 Timolol Uptake Experiments

The polymers were synthesized in the same manner as the propranolol and naproxen imprinted polymers discussed above, with the exception of the template molecule. These polymers used 0.043 g of timolol to create the 4:1 ratio of monomer to template, which readily dissolved in the monomer system. After synthesis, the polymers were cleaned by Soxhlet extraction in ethanol for 5 days, and tested for fluorescence in

the same manner as the propranolol and naproxen imprinted polymers using the front face attachment of the fluorimeter. No fluorescence was detected from the solid polymer.

Figure 3.22 shows the isotherms for timolol uptake. Because timolol is a weakly fluorescent molecule, the amount of timolol adsorbed to the polymer was measured using an absorbance calibration curve (Figure 3.25), and by subtracting the concentration of the solution at equilibrium at 37°C from the original concentration of the solutions ranging from 5 – 65 μM .

The isotherms for timolol uptake have a curvature that is much different from the propranolol isotherms. The curvature is indicative of a less frequently encountered situation in which previously sorbed molecules lead to a modification of the sorbent which favours further sorption.⁸⁵ However, the curves can still be modelled with Langmuir (Figure 3.23) and Freundlich (Figure 3.24) fittings. Results indicate that the MMA-DMAA timolol imprinted polymer (TimMIP) was able to uptake timolol from solution at a level nearly 2-fold greater than the corresponding NIP. The Langmuir model produced negative values for Γ_{max} and K_L , and therefore this value is representative of Freundlich modelling. These data are summarized in Tables 3.10 and 3.11. Statistics indicate that there is a significant difference for timolol uptake with the TimMIP compared to the NIP for the MMA-DMAA polymer system.

The HEMA polymers could not be modelled with the Langmuir equation; however, the Freundlich model indicated that the TimMIP was able to uptake timolol with a 25-fold increase compared to the HEMA NIP.

It is important to note that the K_{id} values for both MMA-DMAA and HEMA polymers to uptake timolol are significantly lower than those for the propranolol uptake.

However, it can be said that there is statistical difference between the uptake of timolol for the TimMIP and the NIP.

a)

b)

Figure 3.21: (a) Absorbance and (b) emission spectra for 100 μM drug solutions of timolol in water. Maximum absorbance at 295 nm with slit width 2 nm and scan speed 240 nm/minute. Excitation wavelength at 295 nm, $\lambda_{\text{max}} = 412$ nm, excitation and emission slit widths of 10 nm, scan speed of 100 nm/minute.

Figure 3.22: Isotherm for timolol uptake of (a) MMA-DMAA and (b) HEMA polymers at an elevated temperature (37°C).

a)

b)

Figure 3.23: Langmuir modelling for timolol uptake of (a) MMA-DMAA and (b) HEMA polymers at an elevated temperature (37°C).

Figure 3.24: Freundlich modelling for timolol uptake of (a) MMA-DMAA and (b) HEMA polymers at an elevated temperature (37°C).

Figure 3.25: Timolol calibration curve, standard solutions from 5 – 65 µL.

Table 3.10: Binding constants for timolol uptake with (a) Langmuir and (b) Freundlich isotherms for MMA-DMAA polymers at 37°C.

$R^2 > 0.29$, $\alpha = 0.05$, p-value = 0.631

Polymer Type	$\Gamma_{\max}$, $\mu\text{mol/kg}$	K_L , L/ μmol	K_{id} , L/kg
NIP	-2.114×10^3	-72.86	34.46
TimMIP	-347.0	-17.87	51.50

$R^2 > 0.65$, $\alpha = 0.05$, p-value = 0.631

Polymer Type	n	K_F , ($\mu\text{mol/g})(\mu\text{M})^{-n}$	n - 1	K_{id} , L/kg
NIP	1.4067	0.0146	0.4067	28.12
TimMIP	1.9449	0.0127	0.9449	58.01

Table 3.11: Binding constants for timolol uptake with (a) Langmuir and (b) Freundlich isotherms for HEMA polymers at 37°C.

$R^2 > 0.78$, $\alpha = 0.05$, p-value = 0.296

Polymer Type	$\Gamma_{\max}$, $\mu\text{mol/kg}$	K_L , L/ μmol	K_{id} , L/kg
NIP	-80.13	-44.66	19.43
TimMIP	83.13	-30.01	35.45

$R^2 > 0.60$, $\alpha = 0.05$, p-value = 0.296

Polymer Type	n	K_F , ($\mu\text{mol/g})(\mu\text{M})^{-n}$	n - 1	K_{id} , L/kg
NIP	2.5618	0.00026	1.5618	3.22
TimMIP	0.6343	0.1351	-0.3657	75.00

CHAPTER 4

DISCUSSION

4.1 Comparing Polymer Composition and the Influence of Imprinting

It is evident from the numerous characterization studies performed, that the composition of the backbone monomers (the copolymer of MMA-DMAA versus the homopolymer of HEMA) within the polymer network significantly alters certain polymer properties. At the same time, this research has also demonstrated that the imprinting process within hydrogels of one type, either MMA-DMAA or HEMA, shows little, to no effect on the polymer morphology.

4.1.1 Templates

The fact that solid polymers prepared in the presence of templates (propranolol or naproxen) exhibit template fluorescence demonstrates that there is some association of the drug compounds with the polymer networks. The fact that this fluorescence can be washed away shows that the association is non-covalent. We suggest that in most cases the association is probably non-specific. However, we have been able to successfully imprint the MMA-DMAA polymer type with the β -adrenergic drug, propranolol, as the template. We know this through the SEM images of propranolol imprinted MMA-DMAA polymers showing the visible holes/pores within the network, and also through the complexation studies indicating that propranolol will interact with MAA prior to polymerization as well as through the enhanced binding observed for propranolol with its own MIP. The imprinted propranolol polymers, ProMIPs, have the same polymer

characteristics as the non-imprinted polymers, NIPs, within each group (MMA-DMAA or HEMA) with respect to RI, water content, FTIR, DSC, and XRD.

Naproxen has proven to be unsuccessful as an imprint molecule with MAA as the functional monomer. This may be because both naproxen and MAA are acids, and are unlikely to interact strongly enough to allow the templating process to be effective. In order to achieve imprinting with naproxen, the polymerization system should include a functional monomer such as 4-vinylpyridine (4-VYP) as it has been shown to interact with naproxen to a larger extent than MAA.^{45, 74, 76}

4.1.1.1 Side Reactions

Preliminary studies were conducted due to the concern that a Michael addition reaction would be possible between propranolol and DMAA during the polymerization reaction, thereby creating covalent imprinting rather than non-covalent imprinting. In order to determine whether the Michael addition occurs between DMAA and propranolol during the MMA-DMAA polymer synthesis, TLC and ¹H-NMR experiments were performed on appropriate reaction mixtures. The TLC indicated that after propranolol and DMAA were mixed and exposed to the same polymerization conditions as used to prepare the test polymer, two separate species were present on the silica, with retention times corresponding to the individual components after eluting with 85:15 DCM: acetone. In other words, the TLC results suggest there is no reaction between DMAA and propranolol.

To further verify this conclusion, both components were mixed together and heated to 70°C for 1 hour to determine whether a thermal reaction might occur. We were

concerned about a thermal reaction because the overall polymerization of this polymer is a highly exothermic process. The ^1H -NMR spectrum of this mixture was measured before and after the heating process; no new peaks were observed, no peaks from the starting materials were decreased in intensity and none of the peaks from the starting materials were shifted. These results indicate that DMAA and propranolol do not undergo the Michael addition. This means the observed molecular imprinting process is non-covalent in nature.

4.1.1.2 Complexation

The nature of the template and monomers and the polymerization reaction itself determine the quality and performance of the polymer product.⁴⁵ Moreover, the quantity and quality of the molecularly imprinted polymer recognition sites are a direct function of the mechanisms and extent of the monomer-template interactions present in the pre-polymerization mixture.⁴⁵

Titration experiments were performed to measure the change in UV/Vis absorption of the template when increasing aliquots of functional monomer were added to a solution of the former. These experiments were done in chloroform to mimic the non-polar environment that is present during polymerization. They indicate that propranolol and naproxen complexation with MAA is negligible.

^1H -NMR experiments were also conducted. These tests show some evidence that propranolol and MAA do complex prior to polymerization. The secondary amine ($-\text{NH}$) proton at approximately 8 ppm undergoes a slight shift upfield when increasing the concentration of MAA from 0:1 to 1:1 to 4:1 (MAA:propranolol). Propranolol alone has

the -NH peak at 8.65 ppm, the 1:1 complex has a shift upfield to 8.55 ppm, and the 4:1 complex has a further shift upfield to 8.52 ppm. Since the proton on the amino group is exchangeable, its resonance position is subject to some uncertainty at the best of times. It will depend on, for example, concentration, temperature etc. In the present case, however, the observed location of the resonance does vary systematically with the concentration of MAA. While not conclusive, this shift (Figure 3.20) is suggestive of propranolol-MAA complexation.

4.1.2 Polymer Washing

We have demonstrated that the currently used method of cleaning these polymers is inefficient, and results in template molecules remaining in the polymer network. A more effective method of cleaning the polymers has been developed; Soxhlet extraction with ethanol. All three templates used in this study, propranolol, naproxen and timolol are more soluble in ethanol than in water and the ethanol allows the polymers to swell to a higher degree than simply water. Also, the polymers are continuously being washed with clean solvent. The majority of the literature suggests that washing both imprinted and non-imprinted polymers in the series water-saline-water for a period of 7 days with changing the washing solutions 4 times daily is sufficient to remove all unreacted species as well as to wash out the templated species. Our tests on these polymers indicated that there is still a measureable level of template within the polymer network using this washing technique. Through Soxhlet extraction with ethanol for a period of 5 days, we have dramatically decreased the fluorescence within the polymer network. In principle this allows for higher template uptake during binding. Other studies imply that the

current method used in the literature will result in 40-90% of the template being washed away,¹ however, our method results in much more template removal. Although we were unable to quantify the level of removal, the level of fluorescence signal from the solid MIPs after Soxhlet washing is extremely low, suggesting negligible amounts of residual template.

The front-face spectra (i.e., the fluorescence of the solid polymer, Figure 3.7) demonstrate that there is a difference in the spectrum for propranolol in water compared to propranolol trapped within the polymer network. The shape of the curve seen for propranolol in the polymer is the same as propranolol in ethanol solution. This indicates that propranolol's fluorescent state may have some utility as a probe of nano-scale environments. It also shows that the environment experienced by propranolol in both dry, solid polymers resembles that of ethanol either in terms of polarity or hydrogen bonding character or both.

4.1.3 Refractive Index (RI)

The refractive indices were recorded for individual polymer components as well as for polymer mixtures, with and without template components. The purpose was to determine whether the optical properties of the reaction mixtures changed when the templates were added. It was determined that the RI values of all individual polymer components matched the literature values, (Table 3.1) which is a reflection of the purity of these components. It was also determined that upon mixing all polymer components, the template had little to no effect on the RI of the resulting solution, and values for MIPs and NIPs were very similar. This indicates that incorporating templates within the

polymer matrix has no affect on the optical properties of the polymer beyond the increase in system absorbance due to their presence.

4.1.4 Water Content

The water content data are important because they indicate the degree of swelling of the individual polymers. A polymer with a large degree of swelling may not maintain the imprinting specificity as well as a polymer with moderate swelling due to the dislocation of the functional groups of the polymer network away from their original positions.²⁰ A moderate degree of swelling is desirable to allow for the drug templates to penetrate the polymer once swollen, and also for release when the drug needs to break the non-covalent interactions that have formed between itself and the polymer. This is the theoretical advantage of using hydrogels for drug delivery. Highly cross-linked polymers are very rigid, and although they may be efficiently templated, the subsequent drug release is expected to be very slow. Low cross-linked polymers are very flexible, however imprinting is much more relaxed and most binding would be unspecific, releasing the template very quickly and/or realizing only poor and non-specific uptake to begin with. Hydrogels are a compromise between these two extremes.

The 1:1 mixture of MMA and DMAA as the polymer backbone components allows for a greater swelling capacity (by nearly a factor of 2) than the HEMA polymer when exposed to moisture (Table 3.2). The high degree of swelling for the MMA-DMAA polymer causes the network to have large pore sizes which allows for template diffusion into the network to be easier but also disrupts the polymer's initial conformation.²⁰ The HEMA polymer has a lower degree of swelling and thus smaller

pore sizes for template diffusion. However, because of the lower water content values the internal structure conformation of the HEMA polymer will remain more intact upon swelling than that for the swollen MMA-DMAA polymer. Reasons for this difference in swelling could be that water molecules are less likely to interact with the functional groups on the HEMA molecule. The HEMA molecule has 3 hydrogen bondable groups whereas the combination of MMA and DMAA has a total of 4 hydrogen bondable groups. An increase in hydrogen bonding capability results in increased hydration. Thus, the water would interact more favourably with the MMA-DMAA polymer than with the HEMA polymer.

The swelling of the MMA-DMAA polymer is also temperature dependent with the extent of swelling, increasing as temperature decreases.²⁰ At 21°C, the swelling of this polymer type is at its maximum amount, with the largest surface area and the largest extension/expansion of the cavities formed by imprinting. This allows for the template to readily enter the pores and may influence the rebinding of template to the functional monomers and cavities within the polymer. At 37°C the swelling is much less, and thus the surface area is decreased. Generally, the amount of swelling in the HEMA polymer network is independent of temperature.²⁰

With respect to the hydrated non-imprinted polymers, the functional monomer (MAA) groups are expected to be too far apart and disordered (because there is no pre-organization) to form specific binding sites for the templates. However, the imprinted polymers are expected to have MAA groups that are closer and more ordered creating specific binding sites.²⁰

The water content data for imprinted (cleaned via the conventional washing method) and non-imprinted polymers did not differ within either group, MMA-DMAA or HEMA. The imprinting process is one that should create polymers with small spaces within the matrix, which would imply that there is the possibility for imprinted polymers to have a larger capacity for swelling upon exposure to moisture, however, this is not observed.

4.1.5 FTIR

The FTIR data indicates that there is only one difference between the two types of polymers with respect to functional groups. The MMA-DMAA polymer has a second carbonyl stretch that has been shifted to a lower wavenumber (1640 cm^{-1}) due to the tertiary amide of DMAA.⁹⁹ The range for carbonyl groups conjugated to a tertiary amide group is $1680 - 1630\text{ cm}^{-1}$, and our stretch is within this range.⁹⁹ It is also the second carbonyl group which provides an additional capability for the MMA-DMAA polymer to hydrogen bond with the templates. Otherwise, both polymer types, MMA-DMAA and HEMA, have a large hydroxyl (OH) stretch centered at 3440 cm^{-1} , a CH stretch at 2900 cm^{-1} , and a carbonyl (C=O) stretch from the ester linkage at 1735 cm^{-1} (Figure 3.2).^{20, 99} A typical ester linkage, as seen in MMA and HEMA will produce a stretch in the range of $1750 - 1735\text{ cm}^{-1}$, which is the exact location where we see our carbonyl stretch.⁹⁹ These functional groups are all able to interact with the templates via hydrogen bonds, van der Waals forces and/or electrostatic forces such as dipole-dipole interactions. As such, we expect that there should be significant interaction between the polymer and the template molecules.

FTIR scans were preformed for both imprinted and non-imprinted polymers.

Figure 3.2 shows the results for the non-imprinted polymers only because there were no differences between imprinted and non-imprinted polymers with respect to functional groups observed within the matrix. This is expected because the templates were non-covalently bound to the polymer network and did not interfere with the polymerization process. Once they are removed, all of the same functional groups exist in the imprinted and non-imprinted case.

4.1.6 DSC

Differential Scanning Calorimetry (DSC) provided information regarding the glass transition temperatures (T_g) of the two types of polymers. The mechanical properties of amorphous polymers (*vide infra*) change profoundly as the temperature is increased/decreased through the glass transition region.⁸⁰ The T_g onset of polymers can provide information about the miscibility of co-monomers during gel synthesis and interactions involving hydrogen bonding between the chains.²⁰ The T_g for the MMA-DMAA polymer averaged to be 130°C, whereas the T_g for the HEMA polymer averaged to 96°C (Table 3.3). The literature value for MMA-DMAA polymers synthesized by the same method was found to lie in a range between 108-137°C.²⁰ Our value falls within this range. The literature provides a range of 102 – 113°C for the glass transition temperature for the HEMA polymer^{20, 95} and again, our measured value is close to this range.

It has been established that compatible blends of amorphous materials will exhibit a single T_g that is intermediate to the T_g values of the component materials, and the value

of which reflects a relationship to the mixture composition.¹⁰⁰ Pure PDMAA has a T_g between 125 - 135°C,¹⁰¹ where PMMA has a T_g of 100°C.¹⁰² Our value of 130°C indicates that the DMAA component of this co-polymer dominates the value obtained. A reason for the higher T_g value could be because DMAA may act as a bulky, inflexible substituent on the carbon chain and it would prevent rotations about the single bonds in the main chain thereby raising the glass transition temperature.⁸⁰ Another reason for the DMAA component to dominate the T_g of the polymer could be due to differential polymerization rates of the individual components. If the polymerization rate for DMAA is faster than that of MMA, it could be the dominant component of the polymerization product.

A higher T_g value is indicative of lower chain flexibility, whereas a lower T_g value suggests higher flexibility. We may therefore conclude that the MMA-DMAA system is inherently less flexible than the HEMA system.

The melting temperatures for these polymers could not be determined because the polymers do not melt, but at certain temperatures, above 275°C, they simply degrade. This was determined by watching the phase changes of approximately one milligram of polymer with manual observation as the temperature was increased from 20 to 300°C. Neither polymer turned into the liquid phase, but the white crystals began to turn brown in colour.

The glass transition temperatures, T_g , did not vary for imprinted (cleaned via the conventional washing method) and non-imprinted polymers. This is expected because once the templates are removed; the material that remains is similar in composition to the non-imprinted polymer.

4.1.7 XRD

Powder X-Ray Diffraction (XRD) spectroscopy was run for both MMA-DMAA and HEMA polymers equilibrated to the moisture content of air. In both cases the XRD indicates that the polymers are generally semi-crystalline, with few regions of crystallinity. The XRD pattern of a completely amorphous polymer is a broad “halo” that represents the average separation of polymer chains, whereas the XRD pattern of a semi-crystalline polymer is a superposition of crystalline reflections on an amorphous halo.¹⁰³ The semi-crystalline pattern is what was observed from the polymers in this study. The shoulders that are seen in the spectra (Figure 3.3) are representative of the crystalline regions within the generally amorphous polymer. XRD scans from polymers frequently contain poorly resolved peaks.¹⁰⁴ When a solid is amorphous, the interaction between the solid and the X-ray is best described as scattering, rather than diffraction.⁸⁹ However, the scattering is still able to provide information about the internal ordering of the analyzed solid.

The dense regions of ordered macromolecular chains in semi-crystalline hydrogels are thought to make an imprinting effect possible because these macromolecular chains are what provide the network stiffness and stabilization necessary for recognition sites.⁷ The observation of semi-crystallinity in the XRD of the polymers in this study is thus consistent with the properties of other hydrogels used for molecular imprinting.

The pattern of peaks is related to the existence of ordered clusters that have one-, or two-dimensional inter-molecular order.⁹⁰ The most frequently existing inter-molecular distances in these clusters afford these broad peaks and they are commonly considered to

represent short range order in the material. The HEMA polymers are more ordered than the MMA-DMAA polymers as indicated by the observation of a higher number of crystalline regions. The scans for the HEMA polymers show five peaks at 3.87, 8.50, 12.89, 18.13 and 30.48 2Θ , whereas the MMA-DMAA polymers only have two peaks at 11.55 and 22.84 2Θ . These 2Θ values were converted to d-spacing values to provide more applicable information (Table 3.4).

The d-spacing value indicates the distance between polymer chains, or specific atoms within the polymer network. With respect to the MMA-DMAA polymer, the most predominant d-spacing value of 7.9 Å indicates the distance between chains⁹², and 3.94 Å indicates the distance between the heaviest atoms within the polymer⁹², in this case the oxygen atoms. These values correspond closely to a PMMA polymer where the first maximum is situated at 8 Å and is responsible for the packing of long polymer chains, and the second, at 3.2 Å, is mainly related to the effect of atom ordering inside the chains.⁹² They are also similar to a PDMAA polymer which has d-spacings at 7.8 and 5.0 Å.⁹³ However, the literature also provides a value for the 1:1 mixture of MMA: DMAA which is 5.9 Å in the dried state and 7.4 Å in the hydrated state (also providing a water content value of 64 and a T_g of 108°C, which is very similar to our water content value and glass transition temperature for this polymer).⁹¹ Because the literature values for pMMA and pDMAA are quite similar, it makes sense that the d-spacing values for the mixture of the two components is somewhere between those values.

The HEMA polymer has its most dominant d-spacing at 5.01 Å which indicates the distance between its chains, and the other four values could be indicative of atom

ordering. The values obtained for these polymers are very similar to that seen in the literature (Table 3.4).^{94, 105}

This XRD data is consistent with the polymer swelling data, that the HEMA polymer has smaller d-spacings (5 Å), between polymer chains, and also swells to a lower degree when exposed to moisture. The MMA-DMAA system has a larger predominant d-spacing value (7.9 Å, moisture equilibrated), and swells nearly twice as much as HEMA when exposed to moisture. In fact, the more open structure of MMA-DMAA implied by the XRD data may permit better water penetration in this polymer and facilitate the swelling process.

The glass transition temperatures do not reflect the trend observed in the spacing data. It might be expected that the polymer which is more compact, the HEMA polymer, would require a higher temperature before undergoing a phase transition. However, T_g ultimately reflects the mobility of polymer chains which in turn reflects factors such as inter-chain attraction, the presence of pendant groups and cross-linking. The pendant groups on the MMA-DMAA backbone provide greater opportunities for dipole-dipole attraction than in the HEMA case. Greater attractive forces between chains tend to increase T_g . Also, while the HEMA pendant group is longer than either of those in the MMA-DMAA system, it is also more flexible. Flexible pendant groups tend to lower T_g .

Profile fitting involves a procedure to optimize cell parameters, peak profiles, integrated Bragg intensities, and background coefficients. These parameters are refined in order to minimize the weighted R-factor, R_{wp} , used to measure the similarity between the simulated and experimental powder patterns. The profile fitting applied to our measured spectra showed that the 2 visible peaks for the MMA-DMAA polymer

correspond to an Rwp value of 3.5, and the 5 visible peaks for the HEMA polymer correspond to an Rwp value of 3.8. These Rwp values represent the goodness of fit, and a value less than 10 is considered to be an acceptable match. The XRD scans and profile fits are seen in Figures 3.3 and 3.4.

The powder X-Ray Diffraction scans seen in Figure 3.3 show that no differences exist between imprinted and non-imprinted polymers. The addition and removal of a template molecule does not have any measureable effect on the crystallinity of either type of polymer. Imprinted polymers remain generally amorphous, and the imprinting technique does not affect the internal arrangement of the polymer.

4.1.8 GPC

Gel Permeation Chromatography (GPC) was able to provide us only with a rough molecular weight for one of the polymers. Due to a lack of solubility of either MMA-DMAA or HEMA polymer in organic solvents, alterations were made to the measurement method. Both polymers were made in the absence of cross-linker in hopes to provide a rough estimate of the molecular weight of these polymers, and yet, the only one to dissolve was the MMA-DMAA polymer without cross-linking. GPC results indicated that this polymer is composed of two different components, with weights of 47 500 and 30 000 Daltons respectively. These two values could indicate the self polymerization of both the MMA and DMAA portions of this polymer, because without cross-linker, the two components may or may not interact with one another under these particular conditions. Therefore, GPC was of limited value for this study.

4.1.9 SEM

Scanning Electron Microscopy (SEM) revealed few morphology differences between the types of polymers. It is not possible to distinguish one polymer type (MMA-DMAA or HEMA) from the other by this method.

SEM performed at magnifications of 40X and 10 000X (the samples for higher magnification were coated with gold to avoid charging effects) indicates that the imprinting process can be visualized. The MMA-DMAA (Figure 3.5) ProMIP has pores within the network that can be seen at the higher magnification, while the NIP and NapMIP have a similar surface morphology to one another. However, the HEMA polymer samples all appear similar (Figure 3.6). This may indicate that the only polymer which was successfully imprinted was the MMA-DMAA ProMIP.

Figure 3.5 shows that the size of the pores in the ProMIP is on the order of 0.25 to 0.50 μm . The dimensions of the propranolol molecule are on the order of 1.0 to 1.5 nm. Therefore the pores observed must reflect spaces formed by aggregates of propranolol, rather than template sites due to individual molecular imprinting events. This raises the question of whether molecular imprinting is actually occurring in the way the literature suggests; that is, creation of imprint cavities by individual molecules, or clusters of template molecules.

Another possibility is that there may be smaller pores extending from the larger pores that can not be visualized at 10 000X magnification. It would be useful to examine the polymers at a higher magnifications (e.g., 20 000X) to explore this possibility. It would also be useful to be able to measure the surface area of the polymers as a method of determining whether the polymers show signs of imprinting.

4.2 Propranolol Uptake Experiments

Propranolol uptake experiments were modelled to Langmuir and Freundlich isotherms to determine binding data of the system. For the imprinted polymers (i.e., those prepared with the templates), both models showed similar trends with respect to propranolol uptake with correlation coefficients ranging from poor to acceptable. The Langmuir data most often produced negative values for $\Gamma_{\max}$ and K_L , and therefore was unreliable and the data could not be trusted (non-physical parameters were obtained from the fits).¹⁰⁶⁻¹⁰⁹ For this reason, the Freundlich binding data is what will be used for evaluating the performance of the polymers.

The Freundlich model assumes heterogeneous binding. It assumes that there are multiple types of sorption sites acting in parallel, with each site type exhibiting different sorption free energies and total site abundance.⁸⁴ Two types of binding are expected, specific and non-specific, to occur within all of our imprinted polymer systems. The specific binding refers to the binding of the drug molecule in the templated sites, and the non-specific would be any other binding onto the excess functional monomers present within the polymer. We would assume that the non-imprinted polymers would only result in non-specific binding, but the Freundlich model results suggest otherwise. This model consistently works with all polymers so we can assume that there may be more than one type of non-specific binding possible.

From the Freundlich data, the propranolol imprinted MMA-DMAA polymers were able to achieve uptake binding constants that are 4-fold greater than the corresponding non-imprinted polymer and 5-fold greater than the inappropriate imprinted polymer at 21°C, but at 37°C propranolol uptake is invariant among imprinted and non-

imprinted polymers of the MMA-DMAA type. The results at the lower temperature would be described in the literature as an example of successful imprinting. In light of the large pores observed for this particular system in SEM, we question whether the current model used to describe imprinting in the literature is applicable in all cases.

The increased rebinding and lack of variation between MIP and NIP at 37°C is also noteworthy. This can be explained by the fact that as the temperature increases the swelling of the MMA-DMAA polymers decreases.²⁰ At 37°C the swelling of the polymer is moderate. The moderate swelling allows for the imprinted polymer (where, following the conventional view of imprinting, the functional groups are oriented to maximize propranolol uptake) and the non-imprinted polymer (where the functional groups are randomized throughout the polymer) to rebind propranolol with similar effectiveness. Thus, at higher temperatures, the limited swelling results in the functional groups of the NIP being located relatively close to one another and, although not necessarily in the proper orientation, in the proper ratio (4:1) for high binding between functional monomer and template. When the temperature is decreased to 21°C the swelling of the polymer is the greatest. In this case high binding constants were observed for the ProMIP because the templating effect has ensured that the cavities will remain in the proper orientation. There is considerably lower binding with the NIP and inappropriate MIP at this lower temperature because when the swelling is increased for these two polymers, the functional groups that are randomized within these polymers are much further apart, and cannot provide the same binding parameters. Putting it another way, at higher temperature, the more compressed state of the MMA-DMAA polymer (MIP or NIP) enhances template binding because of an abundance of complementary

binding sites which are in close proximity to one another. This enhances non-specific interactions while not dramatically altering the effectiveness of specific binding to template sites which in turn masks differences between MIP and NIP rebinding behaviour. At the lower temperature, MMA-DMAA polymers are more swollen which weakens the non-specific interactions due to spatial displacement of the randomly distributed functional monomer groups, responsible for non-specific binding. At the same time, template sites, which are likely to be associated with more crystalline domains within the polymer network, are less distorted by swelling and therefore there is less impact on their binding ability. It is interesting to note in this context that the equilibrium binding constant of the ProMIP at 21°C is very similar to the three values observed at 37°C.

Propranolol uptake was also consistent among imprinted and non-imprinted HEMA type polymers at both temperatures, 21 and 37°C, and similar to the equilibrium binding constants for naproxen with the MMA-DMAA NIP and NapMIP at 37°C. The reason for the lack of difference between HEMA polymers at 21 and 37°C is that the HEMA polymers are minimally affected by temperature change, swelling to essentially the same extent in both temperature environments. Due to their lower swelling ability (Table 3.2) the HEMA polymers are less hydrated than the MMA-DMAA polymers. This lower hydration may inhibit template diffusion into the imprinted pores. For this reason, the template sites are not a factor in the uptake process and all binding that is occurring is non-specific and somewhat weak.

The highest correlation coefficients observed with the Freundlich analysis are for the NIPs which solely participate in non-specific binding, whereas the ProMIP and

NapMIP exhibit, in theory, both specific and non-specific types of binding. The good Freundlich correlations observed for NIPs indicate that they, in fact, also participate in more than one type of binding, which may be more than one type of non-specific binding.

In all cases relative K_{id} values were calculated to determine the amount of bound sorbate relative to the amount left unbound in solution. The relative (%) K_{id} values were also calculated with the following equation:

$$\text{Relative (\%) } K_{id} = ((K_{id} \text{ MIP} - K_{id} \text{ NIP}) / K_{id} \text{ NIP}) * 100 \quad (9)$$

The relative increase in K_{id} according to the Freundlich model was 76.4% for propranolol uptake at 21°C with the MMA-DMAA polymer and 32.9% for propranolol uptake at 37°C with the HEMA polymer. These are the only two instances where the ProMIP was better able to uptake the drug from solution than the corresponding NIP.

4.3 Naproxen Uptake Experiments

Originally the NapMIP was created to determine the selectivity of these polymers. If both polymers were successfully imprinted, we would have been able to compare each imprinted polymer for template selectivity by comparing the uptake of the inappropriate imprinted polymer with the template of interest for uptake (i.e. the ability of the NapMIP to uptake propranolol from solution and vice versa). However, since naproxen shows no signs of imprinting, we conclude that we have essentially created two NIPs in this case.

The results in Figure 3.15 and Tables 3.8 and 3.9 demonstrate that there are no statistical differences in the binding behaviour of naproxen imprinted polymers and non-imprinted polymers. Also, during the propranolol uptake studies, the naproxen imprinted polymer consistently resulted in similar K_{id} values as the NIP. Further, the SEM images (Figures 3.5 and 3.6) show no apparent pores/holes at the 10 000X magnification for the polymers made in the presence of naproxen. Due to the fact that naproxen (a carboxylic acid) and MAA (another carboxylic acid) are not good template and monomer matches, it can be concluded that a naproxen imprinted polymer was not made. It would probably be better to use a functional monomer to such as 4-vinylpyridine (hydrogen bond acceptor) to imprint naproxen.^{45, 74, 76} Naproxen uptake experiments were performed under a controlled pH of 3 because it was proposed that there would be a better chance of template binding if naproxen was fully protonated (i.e. non-ionized), the protonated state being the same one used for naproxen polymer imprinting during polymerization.

Although the Langmuir treatment for naproxen rebinding did provide results with physical meaning, we prefer to focus our discussion on the Freundlich parameters because none of the other Langmuir fits gave physically meaningful outcomes. The

Freundlich K_{id} values for naproxen are comparable to those found for binding of propranolol to the MMA-DMAA NIP and NapMIP at 21°C and those for propranolol binding to all HEMA systems. We propose that these values, on the order of $\sim 10^2$, reflect non-specific binding of either template to either polymer type.

4.4 Timolol Imprinting

Timolol imprinting was performed to compare the success of our imprinting with literature reports. Timolol is a small drug molecule that has been reported to template both of the polymer systems under consideration here. By imprinting both the MMA-DMAA and HEMA polymer systems with timolol, and following the method of Hiritani et al.²⁰ (which is also the method followed in this research), it should be possible to draw comparisons amongst the small drug molecules that were tested.

4.4.1 Timolol Properties

Timolol maleate salt is able to absorb light at 295 nm, but has very low fluorescence quantum yield. It does fluoresce, however, with a $\lambda_{\text{max}} = 412$ nm. The reported fluorescence quantum yields for propranolol, naproxen and timolol are 0.25¹¹⁰, 0.40¹¹¹ and 0.027¹¹², respectively. The quantum yield for timolol is lower by a factor of 10. For this reason fluorescence was only used to determine the amount of timolol residing within the solid polymer network after cleaning. Absorption spectroscopy was used for quantification of rebinding.

4.4.2 Timolol Uptake Experiments

These studies were only performed at the elevated temperature of 37°C, with shaking as per Hiritani's method.²⁰ The uptake results for the MMA-DMAA polymer system indicate that the MMA-DMAA timolol imprinted polymer was able to uptake timolol more efficiently than the non-imprinted polymer by a modest factor of 2. This data was fit to both isotherms, but the Langmuir isotherm produced negative values and

will be disregarded. The HEMA polymer was also able to bind timolol more effectively, by a factor of 25, based solely on the Freundlich model (Tables 3.10 and 3.11). The literature suggests that the HEMA polymer imprinted with timolol is more effective at timolol uptake than the MMA-DMAA polymer,²⁰ which is the trend seen in this study. Also, the isotherms are much different in shape than the propranolol and naproxen isotherms. The timolol isotherms display an upward curvature (Figure 3.22) which is indicative of previously sorbed molecules leading to a modification of the sorbent which favours further sorption.⁸⁵ The relative percent increase in timolol uptake is 51.5 and 95.7 for MMA-DMAA and HEMA polymers respectively. However, the relative percent increase in the literature is higher for the MMA-DMAA polymer (90) than the HEMA polymer (39),²⁰ but in this study, we see the reverse effect.

Timolol has been used extensively in the literature because it is a molecule that has been reported to template efficiently. However, the K_{id} values obtained in this study are much lower for timolol uptake than for propranolol and naproxen. This being said, timolol may be a molecule that templates well, but its efficiency of uptake is greatly decreased from the other two drug molecules tested here.

4.5 Other Applications for MIPs

Molecularly imprinted polymers have also been templated with a single isomer of an enantiomeric drug. Once washed, these can be used to efficiently separate mixtures of racemic drugs. With respect to propranolol, the S isomer is roughly 100 times more physiologically active than the R isomer for treatment of hypertension and myocardial infarctions.⁷¹ For this reason it is useful to be able to separate the mixtures. The production cost of a single pure isomer is extremely high compared to that for the racemate because of the difficulty pharmaceutical companies have separating the two enantiomers. In principle, MIPs can exert a subtle molecular recognition which can discriminate one enantiomer from the other. MIPs may therefore find application as a much cheaper and more efficient separation technique. Studies have been performed that use MIPs as separation media for High Performance Liquid Chromatography (HPLC) and Thin Layer Chromatography (TLC) and the results show that the MIPs are able to efficiently and effectively separate the two isomers.^{74, 113}

The HPLC experiments used (S)-propranolol imprinted polymers that had been dried and ground as the stationary phase. A variety of structurally similar molecules, as well as racemic propranolol were used as test analytes. These structurally similar molecules included pindolol, alprenolol, oxprenolol, befnolol, bupranolol, bunitrolol, butetolol and atenolol. These studies were done to determine the selectivity of the imprinted polymer for (S)-propranolol as well as the specificity for propranolol over other drug molecules. This method provided a chiral separation factor of 2.12 and a selectivity factor of 4.21.⁷⁴ These results were the most promising in the study, with analogous tests with other drug molecules showing poorer separation and selectivity.

The experiments involving TLC used ground, hydrated polymers that were imprinted with (+)-ephedrine as the main component of the plate. The racemic mixtures of the drugs (propranolol included) were dissolved in methanol, and 1 μ L aliquots of these solutions applied to the plate. The results indicated that this method of separation was able to produce a chiral separation factor of 1.30 for one isomer over the other.¹¹³

Molecularly imprinted polymers therefore show promise as a tool for separating isomers from racemic drug mixtures, and may be of much interest for pharmaceutical companies as an easier method of separating the two forms, making the drugs more effective with a smaller dose and more cost effective to produce.

CHAPTER 5

CONCLUSION

Two types of polymers were synthesized and used for this study; MMA-DMAA and HEMA. These polymers differ with respect to their backbone components, which ultimately affects their properties and morphology. The two types of polymers differ with respect to their water content, FTIR, DSC, and XRD characteristics.

The imprinting process of incorporating a template molecule into the solution prior to polymerization has little effect on the properties of either type of polymer. Imprinting with propranolol or naproxen does not affect the water content, FTIR, DSC, XRD or RI characteristics within the MMA-DMAA or HEMA polymer type. However, differences can be seen through SEM, where pores are visible for the MMA-DMAA ProMIP at very high (10 000X) magnification. The dimensions of these pores (~ 0.1 to $0.5 \mu\text{m}$) rule out the possibility that they are imprint sites due to single template molecules as the imprinting literature would normally suggest.

Template rebinding experiments with the various NIPs and MIPs were carried out. The Langmuir and Freundlich isotherm models were used to describe the adsorption experiments. In many cases, the Langmuir model afforded physically meaningless results. The Langmuir model assumes homogeneous binding, so we conclude that in most, if not all of our systems, even for the NIPs that the template binding observed is not homogeneous. The imprinted polymers are expected to display heterogeneous binding (both specific and non-specific) and that is supported by the Freundlich outcomes we have determined.

There are differences observed between cleaned imprinted and non-imprinted polymers with respect to propranolol uptake. The average K_{id} value for propranolol across all polymer types (excluding the case of MMA-DMAA at 37°C) is ~ 300 L/kg. For the MMA-DMAA polymer template with propranolol and tested at 21°C, the K_{id} is significantly higher at 1060 L/kg. This suggests that there is some sort of imprinting process going on in this system. The MMA-DMAA propranolol results at 37°C are somewhat difficult to understand. Our current explanation rests on the fact that MMA-DMAA is more compact at higher temperatures and this (i) enhances non-specific binding and (ii) masks differences between the effects of non-specific and specific binding on uptake of the template. However, we must point out that this explanation is speculative.

Many experiments were conducted to determine the suitability of the chosen templates to template the functional monomer (MAA) used in polymer synthesis in this study. It has been determined that propranolol and MAA are a suitable match, however, naproxen and MAA are less likely to complex. A more suitable functional monomer for naproxen would be 4-vinylpyridine.

By comparing our templates with one that has been reported to imprint successfully (timolol) we are able to determine the success of our imprinting and polymer synthesis. However, the observed binding constants in all cases are modest at best. This is especially true in the case of timolol uptake. This study has determined K_{id} values for propranolol uptake of ~ 1000 L/kg for MMA-DMAA imprinted polymers, and ~ 270 L/kg for the HEMA polymers. Naproxen uptake was also in the range of 500 to 1000

L/kg based on the Freundlich analysis. Timolol binding was much less efficient with K_{id} ranging from 50 – 75 L/kg.

By comparing propranolol and naproxen imprinting with timolol imprinting, we can conclude that the imprinting process isn't as simple and clear cut as it appears in the literature. It is possible that instead of imprinting individual template molecules, we are actually imprinting clusters of molecules.

Initially we had planned to study the release of template drugs from partially cross-linked hydrogels; however, we were unable to experiment with the release behaviour due to time constraints.

CHAPTER 6

FUTURE DIRECTIONS

The present study indicates that by a careful modification of the backbone components of the polymers, the uptake effectiveness can be altered. Encapsulating a drug molecule within a polymer network may have many advantages for effective drug delivery in the future. The existing synthetic strategies may require some alteration to optimize the potential behind this idea.

Future experiments to continue improving the idea behind polymeric drug delivery vehicles include:

- Determine whether molecular imprinting within hydrogels works in practice as it does in theory. That being the imprinting of template molecules instead of template clusters.
- Increase the magnification of the SEM images to determine whether there are smaller pores within the polymer network.
- Use Atomic Force Microscopy (AFM) or Transmission Electron Microscopy (TEM) to determine the surface area of the polymers and make a comparison between MIPs and NIPs. Also, we could add another type of guest as a fluorescent probe which could be visualized by confocal or fluorescence microscopy.
- Optimize the uptake conditions of the imprinted polymers.
- Determine the binding parameters for naproxen uptake at room temperature to compare with the trends seen with propranolol.

- Determine release conditions which are suitable for drug delivery. These investigations should include release into aqueous environments under different temperatures and pHs, to mimic the conditions within the human body.
- Study the same polymer characteristics of the timolol imprinted polymer and compare to those already measured in this study. This would determine if a known imprintable template affects any of the factors previously mentioned.
- Change the functional monomer to 4-VYP in the naproxen imprinted polymer. This would determine if the uptake capabilities of that polymer are comparable to those using MAA.
- Compare the characteristics of the naproxen and 4-VYP polymer to those already studied to determine how changing the functional monomer affects the polymer as a whole. The functional monomer within the polymer only accounts for approximately 1 mol% of the entire polymer, however, its effects could be significant.

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